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Evaluation of Microalgae Utilization as an Ingredient for Broiler Feeding: A Meta-analysis

Giovani Giovani [1], Raihani Indah Kusuma [1], M. Sulaiman Daulai [1], Arif Darmawan [1], Muhammad Ridla [1], Hasliza Abu Hassim [2], Nor Dini Rusli [3], Yuan-Yu Lin [4], Agung Irawan [5] and Anuraga Jayanegara [1] *

[1]Department of Nutrition and Feed Technology, IPB University, Bogor 16680, Indonesia
[2]Department of Veterinary Medicine, University Putra Malaysia, Selangor 45000, Malaysia
[3]Department of Agriculture Science, Faculty of Agro-based Industry, University Malaysia Kelantan, Jeli Campus, Jeli 17600, Malaysia
[4]Department of Animal Science and Technology, National Taiwan University, Taipei 10607, Taiwan
[5]Vocational Program of Animal Husbandry, Universitas Sebelas Maret, Surakarta 57126 Indonesia
***Corresponding author:** anuraga.jayanegara@gmail.com

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ABSTRACT

This meta-analysis evaluated the effects of dietary inclusion of microalgae on performance, health and quality of broiler chickens. A total of 18 journal articles obtained from the Scopus search engine were used as the database. Data of production performance, internal organs, blood hematological and serum metabolites, and fatty acid profiles in breast muscle were analyzed using linear mixed model methodology. Elevating microalgae levels in the diets did not affect feed intake, daily gain, organ weights, and health parameters of the blood. Dietary levels of microalgae had positive linear relationship ($P<0.05$) with C22:5n-3, C22:6n-3, and total omega-3 fatty acids levels in the breast meat of broilers while negatively affected ($P<0.05$) certain types of n-6 fatty acids. According to the type of microalgae, supplementing brown microalgae decreased the omega-6/omega-3 ratio ($P<0.05$), but no effects were found for the daily weight gain and feed conversion ratio (FCR) in broilers. In conclusion, microalgae supplementation in diet of broilers could be a promising approach to improve fatty acid profiles of the meat by elevating the omega 3 fatty acids while decreasing the omega 6, thus offers health benefits for human. The supplementation only limitedly affects production performance, internal organs and blood profiles of broilers.

Key words: Broiler meat, Fatty acid, Meta-analysis, Microalgae.

INTRODUCTION

Efforts to improve production efficiency and product quality have been the main topic of research for broiler nutritionist for decades. This includes assessing potential use of various feed ingredient posing nutritional and functional benefits for broiler chickens, such as microalgae. Microalgae are recognized as single-cell proteins (SCP) due to their high protein content and well-balanced amino acid profile. Certain species, including *Spirulina platensis* and *Chlorella vulgaris*, contain up to 60% protein on a dry matter basis (Nerom et al. 2024). Microalgae are also essential sources of vitamins, including A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid, and pantothenic acid. Macroalgae also contains functional carbohydrates in the form of starch, glucose, sugar, and other polysaccharides. Furthermore, microalgae contain significant levels of omega-3 fatty acids and carotenoids, which have been associated with enhanced immune function and improved broiler meat quality (Dinalli et al. 2024). Such nutritional profile has sparked growing interest to be used as a feed ingredient for broiler chickens. The nutritional composition of microalgae is largely determined by their capacity to capture and convert solar energy into biomass, primarily consisting of proteins, lipids, carbohydrates, and pigments (Alavianghavanini et al. 2024). This indicates that microalgae have great potential as an alternative feed ingredient, particularly as a source of plant-based protein.

The inclusion of microalgae in broiler diets has been shown to improve growth performance and modify fatty acid composition (Zanaty et al. 2024). Inclusion of microalgae in the rations may also increase the percentage of internal organs that produce valuable substances for the

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broiler's immune system (El-Kaiaty et al. 2022). Furthermore, adding microalgae to feed can stimulate antioxidant activity (Mavrommatis et al. 2023), which is important for improving livestock health and immune systems. This shows that microalgae have been extensively researched and have shown many positive impacts as a feed ingredient, especially for broilers. However, despite some potential beneficial effects, investigation of microalgae feeding on broilers has generated inconsistent results on production performance, internal organs, blood hematological and metabolite profiles and fatty acid compositions in the muscle.

Utilizing microalgae as feed is expected to become an alternative renewable natural resource with high biodiversity. Considering their species diversity and various levels when being fed to broilers, therefore, evaluating microalgae as a supplemental feed ingredient requires a meta-analysis to understand their effects. Meta-analysis provides a robust statistical approach to synthesizing data from multiple studies, identifying sources of heterogeneity, and establishing more reliable conclusions regarding the impact of microalgae-based diets (Ahmad et al. 2024). Therefore, in this study we aimed to evaluate the effects of dietary type and levels of microalgae as feed ingredients for broilers by integrating relevant scientific articles using a meta-analysis method.

MATERIALS AND METHODS

Database development

Data were collected by searching for peer-reviewed journals through the Scopus platform using the keywords “microalgae” and “broiler“. A total of 68 potential articles published from 2013 to 2023 were retrieved. After screening the abstracts, 42 articles were selected. Following the full-text review, 26 articles remained. The selected articles had to specify the microalgae species used in broiler rations. Consequently, a total of 18 articles were deemed adequate for use in the database. Table 1 presents the articles used in the meta-analysis of microalgae in broiler feeding that included information on broiler strain, duration of experiment, microalgae source, color, and level. The study selection process is schematically presented in Fig. 1.

Parameters integrated in the database were categorized into four groups, i.e., production performance, internal organs, hematology and serum metabolites, and fatty acid profiles in broiler breast meat. The production performance parameters included body weight gain (g), daily weight gain (g/d), feed intake (g), daily feed intake (g/d), and feed conversion ratio (FCR). The internal organ parameters consisted of the pancreas (%), proventriculus (%), heart (%), liver (%), spleen (%), gizzard (%), bursa of Fabricius (%), breast meat (%), thigh meat (%), duodenum (cm/kg),

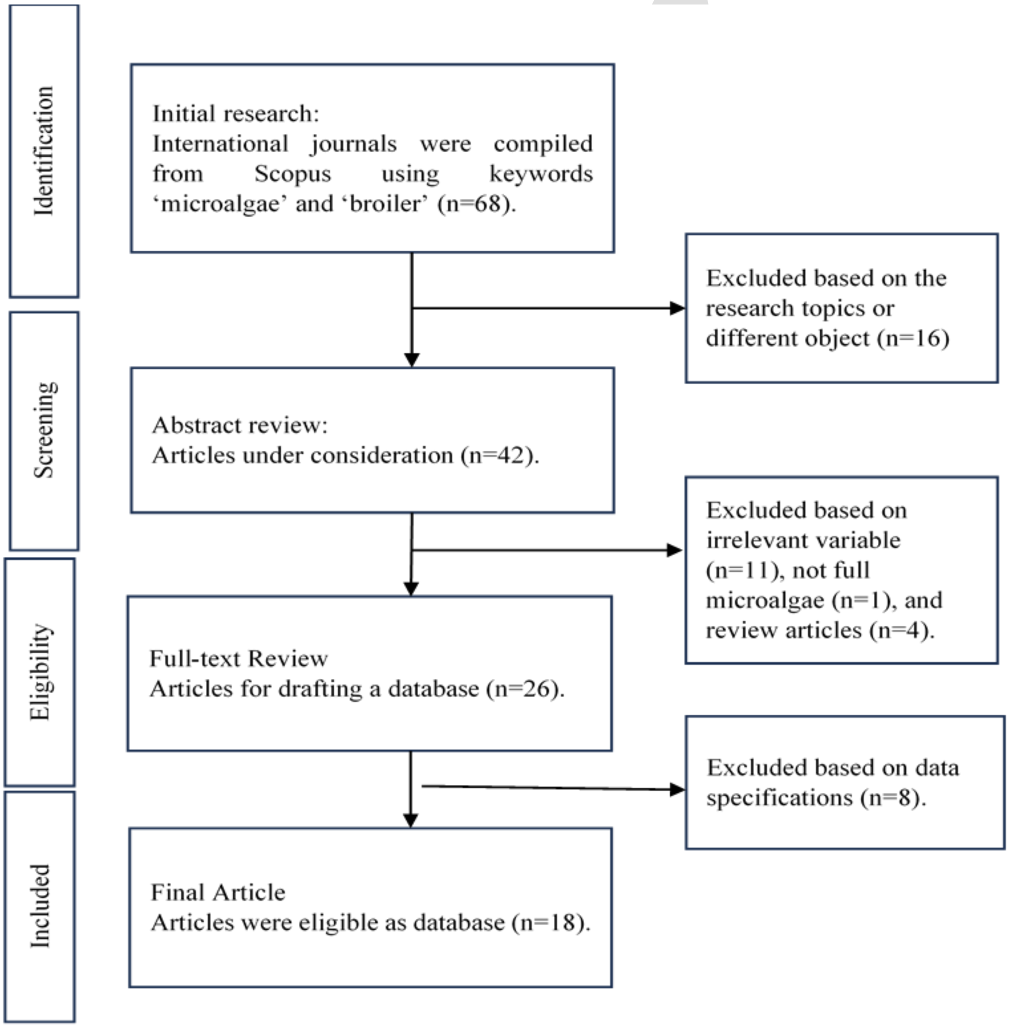

Fig. 1: Study selection process in the meta-analysis of microalgae feeding on broiler.

Table 1: Articles used in the meta-analysis of microalgae in broiler feeding

No	Article	Broiler strain	Final days	Microalgae source	Color	Level (%)
1	Swati et al. (2022)	Unspecified	56	Spirulina sp.; Chlorella sp.	Green	0; 2.5; 5
2	Lee et al. (2023)	Ross 308	130	Chlorella sp.	Green	0; 0.5
3	Yan & Kim (2013)	Ross 308	35	Schizochytrium sp.	Brown	0; 0.1; 0.2
4	Hassan et al. (2022)	Fayoumi	56	Spirulina sp.	Green	0; 0.25; 0.5; 1
5	El-Bahr et al. (2020)	Cobb 500	32	Chlorella sp.; Spirulina sp.; Amphora sp.	Green; Brown	0; 0.1
6	Moran et al. (2018)	Ross 308	42	Aurantiochytrium sp.	Brown	0; 0.5; 2.5; 5
7	Semenova et al. (2021)	Cobb 500	38	Chlorella sp.	Green	0; 0.5
8	Šefcová et al. (2021)	Ross 308	30	Tysochrysis sp.; Tetraselmis sp.; Porphyridium sp.;	Brown; Green; Red	0; 0.2
9	Jeon et al. (2022)	Ross 308	35	Schizochytrium sp.	Brown	0; 2
10	Hajati et al. (2021)	Ross 308	28	Spirulina sp.	Green	0; 2.5; 3.5
11	Mishra et al. (2023)	Cobb 500	35	Spirulina sp.	Green	0; 3
12	Sun et al. (2021)	Cobb 500	14	Desmodesmus sp.	Green	0; 5
13	Khalilnia et al. (2023)	Ross 308	42	Spirulina sp.	Green	0; 0.02; 0.03
14	El-Bahr et al. (2021)	Ross 308	35	Amphora sp.	Brown	0; 0.1
15	Chang et al. (2021)	Arbor Acres	30	Spirulina sp.	Green	0; 0.05; 0.1
16	Long et al. (2018)	Arbor Acres	42	Schizochytrium sp.	Brown	0; 1; 2
17	Levine et al. (2018)	Cobb 500	28	Euglena sp.	Green	0; 0.005; 0.01; 0.02
18	Fuentes et al. (2023)	Cobb 500	42	Spirulina sp.	Green	0; 0.25; 0.5; 1

jejunum (cm/kg), and ileum (cm/kg). The hematology and serum metabolite parameters included red blood cell (RBC) count (10^6/µL), hemoglobin (g/dL), mean corpuscular volume (MCV) (fL), mean corpuscular hemoglobin (MCH) (pg), white blood cell (WBC) count (10^3/µL), lymphocytes (%), monocytes (%), basophils (%), eosinophils (%), heterophils (%), triglycerides (mg/dL), glucose (mg/dL), and cholesterol (mg/dL). The fatty acid content profiles of breast meat consisted of C14:0 (myristic acid) (%), C16:0 (palmitic acid) (%), C16:1n-7 (palmitoleic acid) (%), C18:0 (stearic acid) (%), C18:1n-9 (oleic acid) (%), C18:2n-6 (linoleic acid) (%), C18:3n-6 (gamma-linolenic acid) (%), C18:3n-3 (alpha-linolenic acid) (%), C18:4n-3 (stearidonic acid) (%), C20:2n-6 (eicosadienoic acid) (%), C20:3n-6 (dihomo-gamma-linolenic acid) (%), C20:4n-6 (arachidonic acid) (%), C20:3n-3 (eicosatrienoic acid) (%), C22:5n-3 (docosapentaenoic acid, DPA) (%), C20:5n-3 (eicosapentaenoic acid, EPA) (%), and C22:6n-3 (docosahexaenoic acid, DHA) (%).

Statistical Analysis

The present meta-analysis employed a mixed model methodology as described by Sauvant et al. (2008). The statistical analysis was performed using the PROC MIXED method of SAS software. In the analysis, the studies were considered as random effects, while the microalgae levels and types were considered as fixed effects. The levels of microalgae were taken as the continuous predictor variable and were analyzed using the following model:

$$Y_{ij} = A_0 + A_1X_{ij} + A_2X_{ij}^2 + P_i + a_iX_{ij} + e_{ij}$$

where Y_{ij}: Response variable (dependent), A_0: Total intercept from all experiments, A_1: Linear regression constant Y on X, A_2: Quadratic regression constant Y on X, X_{ij}: Value of continuous forecaster variable (levels of microalgae), P_i: Random effect from study i, a_i: Random effect from study i on regression constant Y on X, and e_{ij}: residual error. The study statistical model relied on the P-values. A variable was deemed significant if its P-value was less than 0.05. The effect was tended to be significant if the P-value was between 0.05 and 0.1.

In addition. data analysis of the effect of types of microalgae on the response variable used the following statistical model:

$$Y_{ij} = \boldsymbol{\mu} + P_i + \boldsymbol{\tau}_j + e_{ij}$$

where Y_{ij}: Response variable (dependent), µ: Overall mean, P_i: Random effect from the study, τ_j: Fixed effect of various microalgae types, and e_{ij}: Study error. When there was significant effect for a particular parameter (P<0.05), the Tukey's test was employed to compare among different treatment means. The interaction between the levels and types of microalgae was also tested by combining both models above.

RESULTS

Supplementation of microalgae at various levels had no significant effects on the production performance parameters (body weight gain, daily weight gain, feed intake, daily feed intake, and FCR) of broiler chickens (Table 2). The supplementation of microalgae significantly increased spleen weight (P<0.001) but did not affect other internal organ parameters such as pancreas, proventriculus, heart, liver, gizzard, bursa of fabricius, breast meat, thigh meat, duodenum, jejunum, and ileum (Table 2). All blood hematological (RBC, hemoglobin, MCV, MCH, WBC, lymphocytes, monocytes, basophils, eosinophils, and heterophils) and serum metabolite parameters (triglycerides, glucose, and cholesterol) were not affected by dietary supplementation of microalgae (Table 3). Microalgae feeding elevated (P<0.05) several omega-3 fatty acid profiles (C22:5n-3, C22:6n-3, and total omega-3) in breast meat of broiler chickens, while decreased the omega-6 fatty acids (Table 4).

Concerning the different sources of microalgae, such differences mostly did not have any significant effects on production performance and internal organs, except pancreas (Table 5), hematological and serum metabolites (Table 6), and breast meat fatty acid profiles (Table 7). However, supplementing brown microalgae decreased (P<0.05) the omega-6/omega-3 ratio in the breast muscle.

Table 2: Effects of dietary levels of microalgae feeding on production performance and organ weight of broilers

Parameter	Unit	N	Model	Intercept	SE Intercept	Slope	SE Slope	P-value	L×C
Production Performance									
Body Weight Gain	g	51	L	1804	217	1.99	9.38	0.834	0.440
Average Daily Gain	g/d	13	L	84.1	141	1.96	0.856	0.056	0.487
Feed Intake	g	32	L	3672	174490	-1.34	39.7	0.973	0.803
Daily Feed Intake	g/d	16	L	122	220	1.32	1.09	0.259	0.100
FCR		50	L	1.70	0.081	-0.029	0.018	0.123	0.834
Organ Weight									
Pancreas	%	6	L	0.207	0.028	0.003	0.023	0.891	na
Proventriculus	%	9	L	0.540	0.155	-0.005	0.016	0.757	na
Heart	%	17	L	0.716	0.068	0.012	0.011	0.313	0.750
Liver	%	22	L	2.84	0.380	0.012	0.047	0.800	0.131
Spleen	%	17	L	0.337	0.151	0.304	0.052	<0.001	0.017
Gizzard	%	16	L	2.17	0.192	-0.066	0.041	0.140	0.306
Bursa of Fabricius	%	10	L	0.163	0.063	-0.005	0.006	0.463	0.299
Breast Meat	%	12	L	21.2	5.66	0.520	0.319	0.150	na
Thigh Meat	%	8	L	10.4	4.39	0.103	0.141	0.505	na
Duodenum	cm/kg	6	L	14.8	0.494	0.345	0.377	0.428	na
Jejunum	cm/kg	9	L	12.6	0.591	-0.279	0.552	0.635	na
Ileum	cm/kg	6	L	8.22	2.11	0.206	0.160	0.288	na

P≤0.01: highly significant, P≤0.05: significant, P>0.05: not significant, N: number of data points, L: linear, Intercept: average value of the response parameter when the microalgae level is zero, Slope: gradient value, FCR: feed conversion rate, LxC: Level x Color.

Table 3: Effects of microalgae feeding on blood hematological and some serum metabolites of broilers

Parameter	Unit	N	Model	Intercept	SE Intercept	Slope	SE Slope	P-value	L×C
Hematological									
RBC	10^6/μL	16	L	3.08	0.534	-0.036	0.035	0.329	0.287
Hemoglobin	g/dL	13	L	12.3	3.72	-0.220	0.219	0.340	0.274
MCV	fL	13	L	118	36.1	0.103	0.892	0.911	0.002
MCH	Pg	13	L	25.7	16.2	-0.148	0.198	0.472	0.688
WBC	10^3/μL	13	L	20.0	9.29	-0.075	0.290	0.801	0.042
Lymphocytes	%	16	L	52.9	17.0	-2.00	1.25	0.137	0.242
Monocytes	%	13	L	11.4	11.1	0.342	1.64	0.840	0.956
Basophils	%	9	L	3.16	5.01	-0.042	1.55	0.979	0.346
Eosinophils	%	9	L	0.442	0.109	0.042	0.032	0.233	0.094
Heterophils	%	9	L	9.93	6.95	2.07	1.22	0.139	0.113
Serum metabolites									
Triglycerides	mg/dL	14	L	56.9	29.7	-0.115	0.713	0.875	0.360
Glucose	mg/dL	14	L	67.1	14.5	-1.55	6.71	0.822	0.026
Cholesterol	mg/dL	10	L	285	19.5	0.139	5.16	0.979	0.670

P≤0.01: highly significant, P≤0.05: significant, P >0.05: not significant, N: number of data points, L: linear, Intercept: average value of the response parameter when the microalgae level is zero, Slope: gradient value, RBC: red blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, WBC: white blood cell, LxC: Level x Color.

Table 4: Effects of microalgae feeding on fatty acid of breast muscle in broilers

Parameter	Unit	N	Model	Intercept	SE Intercept	Slope	SE Slope	P-value	L×C
C14:0	%	18	L	0.464	0.068	0.152	0.017	<0.001	na
C16:0	%	18	L	19.0	4.32	0.151	0.777	0.849	na
C16:1n-7	%	14	L	2.51	0.582	0.269	0.126	0.066	na
C18:0	%	14	L	7.20	1.79	0.077	0.639	0.908	na
C18:1n-9	%	10	L	26.5	3.39	-1.17	0.779	0.182	na
C18:2n-6	%	14	L	24.8	3.88	-1.76	0.640	0.022	na
C18:3n-6	%	7	L	0.286	0.027	-0.038	0.012	0.035	na
C18:3n-3	%	11	L	1.90	0.210	-0.083	0.067	0.255	na
C18:4n-3	%	8	L	0.487	0.421	-0.0009	0.011	0.942	na
C20:2n-6	%	4	L	0.813	0.015	-0.028	0.005	0.033	na
C20:3n-6	%	4	L	1.18	0.040	-0.099	0.014	0.021	na
C20:4n-6	%	7	L	4.11	1.22	-0.432	0.117	0.021	na
C20:3n-3	%	4	L	0.083	0.014	0.003	0.005	0.661	na
C22:5n-3	%	4	L	0.903	0.021	0.033	0.007	0.047	na
C20:5n-3	%	11	L	0.840	0.333	0.114	0.098	0.284	na
C22:6n-3	%	14	L	0.986	0.417	2.55	0.123	<0.001	na
Omega 6	%	10	L	31.5	6.92	-2.37	0.865	0.034	na
Omega 3	%	14	L	3.77	1.05	2.64	0.175	<0.001	na
Omega 6/3		10	L	9.90	1.32	-2.06	0.620	0.016	na
MUFA	%	8	L	13.8	5.03	-4.31	9.74	0.681	na
PUFA	%	11	L	18.7	5.50	-0.716	1.17	0.564	na
SFA	%	14	L	25.1	6.28	1.57	1.21	0.230	na
PUFA/SFA		11	L	0.895	0.062	-0.038	0.074	0.629	na

P≤0.01: highly significant, P≤0.05: significant, P >0.05: not significant, Intercept: average value of the response parameter when the microalgae level is zero, Slope: gradient value, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, LxC: Level x Color, na: not available.

Table 5: Influence of various microalgae sources on production performance of broilers

Parameter	Unit	Control	Green	Brown	P-value
Production Performance					
Body Weight Gain	g	1781±216	1864±217	1749±219	0.329
Daily Weight Gain	g/d	82.3±45.5	91.0±45.5	86.0±45.8	0.058
Feed Intake	g	3682±874	3631±876	3705±876	0.864
Daily Feed Intake	g/d	121±57.6	127±57.6	120±58.0	0.342
FCR		1.73±0.087	1.62±0.089	1.67±0.098	0.094
Organ Weight					
Pancreas	%	0.225±0.055	0.151±0.055	0.250±0.055	0.024
Proventriculus	%	0.557±0.169	0.525±0.169	na	0.204
Heart	%	0.690±0.078	0.674±0.078	0.738±0.080	0.129
Liver	%	2.58±0.346	2.54±0.349	2.74±0.353	0.367
Spleen	%	0.391±0.229	0.390±0.245	0.516±0.235	0.514
Gizzard	%	2.16±0.224	2.07±0.229	2.10±0.236	0.604
Bursa of Fabricius	%	0.160±0.046	0.165±0.047	0.159±0.046	0.917
Breast Meat	%	18.5±6.80	21.2±6.84	18.5±6.80	0.068
Thigh Meat	%	10.4±3.61	10.9±3.64	10.5±3.61	0.632
Duodenum	cm/kg	14.9±0.666	14.5±0.666	na	0.761
Jejunum	cm/kg	11.4±0.762	13.0±0.470	na	0.157
Ileum	cm/kg	8.10±1.93	8.62±1.93	na	0.160

P≤0.01: highly significant, P≤0.05: significant, P>0.05: not significant, FCR: feed conversion rate.

Table 6: Influence of various microalgae sources on blood hematological and some serum metabolites of broilers

Parameter	Unit	Control	Green	Brown	P-value
Hematological					
RBC	10^6/µL	3.02±0.425	3.03±0.433	3.04±0.436	0.997
Hemoglobin	g/dL	12.2±4.39	11.7±4.39	11.9±4.54	0.924
MCV	fL	121±34.0	119±34.0	114±34.2	0.384
MCH	pg	25.6±16.3	25.3±16.3	25.6±16.3	0.951
WBC	10^3/µL	19.8±10.9	19.8±10.9	20.0±11.0	0.996
Lymphocytes	%	51.2±19.3	46.3±19.5	53.6±19.6	0.701
Monocytes	%	9.90±12.8	14.8±12.6	8.16±14.8	0.809
Basophils	%	0.14±0.04	0.14±0.04	0.14±0.04	0.999
Eosinophils	%	0.485±0.325	0.297±0.331	0.840±0.334	0.126
Heterophils	%	13.1±4.74	14.0±5.61	2.45±5.61	0.231
Serum metabolites					
Triglycerides	mg/dL	57.3±23.6	57.2±23.6	56.0±23.6	0.884
Glucose	mg/dL	58.6±18.8	85.4±19.9	52.1±19.9	0.419
Cholesterol	mg/dL	272±23.2	288±28.7	291±23.1	0.505

P≤0.01: highly significant, P≤0.05: significant, P>0.05: not significant, RBC: red blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, WBC: white blood cell, na: not available.

Table 7: Influence of various microalgae sources on fatty acid of breast muscle in broilers

Parameter	Unit	Control	Green	Brown	P-value
C14:0	%	0.546±0.131	0.611±0.202	0.619±0.121	0.781
C16:0	%	22.7±4.86	21.6±4.81	16.6±5.38	0.144
C16:1n7	%	2.84±0.710	3.01±0.730	2.97±0.708	0.531
C18:0	%	8.51±2.16	8.30±2.26	7.83±2.15	0.409
C18:1n9	%	26.1±3.08	26.5±3.23	25.8±3.04	0.892
C18:2n6	%	26.2±3.52	22.2±4.26	22.2±3.35	0.171
C18:3 n-6	%	0.328±0.033	na	0.198±0.018	0.051
C18:3n-3	%	2.12±0.218	1.79±0.267	1.67±0.195	0.113
C18:4n-3	%	0.507±0.440	0.430±0.441	0.503±0.440	0.172
C20:2n-6	%	na	na	na	na
C20:3n-6	%	na	na	na	na
C20:4n-6	%	4.12±1.16	na	3.21±1.05	0.285
C20:3n-3	%	na	na	na	na
C22:5n-3	%	na	na	na	na
C20:5n3	%	0.613±0.298	1.32±0.383	0.961±0.248	0.289
C22:6n3	%	0.700±1.91	2.22±2.82	4.45±1.56	0.222
Omega 6	%	33.3±6.34	na	27.2±6.07	0.082
Omega 3	%	3.41±2.36	5.65±3.32	7.25±2.07	0.237
Omega 6/3		$12.3±2.12^a$	na	$5.85±2.02^b$	<0.001
MUFA	%	16.1±7.20	16.6±7.25	15.3±7.20	0.650
PUFA	%	21.0±6.83	20.6±6.95	22.2±6.82	0.584
SFA	%	29.6±7.45	28.3±7.59	28.9±7.43	0.709
PUFA/SFA		0.803±0.076	0.839±0.119	0.890±0.089	0.817

P≤0.01: highly significant, P≤0.05: significant, P>0.05: not significant, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, na: not available.

DISCUSSION

Supplementation of microalgae apparently has marginal effect on the production performance of broiler chickens. Although microalgae are generally rich in protein contents, cell walls of some microalgae species can be difficult for the chicken to digest, which may potentially limiting the bioavailability of the protein. This is consistent with Wiaętkiewicz et al. (2015) who found that various types of microalgae had not been able to improve broiler performance. In previous study, it was also suggested that high protein content of microalgae might result in lower AME value and energy utilization efficiency in broiler chickens due to the lower contribution of protein toward metabolizable energy utilization coefficient (0.60) compared to carbohydrates (0.75) and lipids (0.95) (Tavernari et al. 2018). This was supported by an absence effect of replacing soybean meal with microalgae that had higher CP content on broiler performance (Evans et al. 2015). In addition, some microalgae contain anti-nutritional factors such as phycotoxins and indigestible carbohydrates that can impair nutrient digestion and absorption, counteracting their potential benefits. The inclusion of microalgae may also cause a decrease in palatability, thereby reducing feed consumption in broiler chicken (Abdelnour et al. 2019).

Microalgae feeding increased spleen weight but had no effects on other internal organs of broilers. A direct study has shown that microalgae supplementation was significantly better than the control and increased spleen percentage with higher levels of supplementation (Fathi et al. 2018). The spleen is the largest peripheral lymphoid organ in chickens and plays a role in the immune response to bacterial and viral infections acquired by antigens (Zhang et al. 2019). Similarly, El-Katcha et al. (2014) described that the spleen and bursa of fabricius are the main internal organs in poultry responsible for immunity. Microalgae are known to contain various bioactive compounds such as polysaccharides, carotenoids, and polyunsaturated fatty acids (Šefcová et al. 2021), which can enhance the immune system. Since spleen is a key organ in the immune system, an increase in spleen weight may indicate an enhanced immune response or immune organ development of the birds due to microalgae supplementation. In addition, microalgae are rich in antioxidants, such as beta-carotene, astaxanthin, and phycocyanin. These antioxidants may reduce oxidative stress and improve immune function of broiler chicken.

The results showed that the supplementation of microalgae had no significant effect on all blood parameters and some serum metabolites ($P>0.05$; Table 3). The addition of different levels and sources of microalgae color did not significantly affect these parameters. However, the data indicated an interaction between the level and color of microalgae on the parameters of mean corpuscular volume (MCV), white blood cell (WBC) count, and glucose ($P<0.05$; Table 3). Increasing the level of microalgae supplementation had a negative effect on WBC count and glucose levels, as shown in Table 3. Microalgae contain bioactive components that play an important role in the development and maturation of white blood cells, which can help enhance both humoral and cellular immune responses in chickens (Hassan et al. 2022). The treatment of microalgae color groups was not significantly different from the control, with brown microalgae having slightly better WBC values than the control (Table 6). Serum glucose is an important energy source for livestock and can affect tissue growth (Long et al. 2018). Increasing the level of green microalgae reduced blood glucose (Moran et al. 2018), while brown microalgae supplementation resulted in better blood glucose levels than the control (Long et al. 2018).

The data from this study show a different interaction between color and the level of microalgae on MCV results in broilers. At lower levels, green microalgae had better MCV values compared to brown. Brown microalgae showed a more significant potential for MCV than the green group at higher levels. Increasing the level of microalgae could decrease MCV content in broilers, but the combination of different levels and types of microalgae resulted in better MCV values than the control (Swati et al. 2022). Although not significantly different, green microalgae supplementation tended to lower MCV values (Hassan et al. 2022), whereas increasing brown microalgae relatively increased MCV values but did not surpass the control (Moran et al. 2018).

The supplementation of microalgae significantly influenced several types of fatty acids in broiler breast muscle ($P<0.05$; Table 4). The majority of the increase was observed in omega-3 fatty acids, while omega-6 fatty acids showed a decrease. Both omega-6 and omega-3 fatty acids are types of polyunsaturated fatty acids (PUFA). This study showed that increased microalgae supplementation led to a considerable reduction in omega-6, significantly decreasing PUFA values.

The specific fatty acids positively affected were C14:0 (myristic acid), C16:1n7 (palmitoleic acid), C18:3n3 (alpha-linolenic acid), C22:5n3 (docosapentaenoic acid; DPA), and C22:6n3 (docosahexaenoic acid; DHA). Conversely, the fatty acids negatively affected were C18:2n6 (linoleic acid), C18:3n6 (gamma-linolenic acid), C20:2n6 (eicosadienoic acid), and C20:3n6 (dihomo-gamma-linolenic acid) (Table 4). The change in fatty acid content depended on the level of microalgae supplementation. Different microalgae colors mostly did not significantly affect fatty acid changes in broilers ($P>0.05$; Table 7). Only the omega-6/3 ratio parameter was significantly affected, with brown microalgae reducing the ratio more effectively than the control. This is consistent with field studies showing that brown microalgae supplementation significantly reduces the omega-6/3 ratio to more desirable levels (Keegan et al. 2019). An omega-6/3 balance of 1:1 is highly desirable for reducing health issues (Moran et al. 2018).

Microalgae are microscopic aquatic plants that contain several biologically active components like omega-3 (El-Bahr et al. 2020). Feeding broilers with omega-3 PUFA-enriched diets can enhance the nutritional value of the meat, benefiting consumers (Yan and Kim 2013). Increasing the microalgae level up to 5% still had a positive impact on increasing omega-3 linearly. As shown in Table 4, there was a linear increase in omega-3 values with increased levels of microalgae supplementation. Microalgae supplementation significantly reduced omega-6 fatty acids in a linear. Linearly, higher levels of microalgae supplementation correlated with lower omega-

6 content in broilers. High consumption of omega-6 fatty acids is associated with increased health issues such as diabetes, obesity, and cardiovascular diseases (Alagawany et al. 2019). Therefore, reducing omega-6 is essential to enhance broiler meat quality and promote consumer health. Microalgae can effectively lower n-6 PUFA concentrations in feed, as they contain negligible amounts of n-6 PUFA (Long et al. 2018).

Conclusion

The meta-analysis study on microalgae indicated that different supplementation levels and color (type) did not significantly affect the production performance (feed intake, daily weight gain, and FCR), hematological parameters and serum metabolites of broiler chickens. The supplementation of microalgae at different levels had positive effects on spleen size, mean corpuscular volume, and various types of n-3 fatty acids. This study suggests that microalgae supplementation can effectively reduce the n-6/n-3 fatty acid ratio of broiler meat, which is advantageous for the health of broilers and consumers.

Conflict of Interest: All authors declare that there is no conflict of interest.

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Data availability statement: The data presented in this study are available on request from the corresponding author.

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