Poultry Science

Effects of microalgae, with or without xylanase supplementation, on growth performance, organs development, and gut health parameters of broiler chickens

Pravin Mishra, Razib Das, Ajay Chaudhary, Birendra Mishra, and Rajesh Jha[1]

Department of Human Nutrition, Food and Animal Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI 96822, USA

ABSTRACT Microalgae are becoming potential sustainable feed ingredients, whereas terrestrial feedstuffs are becoming scarce and costly. They are rich in nutritional and functional values but have lower digestibility. This study evaluated the effects of microalgae with or without xylanase supplementation on growth performance and gut health of broiler chickens. A total of 162-day-old Cobb 500 chicks were raised for 35 d. Birds were fed with either 1 of the 3 dietary treatments: 1) corn−soybean meal-based diet (**CON**), 2) CON + 3% microalgae (**MAG**), and 3) MAG + xylanase (**MAG+XYN**) in 2 phases (starter: d 0−21 and finisher: d 22−35) in mash form. Each dietary treatment had 6 replicates, with 9 birds in each replicate. The level of significance was considered at the P value <0.05. The BW, ADG, and ADFI were significantly higher in MAG by 50%, 52.5%, and 42.4%, respectively, and MAG+XYN by 44.1%, 49.7%, and 38.6%, respectively, compared to the CON group. No significant difference was observed for FCR; however, FCR was reduced by 6.3% in both MAG and MAG +XYN groups compared to the CON group. The carcass and organ weight relative to the total body weight were not significantly different among the treatments. The expressions of *Zonula occludens 1* (***ZO1***), *Cluster of differentiation 56* (***CD56***), and *Solute carrier family 7 member 7* (***SLC7A7***) were significantly modulated, for example, by 3.7, 3.9, and 3.3 folds, respectively, in the MAG group compared to CON and 0.8, 0.6, and 1.1 folds, respectively, in the MAG group compared to MAG +XYN groups on d 35. Villi surface area (**VSA**) of ileum tended to increase on d 3 (P = 0.0725) and d 35 (P = 0.0785) in the MAG and MAG+XYN groups, compared to the CON group. The results suggest that adding microalgae with or without xylanase to broiler's diet could promote growth performance and show a tendency to improve gut health parameters. The nutrient profile and its functional properties make microalgae a valuable resource to the poultry industry as a part substitution of corn and soybean meal and a functional feed supplement to modulate the gut health of broilers.

Key words: broiler, growth performance, gut health, microalgae, xylanase

2023 Poultry Science 102:103056
https://doi.org/10.1016/j.psj.2023.103056

INTRODUCTION

Reducing the production cost of quality meat and eggs for the ever-growing world population is a prime target of the poultry industry. Conventional feed ingredients like corn and soybean meal are widely used in broiler feeding programs, which increase the competition among food and feed and lead to raise production costs (Donohue and Cunningham, 2009; Stefanello et al., 2016). Several strategies were attempted to minimize the cost per unit production, such as feeding different feed additives, supplementation of exogenous enzymes, and alternative terrestrial feed ingredients as a full or partial replacement of corn and soybean meal for energy and protein sources, and these strategies came up with promising response (Yadav et al., 2019; Yadav and Jha, 2021). However, incorporating alternative terrestrial feed ingredients into poultry diets may embrace some challenges, such as the quality, availability, and price of the ingredients. Most of the alternative ingredients contain a high amount of fibers and tannins along with other antinutritional factors like enzyme inhibitors and have low metabolizable energy (Alshelmani et al., 2021). The aqua-based feed ingredients are more promising and sustainable than terrestrial feed ingredients (Liu et al., 2020). The earth's geography is dominated by water bodies that could be utilized for aqua-based feed ingredient production. Among aqua-based feed ingredients,

Received May 19, 2023.
Accepted August 17, 2023.

[1]Corresponding author: rjha@hawaii.edu

Table 1. Common microalgae species used in animal feed and their nutrient profile.

Microalgae species	Composition (% dry matter)			References
	Protein	Carbohydrate	Lipids	
Chlorella vulgaris	51–58	12–17	14–22	(Wolkers et al., 2011)
Haematococcus pluvialis	48	27	15	(Bleakley and Hayes, 2017)
Spirogyra sp.	6–20	33–64	11–21	(Krimpen et al., 2013)
Spirulina maxima	60–71	13–16	6–7	(Milledge, 2011)
Spirulina platensis	46–63	8–14	4–9	(Milledge, 2011)

microalgae are one of the best alternative feed ingredients, and they have a nutrient profile suitable for the poultry feeding program.

Microalgae are single-celled microorganisms found in fresh and seawater (Vale et al., 2020). It can be classified based on the size and pigments it contains. Microalgae are also considered a plant-based superfood as it contains a high amount of proteins, carbohydrates, lipids, different pigments, and unsaturated fatty acids, which are hardly available together in any plant-based food (Vrenna et al., 2021; Terezinha Schneider et al., 2023). Besides these, microalgae are rich in amino acids and other nutrients like vitamins and carotenoids and possess bioactive compounds such as phycocyanin, β-carotene, flavonoid, phycobiliproteins (β-phycocyanin and C-phycocyanin), and phenolic acid with antibacterial, antiviral, antioxidant, and anticarcinogenic properties (Farag et al., 2016; Wu et al., 2016; Saadaoui et al., 2021). However, the nutritional value of microalgae varies from species to species, and the environment in it is cultivated (Table 1). The inclusion of microalgae at a high dose works as a replacement for protein-rich feed ingredients, whereas a low dose of microalgae serves a functional value (Tavernari et al., 2018). The nutritional and functional values of microalgae promote the overall performance of broiler chickens by promoting their health status. Microalgae, such as *Arthrospira*, also known as *Spirulina*, improve the broiler's immune system, protect against different pathogens, and increase T-cell activity in the body (Sugiharto, 2020; Fries-Craft et al., 2021). The inclusion of *Arthrospira platensis* at different doses in the broiler diet also improves villi height (**VH**), crypt depth (**CD**), and VH to CD ratio of the small intestine (El-Hady et al., 2022). The exact mechanistic pathway of microalgae is still unclear; however, we have outlined the plausible pathways (Figure 1) of how microalgae may improve broiler chickens' gut health and growth performance.

Microalgae contain 47.7% hemicellulose, 37.8% cellulose, and 13.3% lignin (Kusmiyati et al., 2020). Due to the abundance of these nonstarch polysaccharides (**NSP**), microalgae are less digestible. The poultry lacks the endogenous enzymes required to digest the NSP of microalgae. However, digestibility can be enhanced by adding xylanase, an exogenous enzyme, to the diet.

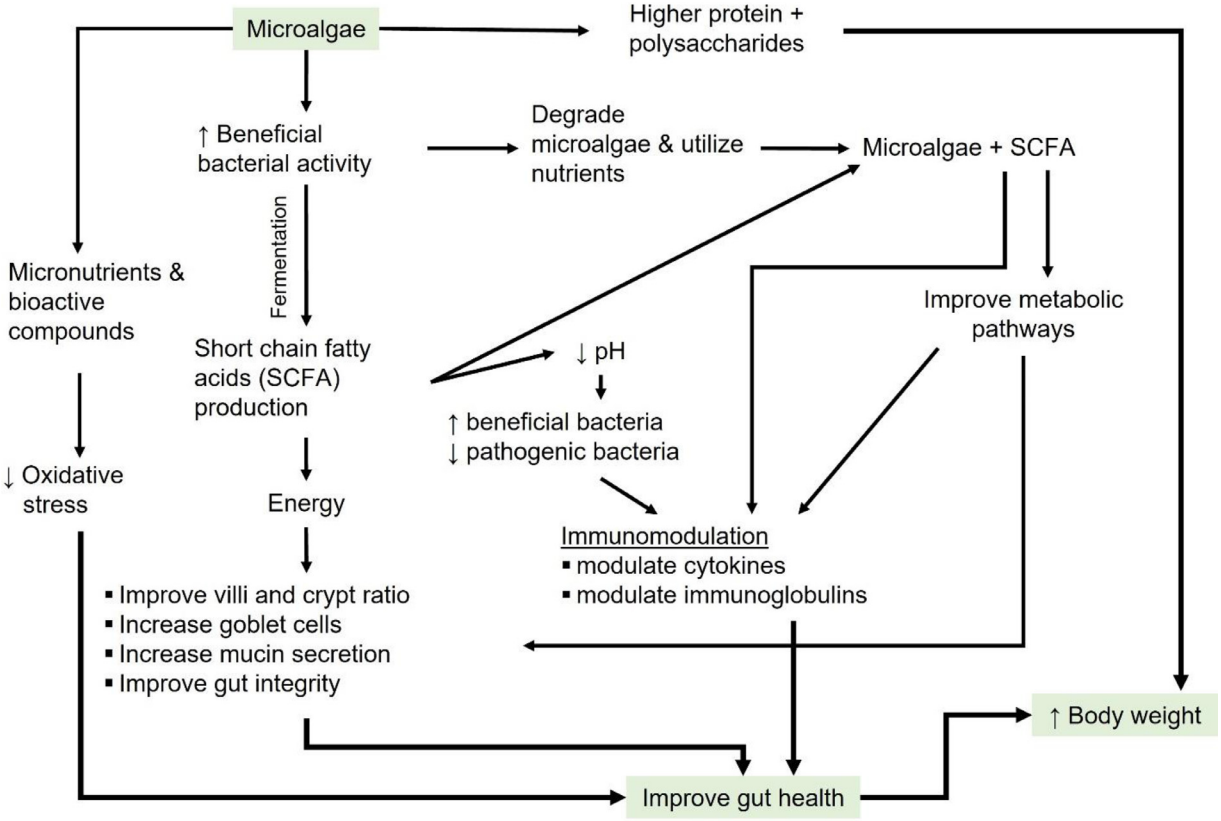

Figure 1. Probable working pathways of microalgae to improve gut health and body weight of broiler chickens.

Xylanase converts xylan (complex polysaccharide) into xylose (monosaccharide). Previous studies suggested that adding xylanase to a fiber-enriched diet improved digestibility and nutrient utilization in broilers (Singh et al., 2021; Van Hoeck et al., 2021).

Therefore, we hypothesized that including microalgae (*A. platensis*) with or without xylanase in the broiler's diet improves growth performance, supports the development of intestinal morphology, and boosts the immune system. The objective of this study was to assess the effect on growth performance, intestinal histomorphology, and immunity in broiler chickens when fed a diet supplemented with microalgae, with or without xylanase.

MATERIALS AND METHODS

All research animal care procedures were approved by the Institutional Animal Care and Use Committee of the University of Hawaii (**UH**), HI (Protocol #15-2274-7).

Experimental Location, Birds, and Management

A total of 162-day-old Cobb500 broiler chicks were brought from a local commercial hatchery (Asagi Hatchery Inc., Honolulu, HI). All the birds were vaccinated against Marek's disease. The birds were reared for 35 d at the Small Animal Facility of UH Manoa on floor pens, maintaining the standard environment (temperature: 18^0C−24^0C and relative humidity: 50 ± 5%) required for Cobb500 broilers (Cobb-Vantress, 2021), and had ad libitum access to feed and water throughout the experiment. The experimental house had an appropriate ventilation system. The photoperiod cycle was set for 23:1 h of light and dark.

Experimental Design and Diet

Immediately after the arrival of chicks, individual birds were weighed, wing tagged, and randomly allocated into one of the 3 dietary treatment groups. Each group had 6 replicates, with 9 birds in each replicate (n = 54 per group). Three different corn and soybean meal-based mash diets were formulated in 2 phases, starter (d 0−21) and finisher (d 22−35): corn−soybean meal control (**CON**), CON + 3% Microalgae (*A. platensis*, **MAG**), and CON + 3% microalgae with xylanase enzyme (**MAG+XYN**) in mash form. Microalgae, at a rate of 30 g/kg diet, were added as premix, whereas xylanase, at a rate of 16,000 BXU/Kg (i.e., 100 g/ton) of diet, was topped up. All the formulated diets met or exceeded the nutritional requirement of broiler chickens (Cobb-Vantress, 2021). Table 2 shows the nutrient profile of microalgae used in this study. Tables 3 and 4 show the ingredients used in feed formulation and their nutritional contents, respectively. The fatty acid profile of microalgae used in the study is presented in Supplementary Table 1.

Table 2. Nutrient composition of microalgae (*Arthrospira platensis*) used in the study.

Nutrients (%, unless otherwise mentioned)	Value
Dry matter	95.09
Gross energy (Kcal/kg)	8,040
Total ash	7.00
Crude protein	70.21
Crude fat	2.73
Crude fiber	2.16
Ca	0.29

Chemical Analysis and Enzymes Activities

The gross energy of samples was determined according to the standard procedures of the Association of Officials Analytical Chemists (AOAC, 2006) using an oxygen bomb calorimeter (Parr Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL). All other chemical profiles were analyzed by Near Infra-red Reflectance Spectroscopy and enzyme activity by enzyme-linked immunoassay method at the AB Vista Innovation and Technology Center (Tredomen Park, Ystrad Mynach, UK).

Growth Performance and Relative Weight of the Organs

The body weight (**BW**, g) of individual birds, penwise supplied feed, and leftover feed were recorded weekly (on d 0, d 7, d 14, d 21, d 28, and d 35). The collected data were used to calculate the average daily gain (**ADG**, g), average daily feed intake (**ADFI**, g), and feed conversion ratio (**FCR**). The total feed intake by the birds in a pen was divided by the number of birds in the pen to get ADFI. The total body weight gain of the

Table 3. Ingredients composition of experimental diets*.

	Starter (d 0−21)		Finisher (d 22−35)	
Ingredients, %	CON	MAG	CON	MAG
Corn	53.67	52.83	60.84	60.50
Soybean meal, CP 44%	38.00	36.00	31.00	29.00
Microalgae	0.00	3.00	0.00	3.00
Soybean oil	5.00	4.90	5.50	5.00
Limestone	1.35	1.35	1.20	1.10
Monocalcium phosphate	0.75	0.75	0.44	0.44
L-Lysine Monohydrochloride	0.18	0.13	0.10	0.04
DL-Methionine	0.18	0.17	0.13	0.13
L-Threonine	0.04	0.04	0.00	0.00
Sodium chloride	0.20	0.20	0.18	0.18
Sodium bicarbonate	0.12	0.12	0.10	0.10
Vitamin + mineral mix[a]	0.50	0.50	0.50	0.50
Phytase	0.01	0.01	0.01	0.01

*The diets MAG in both phases were top-dressed with 100g xylanase per ton of diet to make MAG+XYN diets in the respective phase. Expected xylanase activity was 16000 BXU/kg of feed.

[a]Provides the following nutrients (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (all-rac-tocopherol-acetate), 30 mg; vitamin B1, 2 mg; vitamin B2, 8 mg; vitamin B6, 4 mg; vitamin B12 (cyanocobalamin), 0.025 mg; vitamin K3 (bisulfatemenadione complex), 3mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; betaíne anhydrous, 80 mg; D-biotin, 0.15 mg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg iron ($FeCO_3$), 60 mg; copper ($CuSO_4{\cdot}5H_2O$), 8 mg; iodine (KI), 2 mg; selenium (Na_2SeO_3), 0.2 mg.

Table 4. Nutrient composition of experimental diets.

Composition, % or as mentioned	CON		MAG		MAG+XYN	
	Starter	Finisher	Starter	Finisher	Starter	Finisher
Analyzed values						
Dry matter	87.55	87.29	87.91	87.66	87.39	87.43
Gross energy (Kcal/kg)	4,254	4,242	4,230	4,314	4,212	4,253
Total ash	5.25	4.44	5.32	4.85	3.40	2.81
Crude protein	23.34	18.75	23.26	19.27	22.42	19.06
Crude fat	6.78	7.47	7.10	8.00	7.60	8.85
Starch	39.84	45.00	36.62	41.07	38.07	41.60
Sugars	6.95	6.93	5.83	6.25	6.12	5.57
NDF	6.49	7.80	7.70	8.09	7.21	7.95
ADF	2.49	2.50	2.83	2.59	3.02	2.80
Phytic P	0.27	0.25	0.26	0.25	0.26	0.24
Phytase activity (FTU/Kg)	~129	~151	~98.4	190	903.00	983.00
Xylanase activity (BXU/Kg)	<2,000	<2,000	<2,000	<2,000	21,800	18,600
Calculated values						
Lys	1.32	1.09	1.32	1.08	1.32	1.08
Met	0.52	0.44	0.55	0.48	0.55	0.48
Cys	0.42	0.40	0.41	0.38	0.41	0.38
Thr	0.87	0.73	0.92	0.78	0.92	0.78
Trp	0.31	0.27	0.33	0.28	0.33	0.28
Met+Cys	0.92	0.82	0.88	0.79	0.88	0.79
Arg	1.55	1.35	1.48	1.28	1.48	1.28
Val	1.18	1.05	1.13	1.00	1.13	1.00
Ile	0.90	0.78	0.95	0.84	0.95	0.84
Leu	1.82	1.66	1.90	1.74	1.90	1.74
Na	0.16	0.14	0.16	0.14	0.16	0.14
Cl	0.16	0.15	0.16	0.15	0.16	0.15
Choline (mg/kg)	1371	1224	1311	1167	1311	1167
Ca	0.91	0.77	0.91	0.74	0.91	0.74
Total P	0.71	0.61	0.71	0.62	0.71	0.62
AvP	0.45	0.37	0.45	0.37	0.45	0.37
dig Lys	1.17	0.95	1.08	0.86	1.08	0.86
dig Met	0.48	0.40	0.46	0.39	0.46	0.39
dig Thr	0.67	0.55	0.64	0.52	0.64	0.52

birds in a pen was divided by the bird-pen days to calculate the ADG for the birds in individual pens. The FCR was calculated as the ratio of ADG and ADFI for each pen. The number of birds in a pen during a week was corrected for any mortality. The number of days birds were alive and consumed feed in a pen was considered as the pen-days number for a pen during a week.

On d 35, one bird per replicate was randomly selected to record the relative organs' weight against total body weight and to measure the length of intestinal segments. All the birds were euthanized using CO_2 gas. After euthanization, the birds were dissected, and the weight of the proventriculus, gizzard, liver, intestine, breast muscle, and drumstick, and the length of the small intestine were measured.

Intestinal Histomorphology

Approximately 1 cm of the duodenum, jejunum, and ileum (from a position that is located at half of the length between Meckel's diverticulum and ileocecal junction) sections were collected, digesta was flushed with distilled water and then stored in 10% Neutral Buffered Formalin for histomorphological analysis on d 3 and d 35 (one bird per replicate). The stored tissue samples were processed for paraffin block embedding and sectioned at 5 μm thickness to prepare slides (3 replicates/sample) in the Histopathology core facility, John A. Burns School of Medicine, UHM. Later the prepared slides were processed with Hematoxylin and Eosin staining. The stained slides were observed to measure VH, CD, and villi width (**VW**) using a microscope (Olympus BX43, Olympus Co, Tokyo, Japan) with 8× objective lens for the ileum and 4× objective lens for the duodenum and jejunum. The microscope was fitted with a camera and image processing system (Infinity Analyze software, Lumenera Corporation, Ottawa, ON, Canada). The measurement of VH (distance from the tip of the villus to the crypt) and CD (distance from the villus base to the submucosa) were measured following the method described by Berrocoso et al. (2017). After measuring the VH and CD, the ratio between VH and CD was calculated. The villi surface area (**VSA**) was calculated from VH and VW, using the formula $VSA = 2\pi \times (VW/2) \times VH$, described by Sakamoto et al. (2000).

RNA Extraction, Reverse Transcription, and Real-Time Quantitative PCR

Approximately 1 cm of ileum section was collected in cryovial from each pen (1 bird per replicate) and immediately snap-frozen into liquid nitrogen and transferred to −80°C to prevent degradation. Total RNA was extracted using Trizol reagent (ThermoFisher Scientific, Carlsbad, CA, Catalog number: 15596018) following the

manufacturer's instructions. RNA concentration was determined using nanodrop (ThermoFisher Scientific, Madison, WI, Catalog Number: ND-ONE-W).

The High Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, Foster City, CA, Catalog Number: 4368814) was used for cDNA synthesis from the template of 1,000 ng total RNA extracted from each sample. The standard time and temperature for incubation were as such the company protocol describes.

Real-time quantitative PCR (**qPCR**) was conducted on QuantStudio 3 (ThermoFisher Scientific, Foster City, CA, Catalog number: A28567) using PowerUp SYBR Green Master Mix (ThermoFisher Scientific, Foster City, CA, Catalog number: A25742). For amplification, synthesized cDNA was used as a template. The qPCR amplification condition was set as previously described by Singh et al. (2022). The cycle threshold (**Ct**) value was determined, and the expression of target genes was analyzed using the $2-\Delta\Delta$Ct method (Livak and Schmittgen, 2001) with reference gene *TATA-binding protein* (***TBP***). However, we compared the Ct values from 2 other reference genes *Glyceraldehyde 3-phosphate dehydrogenase* (***GAPDH***) and *Beta* (***β****)-Actin,* aside *TBP*. The qPCR showed the most consistent Ct values for *TBP*. Finally, *TBP* was chosen as the housekeeping gene for all the gene expression analysis in this study. The oligonucleotide primers used for amplification are shown in Table 5.

Statistical Analysis

The data of growth performance, relative organs weight, and histomorphology were analyzed in GraphPad Prism version 8.4.2 for Windows (GraphPad Software, San Diego, CA). The relative expression of genes was analyzed in RStudio version 2022.07.2. The data were checked for normality and log transferred prior to

Table 5. Primers used to quantify the expression of genes using qPCR.

Function	Gene	Sequences (F: Forward, R: Reverse)	Accession number	Amplicon size (bp)
Gut barrier	*OCLN*	F: CCGAGGACAGCCCTCAATAC R: CTTTGGTAGTCTGGGCTCCG	NM_205128	82
	MUC2	F: TGAGTCAGGCATAAATCGTGT R: CAGGTCTAAGTCGGGAAGTGTA	XM 421035	
	CLDN1	F: TACCCCAAAAATGCCCCCTC R: GCGGCATTGTAGTGTCCTCT	NM_001013611	109
	ZO1	F: CTTCAGGTGTTTCTCTTCCTCCTC R: CTGTGGTTTCATGGCTGGATC	XM_015278981	131
	Cadherin	F: GACAGGGACATGAGGCAGAA R: GCCGTGACAATGCCATTCTC	NM_001039258.2	57
Immunity	*IL4*	F: TGTGCCCACGCTGTGCTTACA R: CTTGTGGCAGTGCTGGCTCTCC	NM_001030693	155
	IL6	F: GCTCGCCGGCTTCGA R: GGTAGGTCTGAAAGGCGAACAG	AJ250838	71
	IL10	F: TGTCACCGCTTCTTCACCTG R: CTCCCCCATGGCTTTGTAGA	NM_001004414	105
	IL1β	F: CGCTTCATCTTCTACCGCCT R: GATGTTGACCTGGTCGGGTT	NM_204524	144
	TLR4	F: AGTCTGAAATTGCTGAGCTCAAAT R: GCGACGTTAAGCCATGGAAG	NM_001030693	190
	NF-kB p65	F: GTGTGAAGAAACGGGAACTG R: GGCACGGTTGTCATAGATGG	NM_205129	
	chB6	F: TACTTTGTCGGCCGAGTGTC R: AGTCTGCAGTTCCATTGGGG	NM_205182	197
	AvBD4	F: TTCTCTGCAGTGACACGATTTCC R: AAGCCCACAGCTCCATGAACT	NM_001001610.2	101
	CD3	F: GGACGCTCCCACCATATCAG R: TGTCCATCATTCCGCTCACC	NM_205512	180
	CD45	F: TATTCTTGGTGTTCTTGATTGTTGTG R: CTGCTACAAGGCTGATGACTTCA	NM_204417	120
	CD56 (NCAM1)	F: GTTCATGAGCAGAGGGTGCT R: ACATGGCCTGGATGATGCAA	NM_001242604	196
Antioxidant	*SOD1*	F: CAACACAAATGGGTGTACCA R: CTCCCTTTGCAGTCACATTG	NM_205064.1	119
	SOD2	F: CCTTCGCAAACTTCAAGGAG R: AGCAATGGAATGAGACCTGT	NM_204211.1	160
	Nrf2	F: CCCTGCCCTTAGAGATTAGAC R: CAAGTTCATGTCCTTTTCTCTGC	NM_205117.1	248
Nutrient transporter	*SLC2A1*	F: TCCCAGACAGGTGATCTACA R: AAAGGAGATGAGGAAGACGG	NM_001198927.2	130
	SLC7A7	F: GAAAACCTCAGAGCTCCCTT R: GAGGTAAATTCCTCTCGGGG	XM_015282844.2	148
Reference	*GAPDH*	F: AGCTTACTGGAATGGCTTTCCG R: ATCAGCAGCAGCCTTCACTACC	NM_204305	122
	β-Actin	F: GAGAAATTGTGCGTGACATCA R: CCTGAACCTCTCATTGCCA	NM_205518.1	139
	TBP	F: TAGCCCGATGATGCCGTAT R: GTTCCCTGTTCGCTTGC	NM_205103	147

Table 6. Effect of diets on growth performance of broilers.

	CON[1]	MAG[2]	MAG+XYN[3]	Pooled SEM	*P* value
Body weight, g					
Starter (d 0–d 21)	490 b	705 a	728 a	26.71	<0.0001
Finisher (d 22–d 35)	719 b	1100 a	1005 a	54.91	0.0004
Overall (d 0–d 35)	1203 b	1805 a	1733 a	60.11	<0.0001
Average daily gain, g					
Starter (d 0–d 21)	23.3 b	32.9 a	33.9 a	1.25	<0.0001
Finisher (d 22–d 35)	46.5 b	74.9 a	71.2 a	4.67	0.0012
Overall (d 0–d 35)	32.6 b	49.7 a	48.8 a	2.17	<0.0001
Average daily feed intake, g					
Starter (d 0–d 21)	35.8 b	45.3 a	48.3 a	1.36	<0.0001
Finisher (d 22–d 35)	79.6 b	121.9 a	112.3 a	5.74	0.0003
Overall (d 0–d 35)	53.3 b	75.9 a	73.9 a	2.56	<0.0001
Feed conversion ratio					
Starter (d 0–d 21)	1.5	1.4	1.4	0.05	0.0791
Finisher (d 22–d 35)	1.7	1.6	1.6	0.05	0.2586
Overall (d 0–d 35)	1.6	1.5	1.5	0.03	0.1056

[1]Control.
[2]3% Microalgae.
[3]3% Microalgae + Xylanase.Data are presented as the least square means of 6 replicate pens.Different letters within a row represent significant difference at $P < 0.05$.

conducting one-way ANOVA. Tukey's multiple comparison test was performed to compare the means among the treatment groups. The significance level was set at $P < 0.05$, and the $P < 0.10$ was considered a trend. The results were expressed as the mean and pooled standard error of the mean (**SEM**).

RESULTS

Growth Performance and Relative Organ Weight

The phase-wise growth performance is presented in Table 6. The BW, ADG, and ADFI were significantly higher ($P < 0.05$) in MAG and MAG+XYN compared to the CON group. However, there was no significant difference in the growth performance variables ($P > 0.05$) between MAG and MAG+XYN groups. Though the diets did not show significant differences for FCR ($P > 0.05$), a trend in improving FCR was observed in the MAG group, followed by MAG+XYN and CON groups during the starter phase.

The effect of diets on relative organs' weight and length and carcass weight is presented in Table 7. Although the results were not statistical difference ($P > 0.05$), there was a substantial numerical difference among treatments. The weight of breast muscle and drumstick was the highest in MAG+XYN, followed by MAG and CON. The length of the small intestine also followed a similar trend. However, regarding liver weight and intestinal length, MAG+XYN showed an increase in the values, followed by CON and MAG groups. The proventriculus and gizzard were heavier in CON, followed by MAG+XYN and MAG groups.

Intestinal Histomorphology

The effect of diets on intestinal histomorphology is presented in Table 8. On d 3 (for the ileum) and d 35 (for duodenum, jejunum, and ileum), compared to CON diet MAG and MAG+XYN diets had a higher ratio of villi height and crypt depth except for MAG group of the jejunum on d 35, though the changes were not statistically significant ($P > 0.05$). There was a trend for higher villi surface area of the ileum on d 3 and d 35.

Ileal Gene Expression

The relative expression of different genes related to the gut barrier, immunity, antioxidant, and nutrient transportation from ileal tissue are presented in Figures 2 to 5 (d 3) and in Figures 6 to 9 (d 35). The expression

Table 7. Effect of diets on relative organs' weight, carcass weight, and length of small intestine of broiler, compared to the final body weight on d 35.

Organs	CON[1]	MAG[2]	MAG+XYN[3]	Pooled SEM	*P* value
Proventriculus, %	0.4	0.4	0.4	0.03	0.6631
Gizzard, %	2.8	2.5	2.6	0.21	0.6709
Liver, %	2.2	2.2	2.4	0.14	0.7878
Intestine, %	4.1	4.0	4.2	0.29	0.8679
Breast muscle, %	26.4	29.1	29.6	1.45	0.2774
Drumstick, %	18.3	18.8	20.6	1.40	0.4880
Small intestine (length), inch/g	0.0353	0.0357	0.0402	0.0017	0.1159

[1]Control.
[2]3% Microalgae.
[3]3% Microalgae + Xylanase.Data are presented as the least square means of 6 replicate pens. All the values are calculated relative to the final body weight of the birds.

Table 8. Effects of diets on intestinal histomorphology of broilers on d 3 and d 35.

Parameters	CON[1]	MAG[2]	MAG+XYN[3]	Pooled SEM	*P* value
d 3 - Ileum					
Villi height (VH), μm	768	822	869	50.87	0.3924
Crypt depth (CD), μm	163.7	154.8	168.4	9.03	0.5705
VH:CD	4.7	5.4	5.3	0.49	0.5643
Villi surface area, sq. μm	550,055	525,782	701,855	53,834	0.0725
d 35 - Ileum					
Villi height (VH), μm	625	661	706	29.47	0.1829
Crypt depth (CD), μm	60	62.8	60.9	2.29	0.6787
VH:CD	10.4	10.5	11.7	0.51	0.168
Villi surface area, sq. μm	202,056	238,943	272,916	20,361	0.0785
d 35 - Duodenum					
Villi height (VH), μm	1,472	1,557	1,566	106.57	0.6002
Crypt depth (CD), μm	97.8	94.8	94.3	4.49	0.8339
VH:CD	14.5	16.6	16.8	1.10	0.3187
Villi surface area, sq. μm	913,146	969,669	897,800	111,320	0.8916
d 35 - Jejunum					
Villi height (VH), μm	1156	1124	1200	64.71	0.7112
Crypt depth (CD), μm	103.4	102	98.6	8.82	0.7561
VH:CD	11.2	11.1	12.3	0.72	0.4530
Villi surface area, sq. μm	591,348	547,778	609,520	75,157	0.8385

[1]Control.
[2]3% Microalgae.
[3]3% Microalgae + Xylanase.Data are presented as the least square means of 6 replicate pens.

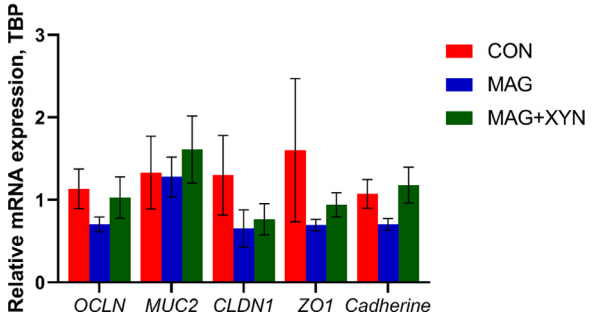

Figure 2. Relative gene expression of gut barrier genes in ileum on d 3 based on reference gene *TBP*. X-axis represents the name of gut barrier-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Abbreviations: *CLDN1*, *Claudin-1*; *MUC2*, *Mucin 2*; *OCLN*, *Occludin*; *TBP*, *TATA box binding protein*; *ZO1*, *Zonula occludens-1*.

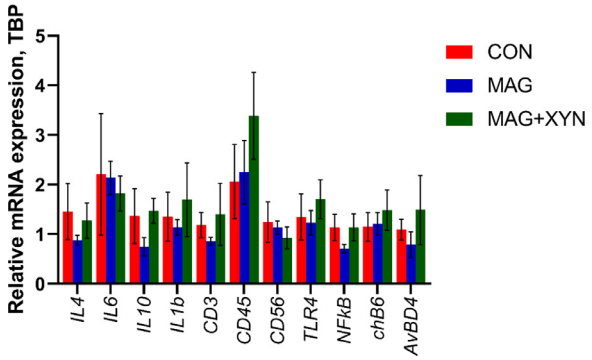

Figure 3. Relative gene expression of immune genes in ileum on d 3 based on reference gene TBP. X-axis represents the name of immune-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Abbreviations: *AvBD4*, *avian beta-defensin 4*; *CD3*, *cluster of differentiation 3*; *CD45*, *cluster of differentiation 45*; *CD56*, *cluster of differentiation 56*; *chB6*, *chicken B-cell marker 6*; *IL10*, *Interleukin-10*; *IL1β*, *Interleukin-1beta*; *IL4*, *Interleukin-4*; *IL6*, *Interleukin-6*; *NF-kB p65*, *nuclear factor-kappa B*; *TBP*, *TATA box binding protein*; *TLR4*, *toll-like receptor 4*.

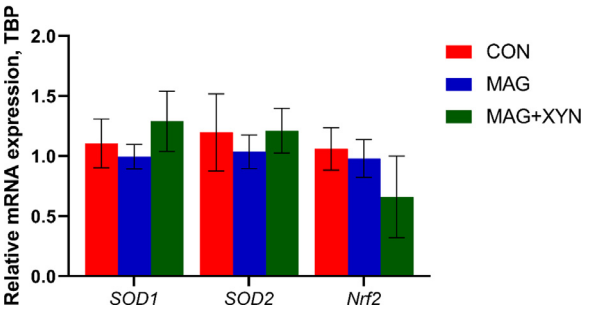

Figure 4. Relative gene expression of antioxidant genes in ileum on d 3 based on reference gene TBP. X-axis represents the name of antioxidant-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Abbreviations: *Nrf2*, *nuclear factor erythroid 2-related factor 2*; *SOD1*, *superoxide dismutase 1*; *SOD2*, *superoxide dismutase 2*; *TBP*, *TATA box binding protein*.

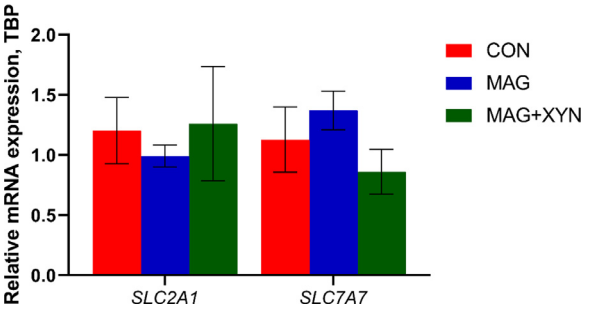

Figure 5. Relative gene expression of nutrient transporter genes in ileum on d 3 based on reference gene TBP. X-axis represents the name of nutrient transport-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Abbreviations: *SLC2A1*, *solute carrier family 2 member 1*; *SLC7A7*, *solute carrier family 7 member 7*; *TBP*, *TATA box binding protein*.

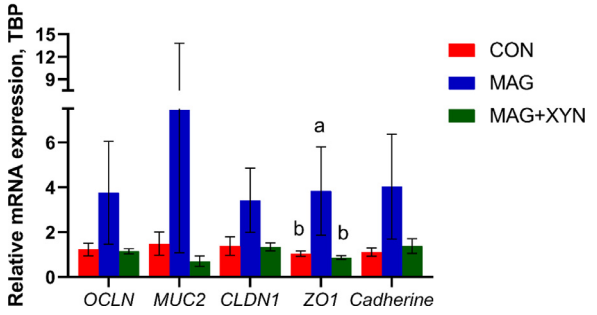

Figure 6. Relative gene expression of gut barrier genes in ileum on d 35 based on reference gene *TBP*. X-axis represents the name of gut barrier-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Different letters above the bars represent significant differences between treatments at $P < 0.05$. Abbreviations: *CLDN1*, *Claudin-1*; *MUC2*, *Mucin 2*; *OCLN*, *Occludin*; *TBP*, *TATA box binding protein*; *ZO1*, *Zonula occludens-1*.

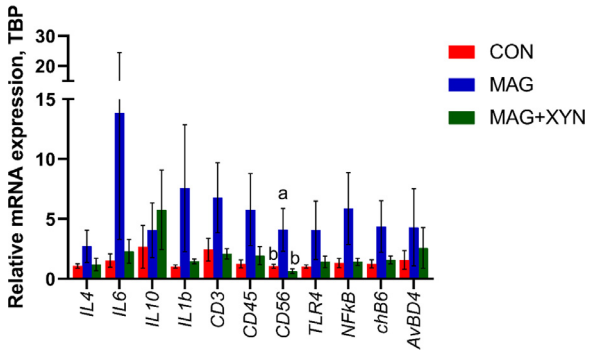

Figure 7. Relative gene expression of immune genes in ileum on d 35 based on reference gene *TBP*. X-axis represents the name of immune-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Different letters above the bars represent significant differences between treatments at $P < 0.05$. Abbreviations: *AvBD4*, *avian beta-defensin 4*; *CD3*, *cluster of differentiation 3*; *CD45*, *cluster of differentiation 45*; *CD56*, *cluster of differentiation 56*; *chB6*, *chicken B-cell marker 6*; *IL10*, *Interleukin-10*; *IL1β*, *Interleukin-1beta*; *IL4*, *Interleukin-4*; *IL6*, *Interleukin-6*; *NF-kB p65*, *nuclear factor-kappa B*; *TBP*, *TATA box binding protein*; *TLR4*, *toll-like receptor 4*.

of *Occludin* (***OCLN***), *Mucin 2* (***MUC2***), *Claudin-1* (***CLDN1***), *Interleukin-4* (***IL4***), *Interleukin-6* (***IL6***), *Interleukin-10* (***IL10***), *Interleukin-1beta* (***IL1β***), *Toll-like receptor 4* (***TLR4***), *Nuclear factor-kappa B* (***NF-kB p65***), *Chicken B-cell marker 6* (***chB6***), *Avian beta-defensin 4* (***AvBD4***), *Cluster of differentiation 3* (***CD3***), *Cluster of differentiation 45* (***CD45***), *Superoxide dismutase 1* (***SOD1***), *Superoxide dismutase 2* (***SOD2***), *Nuclear factor erythroid 2–related factor 2* (***Nrf2***), *Solute carrier family 2 member 1* (***SLC2A1***) genes showed no significant differences among the treatments. However, the expressed values showed that the MAG and MAG+XYN-fed birds had higher expression of most of these genes than the birds from the CON group. *Zonula occludens-1* (***ZO1***), *Cluster of differentiation 56* (***CD56***), and *Solute carrier family 7 member 7* (***SLC7A7***) genes were significantly changed ($P < 0.05$) in MAG group.

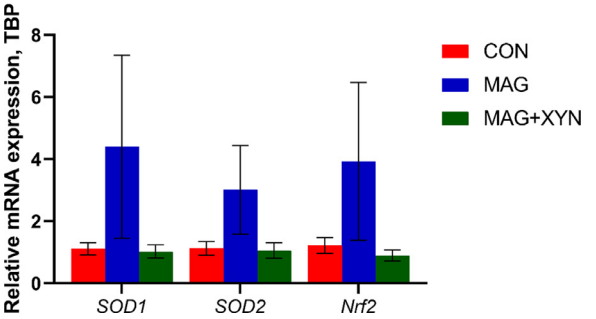

Figure 8. Relative gene expression of antioxidant genes in ileum on d 35 based on reference gene TBP. X-axis represents the name of antioxidant-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Abbreviations: *Nrf2*, *nuclear factor erythroid 2-related factor 2*; *SOD1*, *superoxide dismutase 1*; *SOD2*, *superoxide dismutase 2*; *TBP*, *TATA box binding protein*.

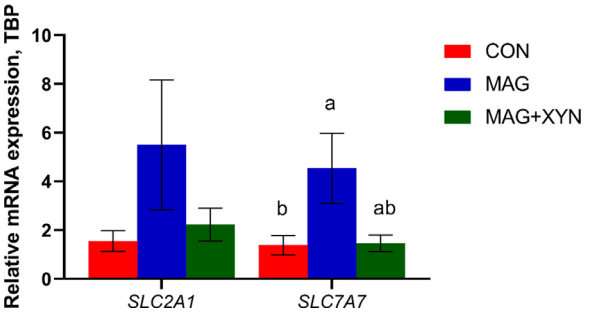

Figure 9. Relative gene expression of nutrient transporter genes in ileum on d 35 based on reference gene *TBP*. X-axis represents the name of nutrient transport-related genes, and Y-axis represents their expression in the ileum. The error bar in the graph represents the standard deviation. Different letters above the bars represent significant differences between treatments at $P < 0.05$. Abbreviations: *SLC2A1*, *solute carrier family 2 member 1*; *SLC7A7*, *solute carrier family 7 member 7*; *TBP*, *TATA box binding protein*.

DISCUSSION

Microalgae are potential alternative animal feed ingredients. Due to their nutritional and functional properties, they have been prioritized to be investigated for suitability in poultry production. In this experiment, either alone or with xylanase, the addition of microalgae showed a significant increment in the body weights in both the starter and finisher dietary phases. However, there was no additional benefit for body weight gain due to xylanase addition. The nutritional richness and functional components of microalgae might be a reason for better growth in treatment groups (Farag et al., 2016; Patel et al., 2021). Other studies also found an improvement in final BW when *A. platensis* was added to the broiler's diet (El-Bahr et al., 2020; Ismita et al., 2022). The interaction of microalgae in poultry feed with xylanase enzyme is still unclear. We expected that the addition of xylanase would further increase the body weight of birds, but the expectation was not met in this study. It might be due to the minimal presence of cellular xylan and minimal conversion of it to xylose, a desired sugar by the microbes for energy production. Bobin-Dubigeon et al. (1997) showed that endo-xylanase releases only

69% of glucose and xylose residue from the resistant cell wall of microalgae *Ulva intestinalis*. Moreover, xylanase increases digesta viscosity preventing its further action (Pestana et al., 2020), and also nonprotein components of microalgae inhibit lipase activity (Kishibuchi et al., 2019). It also suggests that xylanase and β-glucanase have a lower tendency to break down the cell wall of microalgae. This might be a possible reason for no significant changes in BW after using xylanase in the microalgae diet.

The green color of the feed that resulted from the addition of microalgae could influence the birds' appetite and increase the feed intake, which could reflect in increased body weight, as previously reported by Farghly and Abdelfattah (2017), and the higher feed intake in MAG and MAG+XYN groups compared to the CON group aligns the aforementioned findings. Better feed consumption response has been recorded in a poultry house with white room light and a red- and green-colored diet (Rierson, 2011), which aligns with the light and dietary reference of our study. In contrast to the CON group, the FCR of MAG and MAG+XYN treatments was improved, although statistically nonsignificant. The past result showed that green feed significantly increases FCR (Gulizia and Downs, 2021). Though xylanase had no apparent effect in the particular group, the nutritional richness of microalgae and bioactive components might affect the underlying physiological mechanisms, improving the FCR (Chaudhary et al., 2023).

This study found no significant changes in relative carcass weight, gastrointestinal tract weight, and ileal length among the treatment groups. However, there was a numerical increase in the weight of breast and drumstick in the MAG and MAG+XYN groups compared to the CON group. The increase in the weight of muscles may be due to the presence of a higher amount of protein along with other bioactive compounds and fatty acids in *A. platensis* (Peiretti and Meineri, 2011; Farag et al., 2016). The weight of the proventriculus, liver, intestine, and length of the ileum were increased numerically in the MAG+XYN group, followed by CON and MAG groups. Studies found that adequate dietary fiber, a fermentable carbohydrate with prebiotic functions, plays a critical role in the development of the digestive system (Jha and Mishra, 2021). But the weight of gizzard was higher in the CON diet than in the diets with microalgae. An increase in feed particle size enhances the size of the gizzard (Jha and Mishra, 2021). In our study, we fed broilers in mash form, and the microalgae were in powdered form, so the CON diet had comparatively bigger particles.

The major digestion and nutrient absorption in broilers occur in the small intestine. So, understanding the quality of the small intestine is critical. The study found a numerical improvement in villi height and a decrease in crypt depth in MAG and MAG+XYN groups compared to the CON group. The improvement of villi height results in the better absorption of nutrients, and as a result, the study found a significant improvement in BW gain. A study on broiler chickens with different microalgae species (*Tysochrysis lutea, Tetraselmis chuii, Porphyridium cruentum*) also reported the improvement of gut morphology, especially of duodenum and ileum (Šefcová et al., 2021). The potential reason for the improvement in gut morphology in the study might be due to the presence of oligosaccharides in *A. platensis*. Oligosaccharides bind with a side chain of mucin that prevent bacterial adhesion to the gut epithelium and protect against epithelial damage (Cornick et al., 2015).

This study reports the relative expression of different genes related to the gut barrier (*OCLN, MUC2, CLDN1, ZO1*, and *Cadherin*), immunity (*IL1b, IL4, IL6, IL10, TLR4, NFkB, chB6, AvBD4, CD4, CD45*, and *CD56*), antioxidant (*Nrf2, SOD1*, and *SOD2*), and nutrient transportation (*SLC2A1* and *SLC7A7*) on d 3 and d 35. On d 3, the expression of all genes related to the gut barrier, immunity, antioxidant, and nutrient transport was inconsistent, even within the same category. Such variation in the expression of genes might be due to the early age and biological variation of the birds. Perhaps, the period observed is not long enough to conclude the effect of microalgae on gene expression. The study found a significantly higher expression of gene *ZO1* in the MAG group compared to CON and MAG +XYN groups. The ZO1 gene helps maintain the integrity of the tight junctions and prevents any passing of bacteria and toxins into the bloodstream. In addition, *ZO1* also interacts with other proteins to regulate the permeability of epithelium. The change in the relative expression of the *MUC2* gene in the MAG group also represents the positive effects on the gut health of the broiler. The presence of mucins in the gut is vital for the lubrication of feed and the prevention of pathogens, toxins, or environmental irritants (Grondin et al., 2020). The study also found a higher expression of the *AvBD4* gene in the MAG group, followed by the MAG+XYN and CON group. *AvBD4* is a 14-member family of antimicrobial peptides and well known for broad antimicrobial activity in the innate immune system (Sugiharto, 2020). The expression of gene *AvBD4* represents the resistance of broilers against salmonella. The higher expression of *AvBD4* in the microalgae-fed group might result due to the presence of bioactive compounds in microalgae. The study found a significant increase in the expression of *CD56*, also known as neural cell adhesion molecule that maintains cell−cell interaction and cell signaling. An increased expression of *CD56* is associated with reduced inflammatory bowel disease or inflammatory disorders of the gut. *CD56* also acts in regulating immune responses in the gut. Antioxidant gene *Nrf2*, also known as a master regulator of oxidation during environmental stress, was highly expressed in the microalgae-added diets. This expression suggests that microalgae have the potential to fight against oxidative stress. *Nrf2* also plays a critical role in maintaining intestinal integrity and mucosal barrier by regulating reactive oxygen species (Wen et al., 2019). The *SLC2A1* and *SLC7A7* genes, which are linked to nutrient transport,

were also observed. *SLC7A7,* which is responsible for the transportation of L-amino acids, was significantly higher in the MAG group, followed by MAG+XYN and CON groups. The increased expression of *SLC7A7* was attributed to higher nutrient absorption and improved growth performance of broilers in the MAG and MAG+XYN groups. The higher expression of glucose transporter and L-amino acid transporter genes in the microalgae group might be due to the higher villi height in these groups compared to the control group.

In conclusion, the dietary supplementation of microalgae at an adequate level has the ability to improve growth performance (BW, ADG, ADFI, and FCR), relative carcass and organ weight, gut structure (VH and CD), and ileal gene expression related to immunity, gut barrier functions, antioxidant activities, and nutrient transportation. This study did not find any significant changes in the outcomes due to xylanase addition to a diet containing microalgae. Further studies could assess the effect of different concentrations of xylanase inclusion on a diet containing microalgae. Regardless of xylanase addition, microalgae supplementation into a diet has the potential to promote sustainable and healthy broiler production, and the functional properties of microalgae could be credited for the benefits.

ACKNOWLEDGMENTS

This work was supported by the USDA National Institute for Food and Agriculture, Hatch-Multistate Fund, managed by the College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI. The authors thank Prem Lal Mahato and Sadid Al Amaz for their help in the sample collection.

DISCLOSURES

The authors declare no conflict of interest in this manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103056.

REFERENCES

Alshelmani, M. I., E. A. Abdalla, U. Kaka, M. A. Basit, M. I. Alshelmani, E. A. Abdalla, U. Kaka, and M. A. Basit. 2021. Nontraditional feedstuffs as an alternative in poultry feed. Advances in Poultry Nutrition Research. IntechOpen, London, United Kingdom.

AOAC. 2006. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Gaithersburgs, MD.

Berrocoso, J. D., R. Kida, A. K. Singh, Y. S. Kim, and R. Jha. 2017. Effect of in ovo injection of raffinose on growth performance and gut health parameters of broiler chicken. Poult. Sci. 96:1573–1580.

Bleakley, S., and M. Hayes. 2017. Algal proteins: extraction, application, and challenges concerning production. Foods 6:33.

Bobin-Dubigeon, C., M. Lahaye, F. Guillon, J.-L. Barry, and D. J. Gallant. 1997. Factors limiting the biodegradation of Ulva sp cell-wall polysaccharides. J. Sci. Food Agric. 75:341–351.

Chaudhary, A., P. Mishra, S. A. Amaz, P. L. Mahato, R. Das, R. Jha, and B. Mishra. 2023. Dietary supplementation of microalgae mitigates the negative effects of heat stress in broilers. Poult. Sci 102:102958.

Cobb-Vantress. 2021. Cobb broiler management guide. Accessed May 2023. https://www.cobb-vantress.com/assets/Cobb-Files/4d0dd628b7/Broiler-Guide_English-2021-min.pdf (verified 26 April 2022).

Cornick, S., A. Tawiah, and K. Chadee. 2015. Roles and regulation of the mucus barrier in the gut. Tissue Barriers 3:e982426.

Donohue, M., and D. L. Cunningham. 2009. Effects of grain and oilseed prices on the costs of US poultry production. J. Appl. Poult. Res. 18:325–337.

El-Bahr, S., S. Shousha, A. Shehab, W. Khattab, O. Ahmed-Farid, I. Sabike, O. El-Garhy, I. Albokhadaim, and K. Albosadah. 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-Hady, A. M. A., O. A. Elghalid, A. Sh. Elnaggar, and E. A. El-khalek. 2022. Growth performance and physiological status evaluation of *Spirulina platensis* algae supplementation in broiler chicken diet. Livest. Sci. 263:105009.

Farag, M. R., M. Alagawany, M. E. Abd El-Hack, and K. Dhama. 2016. Nutritional and healthical aspects of Spirulina (Arthrospira) for poultry, animals and human. Int. J. Pharmacol. 12:36–51.

Farghly, M., and M. Abdelfattah. 2017. Growth performance and carcass characteristics of broilers as affected by feed color. Egypt. J. Anim. Prod. 54:143–148.

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

Grondin, J. A., Y. H. Kwon, P. M. Far, S. Haq, and W. I. Khan. 2020. Mucins in intestinal mucosal defense and inflammation: learning from clinical and experimental studies. Front. Immunol. 11:2054.

Gulizia, J. P., and K. M. Downs. 2021. The effects of feed color on broiler performance between day 1 and 21. Animals 11:1511.

Ismita, J., K. Md. S. Islam, M. Al-Mamun, and M. R. Debi. 2022. Comparative efficacy of citric acid, Spirulina platensis, and their combination as alternatives to an antibiotic growth promoter on the performances of broilers. J. Adv. Vet. Anim. Res. 9:1–7.

Jha, R., and P. Mishra. 2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. J. Anim. Sci. Biotechnol. 12:51.

Kishibuchi, R., N. Nishibori, T. Sagara, and K. Morita. 2019. Putative effect of Spirulina extract on enzyme activities participating in lipid and carbohydrate digestion processes. J. Diet. Suppl. 16:521–529.

Krimpen, M. M.van, P. Bikker, I. M. van der Meer, C. M. C. van der Peet-Schwering, and J. M. Vereijken. 2013. Cultivation, Processing and Nutritional Aspects for Pigs and Poultry of European Protein Sources as Alternatives for Imported Soybean Products. Wageningen UR Livestock Research, Lelystad, Netherlands.

Kusmiyati, K., A. Heratri, S. Kubikazari, A. Hidayat, and H. Hadiyanto. 2020. Hydrolysis of microalgae Spirulina platensis, Chlorella sp., and macroalgae Ulva lactuca for bioethanol production. Int. Energy J. 20:611–620.

Liu, W.-C., Y. Guo, Z.-H. Zhao, R. Jha, and B. Balasubramanian. 2020. Algae-derived polysaccharides promote growth performance by improving antioxidant capacity and intestinal barrier function in broiler chickens. Front. Vet. Sci. 7:601336.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25:402–408.

Milledge, J. J. 2011. Commercial application of microalgae other than as biofuels: a brief review. Rev. Environ. Sci. Biotechnol. 10:31–41.

Patel, A. K., R. R. Singhania, M. K. Awasthi, S. Varjani, S. K. Bhatia, M.-L. Tsai, S.-L. Hsieh, C.-W. Chen, and C.-D. Dong. 2021. Emerging prospects of macro- and microalgae as prebiotic. Microb. Cell. Fact. 20:112.

Peiretti, P. G., and G. Meineri. 2011. 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.

Pestana, J. M., B. Puerta, H. Santos, M. S. Madeira, C. M. Alfaia, P. A. Lopes, R. M. A. Pinto, J. P. C. Lemos, C. M. G. A. Fontes, M. M. Lordelo, and J. A. M. Prates. 2020. Impact of dietary incorporation of Spirulina (*Arthrospira platensis*) and exogenous enzymes on broiler performance, carcass traits, and meat quality. Poult. Sci. 99:2519–2532.

Rierson, R. D. 2011. Broiler preference for light color and feed form, and the effect of light on growth and performance of broiler chicks. Accessed May 2023. https://krex.k-state.edu/dspace/handle/2097/12037 (verified 13 December 2022).

Saadaoui, I., R. Rasheed, A. Aguilar, M. Cherif, H. Al Jabri, S. Sayadi, and S. R. Manning. 2021. Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. J. Anim. Sci. Biotechnol. 12:76.

Sakamoto, K., H. Hirose, A. Onizuka, M. Hayashi, N. Futamura, Y. Kawamura, and T. Ezaki. 2000. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. J. Surg. Res. 94:99–106.

Šefcová, M. A., F. Santacruz, C. M. Larrea-Álvarez, C. Vinueza-Burgos, D. Ortega-Paredes, G. Molina-Cuasapaz, J. Rodríguez, W. Calero-Cáceres, V. Revajová, E. Fernández-Moreira, and M. Larrea-Álvarez. 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.

Singh, A. K., B. Mishra, M. R. Bedford, and R. Jha. 2021. Effects of supplemental xylanase and xylooligosaccharides on production performance and gut health variables of broiler chickens. J. Anim. Sci. Biotechnol. 12:98.

Singh, A. K., U. P. Tiwari, B. Mishra, and R. Jha. 2022. Effects of in ovo delivered xylo- and mannan- oligosaccharides on growth performance, intestinal immunity, cecal short-chain fatty acids, and cecal microbiota of broilers. J. Anim. Sci. Biotechnol. 13:13.

Stefanello, C., S. L. Vieira, P. S. Carvalho, J. O. B. Sorbara, and A. J. Cowieson. 2016. Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase. Poult. Sci. 95:1881–1887.

Sugiharto, S. 2020. Nutraceutical aspects of microalgae *Spirulina* and chlorella on broiler chickens. Livest. Res. Rural. Dev. 32:84.

Tavernari, F. De. C., L. F. Roza, D. Surek, C. Sordi, M. L. B. D. Silva, L. F. T. Albino, M. J. Migliorini, D. Paiano, and M. M. Boiago. 2018. Apparent metabolisable energy and amino acid digestibility of microalgae *Spirulina platensis* as an ingredient in broiler chicken diets. Br. Poult. Sci. 59:562–567.

Terezinha Schneider, A., M. Costa Deprá, R. Rodrigues Dias, L. Queiroz Zepka, and E. Jacob-Lopes. 2023. Chapter 5 - Microalgae as superfood. Pages 93−102 in Algae Materials. K. Arunkumar (Series Ed.) & A. Arun, R. Raja and & R. Palaniappan, eds. Academic Press, Cambridge, MA.

Vale, M. A., A. Ferreira, J. C. M. Pires, and A. L. Gonçalves. 2020. CO_2 capture using microalgae. Pages 381−405 in Advances in Carbon Capture. M. R. Rahimpour, M. Farsi and M. A. Makarem, eds. Woodhead Publishing, Sawston, United Kingdom.

Van Hoeck, V., D. Wu, I. Somers, A. Wealleans, B. L. Vasanthakumari, A. L. Gonzalez Sanchez, and D. Morisset. 2021. Xylanase impact beyond performance: a prebiotic approach in broiler chickens. J. Appl. Poult. Res. 30:100193.

Vrenna, M., P. P. Peruccio, X. Liu, F. Zhong, and Y. Sun. 2021. Microalgae as future superfoods: fostering adoption through practice-based design research. Sustainability 13:2848.

Wen, Z., W. Liu, X. Li, W. Chen, Z. Liu, J. Wen, and Z. Liu. 2019. A protective role of the NRF2-Keap1 pathway in maintaining intestinal barrier function. Oxid. Med. Cell. Longev. 2019:1759149.

Wolkers, H., M. J. Barbosa, D. M. M. Kleinegris, R. Bosma, R. H. Wijffels, and P. F. H. Harmsen. 2011. Microalgae: The Green Gold of the Future?: Large-Scale Sustainable Cultivation of Microalgae for the Production of Bulk Commodities. Wageningen UR - Food & Biobased Research, Wageningen, Netherlands.

Wu, Q., L. Liu, A. Miron, B. Klímová, D. Wan, and K. Kuča. 2016. The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: an overview. Arch. Toxicol. 90:1817–1840.

Yadav, S., and R. Jha. 2021. Macadamia nut cake as an alternative feedstuff for broilers: effect on growth performance. Anim. Feed Sci. Technol. 275:114873.

Yadav, S., B. Mishra, and R. Jha. 2019. Cassava (*Manihot esculenta*) root chips inclusion in the diets of broiler chickens: effects on growth performance, ileal histomorphology, and cecal volatile fatty acid production. Poult. Sci. 98:4008–4015.