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Effects of dietary *Arthrospira platensis* supplementation on the growth, pigmentation, and antioxidation in yellow catfish (*Pelteobagrus fulvidraco*)

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ABSTRACT

Spirulina, *Arthrospira platensis*, contains high levels of protein and lutein. To evaluate nutritional, pigmentation, and antioxidation effects of *A. platensis*, a total of 900 juvenile yellow catfish (*Pelteobagrus fulvidraco*) were divided into 18 tanks (3 tanks/treatment, 50 fish/tank) and fed a diet supplemented with *A. platensis* for 50 days. *A. platensis* was used in quantities of 0, 57.6, 115.1, 172.7, 230.3, and 287.9 g kg^{-1} for experimental diets to replace fishmeal protein at levels of 0 (AP0), 20% (AP20), 40% (AP40), 60% (AP60), 80% (AP80), and 100% (AP100). Results revealed that substituting up to 80% of fishmeal by *A. platensis* biomass did not have a negative effect on fish growth, feed utilization, or apparent digestibility. However, significantly lower growth rates were observed when 100% of the fishmeal was substituted ($P < 0.05$). *A. platensis* replacement groups led to an increase in skin yellowness and dose-dependent enrichments of total lutein in dorsal skin, abdominal skin, and liver tissues ($P < 0.05$). There were similar enhancements observed in glutathione concentrations and glutathione peroxidase activities in plasma and liver ($P < 0.05$). Analyses based on lutein concentration between abdominal skin and experimental diets revealed that the optimal substitution amount was 72.03% (207.4 g kg^{-1} of *A. platensis*), which could ensure growth and pigmentation in yellow catfish.

1. Introduction

Microalgae has received recent recognition as the future of clean energy and industrial material resources, such as food, drugs, and cosmetics. Additionally, due to their nutritional value, *Arthrospira platensis* and *Chlorella vulgaris* (Radhakrishnan et al., 2014), *Nannochloropsis sp.* and *Isochrysis sp.* (Walker and Berlinsky, 2011), *Tetraselmis sp.* (Kiron et al., 2012), and *Navicula sp.* (Patterson and Gatlin, 2013) have been recognized as renewable protein sources in aquiculture feed. *A. platensis* is a cyanobacterium present in free-floating filaments in the form of an open left-hand helix, characterized by cylindrical multicellular trichomes (Nicoletti, 2016), which can be harvested and processed easily (Habib et al., 2008). It is rich in protein, vitamins, minerals, essential amino acids, antioxidant pigments (e.g., carotenoids), and essential fatty acids (e.g., gamma-linolenic acid) (Belay et al., 1996). Antioxidant compounds, such as carotenoids, phycocyanin, and tocopherols have significant effects on scavenging free radicals (Nakagawa et al., 2007; Şimşek et al., 2007). For example, Hany and Riad (2014) found that dietary *A. platensis* (50 g kg^{-1} and 100 g kg^{-1}) supplements could enhance serum antioxidant activity in Nile tilapia (*Oreochromis niloticus*).

The coloration effect of *A. platensis* has been extensively investigated in cultured animals. Dietary *A. platensis* (40 g kg^{-1}) was found to influence both the yellowness and redness of broiler flesh, and the increments in yellowness due to dietary *A. platensis* content could be related to the accumulation of zeaxanthin in the flesh (Toyomizu et al., 2001). Dietary *A. platensis* (80 g kg^{-1}) has also been found to enhance red coloration in the skin of red swordtail (*Xiphophorus helleri*) (James et al., 2006), as well as enhance yellow and blue coloration in the skin of yellow tail cichlid (*Pseudotropheus acei*) that fed on 25 g kg^{-1} dietary *A. platensis* (Güroy et al., 2012). However, to our knowledge, only a few reports are available concerning the effect of *A. platensis* on yellow catfish (*Pelteobagrus fulvidraco*) pigmentation.

A. platensis is rich in carotenoids, including lutein, β-carotene, astaxanthin and so forth, which dissolve in fat and result in yellow, red, orange, and green pigmentation of the eggs, skin, and flesh of many fish species (Fuji, 1969; Kop and Durmaz, 2008). Fish, like other animals, do not have a biosynthetic pathway that synthesizes carotenoids;

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therefore, they must acquire carotenoids from their food (Habib et al., 2008).

Fishmeal is an important protein source in commercial aquaculture feeds due to its high content of essential amino acids, mineral elements, bioactive substances, and low levels of anti-nutritional factors (Glencross et al., 2007; Chiu et al., 2013). However, fishmeal sources are environmentally and economically unsustainable (FAO, 2014). Therefore, it is necessary to investigate the effect of alternative protein sources by replacing fishmeal with supplemented diets.

Yellow catfish is classified as Siluriformes, an omnivorous freshwater fish, which is regarded as an excellent candidate for freshwater culture and is highly preferred by Asian consumers for its flavor (Luo et al., 2011). Yellow catfish possess a stomach that could digest the cellular wall of *A. platensis*, compared to fish with no stomach. The normal coloration of yellow catfish is black with yellow; these pigments are affected by the melanin and carotenoid content, respectively. In this study, the effect of dietary *A. platensis* on growth performance, feed utilization, pigmentation, and antioxidative status of yellow catfish were investigated.

2. Materials and methods

2.1. Experimental diets

A. platensis (Lvfuyuan Co., Ordos, China) was used in quantities of 57.6, 115.1, 172.7, 230.3, and 287.9 g kg^{-1} to replace fishmeal protein at levels of 20% (AP20), 40% (AP40), 60% (AP60), 80% (AP80), and 100% (AP100). A diet without *A. platensis* was used for the control group (AP0). The amount of fish oil and soybean oil was increased while cellulose content decreased in order to maintain the total lipid content in experimental diets (Table 1). All ingredients were completely mixed with appropriate water then made into pellets using an SLP-45 laboratory granulator (Fishery Mechanical Facility Research Institute, Shanghai, China). Diets were oven-dried at 60 °C and stored at 4 °C until used for experimentation.

Table 1
Diet formulation and chemical compositions of the experimental diets (g kg^{-1} dry matter).

Diets	AP0	AP20	AP40	AP60	AP80	AP100
White fishmeal	300.0	240.0	180.0	120.0	60.0	0.0
A. platensis	0.0	57.6	115.1	172.7	230.3	287.9
Soybean meal	196.8	196.8	196.8	196.8	196.8	196.8
Rapeseed meal	210.0	210.0	210.0	210.0	210.0	210.0
Wheat flour	160.0	160.0	160.0	160.0	160.0	160.0
Fish oil	23.1	25.1	27.1	29.1	31.0	33.0
Soybean oil	23.1	25.1	27.1	29.1	31.0	33.0
Vitamin premix	3.9	3.9	3.9	3.9	3.9	3.9
Choline chloride	1.1	1.1	1.1	1.1	1.1	1.1
Mineral premix	50.0	50.0	50.0	50.0	50.0	50.0
Cellulose	11.0	9.4	7.9	6.3	4.9	3.3
Y_2O_3	1.0	1.0	1.0	1.0	1.0	1.0
CMC	20.0	20.0	20.0	20.0	20.0	20.0
Crude protein	425.2	415.0	417.8	412.7	419.1	407.5
Crude lipid	78.8	74.7	79.7	83.5	83.9	83.3
Moisture	117.1	138.3	129.2	112.2	118.8	127.7
Lutein (μg g^{-1})	0.0	0.6	1.2	1.8	2.4	3.0

White fishmeal: Seafood white fishmeal imported from United States;
A. platensis: Lvfuyuan company, Ordos, China.
Soybean meal and Rapeseed meal: Purchased from Wuhan Gaolong Feed Co. Ltd, Wuhan, Hubei, China;
Vitamin premix (mg kg^{-1} diet): Vitamin B_1, 20; Vitamin B_2, 20; Vitamin B_6, 20; Vitamin B_{12}, 0.02; folic acid, 5; calcium patothenate, 50; inositol, 100; niacin, 100; biotin, 0.1; cellulose, 3522; Vitamin A, 11; Vitamin D, 2; Vitamin E, 100; Vitamin K, 10;
Mineral premixes (mg kg^{-1} diet): NaCl, 500.0; $MgSO_4{\cdot}7H_2O$, 8155.6; $NaH_2PO_4{\cdot}2H_2O$, 12,500.0; KH_2PO_4, 16,000.0; $Ca(H_2PO_4)\cdot 2H_2O$, 7650.6; $FeSO_4{\cdot}7H_2O$, 2286.2; $C_6H_{10}CaO_6{\cdot}5H_2O$, 1750.0; $ZnSO_4{\cdot}7H_2O$, 178.0; $MnSO_4{\cdot}H_2O$, 61.4; $CuSO_4{\cdot}5H_2O$, 15.5; $CoSO_4{\cdot}7H_2O$, 0.91; KI, 1.5; Na_2SeO_3, 0.60; Corn starch, 899.7.
Lutein: the values were calculated through the contents of *A. platensis* in diets.

2.2. Fish, experimental conditions, and feeding procedures

Yellow catfish were obtained from the Dengjia State fish farm (Jiangxia, Wuhan, Hubei, China). Two weeks prior to the feeding trials, all fish were temporarily domesticated in a fiber glass cylinder (1500 L) and fed twice a day at 08:30 and 16:30. Feeding trials were conducted in an indoor recirculating system. At the beginning of each trial, fish were fasted for 24 h. Healthy and similarly sized fish (n = 900, 3.1 ± 0.05 g) were randomly selected, batch weighed, and placed into 18 fiber glass tanks (150 L, 3 tanks/treatment, 50 fish/tank). Each tank was provided with continuous aeration. During the experiment, water temperature was maintained at 27 ± 3 °C. Dissolved oxygen content was kept above 7.5 mg L^{-1}, ammonia-N was less than 0.1 mg L^{-1}, and the photoperiod was 12 L:12D with the light period lasting from 08:00 to 20:00. During the trials, fish were fed to apparent satiation twice daily at 08:30 and 16:30. Feeding trials lasted for 50 d.

2.3. Sample collection

At the beginning of the trials, three batches of 10 fish were randomly selected as the initial samples. After feeding trials were conducted, all fish from each tank were bulk weighed. Seventeen fish from each tank were anesthetized with MS-222 (80 mg L^{-1}, tricaine methane sulfonate; Argent Chemical Laboratories Inc., Redmond, W.A., U.S.A.). Three fish were sampled and weighed, then stored at −20 °C to determine final body composition. Five fish from each tank were sampled, weighed, and measured for length in order to calculate the condition factor, then the weight of the liver was measured to calculate the hepatosomatic index. Three fish were selected to measure coloration and photographed. The remaining six fish were randomly selected for blood, liver, dorsal, and abdominal skin samples. Blood samples were collected from the caudal vein using heparinized syringes then centrifuged at 3000 rpm for 15 min to acquire plasma samples, which were stored at −80 °C for future analyses. After blood sampling, fish were immediately sampled to collect liver, dorsal, and abdominal skin tissue samples. Tissue samples were kept on ice until stored at −80 °C for future analyses.

Two weeks after feeding trials began, feces were collected every day 6 h after the last feeding and stored at −20 °C. Feces were freeze-dried in order to determine apparent digestibility coefficients (ADCs) of dry matter, proteins, and amino acids. *A. platensis* feces were observed under a DP80 microscope (Olympus, Japan). The ADC of each nutrient was calculated using the following formula:

$$ADC = 100 \times (1 - (Nf \div Nd) \times (Md \div Mf)),$$

where ADC is the apparent digestibility coefficient of the nutrient (%), Md is the Y_2O_3 content in the diet (g kg^{-1}), Mf is the Y_2O_3 content in the feces (g kg^{-1}), Nd is the nutrient content in the diet (g kg^{-1}), and Nf is the nutrient content in the feces (g kg^{-1}).

2.4. Biochemical assays

Moisture, protein, lipid, ash of the diets, and whole fish bodies were analyzed following AOAC (2003) procedures. Moisture content was determined by oven drying at 105 °C to a constant weight. Ash content was determined following incineration in a muffle furnace at 550 °C for 12 h. A 2300 Kjeltec Analyzer Unit machine (FOSS Tecator, Haganas, Sweden) was used to measure crude protein content. Crude lipid content was determined by ether extraction in a Soxtec System HT6 (Tecator Ltd., Haganas, Sweden). The amino acid of the experimental diets and feces were analyzed by an A300 amino acid analyzer (membraPure, Germany). Samples were processed following the acid hydrolysis

Table 2
ADC values of dry matter, proteins, and amino acids of yellow catfish fed different experimental diets (%).

Diets	AP0	AP20	AP40	AP60	AP80	AP100	Equations	R^2	*P*- value
Dry matter	74.44 ± 1.45	74.33 ± 1.45	76.74 ± 0.67	75.09 ± 1.66	75.76 ± 0.84	73.06 ± 1.27	None	None	0.460
Protein	90.58 ± 0.61	90.24 ± 0.21	90.84 ± 0.45	90.75 ± 0.9	89.85 ± 0.42	88.43 ± 0.17	$y = -6E\text{-}05x^2 + 0.0117x + 90.294$	0.8864	0.039
Essential amino acid									
Thr	91.91 ± 1.34	91.33 ± 1.10	91.87 ± 1.37	92.12 ± 0.49	91.95 ± 0.71	90.60 ± 0.13	None	None	0.933
Val	91.35 ± 1.43	89.82 ± 1.22	91.70 ± 1.13	90.30 ± 0.67	89.92 ± 0.67	88.25 ± 0.66	None	None	0.381
Met[a]	94.73 ± 1.00	91.79 ± 1.05	89.28 ± 2.09	90.26 ± 0.54	89.03 ± 0.67	84.45 ± 0.23	None	None	0.061
Ile	91.98 ± 1.38	92.08 ± 0.98	92.61 ± 1.30	91.64 ± 0.32	91.18 ± 0.56	89.71 ± 0.23	None	None	0.531
Leu	93.05 ± 1.34	92.67 ± 0.90	93.35 ± 1.13	92.19 ± 0.38	91.92 ± 0.53	90.79 ± 0.19	None	None	0.536
Phe	90.65 ± 1.11	89.79 ± 1.33	92.28 ± 0.84	91.58 ± 0.39	92.09 ± 0.54	91.05 ± 0.51	None	None	0.397
Lys	95.11 ± 0.97	95.29 ± 0.49	95.76 ± 0.74	95.30 ± 0.24	95.15 ± 0.50	94.33 ± 0.19	None	None	0.799
His	93.12 ± 1.01	93.15 ± 0.97	94.43 ± 0.75	94.03 ± 0.25	93.70 ± 0.30	93.35 ± 0.40	None	None	0.739
Arg	96.10 ± 0.43	95.62 ± 0.46	96.04 ± 0.64	94.98 ± 0.39	94.74 ± 0.40	93.22 ± 0.34	None	None	0.066
Non-essential amino acid									
Asp	91.66 ± 1.33	91.79 ± 1.02	92.51 ± 1.28	92.13 ± 0.34	91.90 ± 0.62	90.99 ± 0.46	None	None	0.941
Ser	92.09 ± 1.30	91.86 ± 0.95	91.90 ± 1.32	91.16 ± 0.58	90.54 ± 0.87	89.11 ± 0.02	None	None	0.447
Glu	95.04 ± 0.93	95.26 ± 0.49	95.74 ± 0.69	95.06 ± 0.16	95.04 ± 0.37	94.08 ± 0.13	None	None	0.522
Gly	92.55 ± 1.24	92.47 ± 0.97	93.30 ± 0.97	92.32 ± 0.45	91.91 ± 0.60	90.57 ± 0.30	None	None	0.522
Ala	92.45 ± 1.33	91.86 ± 1.08	92.18 ± 1.22	90.76 ± 0.48	89.96 ± 0.78	88.22 ± 0.32	None	None	0.131
Tyr	91.97 ± 1.09	92.64 ± 0.89	93.07 ± 1.02	92.48 ± 0.48	92.36 ± 0.72	90.49 ± 0.56	None	None	0.933
Pro	93.11 ± 1.22	91.98 ± 1.11	93.29 ± 0.73	93.48 ± 0.45	93.80 ± 0.43	93.67 ± 0.16	None	None	0.684

Data are presented as the Means ± SE ($P < 0.05$; n = 3). The *P*-values in the tables were for one-way ANOVA.
[a] The acid hydrolysis method was adopted to analyze amino acid which may lead to inaccurate content of methionine.

Table 3
Growth, feed utilization, and morphological indices of yellow catfish fed different experimental diets.

Diets	AP0	AP20	AP40	AP60	AP80	AP100	Equations	R^2	*P*- value
IBW (g)	3.10 ± 0.05	3.12 ± 0.04	3.10 ± 0.02	3.13 ± 0.01	3.11 ± 0.01	3.05 ± 0.02	None	None	0.530
FBW (g)	10.17 ± 0.60	11.24 ± 0.30	9.89 ± 0.25	10.11 ± 0.66	9.87 ± 0.90	7.54 ± 0.21	$y = -7E\text{-}05x^2 + 0.011x + 10.273$	0.8114	0.013
FR (%BW/d)	4.76 ± 0.14	4.76 ± 0.07	4.79 ± 0.21	4.42 ± 0.07	4.40 ± 0.33	4.86 ± 0.26	None	None	0.794
SGR (%/d)	2.36 ± 0.11	2.57 ± 0.05	2.32 ± 0.06	2.35 ± 0.13	2.29 ± 0.17	1.81 ± 0.07	$y = -1E\text{-}05x^2 + 0.0024x + 2.3775$	0.8404	0.007
FE (%)	43.98 ± 2.38	47.50 ± 0.7	45.54 ± 2.05	47.32 ± 1.38	42.06 ± 1.63	35.19 ± 1.82	$y = -0.0004x^2 + 0.072x + 43.898$	0.9373	0.047
PRE (%)	14.09 ± 0.76	15.05 ± 0.32	14.29 ± 0.84	15.61 ± 0.28	14.26 ± 1.25	10.76 ± 0.83	$y = -0.6525x^2 + 6.5895x - 13.812$	0.9677	0.017
CF (g/cm^3)	2.88 ± 0.18	3.43 ± 0.18	3.19 ± 0.18	3.44 ± 0.20	2.92 ± 0.10	2.29 ± 0.09	$y = -4E\text{-}05x^2 + 0.0088x + 2.9092$	0.9002	0.043
HSI (%)	1.90 ± 0.25	2.75 ± 0.20	2.16 ± 0.19	2.20 ± 0.21	2.50 ± 0.23	0.98 ± 0.22	$y = -4E\text{-}05x^2 + 0.0102x + 1.9673$	0.6466	0.047

IBW: initial body weight.
FBW: final body weight.
FR: feeding rate (% body weight day^{-1}) = 100 × (feed intake in dry matter)/[days × (initial body weight + final body weight)/2].
SGR: specific growth rate (% d^{-1}) = 100 × [ln (final body weight) − ln (initial body weight)]/days.
FE: feed efficiency (%) = (final body weight-initial body weight)/feed intake in dry matter.
PRE: protein retention efficiency = (100 × protein retained in fish body) / protein intake;
CF: condition factor ($g\,cm^{-3}$) = 100 × (body weight)/$(\text{body length})^3$.
HSI: hepatosomatic index (%) = 100 × (liver weight)/(whole body weight).
Data are presented as the Means ± SE ($P < 0.05$; n = 3). The *P*-values in the tables were for one-way ANOVA.

Table 4
Whole body composition of yellow catfish fed different experimental diets.

Diets	AP0	AP20	AP40	AP60	AP80	AP100	Equations	R^2	*P*- value
% Fresh weight									
Crude protein	14.71 ± 0.14	14.79 ± 0.09	14.96 ± 0.26	14.81 ± 0.09	14.47 ± 0.11	14.87 ± 0.02	None	None	0.305
Crude lipid	7.65 ± 0.15	8.42 ± 0.27	8.51 ± 0.21	7.67 ± 0.25	7.39 ± 0.27	6.68 ± 0.43	$y = -5E\text{-}05x^2 + 0.0094x + 7.8186$	0.8823	0.014
Ash	14.11 ± 0.10	13.80 ± 0.03	14.26 ± 0.24	14.47 ± 0.04	14.31 ± 0.13	15.15 ± 0.12	$y = 2E\text{-}05x^2–0.0016x + 14.049$	0.7960	0.027
Moisture	73.00 ± 0.10	73.39 ± 1.09	73.05 ± 0.10	73.60 ± 0.49	73.75 ± 0.45	74.37 ± 0.60	None	None	0.830

Data are presented as the Means ± SE ($P < 0.05$; n = 3). The *P*-values in the tables were for one-way ANOVA.

method described by Liu et al. (2016). The Y_2O_3 content of the diet and feces samples were determined by an OPTIMA 8000DV Inductively Coupled Plasma-mass Spectrometry machine (PekinElmer, U.S.A.) at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, Hubei, China).

2.5. Fish skin color determination

A Konica Minolta CR-400 tristimulus colorimeter (Minolta, Osaka, Japan) was used to measure the dorsal and abdominal skin color parameters of the fish. The L value represents lightness (0 for black, 100 for white), a* represents the red/green dimension, and b* represents the yellow/blue dimension, in accordance with the recommendations of the International Commission on Illumination (CIE, 1976). Fish from different experimental groups were photographed using a Nikon D5100 camera (Japan).

Table 5
Body color of yellow catfish fed different experimental diets.

Diets	AP0	AP20	AP40	AP60	AP80	AP100	Equations	R^2	*P*- value
Abdominal skin									
Lightness (L)	78.36 ± 0.78	76.25 ± 0.25	76.79 ± 0.54	77.07 ± 0.82	77.54 ± 0.8	77.47 ± 1.16	None	None	0.374
Redness (a*)	−0.95 ± −0.44	−3.87 ± −0.22	−3.24 ± −0.88	−4.21 ± −0.52	−3.59 ± −0.38	−2.33 ± −0.61	$y = 0.0001x^2 - 0.0358x - 1.2875$	0.8097	0.003
Yellowness (b*)	12.83 ± 0.61	35.93 ± 1.78	38.6 ± 1.33b	40.95 ± 1.76	42.94 ± 0.97	33.84 ± 1.43	$y = -0.0009x^2 + 0.3177x + 15.26$	0.9230	< 0.001
Dorsal skin									
Lightness (L)	49.64 ± 4.28	45.51 ± 1.54	43.32 ± 2.84	38.58 ± 1.14	44.61 ± 1.31	41.15 ± 2.52	None	None	0.140
Redness (a*)	−1.79 ± 0.22	−3.88 ± 0.2	−2.84 ± 0.48	−3.16 ± 0.49	−3.42 ± 0.53	−2.36 ± 0.52	$y = 6E\text{-}05x^2 - 0.0173x - 2.1522$	0.4854	0.015
Yellowness (b*)	7.65 ± 1.83	20.84 ± 1.7	22.91 ± 2.32	19.94 ± 1.77	22.49 ± 1.9	17.44 ± 1.27	$y = -0.0005x^2 + 0.1637x + 9.5919$	0.8158	< 0.001

Data are presented as the Means ± SE ($P < 0.05$; n = 9). The *P*-values in the tables were for one-way ANOVA.

2.6. Pigment content

Skin lutein concentration was determined following the methods described by Karadas et al. (2006). Skin and liver samples (200–300 mg) were separately mixed into a 0.7 mL solution consisting of 5% sodium chloride; 1 mL ethanol was added to homogenize samples. During homogenization, 2 mL hexane was added. Then, samples were centrifuged (4 °C, 4600 rpm, 10 min) and the hexane phase was collected. Extraction with hexane was performed twice and the combined phase was evaporated under nitrogen, then the solution was dissolved in a methanol mixture consisting of trichloro methane (3:1 *v/v*, 1 mL). The obtained solution was used for HPLC (Waters e2695, U.S.A.) analyses immediately after passing through a 0.22 μm membrane filter. The HPLC was equipped with a Waters YMC Carotenoid C18 column (5 μm, 4.6 × 250 mm); the mobile phase consisted of solvent A (dichloromethane: methanol: acetonitrile: H_2O = 50:850:55:45) and solvent B (dichloromethane: methanol: acetonitrile: H_2O = 250:280:425:45). All steps were conducted under dim light. The lutein content of *A. platensis* was determined following the methods described by Ferreira et al. (2007).

2.7. Determination of MDA, GSH, and antioxidant enzymes

The contents of malondialdehyde (MDA) and reduced glutathione (GSH) in the plasma and liver were determined using A003-1 and A006-2 kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). The activities of glutathione peroxidase (GSH-PX), catalase (CAT), and superoxide dismutase (SOD) of the plasma and liver were determined using A005, A007-1, and A001-3 kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.8. Statistical analyses

Results are presented as the mean ± standard error (S.E.M). All data were analyzed using SPSS 19.0. *A. platensis* response to the experimental variables were evaluated based on linear or quadratic regression analyses and subjected to a one-way analysis of variance (ANOVA) using diet as the independent variable. Duncan's new multiple range post-hoc test was used to detect significance differences between group means. The significance level was set at $P < 0.05$.

3. Results

In this study, the protein content of *A. platensis* was slightly higher than white fishmeal, and the content of most essential amino acids were higher than white fishmeal, with the exception of lysine (Lys), methionine (Met), and histidine (His). The content of Lys, Met, and His decreased as dietary spirulina increased. The amount of lutein was 10.32 μg g^{-1} in *A. platensis*.

The ADC values of dry matter and amino acids were not significantly different as dietary *A. platensis* increased. The second-order polynomial regression results revealed that there was a significant difference between dietary *A. platensis* level and ADC protein values ($P < 0.05$) (Table 2). Through the examination of fresh feces under a microscope, no complete *A. platensis* was found in any group.

The regression analyses demonstrated that with the dietary increase of *A. platensis*, final body weight (FBW), specific growth rate (SGR), feed efficiency (FE), protein retention efficiency (PRE), condition factor (CF), and hepatosomatic index (HIS) significantly increased at first and then decreased ($P < 0.05$). Additionally, FBW, SGR, FE, and PRE were significantly lower than AP0 when 100% of the fishmeal was substituted (AP100) ($P < 0.05$). Initial body weight (IBW) and feeding rate (FR) were not significantly different across treatments (Table 3). Whole-body lipid content was found to have similar trends as dietary *A. platensis* increased, while ash decreased at first and then increased ($P < 0.05$) (Table 4). The lipid content of the AP80 and AP100

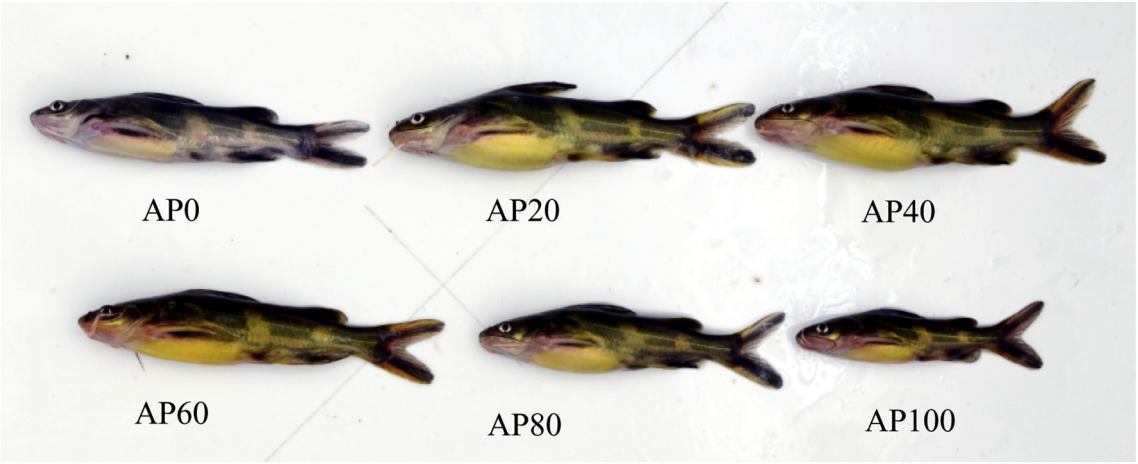

Fig. 1. Effects of dietary *A. platensis* on the pigmentation of yellow catfish.

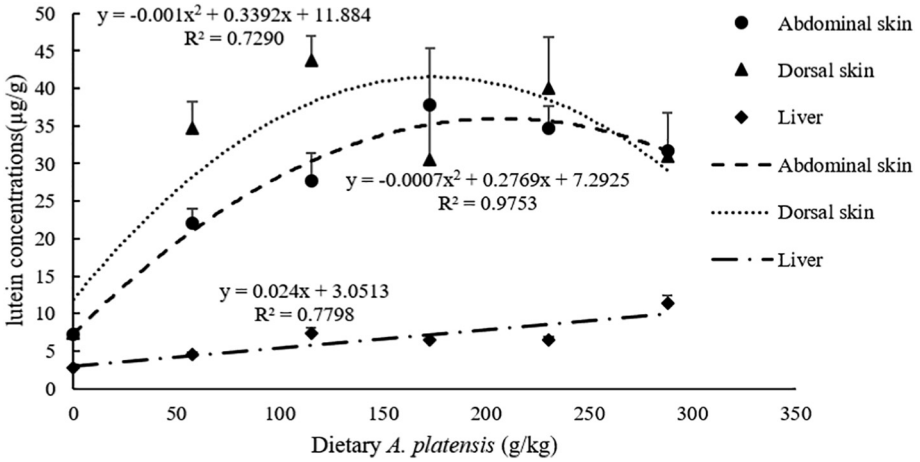

Fig. 2. Regression analysis between the lutein concentration and dietary *A. platensis* of abdominal skin, dorsal skin, and liver tissues in yellow catfish.

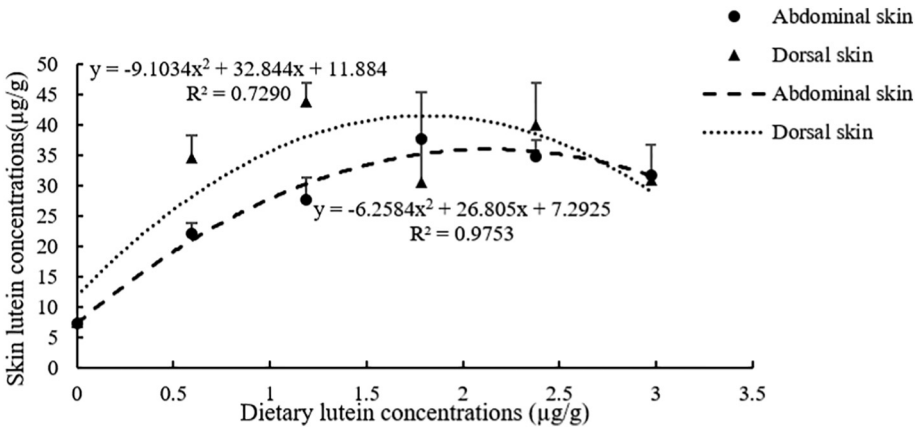

Fig. 3. Regression analysis between the lutein concentration and abdominal and dorsal skin tissues in yellow catfish fed different levels of *A. platensis* experimental diets.

treatments were significantly lower than AP0 ($P < 0.05$). The quadratic regression analyses on specific growth rates (SGR) revealed that yellow catfish could obtain the best growth rate when *A. platensis* replaced 41.68% (120 g kg^{-1} diet *A. platensis*) of the fishmeal protein.

Coloration data revealed that there was no significant difference in the L of skin across groups (Table 5). Regression analyses also revealed that a* of the dorsal and abdominal skin significantly decreased at first then increased as dietary *A. platensis* increased ($P < 0.05$). The b* of these tissues significantly exhibited the opposite trend ($P < 0.05$) (Table 5). The a* of the dorsal skin in the AP20, AP40, AP60, and AP80 groups were significantly lower than AP0 ($P < 0.05$). There was a significant decrease of a* in the abdominal skin compared to AP0 ($P < 0.05$), except for the AP40 and AP100 groups. The b* of AP0 was the lowest among all groups ($P < 0.01$). Compared to AP0, all other groups had better pigmentation as a result of dietary *A. platensis* (Fig. 1).

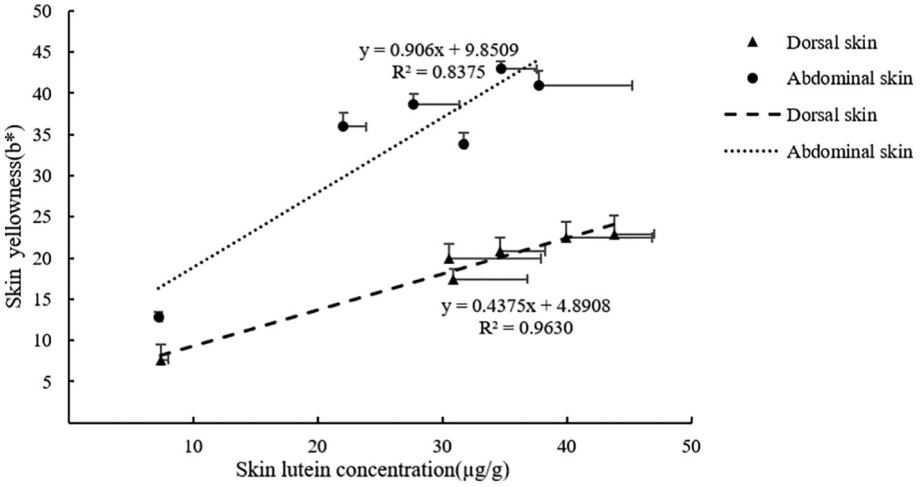

Fig. 4. Regression analysis based on the lutein concentration and yellowness (b*) of abdominal skin and dorsal skin in yellow catfish fed different levels of *A. platensis*.

The second-order polynomial regression analyses revealed significant differences between dietary *A. platensis* level and lutein concentrations of skin tissue ($P < 0.05$), as well as a significantly positive linear effect between dietary *A. platensis* level and lutein concentrations of liver tissue ($P < 0.05$) (Fig. 2). The content of lutein in AP0 was the lowest in both dorsal and abdominal skin tissues ($P < 0.05$). A positive correlation was found between the lutein concentration of different diets and the concentrations of abdominal ($R^2 = 0.9753$) and dorsal skin ($R^2 = 0.7290$) (Fig. 3). Regression analysis also showed a positive liner relationship between yellowness (b*) and the lutein concentrations of skin (Fig. 4), the regression coefficients for dorsal and abdominal skin were 0.9630 and 0.8375, respectively.

With an increase of dietary *A. platensis*, MDA concentrations in the plasma and liver were found to significantly decrease at first then increase ($P < 0.05$), while GSH exhibited the opposite trend ($P < 0.05$). MDA concentrations in the plasma and liver were significantly lower in *A. platensis* substitution groups compared to AP0 ($P < 0.05$). Fish fed *A. platensis* supplemented diets had significantly higher plasma concentrations of GSH compared to AP0 ($P < 0.05$). Regression analyses revealed that GSH-PX and SOD activities in plasma increased at first then decreased as dietary *A. platensis* increased ($P < 0.05$). The GSH-PX, CAT, and SOD activities exhibited the reverse trend in liver tissues ($P < 0.05$). GSH-PX activities in plasma significantly increased in the AP40 and AP60 groups compared to AP0 ($P < 0.05$). SOD activities in plasma also significantly increased in the AP60 and AP80 groups compared to AP0 ($P < 0.05$). GSH-PX and SOD activities in the AP100 group were also significantly higher compared to AP0 in liver tissues ($P < 0.05$). Lastly, when *A. platensis* comprised 40% or more of experimental diets, CAT activities were significantly higher than AP0 in liver tissues ($P < 0.05$) (Table 6).

4. Discussion

The addition of *A. platensis* did not have a negative effect on digestion or feed utilization in yellow catfish, which may be due to the thin cellular wall of *A. platensis* that is comprised of 80% pectin and 20% cellulose (Cohen, 1997). Additionally, the stomach of yellow catfish could help digest these cellular walls, which may act as a barrier in other fish species that do not have a stomach. Compared to *Euglena* and *Chlorella*, *A. platensis* is a more acceptable diet for larval tilapia, as it is easier for larvae to assimilate (Lu et al., 2004). Pakravan et al. (2017) found that the activities of trypsin and chymotrypsin significantly declined in shrimp (*Litopenaeus vannamei*) when fed a diet containing *A. platensis* greater than 200 g kg^{-1}. Similarly, in this study, dietary *A. platensis* could act as a dietary substitute for fishmeal up to 80% (230.3 g kg^{-1} diet *A. platensis*) without affecting the ADC of dry matter, proteins, or amino acids. Moreover, a complete cell of *A. platensis* was not found in the feces of yellow catfish, further validating that this species could tolerate *A. platensis* supplemented diets.

The high protein content (60–71%) of *A. platensis* is a primary reason to consider this species as an unconventional source of protein (Spolaore et al., 2006). The incorporation of *A. platensis* has been demonstrated to improve the growth of juvenile rainbow trout (*Oncorhynchus mykiss*) (Hernandez et al., 2012), which suggests that *A. platensis* contains indispensable amino acids required for most fish species (NRC, 2011). However, in this study, the content of Met, Lys, and His in *A. platensis* was lower than fishmeal. The Met and Lys requirements for juvenile yellow catfish are 19.8 g kg^{-1} and 58.0 g kg^{-1}, respectively (Qiu et al., 2015; Elmada et al., 2016). In this study, the Met content of different diets ranged from 18.11–16.44 g kg^{-1}. However, the acid hydrolysis method could have led to an underestimate of Met. The Lys content for the experimental diets (i.e., AP0, AP20, AP40, AP60, AP80, and AP100) were 65.62, 64.58, 60.08, 56.94, 52.97, and 51.29 g kg^{-1}, respectively. When the dietary substitution amount was below 20% (115.1 g kg^{-1} of *A. platensis*), it did meet the requirement of Lys for yellow catfish and if more fishmeal were to be replaced, then additional Lys would be required to meet this species' dietary needs. To confirm this, Cao et al. (2018a, 2018b) previously found after adding 2.80 g kg^{-1} dietary Lys that *A. platensis* could completely replace fishmeal (200 g kg^{-1} diet) in the diets of juvenile gibel carp (*Carassis auratus gibelio* var. CAS III).

The maximum *A. platensis* inclusion level in fish diets may depend on the feeding habits of the fish species. For example, rainbow trout are predators, so in consideration of growth performance, it is feasible to incorporate up to 31.5 g kg^{-1} of *A. platensis* (Teimouri et al., 2013). In this study, yellow catfish are omnivorous fish and it was found that the maximum substitution amount could be up to 80% (230.3 g kg^{-1} of *A. platensis*) without a negative effect on growth performance, as a greater amount could cause a decline in growth. However, Sudaporn et al. (2010) found that fishmeal could be 100% substituted by *A. platensis* (280 g kg^{-1} of *A. platensis*) and enhance the total weight of Mekong giant catfish (*Pangasianodon gigas* Chevey), who feed on filamentous algae.

In this study, dietary supplementation of *A. platensis* affected the whole fish body composition. Lipid content decreased and ash content increased in fish that fed on diets with increasing *A. platensis* levels. Because *A. platensis* contains polyphenol (Casazza et al., 2015), it could possess hypolipidemic activity (Saito et al., 2002; Yang et al., 2007). Rahimnejad (2013) verified that *A. platensis* contains roughly 1.4 g kg^{-1} polyphenol and resulted in decreased body lipid content in

Table 6
Effects of different experimental diets on oxidant and antioxidant activity in the plasma and liver tissues of yellow catfish

Diets	AP0	AP20	AP40	AP60	AP80	AP100	Equations	R^2	*P*- value
Plasma (μmol/L)									
MDA	11.59 ± 1.31	8.52 ± 0.61	6.94 ± 0.71	5.7 ± 0.84	8.34 ± 0.34	6.06 ± 0.73	$y = 0.0001x^2–0.0469x + 11.2$	0.7517	0.009
GSH	47.79 ± 1.84	74.13 ± 3.2	71.22 ± 1.38	69.23 ± 5.52	65.25 ± 3.71	62.8 ± 1.1	$y = -0.0008x^2 + 0.2529x + 52.93$	0.6630	0.012
Liver (μmol/g protein)									
MDA	1.43 ± 0.19	0.56 ± 0.05	0.7 ± 0.13	0.74 ± 0.09	0.72 ± 0.13	0.77 ± 0.14	$y = 2E\text{-}05x^2–0.0075x + 1.25$	0.6283	0.033
GSH	9.98 ± 0.79	12.91 ± 0.98	17.95 ± 0.69	18.29 ± 0.35	22.28 ± 2.46	21.64 ± 1.13	$y = -0.0001x^2 + 0.0773x + 9.67$	0.9617	< 0.001
Plasma (U/ml)									
GSH-PX	145.93 ± 9.44	160.25 ± 5.22	177.46 ± 4.5	184.70 ± 5.24	163.61 ± 5.87	146.07 ± 2.33	$y = -0.0017x^2 + 0.49x + 143.12$	0.9220	0.001
CAT	13.51 ± 0.04	12.37 ± 1.13	17.84 ± 2.48	18.27 ± 2.20	17.54 ± 1.62	16.15 ± 1.02	None	None	0.145
SOD	22.00 ± 1.69	38.92 ± 1.47	43.41 ± 1.60	54.05 ± 2.04	60.81 ± 2.38	30.65 ± 0.32	$y = -0.0012x^2 + 0.4103x + 19.63$	0.7839	0.012
Liver (U/mg protein)									
GSH-PX	201.09 ± 13.52	217.86 ± 13.09	215.58 ± 13.94	255.74 ± 16.29	271.35 ± 14.88	275.73 ± 3.36	$y = 5E\text{-}05x^2 + 0.2701x + 199.13$	0.9214	0.022
CAT	9.79 ± 0.35	10.39 ± 0.16	11.25 ± 0.13	11.47 ± 0.45	16.87 ± 0.72	17.80 ± 0.18	$y = 0.0001x^2–0.0011x + 9.84$	0.9190	< 0.001
SOD	3.40 ± 0.11	3.28 ± 0.14	3.32 ± 0.18	3.77 ± 0.26	3.75 ± 0.26	4.31 ± 0.25	$y = 2E\text{-}05x^2–0.0017x + 3.37$	0.9197	0.049

MDA: malondialdehyde.
GSH: reduced glutathione.
GSH-PX: Glutathione peroxidase.
CAT: Catalase.
SOD: Superoxide dismutase.
Data are presented as the Means ± SE ($P < 0.05$; n = 6). The *P*-values in the tables were for one-way ANOVA.

parrot fish (*Oplegnathus fasciatus*). In this study, the whole-body protein content was not significantly different among experimental groups.

Additionally, it was found in this study that *A. platensis* could significantly increase lutein concentrations in skin and liver tissues of yellow catfish. The composition of carotenoids is dependent on pigment components and fish species. For Siluriformes, such as channel catfish (*Ictalurus punctatus*), lutein accumulates better than other carotenoids (Li et al., 2010). However, in a previous study, a lutein supplemented diet resulted in the lowest value of total carotenoids in the skin tissues of Lake Kurumoi rainbowfish (*Melanotaenia parva* (Allen)) when compared to astaxanthin or canthaxanthin supplemented diets (Meilisza et al., 2017).

The present data reveal that *A. platensis* could enhance the skin color of yellow catfish. Similar results have been found in blue gouramis (*Trichogaster trichopterus* Pallas) (Alagappan et al., 2004), rainbow trout (Teimouri et al., 2013), goldfish (*Carassius auratus*) (Gouveia et al., 2003), and African sharptooth catfish (*Clarias gariepinus*) (Promya and Chitmanat, 2011), in which *A. platensis* supplemented diets improved pigmentation. The yellow body color is decided by xanthophores, which contain carotenoids (Kimler and Taylor, 2002), while black is determined by melanophores, which contain melanin (Bagnara and Matsumoto, 2007). *A. platensis* is rich in lutein, β-carotene, and astaxanthin, which are types of natural carotenoid sources (Ghaeni et al., 2014). Thus, *A. platensis* could be a pigmentation supplement for various fish species. Previous research described the relationship between instrumentally assessed coloration and carotenoid concentration, which can be used to predict carotenoid levels accurately under normal conditions (Storebakken et al., 2004). In this study, the positive correlation between yellowness and lutein content of abdominal and dorsal skin tissues confirm the possibility that lutein concentration can be an indicator for measuring the pigmentation of yellow catfish.

Dietary *A. platensis* supplementation could also increase antioxidant capacity in yellow catfish. Carotenoids and phycocyanin from *A. platensis* are thought to be responsible for some antioxidant properties (Jensen et al., 1998). For example, MDA is the final product of lipid peroxidation, which accelerates damage in cells and tissues (Liu et al., 2010). Moreover, MDA levels are used to evaluate the degree of lipid peroxidation and oxidative stress in fish (Lushchak, 2011). In this study, *A. platensis* supplementation decreased MDA concentrations in the plasma and liver compared to AP0. Additionally, GSH, SOD, and GSH-PX are endogenous antioxidants that are closely related to clean free radicals, and protect important cellular macromolecules and organelles from oxidative damage (Mahmoud and Edens, 2003; Misra and Niyogi, 2009; Fırat et al., 2011). The results of this study indicate that 100% dietary substitution (287.9 g kg^{-1} of *A. platensis*) increased GSH-PX and SOD activities in liver tissues compared to AP0. Additionally, CAT activities in the plasma were not affected by the addition of *A. platensis*, but increased in liver tissues. These results suggest that, with the addition of *A. platensis* in diets, several antioxidases and antioxidant substances in the fish body enhance the elimination of internal free radicals and reduce the production of lipid peroxide. Similarly, in a previous study, the addition of *A. platensis* resulted in increased CAT and SOD activity in African catfish (*Clarias gariepinus*), revealing that *A. platensis* is a potential antioxidant booster for aquatic animals (Raji et al., 2018).

With global fisheries approaching unsustainable limits, current fishmeal production will inadequately support cost-effective demands of aquafeeds (Tacon and Metian, 2008; Shepherd and Jackson, 2013). Therefore, it is important to identify resource-efficient ingredients that will allow the aquafeed industry to keep pace with the rapid expansion of worldwide aquaculture production, while preventing losses due to decreased productivity, increased costs, or compromised product quality (Jiang et al., 2019). Although the price of *A. platensis* is higher than fishmeal, it is critical to investigate its potential as a feed ingredient in different cultured species. In this study, *A. platensis* was demonstrated to be a potential fishmeal replacement in the aquafeeds

of yellow catfish. Furthermore, *A. platensis* could potentially be used as a functional additive in aquacultures, thereby reducing the amount in fish diets and save costs. For example, Cao et al., 2018a verified that only 3.38% of dietary *A. platensis* could enhance the growth and immune response of juvenile gibel carp (*Carassius gibelio*).

In conclusion, supplemental *A. platensis* can increase pigmentation, as well as enhance total antioxidant capacity and improve the quality of yellow catfish. There were no negative effects observed in the partial replacement of fishmeal on growth rate, digestion, or feed utilization in yellow catfish. These results demonstrate that *A. platensis* is a suitable and sustainable alternative source of protein for the aquaculture industry. Based on the regression analyses on SGR and dietary *A. platensis* content, the optimal substitution amount was found to be 41.68% (120 g kg^{-1} of *A. platensis*) to attain the best growth rate, while the maximum substitution could be up to 80% (230.3 g kg^{-1} of *A. platensis*) without having a negative effect on growth performance. Lastly, based on the regression analyses of lutein concentration between the abdominal skin tissues and experimental diets, the optimal substitution amount was found to be 72.03% (207.4 g kg^{-1} of *A. platensis*), which could ensure quality of growth and pigmentation in yellow catfish.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.05.067.

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