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The role of dietary methionine concentrations on growth, metabolism and N-retention in cobia (*Rachycentron canadum*) at elevated water temperatures

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Abstract

This study determined impacts of dietary methionine concentrations at two temperatures on growth, feeding efficiency and N-metabolites in juvenile cobia. Methionine concentrations of the experimental diets were deficient (M9; 9 g/kg), sufficient (M12; 12 g/kg) and surplus (M16, 16 g/kg). Water temperature was normal (30°C) or elevated (34°C). Twenty cobia in triplicate tanks were fed the experimental diets for 6 weeks. Both methionine and temperature affected cobia's growth and feeding efficiency. Cobia fed M9 performed lower than the fish fed M12 and M16 diets. Additionally, cobia reared at 34°C performed poorer than at 30°C, probably due to lower voluntary feed intake in the fish reared at 34°C. Protein efficiency ratio and protein productive value in cobia fed M9 diet were less than M12 or M16 diets. This was confirmed with the improved retentions of indispensable amino acids (AAs). No interactions between methionine and temperature were observed in growth and protein accretion. At 30°C, CF improved, while HSI and VSI declined upon methionine supplementation levels. Of which an interaction between temperature and methionine was present. Plasma, muscle and liver free AA and N-metabolites were affected by methionine and temperature. Furthermore, temperature affected cobia's lipid class composition, resulting in increased phospholipids and cholesterol at 34°C.

KEYWORDS
amino acid, cobia, metabolism, methionine, N-retention, temperature

1 | INTRODUCTION

Scenarios for changes in global temperature suggest the average increase may be as high as from 4 to 6°C by 2,100 (IPCC, 2015). Vietnam is one of the most vulnerable countries to the predicted climate changes, and there is great concern how aquaculture in the region will be affected. Due to their ectothermic nature, fish will be directly affected by elevated temperature, although the consequences depend on the sensitivity/robustness of each species and to what extent there are alternative locations where the temperature increase is less pronounced. Cobia (*Rachycentron canadum*) is one of the main fish marine species in large-scale commercial aquaculture in Vietnam (Nhu et al., 2011) where during the main season for cobia's larvae and juveniles production seawater temperature in hatcheries often varies

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between 28°C (night-time) and 34°C (daytime). Cobia is a fast growth species (Chou, Su, & Chen, 2001; Su, Chien, & Liao, 2000) with white, firm and good flavoured flesh that is suitable for the sashimi industry. Growth is mainly accretion of body protein, and proper feeding protocols, together with a balanced feed composition as well as optimized amino acid (AA) metabolism, are key components to optimize growth and reduce N-waste. We have previously demonstrated that a dietary lysine-to-arginine ratio close to 1 (one) would result in the best performance of juvenile cobia and that cobia tolerate high inclusion of plant protein, given that the dietary AA profiles are balanced (Nguyen, Rønnestad, Buttle, Lai, & Espe, 2014). We have also started to explore the endocrine control of appetite in cobia and demonstrated that as seen for many vertebrates, neuropeptide Y acts as an orexigenic signal in the regulation of feed intake (Nguyen et al., 2013).

Methionine is often the limiting AA in many protein sources, including plant proteins, the nowadays dominant protein ingredient in aquaculture. This indispensable amino acid is the precursor for S-adenosylmethionine (SAM) that serves as the main methyl donor within the metabolism of animals (Bender, 2012), including fish (Espe, Veiseth-Kent, Zerrahn, Rønnestad, & Aksnes, 2015). Thus, methionine availability affects several different metabolic pathways including fatty acid oxidation, phospholipid status, creatine synthesis, bile acid production and polyamine availability (Espe et al., 2015). Further, methionine is the precursor for taurine and is one of the three constituting amino acids of glutathione after methionine is metabolized to cysteine. Both of the latter are associated with oxidation status and metabolism within the cells (Bauchart-Thevret, Stoll, & Burrin, 2009; Espe & Holen, 2013; Zhang, 2018). With elevated temperature, the rates for all metabolic and physiological processes will increase up to a certain level, but with different effects on different pathways (Hevroy et al., 2011). Although it is unknown to what degree and how strongly the metabolism will adapt to a reduced availability of nutrients, there may be altered availability of metabolites such as methionine that may modulate metabolism, energy allocation, appetite, amongst others. It has been reported that thermal stress induces protein degradation, but reduces protein synthesis resulting in impaired growth in fish (Glencross & Bermudes, 2010; Sun & Chen, 2014). Meanwhile, dietary methionine supplementation might alleviate the negative impact of thermal stress, as methionine might involve immunological functions (Pan et al., 2017), and could suppress the expression of genes related to protein degradation, but induce the expression of genes related to protein synthesis, and thus improve the protein deposition (Del Vesco et al., 2015). It therefore is vital to have a proper understanding on how elevated temperatures affect the requirements for methionine, both with regard to growth and oxidative stress conditions. The requirement of methionine for juvenile cobia is 11.9 g/kg dry diet (26.4 g/kg dietary protein), in the presence of 6.7 g/kg cysteine (Zhou, Wu, Tan, Chi, & Yang, 2006). In this work, we have explored to what extent elevated water temperatures affect the requirement for dietary methionine in cobia using three concentrations of dietary methionine (9.1, 12.8 and 16.8 g/kg diet).

TABLE 1 Formulation (g/kg dry matter basis) and proximate analysis of the experimental diets

	Diets		
Ingredients	**M9**	**M12**	**M16**
Krill meal	50.0	50.0	50.0
Wheatmeal	175.3	175.3	173.3
Fish meal	250.0	250.0	250.0
Soy protein concentrate	100.0	100.0	100.0
Pea protein concentrate	139.0	134.0	132.0
CPSP 90	50.0	50.0	50.0
DL-methionine	0.5	5.5	9.5
Betaine HCl	5.0	5.0	5.0
Encapsuled taurine	5.0	5.0	5.0
Encapsuled tryptophane	5.0	5.0	5.0
Fish oil	28.0	28.0	28.0
Krill oil	30.0	30.0	30.0
Pea starch	100.0	100.0	100.0
Vitamin and mineral mix	20.0	20.0	20.0
Lutavit E50	0.2	0.2	0.2
Calcium carbonate	10.0	10.0	10.0
Monoammonium phosphate	30.0	30.0	30.0
Antioxidant (Paramega)	2.0	2.0	2.0
Proximate composition			
Dry matter	962.0	958.0	968.0
Energy (MJ/kg)	20.0	20.1	20.1
Crude protein	478.0	465.0	464.0
Crude fat	104.0	103.0	103.0

2 | MATERIALS AND METHODS

2.1 | Experimental diets

Three extruded isoenergetic diets with approximated protein levels and contained different levels of methionine (9.1, 12.8 and 16.8 g/kg) were produced at SPAROS Lda. (Olhão, Portugal). The experimental diets (Table 1) were formulated based on previous results in cobia (Nguyen et al., 2014; Zhou et al., 2006). In the present study, crystalline DL-methionine was added with different amount to make a diet deficient in methionine M9 (9.1 g/kg), a diet with sufficient methionine M12 (12.8 g/kg) and a diet with surplus methionine M16 (16.8 g/kg; Table 2). No cysteine was added, therefore there was a slightly higher amount of dietary methionine in M12 than the requirement estimated for cobia (Zhou et al., 2006), in order to eliminate the sparing effect of cysteine on methionine if present as in previous reports (Poppi, Moore, & Glencross, 2017; Toennies & Callan, 1939). About 30% of deficiency or excess of dietary methionine compared to the predicted requirement as in M12 diet was assigned (i.e., 2.8 g/kg of methionine below the requirement in M9 diet and 4.9 g/kg above the requirement in M16 diet). To ensure a low baseline of methionine (as in M9 diet), wheatmeal and soy and pea protein concentrates were used as the main protein sources (Espe et al.,

TABLE 2 Amino acid concentration (g/kg dry matter basis) of the experimental diets

Amino acids	Diets		
	M9	M12	M16
OH-pro	2.6	2.3	2.6
Tau	5.5	5.1	5.6
Tyr	13.7	12.8	13.6
Ser	18.8	18.3	18.4
Pro	18.9	18.4	18.6
Ala	21.5	21.0	20.7
Gly	22.9	21.6	22.4
Asp	41.5	41.5	39.8
Glu	62.2	62.2	60.3
Met[i]	9.1	12.8	16.8
His[i]	9.1	8.6	9.1
Thr[i]	16.4	16.0	16.1
Ile[i]	17.3	16.6	17.0
Phe[i]	20.0	18.6	19.8
Val[i]	20.9	20.1	20.5
Arg[i]	26.5	25.3	26.1
Lys[i]	28.3	28.8	27.2
Leu[i]	31.5	30.5	30.6
Sum AA	381.2	378.2	382.6

Note. Trp and Cys were not analysed; amino acids (AA) followed by i letter are indispensable for fish (NRC, 2011; Wilson 2002); M9, M12 and M16 were test diets with different dietary methionine; taurine was not included in Sum AA.

2014). The other dietary indispensable AAs in the experimental diets were kept at sufficient levels according to the ideal protein concept and based on recent knowledge in juvenile cobia (NRC, 2011).

2.2 | Fish acclimation

Juvenile cobia (1,200 juveniles of 2.5–4.0 g body weight), purchased from a local hatchery in Nha Trang, Vietnam, were transported and acclimatized in two fibreglass tanks (5 m^3; 600 individuals per tank) at the Center for Aquatic Animal Health and Breeding Studies (Nha Trang University) for a period of 1 week. During the period of acclimatization, water temperature in one tank increased at a rate of 1°C per day up to 34°C, while temperature in the other tank was kept constant at 30°C. The fish were fed to near satiety (until most of the fish losing their appetite) by hand at 8:00 and 16:00 by a locally commercial pellet (INVE, Ltd.).

2.3 | Experiment design

Eighteen experimental tanks were set up in two water recirculation subunits maintained at 30 ± 0.5°C and at 34 ± 0.1°C, considered as control and elevated temperature treatments, respectively. Thermal controllers (Chuan Kang Ltd., Taiwan) were used to maintain water temperature in the two rearing subunits, accordingly.

The experimental tanks were fibreglass rectangular tanks (80 × 50 × 60 cm), with 200 L water filled, in two water recirculation subunits with continuous aeration (as described in Nguyen et al., 2014). Salinity was 29 ± 3.1 ppt, pH 7.8–8.3, oxygen 4.6 ± 0.5 mg/L and NH_3 < 0.03 mg/L. Seawater in the recirculation system was renewed every 2–3 days, depending on water quality analyses.

After acclimatizing, the juveniles were graded, and 180 cobia from each of the acclimation tanks (3.7 ± 0.0 and 3.8 ± 0.0 g of body weight) were randomly distributed into the 30 and 34°C experimental tanks (20 fish per tank), accordingly. Each of the three experimental diets was randomly assigned to triplicate tanks. The feeding trial lasted for the 6-week period, and the fish were fed using the same feeding regime as described above.

Uneaten feed and removed fish were recorded for the calculation of feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV).

2.4 | Sampling and sample preparation

Cobia juveniles were individually weighed, and length was measured at the beginning and at the end of the experiment. Prior to any handling, the fish were anesthetized by ethylene glycol monophenyl ether solution (150–200 ppm). A pooled whole-body sample of six 24-hr starved fish was collected at the beginning of the experiment. At the end of the feeding trial, the fish were starved for 24 hr before three cobia from each tank were randomly sampled and pooled for whole-body proximate and amino acid composition analysis. The pooled whole-body samples were homogenized and dried at 105°C for 24–36 hr, until constant dry weight in an oven, then kept in NUNC boxes and frozen until analysed.

Another three cobia from each tank were sampled, and blood was collected into heparinized syringes from the caudal vein and dissected for visceral organ and liver weight for calculation of VSI and HSI. Pooled blood plasma from each tank was prepared after centrifugation at 1,800 *g* for 10 min, then treated with 10% sulfosalicylic acid (100% v/v), containing 1 mM internal standard, and kept at −80°C until analysed. Lateral muscle samples were standardized collected from both sides of the body, above the lateral lines and between the pectoral and anal fin. Pooled livers and muscles from three latter cobia were kept overnight in the freezer (−80°C) before pretreated for N-metabolites and S-adenosylmethionine (SAM) and S-adenosylhomocysteine SAH (for liver samples).

For muscle and liver N-metabolites, frozen pooled samples were thawed on ice and homogenized by cutting off the samples into small pieces and then twice squeezing through a 5-ml syringe. The samples were mixed thoroughly by using forceps at each step during homogenization. An amount of 0.3 g homogenized samples was added into Eppendorf tubes filled with 600 µl 10% sulfosalicylic acid, stirred (fast vortex) and then kept at room temperature for 1 hr before being centrifuged at 7,168 *g* for 15 min. The supernatants were collected and transferred into new Eppendorf tubes and stored in the freezer until analysed (−80°C). Similar procedure was applied for SAM and SAH from the liver samples that used 800 µl ice-cold

0.4 M perchloric acid added into 0.2 g homogenized samples and centrifuged at 16,128 *g* for 15 min.

All samples were stored frozen (−80°C) until transported to Institute of Marine Research (Bergen, Norway) where the chemical analyses were performed.

2.5 | Chemical analyses

The dietary crude composition of dry matter, protein, lipids, ash and energy as well as whole-body lipid and protein was analysed as described in Espe, Lemme, Petri, and El-Mowafi (2006). The lipid classes in whole body were analysed after lipid extraction with 2:1 chloroform:methanol (v:v) and HPTLC as previously described (Espe, Liaset, Hevrøy, & El-Mowafi, 2011). Dietary AA composition was determined after hydrolysis for 22 hr at 110°C in 6 N HCl containing 3.125 mM norvaline (internal standard) and 3 mM DDT (to protect the sulphur amino acids against oxidation), and precolumn derivatized with AccQT$_{agTM}$ at 55°C as described by Waters. The AAs were separated on a UPLC system (Waters Acquity UPLC BEH C18 Column internal diameter of 1.7 μM at a flow rate of 0.7 ml/min using the gradient offered by the supplier). The AA concentration was calculated using external standards supplied by Sigma (Sigma-Aldrich, StLouis, MO, USA).

Amino acids in deproteinized liver, plasma and muscle were determined on an amino acid analyser Biochrom 30 Plus Amino Acid Analyzer (Biochrom, Cambridge, UK) equipped with a lithium column using postcolumn derivatization with ninhydrin and quantified using standards from Sigma. SAM and SAH were determined on a reverse phase HPLC after deproteinization in 0.4 M $HClO_4$ as described (Espe et al., 2015). The concentration of SAM and SAH was quantified using standards of the respective metabolites (Sigma).

2.6 | Calculations

Condition factors were calculated as:

$$\mathrm{CF} = W \times L^{-3} \times 100,$$

where W and L represent body weight (g) and total length (cm), respectively.

Feed conversion ratio (FCR) was calculated from the amount of diet consumed (kg) and the total biomass (kg) gained:

$$\mathrm{FCR} = (\text{diet consumed}) \times (\text{biomass gained})^{-1}.$$

Daily growth coefficient (DGC) was calculated as % daily growth increase:

$$\mathrm{DGC} = \left(W_2^{(1/3)} - W_1^{(1/3)}\right) \times \Delta t^{-1} \times 100.$$

Thermal unit growth coefficient (TGC) was calculated as:

$$\mathrm{TGC} = \left(W_2^{(1/3)} - W_1^{(1/3)}\right) \times T^{-1} \times \Delta t^{-1} \times 1{,}000,$$

where W_1 and W_2 represent the initial and final body weight in grams, respectively; T is the water temperature (°C); and Δt is the duration of the experiment ($T \times \Delta t$ is the thermal sum in degree days).

Viscerosomatic (VSI) index and hepatosomatic index (HSI) were calculated as follows:

$$\mathrm{VSI} = V \times (W)^{-1} \times 100,$$

$$\mathrm{HSI} = H \times (W)^{-1} \times 100,$$

where V and H represent viscera and liver in gram (g), respectively.

Protein efficiency ratio (PER) was calculated as biomass gain (g) for each gram protein consumed:

$$\mathrm{PER} = (\text{biomass gained}) \times (\text{protein consumed})^{-1}$$

Protein productive value (PPV) was calculated as retained protein (g) of consumed protein (g).

$$\mathrm{PPV} = (\text{final protein content} - \text{initial protein content}) \times (\text{protein consumed})^{-1}.$$

Retention of indispensable amino acids (IR) was calculated as follows:

$$\mathrm{IR} = (\text{amino acid gained in fish}) \times (\text{ingested amino acids})^{-1} \times 100.$$

2.7 | Statistical analysis

Data were analysed by the statistical program SPSS for Windows (IBM® SPSS® Statistics version 24). Values are given as tank means ± *SEM*. Two-way ANOVA was used to test any differences and/or the interaction between dietary methionine level and temperature using tanks as the statistical unit. If differences were obtained ($p < 0.05$), the Tukey's test was used to evaluate the differences between treatments. Prior to applying ANOVA, all data were tested for homogeneity in variance by Levene's test and normal distribution by Kolmogorov–Smirnov test. When the data were found inhomogeneous in variance, a Welch's test was used to determine any differences between treatments.

3 | RESULTS

3.1 | Growth performance and feeding efficiency

The fish accepted well all the diets, and survival rate was 95%–98% amongst the treatments. All treatment groups grew well and increased their initial body more than seven times during the 6-week growth trial. Growth performance and feed utilization were higher in cobia fed the sufficient (M12) and surplus (M16) methionine as compared to those fed the methionine deficiency (M9) diet. Also, elevated temperature led to decreased growth performance (a reduction of about 28% in body mass, due to elevated temperature).

TABLE 3 Body weight (BW) and length (TL), specific growth rate (SGR), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), feed intake (FI), feed conversion ratio (FCR), whole-body protein in wet weight basis and dry matter, protein efficiency ratio (PER) and protein productive value (PPV) of the cobia fed the test diets containing different methionine at two temperatures (30 and 34°C)

Temperature	30°C			34°C			*p*-Value		
Diet	M9	M12	M16	M9	M12	M16	Met	Temp	Met × Temp
Initial TL (cm)	9.7 ± 0.0	9.7 ± 0.0	9.7 ± 0.0	9.7 ± 0.0	9.7 ± 0.0	9.7 ± 0.0	NS	NS	NS
Initial BW (g)	3.7 ± 0.0	3.7 ± 0.0	3.7 ± 0.0	3.8 ± 0.0	3.8 ± 0.0	3.8 ± 0.0	NS	NS	NS
Final TL (cm)	20.6 ± 0.1^{Ab}	22.3 ± 0.1^{Aa}	22.0 ± 0.1^{Aa}	18.2 ± 0.1^{Bb}	19.6 ± 0.0^{Ba}	19.6 ± 0.1^{Ba}	<0.0001	<0.0001	NS
Final BW (g)	46.0 ± 1.2^{Ac}	66.2 ± 1.6^{Aa}	60.5 ± 0.8^{Ab}	33.4 ± 1.0^{Bc}	46.0 ± 0.3^{Ba}	45.2 ± 0.5^{Bb}	<0.0001	<0.0001	0.008
DGC	4.85 ± 0.07^{Ac}	5.95 ± 0.08^{Aa}	5.66 ± 0.04^{Ab}	4.00 ± 0.04^{Bc}	4.82 ± 0.02^{Ba}	4.77 ± 0.03^{Bb}	<0.0001	<0.0001	0.04
TGC	1.62 ± 0.02^{Ac}	1.98 ± 0.03^{Aa}	1.89 ± 0.01^{Ab}	1.16 ± 0.02^{Bc}	1.42 ± 0.01^{Ba}	1.40 ± 0.01^{Bb}	<0.0001	<0.0001	0.02
CF (%)	0.52 ± 0.00^{Bb}	0.59 ± 0.01^{Ba}	0.56 ± 0.00^{Ba}	0.53 ± 0.01^{Ab}	0.61 ± 0.00^{Aa}	0.59 ± 0.00^{Aa}	<0.0001	0.0001	NS
VSI (%)	12.10 ± 0.15^{a}	10.14 ± 0.28^{b}	11.51 ± 0.20^{b}	11.63 ± 0.15^{a}	11.38 ± 0.06^{b}	10.56 ± 0.15^{b}	0.0001	NS	0.0002
HSI (%)	2.31 ± 0.11^{B}	1.89 ± 0.04^{B}	1.97 ± 0.06^{B}	2.27 ± 0.04^{A}	2.41 ± 0.12^{A}	2.34 ± 0.05^{A}	NS	0.006	0.010
FCR	1.01 ± 0.04^{Ba}	0.91 ± 0.02^{Bb}	1.00 ± 0.01^{Ba}	1.29 ± 0.02^{Aa}	1.13 ± 0.02^{Ab}	1.23 ± 0.03^{Aa}	0.0007	<0.0001	NS
FI	0.91 ± 0.05^{Ab}	1.31 ± 0.02^{Aa}	1.35 ± 0.04^{Aa}	0.88 ± 0.03^{Bb}	1.12 ± 0.01^{Ba}	1.20 ± 0.03^{Ba}	<0.0001	0.0008	NS
Dry matter (%)	22.7 ± 0.7	23.3 ± 0.3	22.7 ± 0.3	23.7 ± 0.3	23.3 ± 0.3	23.7 ± 0.3	NS	NS	NS
Protein (%)	14.3 ± 0.3	15.3 ± 0.3	14.7 ± 0.3	14.2 ± 0.2	14.7 ± 0.3	14.7 ± 0.3	NS	NS	NS
PER	2.13 ± 0.09^{Ab}	2.37 ± 0.05^{Aa}	2.16 ± 0.03^{Ab}	1.67 ± 0.03^{Bb}	1.91 ± 0.03^{Ba}	1.76 ± 0.04^{Bb}	0.0004	<0.0001	NS
PPV	0.28 ± 0.01^{Ac}	0.35 ± 0.00^{Aa}	0.30 ± 0.01^{Ab}	0.21 ± 0.00^{Bc}	0.25 ± 0.00^{Ba}	0.24 ± 0.01^{Bb}	<0.0001	<0.0001	NS

Note. Value is presented as mean ± *SEM* ($n = 3$); results were based on two-way ANOVA; superscripts after each value on the same row indicate results of pairwise comparisons; different upper case letters indicate significant differences ($p < 0.05$) between temperatures, while different lower case letters indicate significant differences between dietary methionine; NS: not significant; only interactions are indicated.

Significant interactions between dietary methionine and temperature on growth performance were observed ($p < 0.05$; Table 3), which resulted in higher DGC and TGC in cobia fed M12 diet than the fish fed M16 diet reared at 30°C, but not at 34°C.

Feed intake, feed conversion ratio, protein efficiency ratio and protein productive value in cobia were significantly affected by both dietary methionine and temperature, but no interactions occurred (Table 3). Deficiency in dietary methionine and elevated temperature reduced FI in the fish. Cobia fed M12 and M16 showed comparable FI, but higher than those fed M9 diet. The elevated temperature fish group consumed less feed compared to those reared at 30°C. Moreover, FCR in cobia fed M12 is better than those fed either M9 or M16 diets. The best FCR was observed in cobia fed M12 diet, rearing at 30°C.

Cobia fed M12 diet had a higher PER than the fish fed M9 and M16 diets. Also, better PER was observed in cobia reared at 30°C compared to those fish reared at 34°C. The highest PPV was observed in cobia fed M12 diet, while intermediate PPV was in cobia fed M16, and cobia fed M9 showed the least. Similar results were also observed for the retention of indispensable amino acids, except for isoleucine, lysine and methionine which were lower retention in cobia fed M9 or M16 diet (Table 4). In addition, cobia fed the methionine-deficient diet showed lower whole-body indispensable amino acid than those fish fed either the sufficient or surplus methionine diets. Significant interaction between dietary and temperature on the retention of methionine occurred, which resulted in higher methionine retention in cobia fed M12 than the fish fed M9 at 30°C, but comparable in retention of the AA at 34°C.

Dietary methionine significantly affected the condition factor and viscerosomatic index (Table 3; $p < 0.0001$). Cobia fed M9 diet showed lower CF value than those fed either M12 or M16 diets. Higher CF observed in cobia reared at 34°C resulted from slower growth in length as compared to the fish at 30°C. This is useful information for hatchery production, as the price of juvenile cobia at least in Vietnam is based mainly on body length rather than body weight. Viscerosomatic index in the fish fed M9 was higher than in those fed M12 and M16 diets. The interaction between dietary methionine and temperature on VSI of cobia was significant. At 30°C, cobia fed M12 obtained better VSI than those fed M9 or M16 diet, but this was not observed in cobia reared at elevated temperature. Meanwhile, elevated temperature significantly increased hepatosomatic index in cobia. In addition, there was interaction between temperature and methionine on HSI that resulted in better HSI in the fish fed M12 than fish fed M9 than at 30°C, but no difference in HSI observed amongst fish groups at 34°C.

3.2 | Free amino acids and N-metabolites in blood plasma, muscle and hepatic tissues of cobia

Plasma metabolites of urea cycle and key amino acids participating in the methionine metabolism of cobia 24 hr postprandial are presented in Table 5. Plasma urea, serine and anserine were affected

TABLE 4 Retention of indispensable amino acids (%) in the cobia fed different methionine levels at two temperatures

Temperature	30°C			34°C			*p*-Value		
Diet	M9	M12	M16	M9	M12	M16	Met	Temp	Met × Temp
Arg	30.6 ± 0.8^{Ac}	38.7 ± 0.8^{Aa}	32.4 ± 0.8^{Ab}	23.5 ± 1.0^{Bc}	31.2 ± 0.8^{Ba}	27.6 ± 0.8^{Bb}	<0.0001	<0.0001	NS
His	26.3 ± 0.8^{Ac}	35.2 ± 0.8^{Aa}	28.7 ± 0.8^{Ab}	20.8 ± 0.9^{Bc}	29.5 ± 0.8^{Ba}	25.3 ± 0.8^{Bb}	<0.0001	<0.0001	NS
Ile	29.6 ± 0.7^{Ab}	37.6 ± 0.7^{Aa}	32.6 ± 0.7^{Aab}	22.9 ± 0.9^{Bb}	31.1 ± 0.7^{Ba}	27.4 ± 0.7^{Bab}	0.013	0.008	NS
Leu	29.2 ± 0.7^{Ac}	36.5 ± 0.7^{Aa}	32.3 ± 0.7^{Ab}	22.5 ± 0.9^{Bc}	30.0 ± 0.7^{Ba}	27.8 ± 0.7^{Bb}	<0.0001	<0.0001	NS
Lys	34.9 ± 1.1^{Ab}	41.7 ± 1.1^{Aa}	40.3 ± 1.1^{Aa}	25.6 ± 1.3^{Bb}	32.2 ± 1.1^{Ba}	32.6 ± 1.1^{Ba}	0.002	<0.0001	NS
Met	36.4 ± 0.8^{Aa}	34.9 ± 0.8^{Aa}	23.0 ± 0.8^{Ab}	30.7 ± 1.0^{Ba}	28.5 ± 0.8^{Ba}	20.0 ± 0.8^{Bb}	<0.0001	<0.0001	0.012
Phe	24.3 ± 0.8^{Ac}	32.3 ± 0.8^{Aa}	26.2 ± 0.8^{Ab}	19.2 ± 1.0^{Bc}	26.9 ± 0.8^{Ba}	23.2 ± 0.8^{Bb}	<0.0001	<0.0001	NS
Thr	34.8 ± 0.9^{Ac}	43.1 ± 0.9^{Aa}	37.5 ± 0.9^{Ab}	27.0 ± 1.1^{Bc}	35.0 ± 0.9^{Ba}	32.5 ± 0.9^{Bb}	<0.0001	<0.0001	NS
Val	31.0 ± 0.8^{Ac}	38.9 ± 0.8^{Aa}	33.9 ± 0.8^{Ab}	24.1 ± 0.9^{Bc}	31.8 ± 0.8^{Ba}	28.4 ± 0.8^{Bb}	<0.0001	<0.0001	NS
Sum IDA	53.5 ± 0.8^{b}	57.6 ± 0.7^{a}	56.3 ± 0.7^{a}	51.9 ± 1.0^{b}	57.5 ± 0.3^{a}	58.2 ± 0.2^{a}	<0.0001	NS	NS

Note. Value is presented as mean ± *SEM* (n = 3); letters after each value on the same row indicate results of pairwise comparisons. NS: not significant (p > 0.05); different upper case letters indicate significant differences (p < 0.05) between temperatures, while different lower case letters indicate significant differences between dietary methionine.

by temperature. Likewise, both dietary methionine and temperature significantly affected plasma glycine, ornithine and citrulline. Higher concentrations of urea, serine, ornithine and citrulline, but lower concentrations of glycine and anserine, were observed in cobia reared at 30°C as compared to those fish reared at 34°C. In addition, cobia fed M9 diet showed lower plasma glycine, ornithine, and citrulline than the fish fed M16 diet. There were significant interactions between dietary methionine and temperature on plasma urea, ammonium and citrulline. Other plasma-free amino acids (FAA) were also affected by temperature, including glutamine, 1-methyl-L-histidine, aspartic acid, threonine, leucine and isoleucine (Supporting Information Table S1).

Temperature significantly affected muscle concentration of FAA and N-metabolites including urea, taurine, glycine, ornithine, O-Phosphoethanolamine (Pea), O-Phospho-L-serine and methionine (P-ser) (Table 6). Cobia reared at 34°C had higher concentrations of muscle free IDAs arginine, histidine, lysine and methionine than at 30°C (Supporting Information Table S2). Similar results were observed for methionine and taurine, glycine, ornithine and other DAs. There were significant interactions between dietary methionine and temperature on muscle urea and ornithine.

Concentration of liver glycine was affected by both dietary methionine and temperature. Moreover, temperature significantly affected urea, serine, cystathionine and methionine (Table 7). Lower concentrations of urea and glycine, but higher concentrations of serine, cystathionine and methionine, were observed in cobia reared at 30°C as compared to the fish at 34°C. Temperature also influenced the concentration of other liver FAAs, including alanine, aspartic acid, glutamine, proline, tyrosine, asparagine, alfa-amino-n-butyric acid, 1-methyl-L-histidine, threonine, leucine and isoleucine, lysine, valine, arginine, isoleucine, phenylalanine and histidine, while dietary methionine affected liver aspartic acid, threonine and leucine (Supporting Information Table S3). No significant interactions between dietary methionine and temperature on concentration of metabolites of urea cycle nor on muscle FAAs occurred.

3.3 | Liver SAM and SAH

Cobia showed comparable in liver SAM and SAH (Table 7). There were no significant interactions between dietary methionine and temperature on liver SAM and SAH of cobia.

3.4 | Whole-body lipid class composition

Table 8 presents lipid class composition of body lipid (mg/g fat) in cobia fed different dietary methionine at the two temperatures, including phosphatidylethanolamine, free fatty acid, cholesterol, phosphatidylcholine, total phospholipids and triacylglycerol. There were no significant differences in the lipid class composition due to dietary methionine. However, temperature significantly reduced phosphatidylethanolamine, cholesterol, phosphatidylcholine and total phospholipids in cobia. No interactions between dietary

TABLE 5 Plasma-free amino acids and N-metabolites (μmol/100 ml) of the cobia fed the experimental diets as occurring 24 hr postprandial

Temperature	30°C			34°C			*p*-Value		
Diet	M9	M12	M16	M9	M12	M16	Met	Temp	Met × Temp
Amm	29.77 ± 0.81	27.66 ± 0.69	25.77 ± 0.96	27.85 ± 0.51	29.16 ± 1.65	29.70 ± 0.75	NS	NS	0.032
Ans	1.19 ± 0.21^{B}	1.36 ± 0.05^{B}	1.82 ± 0.15^{B}	2.14 ± 0.50^{A}	2.51 ± 0.48^{A}	1.69 ± 0.42^{A}	NS	0.005	NS
Citr	0.71 ± 0.05^{Ab}	2.99 ± 0.35^{Aa}	3.12 ± 0.37^{Aa}	0.40 ± 0.01^{Bb}	0.71 ± 0.12^{Ba}	0.74 ± 0.08^{Ba}	0.0001	0.0001	0.0007
Gly	28.92 ± 0.34^{Bb}	40.01 ± 1.16^{Ba}	41.47 ± 0.89^{Ba}	39.41 ± 2.96^{Ab}	54.98 ± 5.45^{Aa}	56.67 ± 2.99^{Aa}	0.0001	0.0001	NS
Met	8.83 ± 0.14	10.32 ± 0.25	9.29 ± 0.10	10.15 ± 0.42	10.84 ± 1.72	10.02 ± 0.44	NS	NS	NS
Orn	3.92 ± 0.96^{Ab}	4.68 ± 0.64^{Aab}	6.26 ± 0.60^{Aa}	2.71 ± 0.05^{Bb}	4.74 ± 0.73^{Bab}	3.83 ± 0.50^{Bb}	0.045	0.042	NS
P-ser	2.23 ± 0.08	2.14 ± 0.13	1.77 ± 0.62	1.77 ± 0.11	1.94 ± 0.34	2.07 ± 0.24	NS	NS	NS
Ser	9.44 ± 0.09^{A}	9.38 ± 0.71^{A}	8.86 ± 0.62^{A}	6.73 ± 0.44^{B}	6.83 ± 1.69^{B}	7.74 ± 0.18^{B}	NS	0.008	NS
Tau	41.98 ± 3.11	44.56 ± 2.99	47.99 ± 4.95	34.09 ± 7.16	39.28 ± 9.91	57.11 ± 1.49	NS	NS	NS
Urea	295.34 ± 26.22^{A}	206.97 ± 5.83^{A}	215.75 ± 10.34^{A}	134.18 ± 20.31^{B}	141.38 ± 20.74^{B}	139.40 ± 17.40^{B}	NS	0.001	0.04

Note. Value is presented as mean ± *SEM* (n = 3); NS: not significant ($p > 0.05$); Amm (Ammonium); P-ser (O-Phospho-L-serine); Ans (Anserine); different upper case letters indicate significant differences ($p < 0.05$) between temperatures, while different lower case letters indicate significant differences between dietary methionine.

TABLE 6 Muscle-free amino acids and N-metabolites (μmol/g wet weight basis) of the cobia fed the experimental diets as occurring 24 hr postprandial

Temperature	30°C			34°C			*p*-Value		
Diet	M9	M12	M16	M9	M12	M16	Met	Temp	Met × Temp
Amm	5.86 ± 0.60	5.12 ± 0.76	5.21 ± 3.46	5.40 ± 0.51	5.91 ± 0.26	5.93 ± 0.40	NS	NS	NS
Ans	1.42 ± 0.08	1.17 ± 0.10	1.16 ± 0.02	1.24 ± 0.15	1.37 ± 0.11	1.49 ± 0.12	NS	NS	NS
Cysth	0.02 ± 0.00^{b}	0.12 ± 0.00^{ab}	0.27 ± 0.07^{a}	0.05 ± 0.01^{b}	0.15 ± 0.02^{ab}	0.21 ± 0.05^{a}	0.0003	NS	NS
Gly	2.05 ± 0.19^{B}	3.74 ± 0.46^{B}	3.36 ± 0.35^{B}	3.96 ± 0.62^{A}	4.01 ± 0.13^{A}	3.97 ± 0.57^{A}	NS	0.019	NS
Met	0.09 ± 0.01^{Ba}	0.07 ± 0.00^{Bab}	0.05 ± 0.00^{Bb}	0.10 ± 0.01^{Aa}	0.08 ± 0.01^{Aab}	0.07 ± 0.01^{Ab}	0.005	0.002	NS
Orn	0.12 ± 0.04^{b}	0.07 ± 0.01^{b}	0.11 ± 0.04^{b}	0.23 ± 0.06^{ab}	0.41 ± 0.08^{a}	0.18 ± 0.03^{b}	NS	0.0007	0.026
Pea	0.02 ± 0.00^{B}	0.02 ± 0.00^{B}	0.01 ± 0.00^{B}	0.03 ± 0.01^{A}	0.03 ± 0.00^{A}	0.03 ± 0.00^{A}	NS	0.0006	NS
P-ser	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	NS	0.006	NS
Ser	0.21 ± 0.01	0.19 ± 0.01	0.18 ± 0.02	0.21 ± 0.02	0.18 ± 0.02	0.19 ± 0.02	NS	NS	NS
Tau	2.95 ± 0.24^{B}	3.92 ± 0.45^{B}	3.43 ± 0.23^{B}	4.70 ± 0.68^{A}	5.27 ± 0.19^{A}	4.77 ± 0.27^{A}	NS	0.0005	NS
Urea	1.74 ± 0.12^{Aa}	1.27 ± 0.08^{Ab}	1.31 ± 0.05^{Ab}	0.91 ± 0.11^{Ba}	0.93 ± 0.07^{Bb}	0.87 ± 0.09^{Bb}	0.012	0.0001	0.014

Note. Value is presented as mean ± *SEM* (n = 3); NS: not significant ($p > 0.05$); Amm (Ammonium), Cysth (Cystathionine); Pea (O-Phosphoethanolamine); Phser (O-Phospho-L-serine); different upper case letters indicate significant differences ($p < 0.05$) between temperatures, while different lower case letters indicate significant differences between dietary methionine.

TABLE 7 Liver-free amino acids and N-metabolites (μmol/g wet weigh), SAM (nmol/g) and SAH (nmol/g) in the cobia fed the experimental diets as occurring 24 hr postprandial

Temperature	30°C			34°C			*p*-Value		
Diet	M9	M12	M16	M9	M12	M16	Met	Temp	Met × Temp
Cysth	0.07 ± 0.00^{A}	0.06 ± 0.01^{A}	0.05 ± 0.01^{A}	0.04 ± 0.01^{B}	0.03 ± 0.01^{B}	0.02 ± 0.00^{B}	NS	0.001	NS
Gly	2.11 ± 0.13^{Bb}	2.45 ± 0.33^{Bab}	2.51 ± 0.11^{Ba}	2.68 ± 0.12^{Ab}	3.47 ± 0.28^{Aab}	3.59 ± 0.31^{Aa}	0.03	0.0005	NS
Met	0.37 ± 0.07^{A}	0.43 ± 0.04^{A}	0.34 ± 0.01^{A}	0.29 ± 0.02^{B}	0.26 ± 0.03^{B}	0.24 ± 0.05^{B}	NS	0.0005	NS
Orn	0.08 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.00	NS	NS	NS
Pea	0.09 ± 0.03	0.09 ± 0.02	0.08 ± 0.01	0.11 ± 0.01	0.13 ± 0.02	0.12 ± 0.00	NS	NS	NS
P-ser	0.08 ± 0.00	0.08 ± 0.01	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	NS	NS	NS
Ser	1.51 ± 0.18^{A}	1.20 ± 0.18^{A}	1.10 ± 0.06^{A}	0.85 ± 0.05^{B}	0.71 ± 0.09^{B}	0.64 ± 0.16^{B}	NS	0.0003	NS
Tau	27.25 ± 1.64	27.29 ± 1.97	34.31 ± 1.96	26.01 ± 2.76	30.88 ± 2.27	28.45 ± 3.55	NS	NS	NS
Urea	3.72 ± 0.37^{B}	3.33 ± 0.60^{B}	3.22 ± 0.22^{B}	16.11 ± 0.56^{A}	15.04 ± 0.76^{A}	14.83 ± 0.40^{A}	NS	<0.0001	NS
SAH	14.57 ± 2.61	12.90 ± 1.65	14.74 ± 0.02	23.18 ± 4.64	16.15 ± 2.22	13.97 ± 0.70	NS	NS	NS
SAM	7.68 ± 5.27	11.52 ± 7.27	4.22 ± 0.63	2.25 ± 0.41	2.94 ± 0.77	2.42 ± 0.56	NS	NS	NS

Note. Value is presented as mean ± *SEM* (n = 3); NS: not significant ($p > 0.05$); Pea (O-Phosphoethanolamine); Phser (O-Phospho-L-serine); Cysth (Cystathionine); SAM (S-adenosyl methionine); SAH (S-adenosyl homocysteine); different upper case letters indicate significant differences ($p < 0.05$) between temperatures, while different lower case letters indicate significant differences between dietary methionine.

methionine and temperature occurred. There were no differences in TAG due to high variation between tanks.

4 | DISCUSSIONS

In the present experiment, cobia fed M12 showed better growth performance, FCR, PER and PPV than those fish fed M9 or M16 diets. Zhou et al. (2006) reported that optimal dietary methionine requirement in juvenile cobia is 11.9 g/kg dry diet (in the presence of 6.7 g/kg cysteine) for maximum growth and feed utilization. In our study, cysteine was available in the ingredients of the experimental diets, but concentration of this amino acid was not analysed. To ensure methionine fulfilling the requirement, the formulated M12 diet contained slightly higher amount of methionine than the requirement, in order to eliminate sparing effects, if any, on the amino acid from cysteine (Poppi et al., 2017; Toennies & Callan, 1939). Our results indicate that supplemented crystalline methionine was utilized by juvenile cobia, as reported in other fish (Espe et al., 2006; Kim, Kayes, & Amundson, 1992; Niu et al., 2013; Zhou et al., 2006).

A remarkable lower growth (30%) in body weight occurred in cobia fed M9 diet, as compared to the fish fed M12 diet. This should be taken into consideration in practical aquaculture, in order to optimize the pellet formulation of high inclusion of plant-based protein sources for cobia, as methionine is often the first limiting AA in plant proteins (Venero, Davis, & Lim, 2008). Poorer growth performance and feed conversion efficiencies in cobia fed M9 or M16 diets may result from dietary deficiency or excess of methionine in these two diets, respectively.

Dietary deficiency of one indispensable AA will lead to an increased oxidation of other indispensable and dispensable AAs present in the diets, thus reducing the growth in the animals. Dietary deficiency of methionine has been reported to reduced FI, resulting from loss of appetite, reduced growth and feed efficiency in European sea bass, *Dicentrarchus labrax* (Thebault, Alliot, & Pastoureaud, 1985), grouper, *Epinephelus coioides* (Luo et al., 2005), large yellow croaker, *Pseudosciaena crocea* (Mai et al., 2006), and golden pompano (Niu et al., 2013). On the other hand, excessive levels of AA intake may have adverse effect on absorption and utilization of other AAs and thus reduce growth performance (Conceição et al., 2007; Espe, Hevrøy, Liaset, Lemme, & El-Mowafi, 2008; Rønnestad, Jordal, & Gomes, 2016; Rønnestad et al., 2003). Reduced growth performance and feed conversion efficiencies observed in cobia fed M9 or M16 diets from this experiment may be due to negative impacts of deficiency and/or excess dietary indispensable amino acids, as have previously been reported in cobia (Nguyen et al., 2014; Wang et al., 2016; Zhou et al., 2006), as well as in other fish (Murthy & Varghese, 1998; Walton, Cowey, & Adron, 1982; Zhou et al., 2011). Our findings support the statement that either deficient or excessive levels of ingested indispensable AA cause negative impacts on growth performance and feed utilization in fish.

Elevated temperature also negatively affected performance and feeding efficiencies in cobia, with a reduction of about 28% in BW gain due to elevated rearing temperature. This supports that elevated temperature results in lower feed intake and feeding efficiency in this

TABLE 8 Lipid classes (mg/g fat) from whole body of the cobia fed the experimental diets as occurring 24 hr postprandial

Temperature	30°C			34°C			*p*-Value		
Diet	M9	M12	M16	M9	M12	M16	Met	Temp	Met × Temp
Phosphatidylethanolamine (PE)	3.2 ± 0.6^{A}	3.4 ± 0.4^{A}	4.5 ± 0.2^{A}	2.4 ± 0.1^{B}	2.7 ± 0.4^{B}	2.2 ± 0.5^{B}	NS	0.002	NS
Free fatty acid (FFA)	5.0 ± 1.8	8.2 ± 0.8	5.3 ± 2.2	3.5 ± 2.3	2.8 ± 1.6	3.5 ± 0.5	NS	NS	NS
Cholesterol (CHOL)	6.4 ± 0.7^{A}	6.2 ± 0.1^{A}	5.9 ± 0.4^{A}	5.0 ± 0.1^{B}	5.2 ± 0.6^{B}	5.6 ± 0.3^{B}	NS	0.03	NS
Phosphatidylcholine (PC)	17.9 ± 1.3^{A}	16.2 ± 1.6^{A}	18.6 ± 0.6^{A}	14.6 ± 0.2^{B}	15.3 ± 0.7^{B}	14.1 ± 0.7^{B}	NS	0.003	NS
Total Phospholipids (PL)	27.6 ± 2.8^{A}	26.7 ± 3.4^{A}	31.4 ± 1.7^{A}	21.0 ± 0.4^{B}	21.2 ± 0.9^{B}	18.9 ± 1.7^{B}	NS	0.0004	NS
Triacylglycerol (TAG)	317.3 ± 94.8	323.3 ± 70.2	225.0 ± 56.7	188.0 ± 35.4	232.3 ± 33.7	259.7 ± 11.9	NS	NS	NS

Note. Values are tank mean ± *SEM* (n = 3); means followed by different letters are significantly different (two-way ANOVA followed by Tukey)

species with negative effects on performance. Water temperature is a crucial factor for energy budget in fish; too elevated temperature will eventually lead to negative effects on cobia individual performance (Sun & Chen, 2014). Higher costs for routine metabolism, and smaller fraction of energy budget allocated to growth, will result in reduction in growth performance when water temperature is above the optimum (Glencross & Bermudes, 2011, 2012; Sandersfeld, Davison, Lamare, Knust, & Richter, 2015). Also, feed consumption in fish usually increases when temperatures are increasing, peaks at an optimal temperature and then decreases at higher temperature (Brett & Groves, 1979). Sun, Chen, and Huang (2006) studied the effect of various temperature between 23 and 35°C (with an increment of 4°C) on growth and energy budget of juvenile cobia (initial body weight ~22 g). The authors suggested that optimum temperature for cobia at this stage is between 27 and 29°C for maximizing both growth and feed conversion efficiency. However, in a more recent study in cobia with initial body weights between 10 and 200 g for 21 days, Sun and Chen (2014) suggested a higher optimum temperature (33°C) for rearing cobia for these fish sizes, which resulted in the highest SGRs that varied between 2.39 and 6.43. Temperature above the optimum causes increased faecal production, but reduced feed consumption and feed absorption and feeding efficiency in cobia (Sun et al., 2006), as well as in other fish (Bermudes, Glencross, Austen, & Hawkins, 2010; Glencross & Bermudes, 2010; Sun, Zhang, & Tang, 2001). A higher FCR in cobia reared at elevated temperature implies that a larger amount of feed would be needed to support the same growth performance as those fish reared at control temperature. Therefore, elevated temperature results in increased energy demand, especially for routine metabolism, activity and excretion, and thus for whole-animal metabolic rates. Results from our study suggest that elevated temperature negatively affect appetite and feed utilization efficiency of juvenile cobia. Further study however is required to clarify this.

Interestingly, the interaction between methionine and temperature resulted in significant differences in BW and growth between cobia fed M12 diet and the fish fed M16 diet at 30°C, but not at 34°C. This suggests that requirement for dietary methionine, possibly for other indispensable AAs as well, may increase when rearing water temperature beyond the optimum. Considering the global warming scenario with elevated water temperature, further research into the pathways of methionine metabolism in cobia is needed.

Dietary methionine deficiency and elevated temperature both reduced retention of indispensable AAs in cobia, except for methionine. AA retention depends on feed consumed, feeding efficiency, growth and metabolism, digestion and assimilation of the amino acid. Dispensable AAs are used preferentially as an energy substrate, and indispensable AAs are spared for growth (Conceição, Rønnestad, & Tonheim, 2002; Rønnestad, Conceição, Aragão, & Dinis, 2001). Also, feeding the fish with diets of balanced AA profile results in increased protein retention, and thus AA retention, with reduction in the catabolic losses of AA (Aragão et al., 2004). Higher retention of indispensable AAs in cobia reared at 30°C may be due to the fact that these cobia performed better growth and feeding efficiency than those fish reared at 34°C. Methionine deficiency (the

M9 diet) resulted in lower indispensable AA retention in cobia due to lower feed intake, poorer feeding efficiency and slower growth, as it has been reported in turbot *Scophthalmus maximus* (Peres & Oliva-Teles, 2005), gilthead sea bream (Sanchez-Lozano, Martinez-Llorens, Tomas-Vidal, & Cerda, 2011). Further, lower methionine retention in cobia fed M16 suggested that surplus methionine intake will be catabolized rather than used for protein accretion. Interestingly, there was significant interaction between dietary methionine and rearing temperature on retention of methionine in cobia, suggesting the important role of temperature on the catabolism of this amino acid in cobia, though further studies are required to clarify this.

Urea concentration in plasma, as well as muscle and liver urea, was higher than expected. This may indicate that cobia may be partiality or completely ureotelic, what is rare in teleosts, and in particular in pelagic marine fish. However, further studies on enzyme activities involving in urea cycle of this species are required to clarify this. It may also happen the cobia uses the ureotelic pathway to quickly reduce ammonia body concentrations, what may make sense considering its fast growth and its likely active amino acid catabolism to fuel such growth rates.

Concentration of plasma FAA may vary depending on dietary composition, digestion and absorption, and that free amino acid in diets may also be faster assimilated and more efficiently assimilated than protein-bound amino acids (Rønnestad, Conceição, Aragão, & Dinis, 2000). However, at 24 hr postfeeding, most of ingested IDAs from the diets would return back to normal plasma concentrations as reported in cobia by Mach and Nortvedt (2011),whereas plasma FAA did not reflect dietary methionine that was in accordance with those previously reported on cobia (Mach & Nortvedt, 2011), Atlantic salmon (Berge, Sveier, & Lied, 2002) and rainbow trout (Kaushik & Fauconneau, 1984).

In the present study, temperature significantly affected most FAAs in blood plasma, muscle and hepatic tissues. Cobia reared at 34°C generally showed lower FAAs in plasma and livers, but higher in muscle for arginine, histidine, methionine and valine, as well as most of other DAs indicate that at 34°C amino acids from absorption in cobia are more rapidly metabolized by the liver, redistributed to the blood plasma and then channelled to the muscle for protein turnover than at 30°C. Further, higher concentration of FAAs in muscle and metabolites (e.g., phosphoethanolamine taurine and 1-methylhistidine), concomitant with lower growth in cobia reared at 34°C, that elevated temperature altered protein turnover in which slower protein synthesis for tissue growth, but higher protein catabolism occurred, as compared to the fish reared at 30°C. These results suggest that temperature beyond the optimum may change de novo synthesis and/or catabolism of amino acids in cobia. In addition, blood plasma is a pool of FAA delivered via blood fluids to organs that play an important role in physiological and immunological responses. Results from the present study suggested that under long-term exposure to high temperature—stressor, cobia could alter de novo synthesis and catabolism of the amino acids in order to adapt to such changing scenario. To have a full understanding of the mechanism underlining such alterations due to stress thermal in cobia, further study including the enzyme activities is required.

Concentration of SAM and SAH from liver of cobia was not significantly different amongst dietary methionine and temperature treatments. Serving as the precursor, methionine may affect metabolism of SAM and SAH, and DNA methylation and gene expression related to hepatic lipid metabolism in animals (Zhang, 2018). Increased dietary methionine intake would result in higher SAM which would be converted to SAH by DNA methyltransferases. Atlantic salmon showed higher hepatic SAM, but not SAH when fed higher methionine diet (Espe et al., 2008). However, in this study, changes in liver SAM or SAH were not observed may due to the fact that cobia may adapt to fluctuations of dietary methionine.

In the present study, dietary methionine deficiency and elevated temperature increased VSI and HSI in cobia, respectively. Interestingly, there was significant interaction between methionine and temperature on both VSI and HSI. Our results were not in line with those reported by Zhou et al. (2006) that dietary methionine did not affect VSI nor HSI in cobia under diurnal water temperature. Dietary composition has been reported to affect viscera mass as well as HSI in Atlantic salmon (Espe, Ruohonen, & El-Mowafi, 2012) and juvenile turbot (Wei, Liang, Mu, Zheng, & Xu, 2016), as a consequence of energy and lipid accumulation. Poorer VSI and HSI and lower growth performance in cobia fed M9 suggested that more energy was allocated for visceral organs, but less for growth, as compared to cobia fed either M12 or M16 diets. Results from this study also suggested that heat stress may alter hepatic lipid deposition and accumulation, resulting from induced liver uptake of circulating lipids from peripheral fat, similar to cold stress that reported in gilthead sea bream (Ibarz et al., 2005) and Senegalese sole (Arjona et al., 2010). In addition, cobia reared at 34°C had higher cholesterol, phosphatidylcholine, phosphatidylethanolamine and total phospholipids in whole body, but lower muscle phosphoethanolamine as compared to the fish reared at 30°C. This indicates that elevated temperature alters phospholipid synthesis or catabolism in juvenile cobia. However, further study on lipid metabolism under thermal stress in cobia is needed to clarify this.

5 | CONCLUSIONS

In summary, results from the present study indicate that 34°C is too high temperature for juvenile cobia rearing. Water temperature above the optimum leads to an abrupt decline in growth performance, feed intake and feeding efficiency and energy deposition, and altering lipid metabolism, protein turnover and protein accretion in juvenile cobia.

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SUPPORTING INFORMATION

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