



Review

Methionine in fish health and nutrition: Potential mechanisms, affecting factors, and future perspectives

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ABSTRACT

Amino acids do not only work in protein synthesis but also act as molecular signals regulating energy metabolism, and the immune and antioxidant systems in animals. As an essential amino acid, crystalline methionine is widely applied in fish feed, especially when fishmeal is replaced by other alternative protein sources. To systematically understand the present knowledge of methionine application and utilization in fish, this paper reviewed studies conducted on farmed fish, encompassing methionine requirements of various fish species, the potential mechanisms of methionine in regulating growth, feed utilization, lipid and glucose metabolism, anti-oxidative and immune status, and its interactions with cysteine and taurine. This review concludes that the dietary methionine requirement of fish ranges from 0.49% to 2.5% of the diet (1.49% to 4.7% of dietary protein), and the requirement is influenced by species, life stage, methionine forms, and dietary cysteine and taurine levels. The lipid and glucose metabolism responses to dietary methionine differ by species, and inconsistent results were reported. In addition, there is a need for further studies on the impact of methionine on intestinal microbiota, and lipid and glucose metabolism regulated by methionine, and methionine toxicity in different fish species. Furthermore, the current trend of high plant protein and lipid inclusion in fish feed necessitates a careful reevaluation of dietary methionine requirement of certain species.

1. Introduction

Nutrition-balanced feed is essential for the optimal health and growth performance of fish. Protein and amino acids are vital nutrients that affect the physiological condition, immunological status, and growth performance of fish (Li et al., 2009; Teles et al., 2019). Although fishmeal remains the preferred protein source in fish feed, the plateaued global supply makes it more expensive and less available. Economic constraints and the drive for sustainability necessitates a paradigm shift towards higher inclusion levels of other alternative protein sources (processed plant proteins, byproducts from agriculture, fisheries, or the slaughtering of terrestrial production animals), which may result in imbalanced amino acid profiles, especially essential amino acids (EAAs) (Nunes et al., 2014; Rolland et al., 2015). To formulate a balanced amino acid profile feed for fish, crystalline amino acid (CAA) inclusion is

becoming a rational approach rather than simply increasing the dietary inclusion levels of the protein sources that contain ideal levels of the targeted EAAs (Chu et al., 2014; Nunes et al., 2014). Current studies have revealed the important roles of amino acids in physiological functions, protein and hormone synthesis, and immune and anti-oxidative responses. The multiple functions of arginine (Hoseini et al., 2020), taurine (El-Sayed, 2014), methionine hydroxy analog (MHA) (Guo et al., 2020a), and tryptophan (Hoseini et al., 2019) in fish nutrition and health have been reviewed, providing important information for future study and application. Methionine is a nutritionally indispensable amino acid, and it is special among other indispensable amino acids because it contains sulfur atoms. Methionine is the primary methyl donor in organisms through S-adenosylmethionine by the trans-methylation pathway, which is vital for some important metabolites, such as phosphatidylcholine and creatine. Methionine also takes part in

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cysteine and glutathione synthesis by the transsulfuration pathway, which together with methionine are necessary for protein synthesis and the antioxidant system (Elango, 2020). Thus, methionine does not only serve as substructure for protein synthesis but also as signaling molecule for regulating immunity, energy metabolism, and reproduction (Wang et al., 2021).

As an EAA, methionine is the first limiting amino acid in plant and rendered animal proteins, followed by lysine (Chu et al., 2014; Nunes et al., 2014). Additional methionine is necessary for commercial feed when fishmeal is replaced by other protein sources, especially plant proteins. Although many studies have been conducted on methionine affecting the growth and health condition of various fish, a systematic review of the nutritional and health-promoting roles is still lacking. Thus, this paper aims to provide a synopsis of methionine requirements of fish and the affecting factors, as well as the potential mechanisms by which methionine regulates lipid and glucose metabolism, feed utilization, and antioxidant and immune status.

2. Methionine metabolism process

Methionine is normally available in the DL-form. L-methionine is the natural isomer and can be absorbed rapidly and used efficiently by animals (Li et al., 2009), while D-methionine must be transaminated into α -ketoacid by D-methionine oxidase, and α -ketoacid subsequently converted into L-methionine, catalyzed by transaminases (Wu and Thompson, 1989). In organisms, L-methionine must be activated by ATP to produce S-adenosyl-methionine (SAM), which is the principal donor of methyl groups in the body. Then, S-adenosylhomocysteine (SAH) is produced after methionine donates its methyl group to methyl acceptors, which is unstable and quickly converted to homocysteine (Andersen et al., 2016). The remethylation of homocysteine to methionine requires folic acid as the methyl donor under the catalysis of methionine synthase (MS), whereas the transmethylation of homocysteine to methionine requires betaine as the methyl donor by the catalysis of betaine homocysteine methyltransferase (BHMT) (Finkelstein, 1990). Homocysteine can be subsequently converted to methionine via transmethylation or remethylation, or trans-sulfurated to cystathionine. Cystathionine is then metabolized to cysteine, which can be converted into taurine or participate in glutathione synthesis (Andersen et al., 2016) (Fig. 1).

3. Different forms of methionine and their bioavailability

The different forms of methionine used in fish feed have been reviewed by Nunes et al. (2014). Briefly, DL-methionine is the most common crystalline methionine applied in animal feed and contains a mixture of D- and L isomers of methionine (Goff and Dmiii, 2004), whereas DL-2-hydroxy-4-methylthiobutanoic acid (HMTBa), MHA, and DL-methionyl-DL-methionine (Met-Met) are newly developed crystalline methionine forms. The D-isomer of DL-methionine and D- and the L-isomers of HMTBa can be converted into L-methionine (Nunes et al., 2014). MHA has a hydroxyl group on the alpha carbon, which is the main chemical difference from DL-methionine which has an amino group that resembles commonly used organic acids. In organisms, MHA can be converted to L-methionine in the peroxisomes within the liver and kidney, while D-methionine is processed with a mitochondrial enzyme in most tissues once absorbed (Baker, 2006). In addition, Met-Met is a new dipeptide with a methionine substitution that consists of four different methionine stereoisomers (DL-methionine-methionine, LD-methionine-methionine, DD-methionine-methionine, and LL-methionine-methionine) that can be efficiently converted to free D- and L-methionine by digestive enzymes (Façanha et al., 2016; Niu et al., 2017; Wang et al., 2019).

Many studies have estimated the utilization efficiency and bioavailability of these methionine sources in fish. For instance, Powell et al. (2017) reported that D- and L- methionine showed a similar

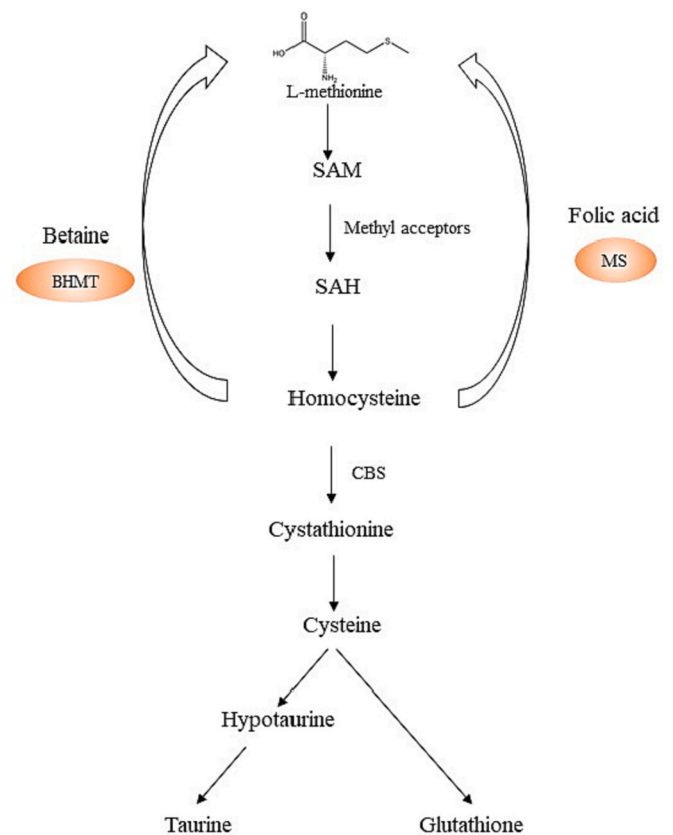


Fig. 1. Schematic figure of methionine pathways producing cysteine and taurine. SAM (s-adenosyl methionine), SAH (s-adenosyl homocysteine), MS (methionine synthetase), BHMT (betaine homocysteine transferase), CBS (cystathionine beta-synthase).

bioavailability in Atlantic salmon (*Salmon salar* L.), whereas D-methionine showed an even better feed utilization efficiency in Atlantic salmon than L-methionine which could be due to the slower uptake of D-methionine from the intestine into the blood (Sveier and Berge, 2001). In addition, studies in fish have demonstrated that the bioefficacy of MHA is lower than that of DL-methionine (Pan et al., 2017; Powell et al., 2017; Zhao et al., 2022; Zhou et al., 2021) and approximately 75% to 80% that of methionine on an equimolar basis based on current experimental evidence (NRC, 2011). Teodósio et al. (2021) compared the metabolism and growth-promoting effects of DL-methionine and MHA-Ca on Nile tilapia (*Oreochromis niloticus*) on equimolar levels of methionine *in vitro* and *in vivo*. The authors found that DL-methionine was more efficient in improving the growth performance of fish than MHA-Ca. Based on the radiolabeled tracers (^{14}C -DL-methionine and ^{14}C -MHA), DL-methionine showed a better retention ratio than MHA, probably accounting for a faster absorption rate as well as greater availability of free methionine in the tissues to be utilized. However, some contradictory results were also reported in some fish. Red drum (*Sciaenops ocellatus*) fed an equal-sulfur diet of L-methionine, DL-methionine, and MHA showed no significant differences in survival, weight gain (WG), feed efficiency (FE), protein efficiency ratio (PER), or protein conversion efficiency (PCE) (Goff and Dmiii, 2004). Similarly, based on percent weight gain (PWG) and FE, the efficacy of MHA relative to DL-methionine was estimated to be 97%, indicating that MHA could effectively be used as a methionine substitute according to the growth performance of grass carp (*Ctenopharyngodon idella*). In addition, MHA is superior to DL-methionine in terms of strengthening immune function and improving the structural integrity of immune organs (Pan et al., 2016). Regarding HMTBa, numerous studies conducted on livestock species revealed that HMTBa presented efficient absorption,

availability, metabolism, and biological efficiency (Nunes et al., 2014). Similarly, Ma et al. (2013) reported that HMTBa supplementation in turbot (*Psetta maxima*) diets had a growth-promoting effect similar to that of L-methionine. Supplementation of HMTBa resulted in a relatively slightly lower methionine requirement but a higher PER than that of L-methionine in trout, indicating that HMTBa is an effective or even better methionine source than L-methionine (Ma et al., 2013). As a novel methionine source, Met-Met has some advantages, such as better absorption and lower water solubility than crystalline methionine. Dietary Met-Met was superior to DL-methionine at enhancing growth performance and improving intestinal immune function in Nile tilapia and grass carp (Guo et al., 2020b; Su et al., 2018).

Thus, with the differences in bioavailability and utilization efficiency, various forms of methionine inclusion may lead to different methionine requirements of a given fish species. Further studies should compare these methionine sources both from bioavailability and economical aspects to achieve the optimal effect of methionine inclusion.

4. Methionine requirements of fish

A total of 52 studies were reviewed that designed more than four methionine levels and used statistical analysis to estimate the methionine requirement of a certain fish. The methionine requirements of farmed fish species range from 0.49% to 2.5% of the diet (1.49% to 4.7% of dietary protein) (Table 1). This is consistent with previous conclusions that the dietary methionine requirement of fish ranges from 0.5% to 1.5% of the diet (1.3% to 4.5% of the dietary protein) (Habte-Tsion, 2020; NRC, 2011; Nunes et al., 2014). It has been illustrated that variations in the dietary amino acid requirements of fish may be linked to different fish species, life stages and sizes, quality of the diet used, feeding levels, and rearing conditions (Hoseini et al., 2020; Lall and Anderson, 2005). In terms of methionine, fish size and age, methionine form, basal diet composition, dietary cystine and taurine levels, and the criteria for requirement seem to be vital factors that affect the dietary methionine requirement of fish (Table 1). For example, the methionine requirement of juvenile grass carp estimated by Met-Met (1.81% dietary protein (Su et al., 2018)) was lower than that estimated by L-methionine (2.67% dietary protein (Ji et al., 2022)) and DL-methionine (2.18% dietary protein (Wu et al., 2017)). In particular, high dietary digestible energy (lipid) (Nile tilapia) (He et al., 2017) and plant protein (channel catfish (*Ictalurus punctatus*)) (Cai and Burtle, 1996) levels cause higher methionine requirements in certain fish species.

The growth-promoting effect of methionine is related to modulation of the somatotrophic axis, improvement of protein synthesis, protein turnover, feed utilization, and enhancement of antioxidant and immune systems. In addition, dietary cystine and taurine levels may also influence the methionine requirement of fish (discussed below). The methionine level in the formulated diet of a given fish should consider both methionine levels in protein sources and supplemented methionine forms and levels.

5. Methionine interaction with cysteine and taurine

Methionine not only participates in protein synthesis but also serves as a methyl donor and source of sulfur which is essential for cystine and taurine biosynthesis (Andersen et al., 2016; Michelato et al., 2018). Metabolic and nutritional interactions of methionine with cystine and taurine have been illustrated in many organisms. First, methionine and cystine are the main sulfur-containing amino acids (SAAs). Cystine can only be synthesized from a methionine precursor, and the requirement for total sulfur amino acids (TSAAs) can be met by either methionine alone or the proper mixture of methionine and cystine; thus, a portion of the methionine requirement can be spared by cystine in some fish species (Chu et al., 2014; Luo et al., 2005; Martínez et al., 2017; Moon and Gatlin, 1991; NRC, 2011). In particular, a study in rainbow trout

(*Oncorhynchus mykiss*) reported that methionine deficiency caused a high methionine to cystine conversion ratio (80%), while increased dietary methionine decreased this ratio (Rumsey et al., 1983). The overlapping effects of methionine and cystine determine that some studies estimated the TSAA requirement of fish. Methionine or TSAA requirements have been reported to vary from 2.20% to 6.50% of dietary protein in different fish species (De Silva and Anderson, 1994), which is in accordance with the present review result (ranging from 0.56% to 3.4% of the diet (2.34% to 6.34% of dietary protein), Table 1). Thus, estimation of the dietary methionine requirement of certain fish should take the species-specific methionine sparing effect of cystine into account. For instance, the methionine requirement of African catfish (*Clarias gariepinus*) in non-cystine inclusion experiment was approximately two times higher than that in a 0.44% cystine experiment (Table 1) (Elesho et al., 2021; Fagbenro et al., 1999). Similarly, cystine was able to save approximately 51% of the methionine requirement in yellow perch (*Perca flavescens*) (Twibell et al., 2000), 42% of the methionine requirement in rainbow trout (Kim et al., 1992), and 40% (Moon and Gatlin, 1991) or 50% (Goff and Dmiii, 2004) of the methionine requirement in red drum. Feng et al. (2020) reported that cystine could spare methionine by a certain level when grouper (*Epinephelus coioides*) were fed diets with different methionine/cystine ratios. On the other hand, cystine could improve muscle growth and overcome the negative impact of methionine deficiency at a certain level in field eel (*Monopterus albus*) (Hu et al., 2021). Normally, dietary cystine excess of 3 g/kg has no methionine-sparing effect (Nunes et al., 2014), and most studies (Table 1) supplied more cystine than this limit. However, some studies used very low dietary cystine levels to estimate the dietary methionine requirement of fish, such as Japanese flounder (*Paralichthys olivaceus*) (0.06% of the diet) (Alam et al., 2007), grass carp (0.15% of the diet) (Fang et al., 2021; Wu et al., 2017), and humpback grouper (*Cromileptes altivelis*) (0.10%–0.18% of the diet) (Irm et al., 2021), which might obtain incredible results.

Taurine is a β -sulphonic amino acid that is derived from the trans-methylation and sulfuration pathway of methionine and cysteine. Taurine is a conditionally essential amino acid for fish, especially when the diet is deficient in cysteine and/or methionine or with high levels of plant-protein feedstuff inclusion (Al-Feky et al., 2016; El-Sayed, 2014). Normally, freshwater fish have a relatively higher taurine synthesis ability than marine water species (El-Sayed, 2014). Dietary methionine increases taurine contents in different organs and spares the taurine requirement in many fish species (Candebat et al., 2020; Marit et al., 2008; Zhou et al., 2021). Notably, dietary taurine can spare the methionine requirement of some fish species. The methionine-deficient diet with taurine supplementation achieved similar growth performance as the methionine-sufficient group in Nile tilapia (Michelato et al., 2018) rainbow trout (Gibson Gaylord et al., 2007), and rice field eel (Hu et al., 2021). However, most studies on the methionine requirement of certain fish species have ignored the taurine content in the diet, especially using fishmeal as the main protein source in the basal diet.

6. Effects of methionine on growth performance

Suitable levels of methionine could improve fish growth performance and have been widely demonstrated in various fish species. However, inconsistent results were observed for fish growth performance and dietary methionine levels. For instance, studies on gibel carp (*Carassius auratus gibeilo*) (Wang et al., 2016a), silver catfish (*Rhamdia quelen*) (Rotili et al., 2018), yellow tail (*Seriola quinqueradiata*) (Ruchimat et al., 1997), European sea bass (*Dicentrarchus labrax*) (Tulli et al., 2010), rockfish (*Sebastes schlegeli*) (Yan et al., 2007), golden pompano (*Trachinotus ovatus*) (Niu et al., 2013), humpback grouper (Giri et al., 2006), gilthead seabream (*Sparus aurata*) (Marcouli et al., 2005), arctic charr (*Salvelinus alpinus*) (Simmons, 1997), and Nile tilapia (Nguyen and Allen Davis, 2009) observed that fish growth increased first and then plateaued with increasing dietary methionine. However, fish growth

Table 1
The methionine requirements of different fish species.

Species	Initial weight (g)	Methionine form	Dietary protein (% of diet)	Methionine requirement (% of dietary protein)	Dietary requirement (%)	Cystine level (% of diet)	Total sulfur amino acids (Met+Cys, % of diet)	Dietary protein source	Estimation mode used	Criteria for requirement determination	References
African catfish	18.7	DL-Met	40	3.2	1.28	0	1.28	Casein and gelatin	BLR	WGR	Fagbenro et al. (1999)
African catfish	78	DL-Met	32	1.92	0.63	0.44	1.07	SPC and wheat	BLR and QRA	WGR and FCR	Elesho et al. (2021)
Arctic charr	20.5	L-Met	40	4.4–4.7	1.76–1.88	NM	/	Herring meal, SBM	QRA	Liver weight, carcass protein, energy gain, Liver size	Simmons (1997)
Atlantic salmon	493	DL-Met	43	2.1–2.3	0.90–0.99	0.60	1.50–1.59	FM, CGM, and SBM	Exponential function		Espe et al. (2008)
Atlantic salmon	1066	DL-Met	41	2.3	0.92	NM	1.53	FM, SPC	QRA	WGR	Sveier and Berge (2001)
Black sea bream	14.21	L-Met	38	4.53	1.72	0.31	2.03	FM, SPC	BLR	SGR	Zhou et al. (2011)
Blunt snout bream	101.8	L-Met	33	2.24–2.30	0.74–0.76	0.22	0.96–0.98	FM, casein	QRA	FER and SGR	Liang et al. (2016)
Carp	569	DL-Met	40.2	2.13	0.855	0.42	1.275	SP, peas	BLR	FW	Schwarz et al. (1998)
Channel catfish	202	L-Met	24	2.34	0.56	0	0.56	Egg protein	NM	NM	Harding et al. (1977)
Channel catfish	14	DL-Met	27.7	3.41	0.94	0.4	1.13	SBM	BLR	Protein gain	Cai and Burtle (1996)
Chinese sucker	1.72	L-Met	44	3.20	1.41	0.37	1.78	FM, FSBM	QRA	SGR	Chu et al. (2014)
Cobia	9.79	DL-Met	46	2.69	1.24	0.4	1.64	FM, SBM,	BLR	WGR	Chi et al. (2020)
Cobia	11.61	L-Met	44	2.64	1.19	0.67	1.86	FM, WG	QRA	SGR	Zhou et al. (2006)
Cobia	150.9	L-Met	46	2.35–2.45	1.04–1.15	0.40	1.44–1.55	FM, SBM	QRA	WGR and FER	Wang et al. (2016c)
Euroupen sea bass	13.4	L-Met	44	2.0	0.91	0.4	1.31	SBM	BLR	WGR	Tulli et al. (2010)
Gibel carp	51.0	DL-Met	43	2.27	0.98	0.399	1.379	FM, BM, PPC	QRA	WGR	Wang et al. (2016a)
Gilthead seabream	3.5	L-Met	45	2.77	1.24	0.65	1.89	FM	BLR	WGR	Marcouli et al. (2005)
Golden pompano	12.4	DL-Met	43	2.46–2.95	1.06–1.27	0.19	1.25–1.36	FM, SBM	BLR	WGR, NRE	Niu et al. (2013)
Grass carp	0.36	L-Met	38	2.67	1.03	0.34	1.37	FM, RM, SBM	BLR	SGR, FCR	Ji et al. (2022)
Grass carp	451.3	DL-Met	28	2.18	0.612	0.15	0.762	FM, casein	QRA	WGR	Wu et al. (2017)
Grass carp	178.47	DL-Met	28	3.33	0.956	0.15	1.106	FM, SPC	QRA	WGR	Fang et al. (2021)
Grass carp	10.10	Met-Met	32	1.81	0.58	0.37	0.95	FM, SBM, CSM	QRA	WGR	Su et al. (2018)
Grass carp	259.70	MHA	27.9	1.79–2.43	0.50–0.68	0.48	0.98–1.16	FM, SBM	QRA	FI, PWG, immune and antioxidant parameters	Pan et al. (2016)
Grouper	13.25	L-Met	48	2.73	1.31	0.26	1.57	FM, SPC	BLR	WGR	Luo et al. (2005)
Humpback grouper	6.57	DL-Met	48.9	2.18	1.07	0.10–0.18	1.17–1.25	FM, amino acid mixture	BLR	WGR	Irm et al. (2021)
Humpback grouper	5.6	L-Met	49	2.37–2.41	1.16–1.28	0.28	1.44–1.56	FM, casein	BLR	WGR, FER	Giri et al. (2006)
Hybrid grouper	10.61	DL-Met	49.6	2.92	1.45	0.69	2.14		QRA	WGR	Li et al. (2020)

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Table 1 (continued)

Species	Initial weight (g)	Methionine form	Dietary protein (% of diet)	Methionine requirement (% of dietary protein)	Dietary requirement (%)	Cystine level (% of diet)	Total sulfur amino acids (Met+Cys, % of diet)	Dietary protein source	Estimation mode used	Criteria for requirement determination	References
Indian catfish	4.33	L-Met	40	2.6–2.87	1.05–1.15	0.40	1.49	FM, amino acid mix	QRA	WGR, FCR, PER, and BPD	Ahmed (2014)
Indian major carp	0.19	L-Met	40	4.1–4.22	1.60–1.69	0.85	2.45–2.54	Amino acid mix, dextrin	BLR and QRA	WGR, FCR and NRE	(Khan and Abidi, 2013)
Indian major carp	0.50	L-Met	40	3.12	1.25	1.00	2.25	Casein	SDPR	FCR	Ahmed et al. (2003)
Japanese flounder	120 d after hatching	L-Met	50	2.88	1.44	0.06	1.5	Casein	BLR	WGR	Alam et al. (2007)
Jian carp	9.9	NM	35	3.43	1.2	0.30	1.5	FM, SCP	QRA	WGR	Tang et al. (2009)
Large yellow croaker	1.23	L-Met	43	3.22–3.34	1.39–1.44	0.29	1.73	FM, SBM, and yeast	QRA	SGR and FCR	Mai et al. (2006)
Largemouth bass	14.49	MHA	42	3.45	1.45	0.62–0.67	2.07–2.12	FM, SBM	QRA	SGR	Zhao et al. (2022)
Largemouth bass	37.88	L-Met	44	2.75	1.22	0.68	1.90	FM, SPC	QRA	SGR	Chen et al. (2010)
Meagre	50.0	DL-Met	42	1.78	0.75	0.60–0.65	1.35–1.40	FM, CGM	NM	WGR	de Moura et al. (2018)
Milk fish	8.0	NM	40–45	1.6–1.8	2.5	0.75	3.25	FM, SBM	BLR	WGR	Borlongan and Coloso (1993)
Nile tilapia	5.62	L-Met	28	1.75	0.49	0.45	0.94	CSM, DSESM	BLR	WGR	Nguyen and Allen Davis (2009)
Nile tilapia	8.95	DL-Met	28.8	2.5–3.4	0.73–0.99	0.46–0.5	2.96–3.9	CSM, SBM, RM	BLR and QRA	WGR	He et al. (2017)
Rainbow trout	505	L-Met	36.6	1.69–1.91	0.59–0.67	0.5	2.19–2.41	CAA	BLR	Plasma methionine	Bae et al. (2011)
Rainbow trout	8.8	L-Met	35	1.57–2.14	0.55–0.75	0.3	0.85–1.05	Gelatin, dextrin	BLR	WGR	Rumsey et al. (1983)
Rainbow trout	11.2	D-Met	35	1.49	0.52	0.5	0.8	CAA, casein, dextrin	BLR	WGR	Kim et al. (1992)
Red drum	0.9	L-Met	35	3.03 (TSAA)	1.06 (TSAA)	0	1.06	Red drum muscle, shrimp meal	BLR	Growth and feed efficiency	Moon and Gatlin (1991)
Rockfish	43.61	L-Met	48.7	2.80	1.37	0.12	1.49	FM	QRA	SGR	Yan et al. (2007)
Seabass	35	L-Met	50	2	1.3	0.6	1.8	FM, SBM, yeast	BLR	WGR	Thebault et al. (1985)
Silver catfish	3.26	DL-Met	37	3.44–3.58	1.27–1.36	0.07	1.34–1.43	Casein, gelatin, FM	QRA	WGR, SGR	Rotili et al. (2018)
Turbot	5.59	L-Met	48	3.27–3.31	1.58–1.59	0.42	2.00–2.01	FM, SBM	QRA	FI and SGR	Ma et al. (2013)
Turbot	5.59	HMTBa	48	3.19–3.25	1.49–1.56	0.42	1.91–1.98	FM, SBM	QRA	FI and SGR	Ma et al. (2013)
Ussuri catfish	0.6	L-Met	43	3.48–3.53	1.41–1.43	0.25	1.66–1.68	FM, CGM, SBM	QRA	WGR	Wang et al. (2016b)
Yellow catfish	2.0	NM	45	2.35	1.15	0.40	1.55	FM, SBM, WGM	QRA	WGR	Elmada et al. (2016)
Yellow Perch	4.7	L-Met	33.6	3.4	1.0–1.1	NM	3.1–3.4	Casein	BLR	WGR, FER	

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Table 1 (continued)

Species	Initial weight (g)	Methionine form	Dietary protein (% of diet)	Methionine requirement (% of dietary protein)	Dietary requirement (%)	Cystine level (% of diet)	Total sulfur amino acids (Met+Cys, % of diet)	Dietary protein source	Estimation mode used	Criteria for requirement determination	References
Yellow River carp	21.56	NM	32.5	3.75	1.22	NM	NM	SBM	QRA	PWG	Twibell et al. (2000) Yun et al. (2022)
Yellow tail	23.3	L-Met	43.3	2.56	1.11	0.31	1.42	FM, SPC	BLR	WGR and FER	Ruchimat et al. (1997)

Abbreviation list: BLR (broken-line regression); BM (blood meal); BPD (body protein deposition); CAA (crystalline amino acids); CGM (corn gluten meal); DL-Met (DL-methionine); DSESM (dehulled solvent-extracted soybean meal); CSM (cottonseed meal); FCR (feed conversion ratio); FER (feed efficiency ratio); FM (fish meal); FI (feed intake); FSBM (fermented soybean meal); FW (final weight); HMTBa (2-hydroxy-4-(methylthio) butanoic acid); L-Met (L-methionine); Met-Met (DL-methionyl-DL-methionine); MHA (methionine hydroxy analogue); NM (not mentioned); NRE (nitrogen retention efficiency); PER (protein efficiency ratio); PPC (pea protein concentrate); PWG (percent weight gain); RM (rapeseed meal); QRA (quadratic regression analysis); SBM (soybean meal); SCP (soybean-condensed protein); SDPR (second degree polynomial regression); SGR (specific growth rate); SP (soya protein); SPC (soybean protein concentrate); WG (wheat gluten); WGR (weight gain rate); WGM (wheat gluten meal).

increased first and then decreased with increasing dietary methionine levels in Chinese sucker (*Myxocyprinus asiaticus*) (Chu et al., 2014), cobia (*Rachycentron canadum*) (Chi et al., 2020; Wang et al., 2016c; Zhou et al., 2006), grass carp (Pan et al., 2016; Wu et al., 2017), largemouth bass (*Micropterus salmoides*) (Chen et al., 2010; Zhao et al., 2022), Ussuri catfish (*Pseudobagrus ussuriensis*) (Wang et al., 2016b), hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂), milkfish (*Chanos chanos* Forsskal) (Borlongan and Coloso, 1993), European seabass (*Dicentrarchus labrax*) (Thebault et al., 1985), Yellow River carp (*Cyprinus carpio haematopterus*) (Yun et al., 2022), and field eel (Hu et al., 2022). Interestingly, Li et al. (2021) reported that low (9.4 g/kg) and high (14.8 g/kg) dietary methionine supplementation in a high lipid diet did not significantly influence the WG and specific growth rate (SGR) of large yellow croaker (*Larimichthys crocea*), but regulated hepatic lipid metabolism. Similar unaffected growth performance by increasing dietary DL-methionine (1.64 g–2.98 g methionine 16 g⁻¹ N) was also reported in Atlantic salmon (Espe et al., 2008). Thus, most studies have demonstrated that optimal dietary methionine could promote growth, but different fish species show various responses to excessive dietary methionine.

Optimal dietary methionine levels promote growth performance and are highly related to its regulation of the somatotrophic axis, whereas growth hormone (GH)/insulin-like growth factor-1 (IGF-1) is the main axis (Gómez-Requeni et al., 2003). In mammals, GH-IGF modified metabolism and growth mainly rely on the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway, which is sensitive to various extracellular stresses and growth factors and is responsible for cell survival and critically regulates cell proliferation, differentiation, and apoptosis (Engelman et al., 2006; Ji et al., 2020). In addition, PI3K-AKT is upstream of mechanistic target of rapamycin (mTOR), which is a major component of a cell-signaling pathway that regulates cytoskeleton remodeling, protein synthesis, and intracellular protein degradation via autophagy (Wu et al., 2014). TOR activated by PI3K-AKT can promote protein synthesis, intermediary metabolism, and the immune response (Alessi et al., 1997; Wang et al., 2021).

In fish, the administration of methionine can elevate free amino acid (FAA) levels in plasma, which are important regulators of body catabolism and anabolism (Wang et al., 2021). Dietary methionine inclusion has been reported to increase plasma FAA content in various fish species (Bae et al., 2011; Gao et al., 2019; Harding et al., 1977; Liang et al., 2016; Ruchimat et al., 1997; Tulli et al., 2010; Zhou et al., 2021). Studies in fish have reported that optimal dietary methionine administration regulates the somatotrophic axis by increasing GH and IGF-1 levels and gene expression in cobia (Chi et al., 2020; He et al., 2019; Wang et al., 2016c), rainbow trout (Rolland et al., 2015), and humpback grouper

(Irm et al., 2021), which was in accordance with the improved growth performance. In addition, it has been widely reported that methionine can activate the TOR signaling pathway to improve protein synthesis and growth in fish (He et al., 2019; Rolland et al., 2015). For instance, methionine inclusion increased amino acid (AA) concentrations by improving the transport efficiency of AA transporters, which promoted systemic nutrient sensing by activating the GH-IGF signaling pathway and upregulating TOR signaling, which increased the nutrient metabolic level and promoted the growth of largemouth bass (Wang et al., 2021). Suitable dietary methionine levels increased hepatic IGF-1 expression and improved the phosphorylated protein and mRNA levels of PI3K, TOR, eukaryotic initiation factor 4E binding protein-1 (4E-BP1), and ribosomal protein S6 kinase 1 (S6K1) (which are downstream of TOR and function in protein synthesis and growth) (Ji et al., 2022). Similarly, suitable levels of dietary methionine enhanced muscle protein content via TORC1/S6K1 signaling in grass carp (Fang et al., 2021) and hybrid grouper (Li et al., 2020). On the other hand, methionine deficiency downregulated the expression of TOR in cobia (Chi et al., 2020), turbot (*Scophthalmus maximus* L.) (Jiang et al., 2017), rainbow trout (Rolland et al., 2015; Skiba-Cassy et al., 2016), and humpback grouper (Irm et al., 2021). Therefore, similar to the mechanism in mammals, there is strong proof that optimal methionine intake promotes growth by the growth hormone-regulated PI3K/Akt/TOR pathway in fish.

Methionine deficiency inhibits fish growth, and in addition to inhibition of the TOR pathway, activation of the amino acid response (AAR) pathway is another important route (Skiba-Cassy et al., 2016). A deficiency in amino acids inhibits protein translation, causing uncharged transfer RNA (tRNA) accumulation in cells. General control non-repressible 2 kinase (GCN2) senses uncharged tRNAs and phosphorylates elongation initiation factor 2 α (eIF2 α), causing a reduction in protein synthesis in both mammals and fish (Anthony et al., 2004; Guo and Cavener, 2007). In addition, activation of GCN2 upregulates its downstream transcription factor 4 (ATF4), and ATF4 then binds to tuberous sclerosis complex 2, an important upstream factor of TOR, which could inhibit TOR (Fig. 2) (Jewell and Guan, 2013). A study in rainbow trout revealed that methionine deficiency activated the GCN2/eIF2 α pathway and upregulated the expression of the ATF4 target genes asparagine synthetase (ASNS), system A amino acid transporter 2 (SNAT2), and cationic amino acid transporter 1 (CAT1), which are responsible for retarded protein synthesis (Skiba-Cassy et al., 2016). Similarly, activation of the GCN2 pathway by methionine deficiency was reported in humpback grouper (Irm et al., 2021), cobia (Wang et al., 2016c), and largemouth bass (Wang et al., 2021). In addition, the primary muscle cells of turbot also supported this hypothesis. Methionine deprivation (MD) suppressed target TOR signaling and activated the

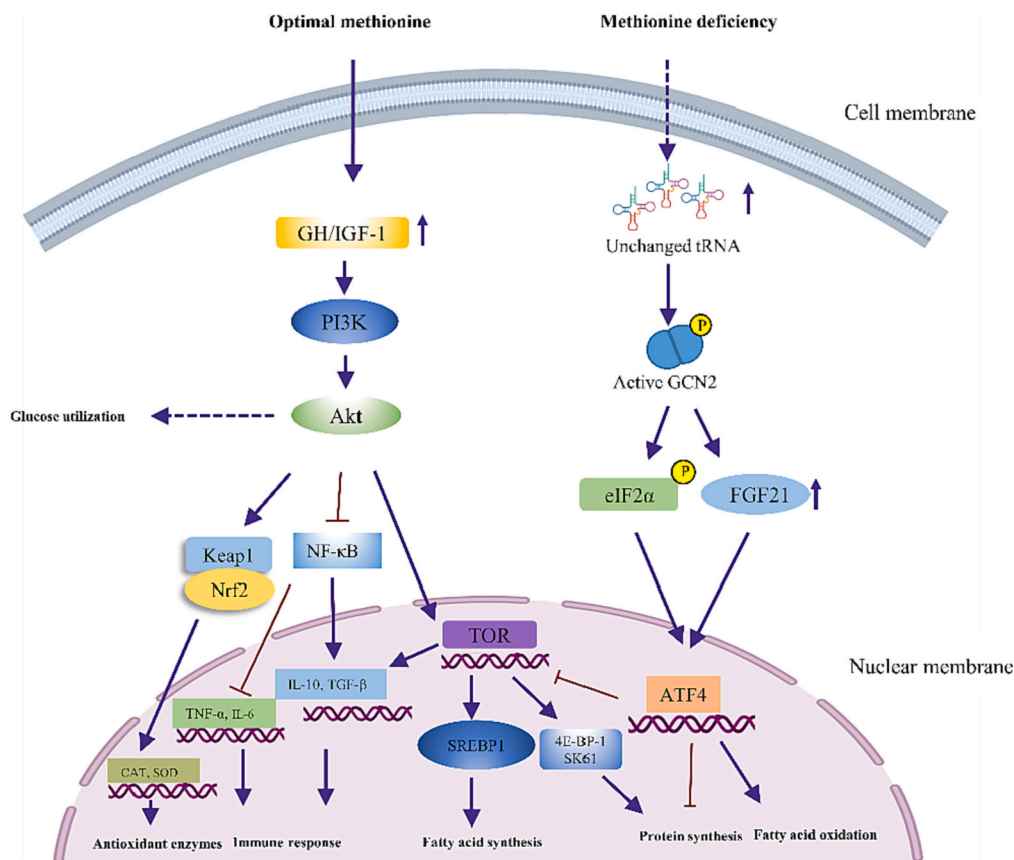


Fig. 2. The potential mechanisms of methionine on protein synthesis, antioxidant status, immune response, fatty acids metabolism, and glucose utilization. Abbreviation list: 4E-BP1 (eukaryotic initiation factor 4E binding protein-1), ATF4 (transcription factor 4), CAT (catalase), eIF2 α (elongation initiation factor 2 α), FGF21 (fibroblast growth factor 21), GCN2 (general control nonderepressible 2 kinase), GH/IGF-1 (growth hormone / insulin-like growth factor-1), IL-6 (interleukin 6), IL-10 (interleukin 10), Keap1 (kelch-like-ECH-associated protein 1), NF- κ B (nuclear transcription factor- κ B), Nrf2 (nuclear factor erythroid 2-related factor 2), PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase B), S6K1 (ribosomal protein S6 kinase 1), SOD (superoxide dismutase), SRBP1 (sterol regulatory element binding protein 1), TGF- β 1 (transforming growth factor β 1), TNF- α (tumor necrosis factor- α), TOR (target of rapamycin).

GCN2 pathway (eIF2 α /ATF4), which inhibited cellular protein synthesis and increased protein degradation, thus leading to increased intracellular free amino acid levels and degradation (Jiang et al., 2017). However, the linkage between the TOR and GCN2 pathways is not sufficiently clear in fish at present, and more studies are warranted.

As mentioned above, excessive dietary methionine may cause retarded fish growth, which could be linked to the toxic effect of methionine. Normally, more energy expenditure is required for the degradation of extra methionine (Baker, 2006; Zhou et al., 2006). In addition, excessive methionine may inhibit lipogenesis (discussed below), elevate endoplasmic reticulum (ER) stress, activate the caspase pathway (Song et al., 2018), and reduce feed intake (FI) (Marit et al., 2008; NRC, 2011). Indeed, the imbalance of dietary methionine may cause various physiological symptoms, endangering fish growth and health. For example, excess dietary HMTBa (10% of the diet) caused inhibition of growth, high mortality, low feed and protein utilization, reduced antioxidative ability, negatively influenced liver histology, reduced intestinal fold height, and impaired integrity of intestinal structures of turbot (Hu et al., 2015). However, different fish species have various responses to excessive dietary methionine (mentioned above), but the mechanisms are not clear in fish.

7. Feed utilization

The improvement of fish growth by methionine inclusion is highly related to enhanced feed utilization efficiency. Optimal dietary methionine led to the lowest feed conversion ratio (FCR) observed in many fish species, such as grass carp (Fang et al., 2021; Ji et al., 2022), gibel carp (Wang et al., 2016a), silver catfish (Rotili et al., 2018), largemouth bass (Wang et al., 2021), cobia (He et al., 2019; Wang et al., 2016c), India major carp (*Cirrhinus mrigala*, (Hamilton)) (Khan and Abidi, 2013), Chinese sucker (Chu et al., 2014), Indian catfish (*Heteropneustes fossilis* (Bloch-1974)) (Ahmed, 2014; Ahmed et al., 2003), Nile tilapia (Urbich

et al., 2022), and large yellow croaker (Mai et al., 2006), indicating that methionine could enhance the feed utilization of fish. The contributing factors are as follows: (1) Crystalline methionine has a faster absorption rate and assimilation ratio than protein-bounded methionine, thus, it will not induce competition for intestinal absorption sites and improve the absorption efficiency of dietary methionine (Rønnestad et al., 2000; Wang et al., 2019). (2) Methionine improves digestive enzyme activity. For instance, methionine increased the intestinal proteolytic enzymes of red sea bream (Mamaug et al., 2012). Reduced WG and feed utilization parameters were in line with the reduced intestinal trypsin, lipase, and amylase activities in grass carp fed insufficient methionine (Wu et al., 2017). (3) Methionine increases the apparent digestibility of nutrients. Methionine supplementation increases hepatic trans-sulphuration and taurine synthesis, which could be conjugated to bile acids and improve biliary secretion, thus improving the digestibility of amino acids (Espe et al., 2010). Plasma bile acid levels linearly increased with increasing dietary DL-methionine levels in Atlantic salmon (Marit et al., 2008). Besides, the apparent digestibility coefficients (ADCs) of dry matter, protein, and gross energy were enhanced by methionine inclusion in black sea bream (*Acanthopagrus schlegelii*) (Zhou et al., 2011). Similarly, methionine supplementation affected almost all ADCs of amino acids in African catfish (Elesho et al., 2021) and improved the ADCs of protein in Chinese sucker (Chu et al., 2014). Fed Atlantic salmon a plant-protein diet with crystalline DL-methionine supplementation resulted in an even higher ADC of amino acids than the fishmeal-based diet (Marit et al., 2008). (4) Methionine inclusion improves intestinal health status. During some infections, methionine can serve as an additional energy source for intestinal cells (Martínez et al., 2017; Yin et al., 2004). Besides, methionine could reduce the intestinal inflammatory response and enhance the intestinal immunological barrier under normal conditions via multiple pathways (discussed below) (Pan et al., 2017; Su et al., 2018; Zhao et al., 2022). (5) Methionine maintains intestinal histomorphology. Improvement of fish intestinal histomorphology is

beneficial for the digestibility of nutrients and subsequent fish growth (Abdel-Latif et al., 2020). Methionine (HMTBa) insufficiency or excess caused a lower intestinal fold height and impaired the integrity of intestinal structures, and methionine deficiency reduced the number of goblet cells and the height of the intestinal villus and microvilli of turbot (Gao et al., 2019). On the other hand, the intestinal fold height, enterocyte height, fold width, and microvillus height of hybrid grouper were improved by suitable methionine supplementation levels (Li et al., 2020). (6) Methionine benefits intestinal bacterial communities. The intestinal microbiota is vital for nutrition harvest, immunity, and resistance to pathogens (Wang et al., 2018). MHA inclusion improved the intestinal microbiota richness and diversity of largemouth bass and increased the abundances of *Bifidobacterium* and *Bacillus* (potential probiotics) but decreased the abundance of *Bacteroides* (potential pathogenic bacteria) at the genus level (Zhao et al., 2022). Similarly, dietary methionine increased intestinal *Lactobacillus* and *Bacillus* richness and decreased *Aeromonas* and *Escherichia coli* counts in Jian carp (*Cyprinus carpio* var. Jian) (Tang et al., 2009). Optimal dietary Met-Met also increased intestinal microbiota diversity, leading to the highest Firmicutes:Bacteroidetes (F:B) ratio in Nile tilapia (Guo et al., 2020b), which indicates improved resistance to pathogens (Mariat et al., 2009), as well as enhanced intestinal energy harvesting ability (Wang et al., 2022).

8. Lipid and glucose metabolism

Lipids are the main energy sources for fish, and the liver is the main organ that modulates lipid metabolism to react with environmental and nutritional signals (Xu et al., 2020). In fish, hepatic lipid accumulation is a consequence of lipid absorption from the diet, *de novo* fatty acid synthesis (lipogenesis), lipid catabolism by β -oxidation (lipolysis), and lipid transport (Tocher, 2003; Zhu et al., 2014). Currently, most studies are concerned with lipogenesis and lipolysis processes regulated by methionine. Acetyl-CoA carboxylase (ACC), sterol regulatory element binding protein 1 (SREBP-1), stearyl-CoA desaturase (SCD), peroxisome proliferator-activated receptor gamma (PPAR γ), and fatty acid synthetase (FAS) are the key factors that regulate *de novo* lipogenesis, while peroxisome proliferator-activated receptor alpha (PPAR α), hormone-sensitive lipase (HSL), and carnitine palmitoyltransferase 1 (CPT1) are the main lipolytic genes (Li et al., 2019; Zheng et al., 2014). Methionine is a source of methyl, and both methionine and its metabolites can modulate lipid metabolism (Martínez et al., 2017; Zhou et al., 2016). A close relationship between dietary methionine and lipid metabolism has been illustrated in mammals (Yin et al., 2018; Zhou et al., 2016). In fish, methionine seems to have tissue-, age-, dose-, and species-specific regulatory effects on lipid metabolism, implying that the mechanisms of methionine regulation of lipid metabolism are complex. (1) Species specific. Compared with methionine deficiency, optimal dietary methionine improved whole-body and hepatic lipid contents and upregulated lipogenic gene (FAS and SREBP-1) expression in cobia (Chi et al., 2020; Wang et al., 2016c). However, both excess methionine and methionine deficiency evoked apoptosis signaling pathways, decreasing lipid transport and oxidation gene expression and eventually increasing hepatic lipid deposition in yellow catfish (*Pelteobagrus fulvidraco*) (Song et al., 2018). Besides, increasing dietary methionine positively increased the whole body, muscle, and liver lipid contents of grouper (Luo et al., 2005) and the whole body and liver lipid contents of tiger puffer (*Takifugu rubripes*) (Xu et al., 2019), whereas increasing dietary methionine negatively influenced the fillet, viscera and residual carcass lipid contents of common carp (Schwarz et al., 1998). (2) Age specific. Increasing dietary methionine did not significantly influence the whole-body and muscle lipid contents of grass carp fry, which might be due to fry using nutrients for development and rapid growth (Ji et al., 2022). (3) Tissue-specific. Methionine deficiency promoted the PI3K/Akt pathway and induced hepatic lipogenesis gene expression of SREBP-1, ACC, and FAS but inhibited the TOR pathway and increased hepatic lipid content. However, methionine deficiency inhibited lipogenesis

(SREBP-1, FAS, and ACC) and decreased muscle lipid content in blunt snout bream (*Megalobrama amblycephala*) (Ji et al., 2021). In addition, increased lipid contents due to methionine inclusion were observed only in the whole body and liver but not in the muscle of tiger puffer (Xu et al., 2019). (4) Dose specific. Deficiency and excess dietary methionine showed different effects on hepatic lipid metabolism in yellow catfish. Methionine deficiency reduced lipolysis and lipid transport via the liver kinase B1 (LKB1)/AMP-activated kinase (AMPK)-forkhead transcription factor (FOXO) pathway, and excess dietary methionine reduced lipid oxidation and transport pathways and increased lipogenesis via the PI3K/AKT-TOR and cyclic AMP (cAMP)/protein kinase A (PKA)/cAMP-response element binding protein (CREB) pathways (Song et al., 2018). In addition, muscle and hepatic lipid contents increased first and then decreased with increasing dietary methionine levels in cobia (Wang et al., 2016c).

In mammals, methionine deficiency can activate GCN2, thus promoting its downstream CCAAT/enhancer-binding protein β (C/EBP β) expression, which could bind to the SREBP-1 promoter that directly regulates SREBP-1 (Payne et al., 2009). The activation of the GCN2 pathway can also increase the β -oxidation of fatty acids by upregulating fibroblast growth factor 21 (FGF21), a critical hormone for the induction of hepatic lipid oxidation during starvation and a target gene for ATF4 (De Sousa-Coelho et al., 2012). In addition, activation of the GCN2 pathway can inhibit the TOR signaling pathway, thus suppressing lipogenesis by inhibiting SREBP-1 (Jewell and Guan, 2013). Methionine restriction inhibits lipid synthesis but promotes oxidation in other animals (Du et al., 2012; Guo and Cavener, 2007; Hasek et al., 2013; Plaisance et al., 2011; Wu et al., 2019). Similarly, methionine deficiency activated the GCN2 pathway and inhibited TOR expression and lipogenesis, but improved fatty acid oxidation (PPAR α and CPT-1) has been reported in cobia (Wang et al., 2016c) and humpback grouper (Irm et al., 2021). Methionine deficiency also inhibited hepatic lipogenesis expression (SREBP1, SCD1, and FAS) in the liver of tiger puffer (Xu et al., 2019) and rainbow trout (FAS and SREBP-1) (Craig and Moon, 2013). Thus, sufficient methionine may improve lipid accumulation by inhibiting the GCN2 pathway and increasing lipogenesis but inhibiting fatty acid oxidation. A study in cobia reported that excessive dietary methionine inhibited hepatic lipogenesis (FAS, SCD-1, ACC1, SREBP-1, and PPAR γ), which might be linked to the repressed GCN2 pathway (Wang et al., 2016c). However, this hypothesis needs more valid proof in fish because methionine deficiency has been reported to promote hepatic fatty acid synthesis in grass carp (Ji et al., 2022), rainbow trout (Latimer et al., 2018), yellow catfish (Song et al., 2018), and blunt snout bream (Ji et al., 2021). In addition, unaffected lipogenesis (FAS, ACLY, and SREBP-1) and fatty acid oxidation (hydroxyacyl-CoA dehydrogenase (HOAD), CPT1, and CPT2) by methionine deficiency were reported in rainbow trout (Skiba-Cassy et al., 2016).

In terms of lipid oxidation, optimal dietary methionine levels can help the liver better utilize lipids by inducing lipid mobilization and fatty acid β -oxidation in some fish species. For instance, compared with the 1.20% methionine group, 0.55%–0.87% methionine increased the expression levels of CPT1, PPAR α , adipose triglyceride lipase (ATGL) and HSL in grass carp (Ji et al., 2022). Methionine also promoted hepatic HSL, ATGL, and CPT1 expression in yellow catfish (Song et al., 2018), and hepatic and muscle CPT1 and PPAR α expression in blunt snout bream (Ji et al., 2021). A suitable dietary level of methionine alleviated hepatic lipid accumulation in large yellow croaker caused by a high lipid diet mainly by upregulating mitochondrial fatty acid β -oxidation genes (PPAR α and CPT1) and alleviating endoplasmic reticulum (ER) stress (Li et al., 2021). However, insufficient dietary methionine also upregulated PPAR α and CPT1 expression in the liver of cobia (Wang et al., 2016c) and rainbow trout (Craig and Moon, 2013). Jiang et al. (2017) found that MD stimulated the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, which enhanced energy expenditure in primary muscle cells of turbot. In addition, excessive dietary methionine increased the hepatic expression level of the fatty acid oxidation gene (HOAD) of

rainbow trout (Skiba-Cassy et al., 2016).

Thus, methionine-modulated lipid accumulation is a complicated balance of lipid absorption, lipogenesis, lipolysis, and lipid transport. For example, the hepatic lipid content of tiger puffer increased with increasing dietary methionine. However, the expression levels of lipolytic genes (acyl-CoA oxidase 1 (ACOX1), apolipoprotein B100 (ApoB100), and HSL) were upregulated by increasing dietary methionine levels, while the expression levels of lipogenic genes (FAS, glycerol-3-phosphate acyltransferase (GPAT), PPAR γ , ATP citrate lyase (ACLY), and SCD1) showed the opposite trend (Xu et al., 2019). Most current studies concern the lipid synthesis and oxidation pathways; only Song et al. (2018) reported that MD reduced lipid transport via the LKB1/AMPK-FOXO pathway. Thus, more studies on methionine-mediated lipid uptake, mobilization, and transport are needed to illustrate the complicated lipid regulatory role of methionine in fish.

It is well known that fish are naturally "diabetic", mainly due to their poor insulin secretion, glucose production, and insulin receptors in muscle, as well as their limited ability to use glucose for lipogenesis (Kamalam et al., 2017). Interestingly, serum glucose content increased with increasing dietary methionine levels and then decreased in Chinese sucker (Chu et al., 2014), grouper (Luo et al., 2005), and cobia (Zhou et al., 2006), indicating that methionine may also regulate glucose metabolism in fish. Craig and Moon (2013) found that methionine restriction could abolish a glucose-intolerant phenotype of trout, shown as reduced plasma and hepatic glucose contents but increased hepatic glycogen concentration, along with repressed hepatic glycolytic activity and gene expression levels (GK and HK), reduced hepatic lipid content and *de novo* lipogenesis (FAS, SREBP-1), lipid oxidation (PPAR γ coactivator 1 (PGC1 α) and PPAR α), indicating that fish might shift direct fuel from lipid to carbohydrate, which might reduce the energy availability and activate the AMPK pathway in the liver and muscle. Similarly, glycolysis inhibition by methionine deficiency was also reported in turbot (Jiang et al., 2017). Besides, a study in cobia reported that pyruvate kinase (PK) expression increased with increasing dietary methionine and peaked at 12.4 g/kg, after which it decreased, while phosphoenolpyruvate carboxykinase (PEPCK) expression showed the opposite trend, suggesting that optimal methionine improved glucose conversion into glucose-6-phosphate and hepatic glucose utilization, as well as suppressed gluconeogenesis, which in all led to the optimal use of energy sources to maintain the maximum growth of fish (Chi et al., 2020). In addition, excessive methionine upregulated postprandial hepatic gluconeogenesis gene expression (G6pase and PEPCK) in rainbow trout (Skiba-Cassy et al., 2016) and promoted hepatic gluconeogenesis (PEPCK, G6Pase) and glycolysis (GK, PFK, PK) but inhibited muscle glycolysis (PFK, PK) in blunt snout bream (Ji et al., 2021). Thus, methionine may improve fish utilization of glucose, which is another contributing factor for improved growth, as well as complicated lipid metabolism regulated by methionine.

9. Antioxidative, immunoregulatory, and disease resistance effects of methionine

Oxidative stress is the consequence of imbalanced antioxidant and prooxidant homeostasis that causes detrimental effects on cells and macromolecules and results in severe damage to the organism (Gonsette, 2008). Reactive oxygen species (ROS) are produced in non-physiological and physiological circumstances, such as mitochondrial dysfunction, cellular respiration, neutrophils, phagocytes, pathologies, and stress. Cellular systems of detoxification can activate endogenous antioxidants to eliminate a relatively low level of ROS (Martínez et al., 2017). However, excessive production of ROS induces oxidative damage and apoptosis, thus causing cell death, an inflammatory response, and eventually tissue injury (Habte-Tsion, 2020). Fish have both non-enzymatic (e.g., vitamins, carotenoids, glutathione, and thioredoxins) and enzymatic antioxidant (e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR))

defenses against oxidative stress, and these enzymes are important in eliminating ROS (Hoseini et al., 2020; Hoseini et al., 2019; Martínez et al., 2017). The transcription of antioxidant enzymes is controlled by nuclear factor erythroid 2-related factor 2 (Nrf2) and its cytosolic repressor kelch-like-ECH-associated protein 1 (Keap1) (Kobayashi et al., 2002). Keap1 is an Nrf2-binding protein that targets Nrf2 for proteasomal degradation primarily by preventing Nrf2 translocation to the nucleus, and Nrf2 is a signaling molecule that promotes the expression of a group of antioxidant enzymes (Muthusamy et al., 2012; Zhang, 2006). Administration of antioxidant compounds is a common approach to reducing fish oxidative stress. Certain amino acids, such as arginine (Hoseini et al., 2020), tryptophan (Hoseini et al., 2019), and histidine (Habte-Tsion, 2020), have been reported to exert antioxidant properties in teleosts. In the case of methionine, even though most studies are concerned with its growth-promoting effect, the antioxidant property of methionine has been widely reported in fish. For example, plasma CAT, total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), and GPx activities were significantly enhanced in the methionine sufficient group of blunt snout bream (Ji et al., 2020) and yellow catfish (Elmada et al., 2016). Feng et al. (2011) reported that supplementation with optimal MHA levels in Jian carp significantly increased SOD, CAT, glutathione-S-transferase (GST), GPx, and GR activities in the intestine and hepatopancreas, along with decreased malonaldehyde (MDA) and protein carbonyl (PC) contents. Dietary methionine improved the hepatic antioxidative status of large yellow croaker under high dietary lipid stress showing reduced serum and liver MDA contents but increased hepatic T-AOC activity (Li et al., 2021). On the other hand, methionine deficiency resulted in decreased SOD and GR activities in the hepatopancreas and intestine of grass carp (Wu et al., 2017). Dietary methionine enhanced antioxidative status mainly by activating the PI3K/Akt/Nrf2 pathway. For instance, MHA supplementation in the largemouth bass diet decreased intestinal protein and lipid peroxidation, increased intestinal antioxidant (SOD, CAT, GPx, GST, and GR) activities and expression via the Keap1/Nrf2 axis, and decreased intestinal MDA and PC contents (Zhao et al., 2022). Compared with the methionine restriction group, sufficient dietary methionine significantly upregulated the genes in the PI3K/Akt/Nrf2 pathway, including CAT, manganese superoxide dismutase (Mn-SOD), heme oxygenase 1 (HO-1) and glutathione peroxidase-1 (GPx-1), in the kidney and liver of blunt snout bream (Ji et al., 2020).

Apart from methionine activating antioxidant pathways, its catabolism leads to the production of glutathione, taurine, and other metabolites, which are low-molecular-weight antioxidants (Blachier et al., 2013; Martín-Venegas et al., 2013; Martínez et al., 2017; Wu et al., 2004). For instance, dietary methionine improved the intestinal glutathione content of trout (Gao et al., 2019), and MHA administration improved glutathione contents and antioxidant enzyme activities in the head kidney, spleen, and skin of grass carp (Pan et al., 2016), while methionine deficiency resulted in decreased glutathione contents in the intestine and liver of grass carp (Wu et al., 2017).

Amino acids are closely related to the immune system, in terms of influencing the activation of lymphocytes, cellular redox state, gene expression, lymphocyte proliferation, and the production of antibodies, cytokines, and other cytotoxic substances. Deficiency in protein or amino acids will impair the immune system and induce susceptibility in animals (Habte-Tsion, 2020; Li et al., 2007). Immune function is related to innate and adaptive immune components (e.g., lysozyme and acid phosphatase (ACP)), antimicrobial peptides, and immunoglobulins in fish (Su et al., 2018). Sufficient dietary methionine is important for the proteins produced in the immune system. Besides, its metabolite, glutathione, is required to support optimal lymphocyte proliferation and the production of cytokines by lymphocytes and macrophages (Blachier et al., 2013; Elango, 2020). Ji et al. (2020) reported that suitable dietary methionine increased plasma immunoglobulin M (IgM) and component 3 (C3) contents compared with the methionine deficiency group in blunt snout bream. Intestinal lysozyme activity, lectin potency, sim-

immunoglobulin M, C3, and C4 contents were increased by methionine supplementation in Jian carp (Tang et al., 2009). Optimal dietary MHA increased lysozyme and ACP activities, and C3, C4, and IgM contents, as well as upregulated hepatic antimicrobial peptide 2 and hepcidin expression in the kidney of grass carp (Pan et al., 2017).

The cytokine-related inflammatory response is an important part of the cellular immune response in fish (Secombes et al., 2001). The cytokines include anti-inflammatory cytokines (transforming growth factor β 1 (TGF- β 1) and interleukin 10 (IL-10)) and pro-inflammatory cytokines (interleukin 1 beta (IL-1 β), interleukin 8 (IL-8), and tumor necrosis factor alpha (TNF- α)) (Rymuszka and Adaszek, 2012). The nuclear transcription factor- κ B (NF- κ B) pathway is a vital regulator of a group of inflammatory cytokines (Rebl et al., 2010). Besides, the TOR signaling pathway is also involved in the inflammatory response of macrophages, monocytes, and peripheral myeloid dendritic cells (Holz et al., 2005; Weichhart and Säemann, 2009). Normally, deficiency of EAAs may inhibit the TOR pathway, thus activating caspase-3, -8, and -9 mRNA expression and increasing DNA fragmentation and apoptosis (Jiang et al., 2016). Dietary methionine regulates the immune system mainly through the NF- κ B and TOR pathways. Pan et al. (2017) reported that MHA improved intestinal immunological barrier function by repressing the p38 mitogen-activated protein kinase (p38MAPK)/I κ B kinase β (IKK β)/inhibitor of κ Ba (I κ Ba)/NF- κ B signaling pathway, potentiating TOR signal cascades to upregulate anti-inflammatory cytokines, and increasing the activities or contents of non-specific immune parameters (C3, C4, ACP, and IgM). Su et al. (2018) reported that Met-Met supplementation inhibited the intestinal NF- κ B pathway but activated the TOR pathway, thus inhibiting the expression of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-8, etc.) but promoting anti-inflammatory cytokine expression (IL-4/13A, IL-6, IL-10, and IL-11). Similarly, decreased proinflammatory cytokine (IL-1 β , TNF- α , and IL-6) expression involved in the NF- κ B pathways by methionine inclusion was also reported in blunt snout bream (Ji et al., 2020). In addition, the myosin light chain kinase (MLCK) and MAPK pathways are also involved in the enhanced immune system induced by MHA administration in grass carp (Pan et al., 2016).

Due to the significant regulation of immune and antioxidant systems, methionine shows potent benefits in stress and disease resistance. As reviewed by Ciji and Akhtar (2021) and Herrera et al. (2019), the synthesis of glutathione, taurine, and polyamines, as well as the maintenance of the antioxidant defense system by methionine are the main factors contributing to stress resistance. Besides, Met-Met supplementation reduced the morbidity of grass carp after infection with *Aeromonas hydrophila* (Su et al., 2018). Methionine supplementation increased the peripheral leucocyte response, complement activity, bactericidal capacity, cellular recruitment to the inflammatory focus, and plasma peroxidase and bactericidal activities of European seabass after injection with inactivated *Photobacterium damsela* subsp. *piscicida* (Phdp) (Machado et al., 2015). Kuang et al. (2012) observed that Jian carp fed optimal MHA had an increased survival rate, leukocyte phagocytic activity, lysozyme activity, acid phosphatase activity, total iron-binding capacity, hemagglutination titre, and C3, C4, and IgM contents after infection with *Aeromonas hydrophila*. Similarly, lysozyme and ACP activities, IgM, C3 and C4 contents in the spleen and head kidney, as well as the structural integrity of the head kidney and spleen of grass carp, were improved by MHA supplementation after challenge with *A. hydrophila* (Pan et al., 2016).

10. Future perspectives

Most studies estimated the methionine requirement of a certain fish using L-methionine or DL-methionine, and with the development of novel methionine sources, some of the requirements may need to be re-estimated both from utilization efficiency and economical aspects. In addition, due to the prevalence of high lipid inclusion in the fish diet and fish having a high ability to use lipids as an energy source, methionine

can regulate lipid metabolism via various pathways and dietary lipid levels may influence methionine requirements that should be taken into consideration. For instance, a study on large yellow croaker observed that different levels of methionine inclusion in a high lipid diet did not significantly influence growth but alleviated hepatic lipid accumulation (Li et al., 2021). Similarly, the methionine requirement of Nile tilapia increased with increasing dietary digestible energy (dietary lipid level) (He et al., 2017). In addition, the high inclusion of fishmeal alternatives (especially plant protein sources that lack methionine and taurine) in the fish feed may require more methionine inclusion levels in the diet and should be estimated carefully. However, de Moura et al. (2018) reported that methionine inclusion in a high plant-protein diet did not alleviate the negative effect of taurine deficiency in meagre (*Argyrosomus regius*), and synergistic effects of dietary methionine and taurine were observed in Nile tilapia fed a plant protein diet (Urbich et al., 2022). Thus, the combination of methionine and taurine levels, as well as their suitable inclusion ratio, deserves further investigation. In terms of the unknown mechanisms, methionine seems to improve glucose metabolism in fish, which is meaningful for the feed industry since fish normally show poor glucose utilization, but the mechanism is not clear now. In addition, both the TOR and GCN2 pathways regulate autophagy in mammals (Gold and Masson, 2022; Tsalikis et al., 2013), but autophagy is seldom associated with methionine-mediated enhanced immunity in fish.

11. Conclusion

Methionine is a limiting essential amino acid in fish feed when high inclusion levels of non-fishmeal protein sources are used. Methionine is involved in various metabolic pathways and participates in many physiological processes. Different forms of methionine are available on the market with different bioavailabilities and they have to be transformed into L-isomers before being used by fish. The methionine requirements of various fish range from 0.49% to 2.5% of the diet (1.49% to 4.7% of dietary protein), which could be affected by dietary cystine and taurine levels, life stage and species of fish, methionine form, the criteria used for methionine requirement, and basal diet composition. Both methionine deficiency and excess may inhibit fish growth, and different fish show an inconsistent growth response to increasing dietary methionine levels. Optimal dietary methionine enhanced fish growth performance mainly by activating the GH/IGF/PI3K/Akt/TOR pathway and inhibiting the GCN2 pathway. Methionine inclusion could improve digestive enzyme activity, nutrients digestibility, intestinal health status, and histomorphology, as well as regulate microbiota composition, which in all contribute to improved feed utilization. The regulation of lipid metabolism by methionine shows tissue-, age-, dose-, and species-specific characteristics, but the TOR and GCN2 pathways may be involved in the processes of lipid uptake, mobilization, and transport. Available data suggest that methionine might improve glucose utilization by fish, but more mechanistic studies are needed. Methionine improves the antioxidant status mainly by activating the Nrf2/Keap1 pathway, while the TOR and PI3K/Akt/NF- κ B pathways are the main routes by which methionine promotes immunity, which is attributed to the improved stress and pathogen resistance of fish.

Author contributions

All authors have read and agreed to the published version of the manuscript. Lei Wang wrote the paper under the supervision of Yunzhi Yan; Chang Gao, Bin Wang, and Chenyang Wang helped collect data. Gladstone Sagada helped revise the manuscript. All authors contributed to the article and approved the submitted version.

Author statement

Lei Wang wrote the paper under the supervision of Yunzhi Yan; Gladstone Sagada revised the manuscript; Chenyang Wang, Ruichen Liu,

Qiang Li, and Chen Zhang collected the data and helped analyze the results.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

No data was used for the research described in the article.

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