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Functional amino acids in fish health and welfare

Synne M. Andersen[1,2], Rune Waagbø[1], Marit Espe[1]

[1]National Institute of Nutrition and Seafood Research (NIFES), PO BOX 2029 Nordnes, 5817 Bergen, Norway, [2]Current address: EWOS AS, Tollbodalmenningen 1B, 5803 Bergen, Norway

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1. ABSTRACT

Protein is the most expensive part of fish diets and supplies amino acids (AA) for energy, growth, protein synthesis and as substrates for key metabolic pathways. Functional AA is a term used to describe AA that are involved in cellular processes apart from protein synthesis. A deficiency, or imbalance, in functional AA may impair body metabolism and homeostasis. Recent years have seen an increased interest in AA to increase disease resistance, immune response, reproduction, behavior and more. This has led to a boost of commercially available functional fish feeds that aim to optimize fish performance and quality of the product. This review aim to collect recent findings of functional AA and of how they may improve fish health and welfare. It will focus on functional properties of some of the most studied AA, namely arginine, glutamine, glutamate, tryptophan, sulfur amino acids (methionine, cysteine and taurine), histidine and branched chain amino acids. Where information is not available in fish, we will point towards functions known in animals and humans, with possible translational functions to fish.

2. INTRODUCTION

Dietary amino acids (AA) are crucial for fish as energy substrates, for endogenous protein synthesis and to regulate metabolic pathways. More than half of the AA consumed by fish may be deposited into body protein, and the requirement of essential amino acids (EAA) corresponds to the AA tissue content (1). Protein is a significant component of fish diets, and is generally higher in carnivorous than herbivore fish. In salmonid diets, protein makes up 35-55% of the diet, with highest inclusion levels at early life stages (2). Most of the AA are protein bound but can also be supplied in the form of crystalline AA to fulfill the AA requirement, as regulated by national legislation of feed additives, especially when using alternative protein sources (3). The National Research Council (NRC) published their latest recommendations for AA requirements in fish and shrimp in 2011 and takes into consideration different requirement between species and developmental stages (Table 1). The NRC data are based on the literature available at the time, on requirement studies performed in these species. However, it has some main drawbacks; I - several of these studies were performed with diets with a high fishmeal inclusion, while fish diets today generally have a high plant protein inclusion. Plant protein diets have been demonstrated to reduce feed intake, growth and protein utilization (4), and requirement of some AA appears to be higher when fish are fed a plant protein based diet (5). This could be related to differences in non-protein nitrogen compounds, as soy bean meal for example is low in taurine, and a minimum fishmeal inclusion of 5% is still required for Atlantic salmon *(Salmo salar)* (6). II - The studies often use growth as the parameter to determine requirement, while overlooking the metabolic need. The metabolic requirement often surpasses the need for growth, especially during stressful and challenging conditions (7). For instance, histidine requirement to support optimal ocular health is higher than the requirement for growth in Atlantic salmon (8).

Table 1. NRC requirements of digestible EAA in % of diet dry matter. Estimated using a factorial model for different weights of Atlantic salmon fed diets with 4.7.8 Mcal DE From (2).

Amino acid	Weight class			
	0.2.-20 g	20-500 g	500-1500 g	>1500 g
	% diet DM			
Arginine	1.7.9	1.8.2	1.7.0	1.4.6
Histidine	0.8.0	0.8.0	0.7.5	0.6.4
Leucine	2.3.1	2.3.1	2.1.4	1.8.2
Isoleucine	1.3.2	1.3.2	1.2.2	1.0.4
Lysine	2.5.5	2.5.4	2.3.5	2.0.0
Met+Cys (TSAA)	1.2.8	1.3.0	1.2.1	1.0.3
Phe+Tyr (AAA)	2.7.1	2.6.8	2.4.6	2.0.9
Threonine	1.5.5	1.6.0	1.5.1	1.3.0
Trypthophan	0.3.5	0.3.7	0.3.5	0.3.0
Valine	1.7.5	1.7.9	1.6.7	1.4.4
TSAA: total sulfur amino acids, AAA: aromatic amino acids				

III - The fish is not always pair fed, leading to differences in feed intake between dietary treatments. Thus, the effects observed may be due to palatability of the feed rather than a growth stimulating/inhibiting effect. IV) Finally, the type of growth is not always reported. The main aim in aquaculture is as high muscle growth as possible, while visceral mass is a byproduct. Increased deposition of fat around the viscera might have negative health effects on the fish, as is the case in humans (9).

AA have traditionally been classified as essential (EAA) or nonessential (NEAA) relating to whether the organism can produce the AA endogenously from the dietary NEAA. Recently, the term functional AA (FAA) have received more attention relating to AA that modulate key metabolic pathways thus affecting immune response, health, reproduction, cell signaling, animal welfare and more (10). In addition, AA classified as NEAA such as glutamine, glutamate and proline have been demonstrated to have functional properties in both fish and mammalian metabolism, suggesting that fish have requirement also for NEAA to obtain maximum performance. In the case for NEAA, both the dietary content of the AA and its substrates are of importance. Cysteine and tyrosine for instance, can be synthesized endogenously from methionine and phenylalanine respectively, both of which are EAA and need to be supplied through the diet. Thus, a deficiency of these AA may occur due to limited dietary supply of the AA and/or its precursor that may again affect growth, metabolism and health.

Moreover, all AA have some degree of functional properties, but this review will focus on some of the most studied AA and their effects on health, disease and metabolism in fish (Table 2). The AA reviewed herein includes arginine, glutamate, glutamine, tryptophan, histidine, sulfur amino acids (SAA; methionine, cysteine and taurine) and branched chained AA (BCAA; leucine, isoleucine and valine). Notably, other AA including glycine, lysine, threonine and aromatic AA are also involved in metabolic pathways but is not the topic for this review. As salmonid species dominate northern European aquaculture, this review will focus on the information available in salmonid species, but will also include general knowledge from studies in other fish species when relevant or when this is not available in salmonids. It will not discuss the requirements of each AA as this has been extensively discussed elsewhere (2).

3. FUNCTIONAL AMINO ACIDS

3.1. Arginine

Arginine is an EAA in fish, while in humans arginine is considered a conditionally EAA, as glutamate, glutamine and proline can be converted to citrulline via the intermediate pyrroline-5-carboxylate (P5C) in the enterocytes (Figure 1). Citrulline is then converted into arginine in the kidneys of healthy individuals and transported to the liver where it is metabolized trough the urea cycle and used for production of polyamines and creatine (11). Citrulline synthesis from glutamine have been indicated in channel catfish (12) but whether this is the case for all fish species as well as the location of this production and how, where and at what rate this citrulline can be converted to arginine in fish still needs to be elucidated. There have been indications of interconversion between arginine and glutamate in channel catfish *(Ictalurus puntatus)* (13,14), where arginine supplementation increased plasma levels of free citrulline, glutamate and glutamine and supplementing glutamine reduced dietary arginine requirement. In Atlantic salmon however, arginine reduced citrulline concentrations in plasma and muscle without affecting glutamine (15), indicating specie differences in arginine metabolism. Chicken and cats are unable to produce arginine endogenously as they lack the enzyme P5C synthase and this is also believed to be the reason for a lack of arginine synthesis in fish. Production of citrulline takes place in the mitochondria and requires carbamoyl phosphate, which in fish is synthesized by carbamoyl phosphate synthase III (CPSIII) requiring glutamine, not ammonia, as a substrate (16). No hepatic activity were observed of CPSIII or ornithine carbamoyl transferase (OCT) in rainbow trout further explaining the low de novo synthesis of arginine (17). CPSIII and OCT activity is shown to be higher in early developmental stages of zebrafish *(Danio reiro)* (18), which has been linked to a higher need for ammonia detoxification through the urea cycle in early life stages. Citrulline production from ornithine may thus be limited in most adult fish, limiting

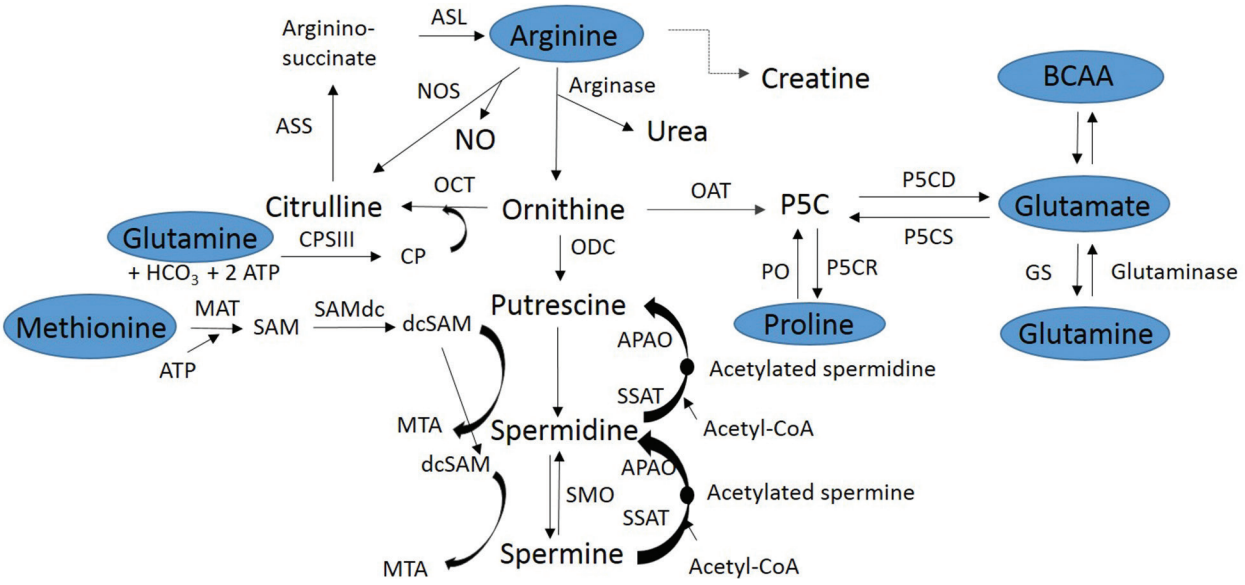

Figure 1. Arginine metabolites and interactions with methionine, proline, glutamine and BCAA metabolism are as described in mammals. Amino acids are highlighted in blue. Production of creatine is via multiple steps also consuming SAM and glycine. Notably, not all of these steps are well described in fish and may take place in different tissues and compartments. NO – nitric oxide, NOS – NO synthase, OCT – ornithine carbamoyltransferase, ASS – argininosuccinate synthase, ASL – argininosuccinate lyase, OAT – ornithine aminotransferase, P5C – pyrroline-5-carboxylate, P5CR – P5C reductase, P5CS – P5C synthase, P5CD – P5C dehydrogenase, PO – proline oxidase, CP – carbamoyl phosphate, CPSIII – CP synthase III, ODC – ornithine decarboxylase, MTA – 5'methylthioadenosine, MAT – methionine adenosyl transferase, SAM – S-adenosylmethionine, SAMdc – SAM decarboxylase, dcSAM – decarboxylated SAM, SAH–S-adenosylhomocysteine, SMO – spermine oxidase, SSAT – spermidine/spermine acetyltransferase, APAO – acetylated polyamine oxidase.

Table 2. Some functional properties of amino acids reviewed herein and their metabolites in fish

Amino acid	Metabolite (if applicable)	Function	Specie	Reference
Arginine	NO	Increased resistance to *Edwardsiella ictaluri* Increased disease resistance during external stress	Channel catfish Senegalese sole	(28) (31)
	Polyamines	Spermine induce intestinal maturation	Sea bass	(48)
Trypthophan	5-HT	Reduced aggressive behavior	Rainbow trout	(72)
	Melatonin	Enhanced innate immune response *in vivo*	Gilthead seabream	(80)
Glutamine	GABA	Affecting secretion of pituitary hormones	Rainbow trout	(59)
	Direct	Improve intestinal structure and activate intestinal enzymes Improve immune response Protect against oxidative damage	Red drum Channel catfish	(46) (29, 65) (63)
Methionine	Direct Taurine	Stimulates protein synthesis Reduce green liver syndrome Increase cell survival Improve digestibility and growth	Rainbow trout Red sea bream Atlantic salmon Rainbow trout	(143) (182) (158) (146)
Histidine		Prevent cataracts	Atlantic salmon	(8)
BCAA	Leucine	Stimulate protein synthesis	Indian major carp	(170)
	HMB	Increase disease resistance	Rainbow trout	(179, 180)

NO: nitric oxide, 5-HT: serotonin, GABA: gamma-aminobutyric acid, BCAA: branched chain amino acids, HMB: beta-hydroxy beta-methylbutyrate acid

the potential of glutamine as a substrate for arginine. Plasma urea is suggested as a better requirement parameter than growth in fish, as it increases once the requirement is met (19).

Arginine is involved in several cellular metabolic pathways, including the urea cycle and synthesis of creatine, nitric oxide (NO) and polyamines (Figure 1). The enzyme arginase catalyzes the production of ornithine and

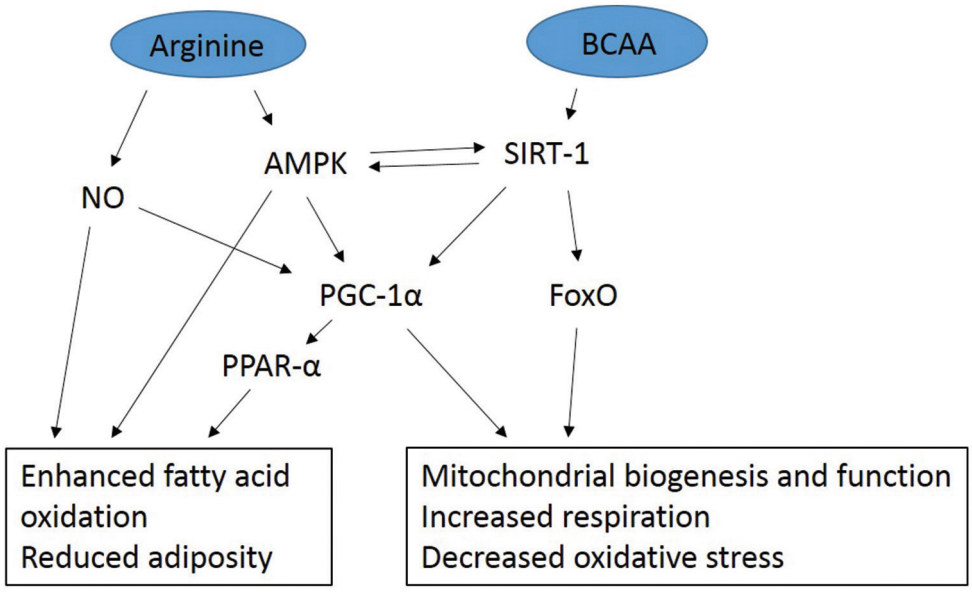

Figure 2. AA also affects metabolism by interacting with several different cellular signaling pathways, and the different AA may have synergistic and/or opposing effects. The outcome of imbalanced diets affects multiple signaling pathways and depends on both concentration and ratios and location of the AA.

urea from arginine. Two isoforms of arginase are known: arginase-1 and arginase-2. In mammals, arginase-1 is known to be primarily expressed in the cytosol of hepatic cells, while arginase-2 is expressed in the mitochondria of several tissues (20). Both isoforms are also expressed in Atlantic salmon, where arginase-1 was also found in muscle tissue, though arginase-2 was the dominant isoform in the muscle (15). Arginase activity varies between fish species, but is generally highest in liver and kidney, and was not detected at all in white muscle of several species including Atlantic salmon (21). Three isoforms of the enzyme nitric oxide synthase (NOS), which produces NO and citrulline from arginine is present in fish: inducible (iNOS), neuronal (nNOS) and endothelial (eNOS) NOS (22). While production of NO from nNOS and eNOS is relatively low and stable, NO production from iNOS upon stimulation from endotoxins, cytokines or nutrients can be significant. NOS has to compete for its substrate arginine with arginase. Increased activity of arginase or a deficiency of arginine availability will lead to uncoupling of NOS leading NOS to produce superoxide instead of NO. Superoxide further stimulates arginase activity and inhibits NOS, creating cellular oxidative stress that has been linked to endothelial dysfunction and related cardiovascular diseases in mammals (23). NO is a potent vasodilator, and can increase blood flow to peripheral organs thus allowing for increased uptake of oxygen and nutrients. In the endothelium, NO diffuses into neighboring smooth muscle cells and activates guanylyl cyclase, increasing intracellular cGMP concentrations, relaxing the muscle tissue. NO thus allows for increased uptake and oxidation of nutrients. As in mammals, NO has also been linked to cardiac function in fish, as inhibiting iNOS in juvenile salmon leads to initial increase in heart rate followed by a decreased heart rate (24). NO further has a role in neurological function and embryological development. Detection of nNOS positive neurons in masu salmon *(Oncorhynchus masou),* indicates that NO is a key modulator of somato-, viscerosensory, and visceromotor systems of the medulla (25). In the gills of Atlantic salmon NOS is colocalized with Na+, K+ATPase, indicating a role of NO in ion transport (26). NOS require NADPH as a cofactor, and increased concentrations of reduced NADPH was observed in the gills during smoltification, suggesting a role for NO in attenuation of increased Na+,K+ATPase activity following seawater transfer. Through NO, arginine stimulates mitochondrial biogenesis and function, thus regulating energy metabolism (27). NO stimulates expression of peroxisome proliferator activator receptor (PPAR)-gamma coactivator1-alpha (PGC-1-alpha), which further stimulates PPAR-alpha, increasing mitochondrial biogenesis and metabolism (Figure 2).

Effects of arginine on both the innate and adaptive immune response have been demonstrated in fish, where arginine may act through NO to combat pathogens, through polyamines, directly by affecting gene expression or by regulating nutrient availability for immune cells by endocrine control. Increased inclusion of arginine in channel catfish diets has shown to correlate with survival when exposed to the bacteria *Edwardsiella ictaluria* (28). This was linked to increased NO production in activated macrophages after increased plasma

arginine (28). Further *in vivo* and *in vitro* experiments in channel catfish confirmed this positive effect on the immune system, as arginine supplementation improved macrophage killing and phagocytosis abilities (29,30). Moreover, arginine increased hematocrit, hemoglobin, erythrocyte count and lysozyme activity, as well as enhanced native T-cells and B-lymphocytes proliferation after mitogenic exposure (29,30). Costas *et al* (31) found that dietary arginine supplementation to Senegalese sole *(Solea senegalensis)* increased the respiratory burst after mitogenic exposure, and this correlated with increased NO production in headkidney leucocytes. In a later paper, they found that plasma cortisol levels were reduced in stressed turbot *(Scophthalmus maximus)* after arginine supplementation (32). Both arginine and lipopolysaccharide (LPS) exposure induce NO production from iNOS in headkidney macrophages (33,34). Substrate availability of arginine may thus be of importance in order to produce a sufficient immune response when required. This highlights the importance of sufficient dietary supply of arginine during exposure to pathogens as plasma arginine is known to decrease in fish during stress (7). Arginine increased abundance of phosphorylated p38MAPK in Atlantic salmon headkidney and liver cells *in vitro* contrary to the effects of LPS, suggesting anti-inflammatory effects of arginine (35). Notably, small concentrations of NO can protect the cell from apoptosis and pathogens by activating heat shock proteins and inducing macrophage activity. However, overproduction of NO is toxic to the cell and can induce apoptosis through DNA damage or activating endoplasmic reticulum stress pathway (20).

Polyamines (PA) are present in all eukaryotic cells and are essential for cell growth and differentiation. Putrescine is produced from ornithine by ornithine decarboxylase (ODC), which is further synthesized into the PA spermidine and spermine. As they are positively charged at physiological pH they can bind to negatively charged RNA, DNA, proteins and phospholipids, affecting gene expression, cell signaling and cell membrane stability. Activity of ODC is almost absent in muscle of Atlantic salmon (36) suggesting liver or intestine as the main sites for PA production. Also, arginase activity rather than ODC activity appears to be regulated by arginine availability in fish, suggesting that arginase rather than ODC is the rate-limiting step for PA production in fish (5,36). Spermidine and spermine synthase consumes decarboxylated s-adenosyl methionine (dcSAM), which is made from methionine in and ATP-dependent process (Figure 1, see section 3.5.). The PA can then be acetylated by spermidine, spermine acetyltransferase (SSAT), enabling them to be transported out of the cell or to be oxidized back to the shorter PA by acetylated polyamine oxidase (APAO). Spermine may also be oxidized to spermidine by spermine oxidase (SMO). Increased turnover of PA also has the potential to induce oxidative stress through production of reactive oxygen species (ROS) as APAO and SMO releases hydrogen peroxide. However, as APAO is located in the peroxisomes and SMO in the cytosol, SMO is expected to be the main producer of cytotoxic ROS (37). Arginine is expected to induce SSAT, thus causing limited oxidative stress. On the contrary, arginine supplementation has been shown to induce antioxidant capacity in pigs (38) and increased lysozyme activity in Japanese flounder *(Paralichthys olivaceus)* (39). How and if arginine can protect against oxidative stress in fish, and at what doses, needs to be further examined. A role for PA in the innate immune system has been postulated, as *in vitro* supplementation of PA increased expression of immune associated genes in gilthead seabream leucocytes and that putrescine, but not spermidine or spermine, increased phagocytic ability (40).

A lipid-reducing effect of arginine has been observed in diet induced obese or diabetic mammals (27), where it reduced white fat mass while maintaining lean muscle mass. Generally, dietary arginine upregulated expression of genes for lipolysis and decreased genes for lipogenesis in the adipose tissue, while increasing lipogenesis and protein synthesis in the muscle. This effect has been linked to activation of SSAT and increased turnover of PA (41). SSAT and MAT consumes acetyl-CoA and ATP, respectively, making PA turnover an energy consuming process. Activation of SSAT will deplete cellular concentrations of acetyl-CoA, further decreasing malonyl-CoA concentrations and thus release the inhibitory effect of malonyl-CoA on carnitine palmitoyl transferase-1 (CPT-1), the rate limiting enzyme for transportation of long chained fatty acids (LCFA) into the mitochondria for ß-oxidation (41). Increased PA turnover and expression of CPT-1 was observed in the liver of juvenile Atlantic salmon fed a high arginine diet, but not in the muscle or adipose tissue (42). An increased capacity of beta-oxidation in the liver could improve their metabolic health and avoid fat accumulation in the liver. NO also upregulates CPT-1 expression, increasing mitochondrial oxidation of LCFA. The effect of arginine on lipid oxidation in fish is still uncertain. Lall *et al* (43) showed that a deficiency of arginine in Atlantic salmon smolts increased lipid retention, while no effect was observed from supplementing surplus arginine. Similarly, no effect of arginine on lipid deposition was observed in later studies in Atlantic salmon (15,44). Arginine increased expression of mammalian target of rapamyacin (mTOR) in muscle and hepatopancreas of Jian carp *(Caprinus carpio var Jian)*, suggesting increased protein synthesis (45). In addition, dietary arginine increased activity of several intestinal enzymes and changed composition of the intestinal microbiota. This is in line with observations in red drum and hybrid striped bass *(Morone chrysops x Morone saxatilis)* where arginine improved intestinal morphology (46,47), suggesting a potential role for arginine to improve intestinal health. These effects may be mediated through PA, as spermine

supplementation improved intestinal maturation in sea bass larvae *(dicentrarchus labrax)*, by activating pancreatic enzymes and increasing activity of brush border membrane enzymes (48).

Creatine is synthesized from arginine, glycine and SAM in a two-step process producing creatine and ornithine and is used as a storage molecule for energy in the muscle in the form of phosphocreatine. Creatine is continuously broken down and excreted as creatinine in the urine, and thus needs to be continuously replaced. Little is known about how dietary arginine can influence creatine in fish, but Chen *et al* (45) showed that creatine kinase activity increased in the intestine of jian carp after arginine supplementation, while in Atlantic salmon no effect was observed on plasma creatinine due to dietary arginine (15). If supplemented arginine could be directed towards creatine production this has the potential to increase muscle mass, though as mentioned this is also dependent on sufficient supply of glycine and the methyl donor SAM.

Arginine is involved in endocrine functions in fish, and is indeed a more potent stimulator for insulin release than glucose in rainbow trout *(Oncorhynchus mykiss)* (49). In coho salmon *(Oncorhynchus kisutch)*, arginine injections increased plasma insulin concentrations in a dose dependent matter concomitantly decreasing plasma glucose, indicating increased glucose uptake (50). A similar effect was observed in isolated liver cells from Atlantic salmon, where cells from salmon fed a high arginine diet appeared to have a higher uptake of glucose (51). Rainbow trout fed diets supplemented with arginine for two months had a higher plasma insulin and a higher weight gain, but this was associated with increased food consumption and FCR (50). Arginine further stimulates release of glucagon and GLP-1, and arginine injections to feeding rainbow trout resulted in decreased plasma fatty acids and liver glycogen (49). Arginine is known to stimulate glucose uptake and oxidation in mammals through NO and activation of 5'activated protein kinase (AMPK), which activates translocation of glucose transporter-4 (GLUT-4) to the cell membrane as well as inactivating acetyl Co-A carboxylase (ACC) (52). NO may also increase GLUT-4 translocation through cGMP, rather than insulin signaling (53,54). Thus, arginine has potential to increase glucose utilization is fish, though further research is required in this area.

3.2. Glutamate and glutamine

While glutamate is a NEAA in fish, glutamine is generally considered a conditionally EAA. Glutamate may be synthesized endogenously from α-ketoglutarate and BCAA, and glutamine is produced from glutamate by the ATP-dependent enzyme glutamine synthase (GS). Glutaminase may hydrolyze glutamine back to glutamate, releasing ammonia. Both AA are important energy substrates in fish, through deamination and transamination reactions. Most of the dietary glutamine and glutamate (more than 60%) is known to be catabolized by the intestinal mucosa in mammals (55), which is probably also the case for fish. Thus, dietary glutamine and glutamate may never pass the intestine in fish. Glutamate can be decarboxylated to gamma-aminobutyrate (GABA) and both glutamate and GABA act as neurotransmitters found in high concentrations in the fish brain (56). GABA is considered to be the main inhibitory neurotransmitter, while glutamate is the main excitatory one (57). GABA is demonstrated to stimulate secretion of lutenizing hormone (LH) from the pituitary of Atlantic croaker *(Micropogonias undulatus)* via the $GABA_A$ receptor (58), and in rainbow trout GABA stimulated secretion of both LH and of follicular stimulating hormone (FSH) (59). However, in both cases this effect appeared to be dependent on the reproductive stage of the fish, as GABA stimulated LH in regressed fish, while it inhibited secretion in mature fish (58,59). The actions of GABA on LH release appears to be mediated through both activation of gonadotropin releasing hormone, by direct stimulation and through inhibition of dopamine (57). Glutamate itself may also stimulate secretion of LH and growth hormone through specific glutamate receptors (56,57). GABA is known to affect muscular tone in humans, and evidence in chinook salmon *Oncorhynchus tshawytscha* supports stimulatory effect of GABA on locomotor acitivity mediated through dopaminergic and seretonergic pathways (60).

Glutamate and glutamine play a crucial role in intestinal health of fish, by modulating intestinal structure, protecting against oxidative damage and acting as energy substrate for the enterocytes. Glutamine supplementation increased growth and intestinal structure in red drum *(Sciaenops ocellatus)* (46), Jian carp (61) and hybrid striped bass (47), as well as increasing growth and differentiation of carp enterocytes *in vitro* (62). The positive growth effect can be explained by a stimulatory effect on protein synthesis as glutamine increased intestinal protein content (61) and *in vitro* protein retention (62). Furthermore, glutamine supplementation upregulated alkaline phosphatase and Na+K+ATPase activity in enterocytes *in vivo* and *in vitro*, demonstrating increased nutrient uptake and cell differentiation. Glutamine may also protect the enterocytes against oxidative damage, as glutamine supplementation to hydrogen peroxide exposed enterocytes reversed the increase in lactic acid dehydrogenase activity, lipid peroxidation and protein oxidation, while restoring ROS induced decreased activity of intestinal enzymes (63). Glutamine also restored the ROS induced decline in enzymes involved in oxidative repair and increased the reduced to oxidized glutathione ratio (GSH:GSSG) (63). Glutamate is together with cysteine and glycine essential to synthesize the antioxidant glutathione.

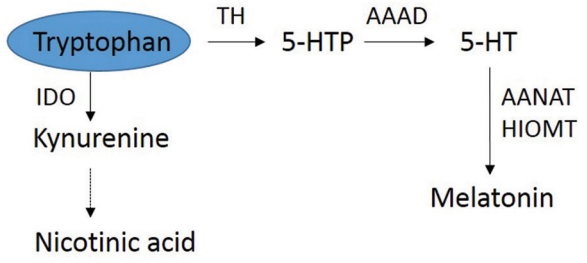

Figure 3. Key steps in tryptophan metabolism. Production of 5-HT is high during the day (light), while melatonin secretion and activity of AANAT is high during the night (dark). Multiple steps not shown are required to convert kynurenine into nicotinic acid. TH – tryptophan hydroxylase, 5-HTP – 5-hydroxytryptophan, 5-HT – serotonin, 5-HIAA – 5-hydroxyindoleacetic acid, AAAD – aromatic amino acid decarboxylase, MAO – monoamine oxidase, AANAT – arylkylamine N-acetyltransferase, HIOMT – hydroxyindole-0-methyltrasnferase, IDO – indole 2,3-dioxygenase.

The role of glutamine in immune response appears to be specie specific. Glutamine has been proposed to aid the immune response by modulating the NO response of macrophages and act as energy substrate for leucocytes. In general plasma levels of glutamine and glutamate drops after exposure to stress or pathogens, increasing the demand of glutamate for energy (64). GS is expressed in the brain, muscle, intestine, liver and kidney of snapper *(Pagrus auratus)* but is absent form lymphoid organs (64). Maintaining sufficient plasma glutamine concentrations during disease is thus important to supply energy for the leucocytes. In mammals, glutamine is essential for proliferation of T and B cell lymphocytes, an effect also observed in fish, as dietary glutamine increased lymphocyte proliferation in channel catfish head-kidney and spleen, but not in peripheral lymphocytes, after vaccination (65). The proportion of IgM+ cells also increased in glutamine supplemented head-kidney tissue after vaccination. Furthermore, *in vitro* glutamine supplementation increased non-specific T and B cell proliferation in channel catfish lymphocytes (29). Glutamine further increased superoxide anion and neutrophil oxidative radical production in kidney macrophages from red drum (46), and improved macrophage superoxide anion production and lysozyme activity in hybrid striped bass (47). In channel catfish macrophages on the other hand, no effect was observed from glutamine supplementation on phagocytosis or bacteriocidal activity after pathogen exposure (29). Glutamine is demonstrated to regulate cytokine production, expression of immune related genes and inhibit apoptosis in mammals (10), while these interactions is yet to be investigated in fish. Synergistic effects of glutamine and arginine on the immune response have been examined in several fish species (29,46,47,65), with various results, showing both synergistic and inhibitory effects.

Glutamine is a precursor for synthesis of purine and pyrimidine nucleotides, and could thus regulate DNA and RNA synthesis and affect lymphocyte proliferation. Nucleotides is commercially used as an immune-stimulant in Atlantic salmon feed, as dietary supplementation is known to increase antibody titers in Atlantic salmon after pathogen exposure (66). Whether dietary glutamine may stimulate the immune response in fish through endogenous nucleotide synthesis is still unknown.

Glutamine plays a key role in nitrogen detoxification, as the enzyme GS binds ammonia to glutamate forming glutamine. This glutamine may then enter the urea cycle as described in section 3.1. As CPSIII activity is present only in the muscle, not in the liver of rainbow trout (17), this pathway may be essential for elimination of ammonia produced in the muscle during exercise. Indeed, urea secretion increased rapidly after exercise without affecting ammonia excretion in juvenile trout and increased muscle activity of GS was observed after 4 days exposure to high-speed swimming (67). GS is also found in high concentrations in the brain (56,64), where it plays a pivotal role in ammonia detoxification. Ammonia exposure increased brain activity and expression of GS in rainbow trout (68), an important adaptation to cope with changes in environmental pH.

Finally, glutamate is used as a flavor enhancer in the form of monosodium glutamate (MSG) in human and animal diets, where glutamate and GABA stimulate feed intake through orexigenic neurons in the hypothalamus. Limited work has been conducted on the role of glutamate to affect hunger and palatability in fish. Dietary glutamate supplementation affected fillet quality in Atlantic salmon (69), without affecting growth or FCR. This was observed in association with decreased hepatosomatic index and fat accumulation in the liver of salmon (69). Further, microarray analysis of gene expression in white muscle demonstrated that glutamate affected several key metabolic pathways.

3.3. Tryptophan

Tryptophan is the precursor for the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT, Figure 3), which implications for fish welfare have recently been well reviewed (70). 5-HT is unable to pass the blood-brain barrier and synthesis is thus dependent on tryptophan uptake into the brain. Dietary tryptophan has shown to correlate with tryptophan concentrations in the brain and with 5-HTergic activity in fish (71,72). Supplementing dietary tryptophan has shown to reduce aggressive behavior in rainbow trout (72), Atlantic cod (71) and fighting fish *(Betta splendens)* (73), through calmative effects of 5-HT. Indeed, inhibitors of monamine oxidase (MAO inhibitors, Figure 3) is a common strategy to treat depression and anxiety in humans.

5-HT is further the precursor for the hormone melatonin produced in the pineal gland. Melatonin is involved in day rhythm regulation, reproduction, immune

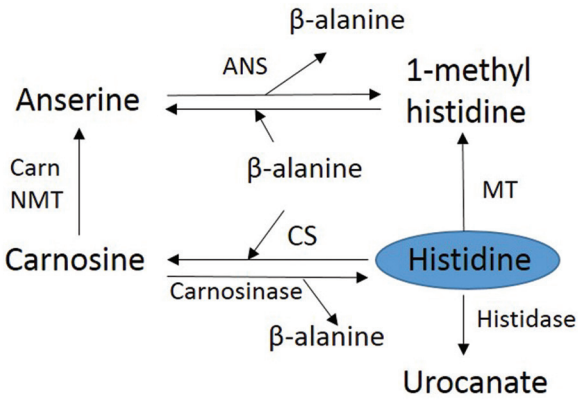

Figure 4. Schematic illustration of the histidine dipeptide metabolism. Anserine can be synthesised by incorporating β-alanine to 1-methyl histidine or by methylation of carnosine in the cells. Excess histidine is degraded to urocanate by the enzyme histididase in the liver. ANS – anserinase, MT – methyl transferase, CS – carnsosine synthetase, carn NMT – carnosine N-methyl transferase.

function and act as an antioxidant (74). Secretion of melatonin is inversely related to light intensity in Atlantic salmon, where secretion is high at night while it is suppressed by light during the day (75). Concentrations are also slightly higher in the summer at high temperatures and melatonin is thought to signal timing of developmental, reproductive and behavioral changes in fish. A role for melatonin to affect reproduction in seasonal breeders by either inhibiting or stimulating gonadal function have been suggested, as light manipulation has successfully been used to induce sexual maturation (76). Maitra *et al* recently reviewed melatonin's effect on reproduction in carp (77). Melatonin may assert its effect directly on peripheral organs or through modulating the hypothalamo-pituitary axis, as melatonin is shown to increase release of growth hormone and decrease release of prolactin from the pituitary gland in rainbow trout in a dose dependent matter (78). Seasonal changes in immune-competence and disease outbreaks have also been linked to diurnal and seasonal changes of melatonin secretion in fish (79). Injecting melatonin to gilthead seabream *(Sparus aurata)* enhanced gene expression of virus and lymphocyte markers and increased respiratory burst, cytotoxic and peroxidase activity of head-kidney leucocytes (80), indicating a role for melatonin in the innate immune defense of fish. Effects of melatonin may vary dependent on specie, age, photoperiod, temperature and dosing and may act trough release of other hormones and these areas all requires further studies. The melatonin system can be manipulated by changing light regime or injecting melatonin, affecting reproduction, immune response, feeding and behavior in fish. Continuous light is shown to halt melatonin secretion and affect cortisol and expression of clock genes in Atlantic salmon smolt (81). Light regimes have successfully been applied for endogenous control of reproduction in both trout (82) and Atlantic halibut *(Hippoglossus hippoglossus)* (76). Continuous light regime is readily applied in commercial aquaculture in order to maximize growth, though the exact mechanisms behind is not clearly understood.

Furthermore, dietary tryptophan may alleviate stress, as dietary tryptophan attenuated stress induced anorexia in brown trout *(Salmo trutta)* (83) and counteracted stress induced increase of plasma cortisol in rainbow trout (84). In Atlantic salmon fed increasing inclusion of tryptophan, cortisol secretion was suppressed at a basal state while during stress an acute stimulatory and long-term inhibitory effect was observed along with altered dopamine concentrations in the hypothalmaus (85). In mammals, most of the tryptophan is metabolized along the kynurenine pathway, which is used for production of niacin and for regulating the immune response (86). Little information is available about this pathways in fish, though Ng *et al* showed that tryptophan was a poor precursor for niacin in channel catfish (87). Dietary tryptophan is shown to advance maturation in male and females ayu *(Plecoglossus altivelis)* (88) and the intermediate tryptophan metabolite l-kynurenine is identified as a sex attractant in female masu salmon (89), suggesting that there is some activity of the kynurenine pathway also in fish.

A deficiency of tryptophan results in scoliosis and an imbalance in mineral metabolism in rainbow trout (90) and some salmonids (91), and is thus crucial for maintaining fish health and welfare. As discussed above, further supplementing tryptophan beyond the requirement for growth could improve fish behavior, disease resistance and alleviate stress. Tryptophan is thus important to avoid production loss due to aggressive behavior and cannibalism. Still, further research is required to elucidate the underlining mechanisms.

3.4. Histidine

Histidine is classified among the EAA for fish. Chemically, the histidine molecule has a functional positively charged imidazole group (therefore also named an imidazole) that can act as an ampholyte. Besides having important functions in the catalytic sites of many proteins (enzymes), histidine is also an important component for histamine formation (signalling), the synthesis of purines, as well as being a precursor for many functional imidazoles in fish (carnosine, anserine, N-acetyl histidine (NAH); Figure 4). Functional aspects of histidine was firstly focused in fish nutrition after recurrent occurrence of high incidences of the eye disorder cataract in farmed Atlantic salmon in Europe in the late nineties (92,93). Cataract formation was connected to periods of rapid growth in Atlantic salmon, and was observed after blood meal was omitted from the salmon feeds in the late nineties due to a potential risk for transmitting Bovine Spongiform Encephalopathy (BSE). Blood meal (haemoglobin) is especially rich in histidine,

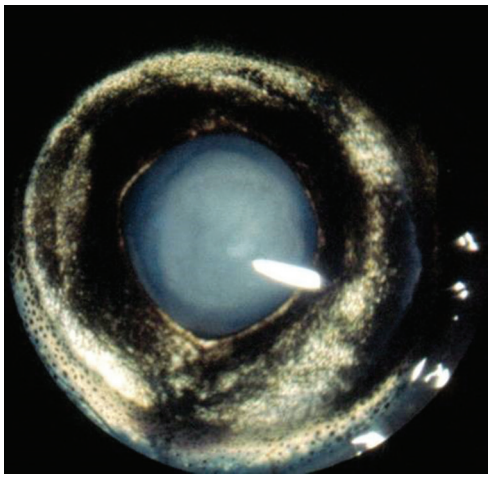

Figure 5. Photo of an Atlantic salmon eye with a complete mature cataract (Photo by Prof. Ellen Bjerkås, reproduced by kind permission of CAB Reviews).

and the marginal dietary histidine supply was identified as a causative factor for cataract development in Atlantic salmon (92). Recent research on this topic has explored novel functions of histidine and related imidazole compounds in osmoregulation (94), pH buffering (95,96), anti-oxidation (97,98), metal chelation (99) and anti-glycation of proteins in salmonids. Histidine has the ability to bind to and modulate the absorption of zinc, copper and iron (99), and may thereby affect the distribution and excretion of essential elements in the fish. The most striking and acute pathology at suboptimal histidine nutrition is observed as cataract development in the salmon lens (Figure 5).

The histidine requirement for growth has been estimated to 7 g histidine/kg feed or 15 g histidine/kg of crude protein in salmonids (100), while freshwater species show variation in requirements between 4 g histidine/kg feed in channel catfish (*Ictalurus punctatus*) to 10 g histidine/kg feed (carp and tilapia species), or 15 to 21 g histidine of crude protein. Most of these studies are from the nineties, while a comment from the recent NRC (2) is that these requirement estimates do not support optimal ocular health in fast growing Atlantic salmon and salmon undergoing parr smolt transformation (smoltification). Breck *et al* (101) showed that supplementation with dietary histidine at levels far above the requirement for growth (~18 g histidine/kg feed) significantly reduced the prevalence of cataracts in salmon smolt. Studying histidine turnover in the salmon lens, Breck *et al* (102) suggested that histidine not only is essential for lens protein synthesis, but also constitutes an important osmolyte in the chemical form of NAH. The cataract alleviation of excess dietary histidine has later been confirmed by Remø *et al* (8) at 13.4. g histidine/kg for Atlantic salmon smolt and at 12.8. g histidine/kg in adult salmon (103). The cataract formation is, however interacted and provoked by several farming conditions and fish genetics (104). The latter is probably mediated through genetic differences in histidine metabolism (101). Recently, a higher requirement of histidine to prevent cataract development in triploid Atlantic salmon versus diploids were demonstrated (105). The reason for this difference in histidine metabolism with ploidity is not presently known.

Quantitative amounts of free histidine is used for cell protein synthesis, for synthesis of imidazole derivatives and for purine synthesis. Minor amounts are used for histamine synthesis in immune cells and paracrine signalling in the stomach, after a decarboxylation. From the requirement studies, the well regulated protein synthesis is of priority for the cellular free histidine. The synthesis of histidine dipeptides and modified histidine compounds seem to be of second priority, with the aim to trap the imidazoles at high concentrations in the cells for physiological purposes (like pH buffering, osmolyte function, antioxidation etc). Thus, the imidazole compounds have been used as markers of the histidine status in salmonids (8). Interestingly, the repertoire of imidazoles change between fish species, as well as between organs in the individual fish species (106). Typically, salmonids have high concentrations of anserine in the trunk muscle, while high concentrations of NAH is present in the lens and heart (8,101). NAH is synthesized in the cells from acetyl-CoA and histidine by the enzyme histidine N-acetyl-transferase. NAH have been identified in the lens, heart and brain of salmonids (107). NAH is hydrolysed by anserinase (108), found in the extracellular fluids and blood (109).

Anserine is synthesized from 1-methyl-histidine (1-MHis) and β-alanine by carnosine synthase or through the formation from carnosine, followed by methylation of carnosine (carnosine–N-methyl-transferase, Figure 4). Carnosine is synthetized by binding of β-alanine to histidine. The low levels of carnosine and 1-MHis and high concentrations of anserine in the salmonid muscle cells show that carnosine and 1MHis are intermediate compounds to anserine (8,101,103). While blood and fluids carnosinase activity hydrolyse carnosine back to histidine and β-alanine, the histidine moiety of anserine cannot be recycled to histidine. Significant levels of anserine is found in marine and animal products, while it is nearly absent in novel plant sources used in fish feeds (110). Muscle anserine seems, however to be homeostatic regulated in rainbow trout and independent on dietary levels of anserine (from marine hydrolysates) between 0.3. to 5.0. g/kg and with 22 g histidine/kg crude protein (110). However, recent data indicate large differences in histidine metabolism (and requirement) between rainbow trout and Atlantic salmon (8). Excess histidine is degraded by the enzyme histidase (histidine ammonia lyase, HAL) to urocanate and further to glutamate. While an imbalanced AA composition in the

diet to rats increased the catabolism of histidine (111), Remø *et al* (8) could not demonstrate changes in the transcriptional level of HAL in the liver of Atlantic salmon smolt fed dietary histidine levels up to 18 g/kg.

N-acetyl histidine was suggested to be an osmolyte in the fish lens (109,112,113) and a dysfunction of the lens osmoregulation was related to cataract formation. *Ex vivo* lens culture studies in hypoosmotic media demonstrated that NAH was rapidly released from lenses to maintain the water homeostasis to prevent lens swelling and subsequently lens fibre lysis and rupture of the lens capsule (112). Baslow (114) suggested that NAH forms a part of an intercompartmental biochemical cycle, with the aim to transport water out of the lens cells. The cycle appears to be gradient driven and depend on a high standing concentration of NAH. Once released from the lens, NAH is hydrolysed by anserinase and histidine re-transported into the lens cells. For the Atlantic salmon, this mechanism may be especially important in the period after seawater transfer, which is an osmotically challenging period for Atlantic salmon smolts (115,116). The high NAH status in the lens depend on dietary histidine, and low NAH concentrations has been directly related to the severity of cataract development in Atlantic salmon (8,101,103). Several studies have shown a correlation between rapid growth rates and cataract development (115,117,118). Waagbø *et al* (103) showed a higher prevalence of cataracts in adult Atlantic salmon after a natural increase in water temperatures between 12 and 18.5.°C. The increased growth in these studies implies less free histidine available for NAH synthesis and optimal lens osmoregulation.

Several studies have implied that histidine and histidine derivatives functions as anti-oxidants or can mitigate the impact of oxidative stress (119-121), low density lipoprotein oxidation (122), oxidative DNA damage and experimentally induced oxidation of liposomes (99), diabetes induced oxidation (123) and heart ischemia (124). The antioxidant activity is connected to the imidazole ring structure in histidine and histidine compounds, with ability to scavenge hydroxyl radicals and singlet oxygen (97-99). The imidazole dipeptide N-acetyl-carnosine (NAC) has been shown to protect against oxidative damages in both canine and human lenses, and eye remedies containing NAC has been suggested as a treatment for cataracts in humans (125). Since neither carnosine nor anserine have been detected in salmon lenses (116), the high concentrations of NAH has been suggested to cover the role as an antioxidant in the salmon lens. Remø *et al* (98) showed that the concentration of NAH was significantly reduced in oxidatively stressed salmon lenses and in culture, and based on this study it was suggested that NAH has a role as antioxidant in the Atlantic salmon lens. The integrated antioxidative defence system in the lens also appeared to be influenced on a transcriptional level by histidine enrichment of the media.

The chemical structure allows the imidazole compounds to act as pH buffers. Histidine and anserine constitute important buffer components in the fish muscle, and are vital for the continuous anaerobic energy production during burst swimming activity (126-128). Histidine and anserine, and the muscular buffer capacity of masu salmon differ in parr and smolts and seem to relate to the bust swimming ability (129). In juvenile Yellowtale (*Seriola quinqueradiata*), the tissue buffer capacity correlated to the level of histidine and anserine (130). Climatic changes imply periodic critical high water temperatures and low water oxygen saturation, which may increase the anaerobic metabolism and challenge the muscle buffer capacity in farmed salmon (8). While the buffering capacity of histidine decreases with increasing temperature, the buffering capacity of anserine does not appear to be sensitive to changes in temperature in the physiological pH range of pH=6.5.-7.5. (96). Like muscle anserine, Remø *et al* (8) showed that NAH in heart tissue of Atlantic salmon also increase after sea transfer, and seem to reach tissue saturation at moderate dietary histidine concentrations. Histidine compounds exert protective mechanism including regulation of intracellular pH (131) and antioxidant functions (120) in rodent and mammalian hearts. In Atlantic salmon heart, anserine and carnosine were not detected, while NAH could possess an analogue buffering function (8, 98).

3.5. Sulfur amino acids

Methionine is an EAA and can be used to synthesize cysteine, which together with methionine constitutes the sulfur AA (SAA). Methionine is in addition to participating in protein synthesis the most common methyl donor in the body (132). Before methionine can transfer its methyl-group it has to be activated by ATP, producing S-adenosylmethionine (SAM, Figure 6). S-adenosylhomocysteine (SAH), produced when methionine donates its methyl group to methyl acceptors within the body, is unstable and quickly converted to homocysteine. Homocysteine is a branch point in sulfur metabolism, as it can be re-methylated or trans-methylated back to methionine or be trans-sulfurated to cystathionine. Cystathionine may be metabolized to cysteine of which may participate as a constituent of glutathione or it may be metabolized to taurine. Re-methylation of homocysteine to methionine occurs in almost all body compartments by the enzyme methionine synthase (MS) that needs folic acid as the methyl donor. While trans-methylation of homocysteine to methionine by the enzyme betaine homocysteine methyltransferase (BHMT) needs betaine as the methyl donor. BHMT is reported to be present in gastrointestinal, liver and kidney only (132,133). Betaine needed for the trans-methylation arrives from food or it may be synthetized from choline by two successive reactions taking act in the mitochondria (134). In addition to being a methyl donor, SAM may be de-carboxylated producing the aminopropyl group used in synthesis of the PA spermine

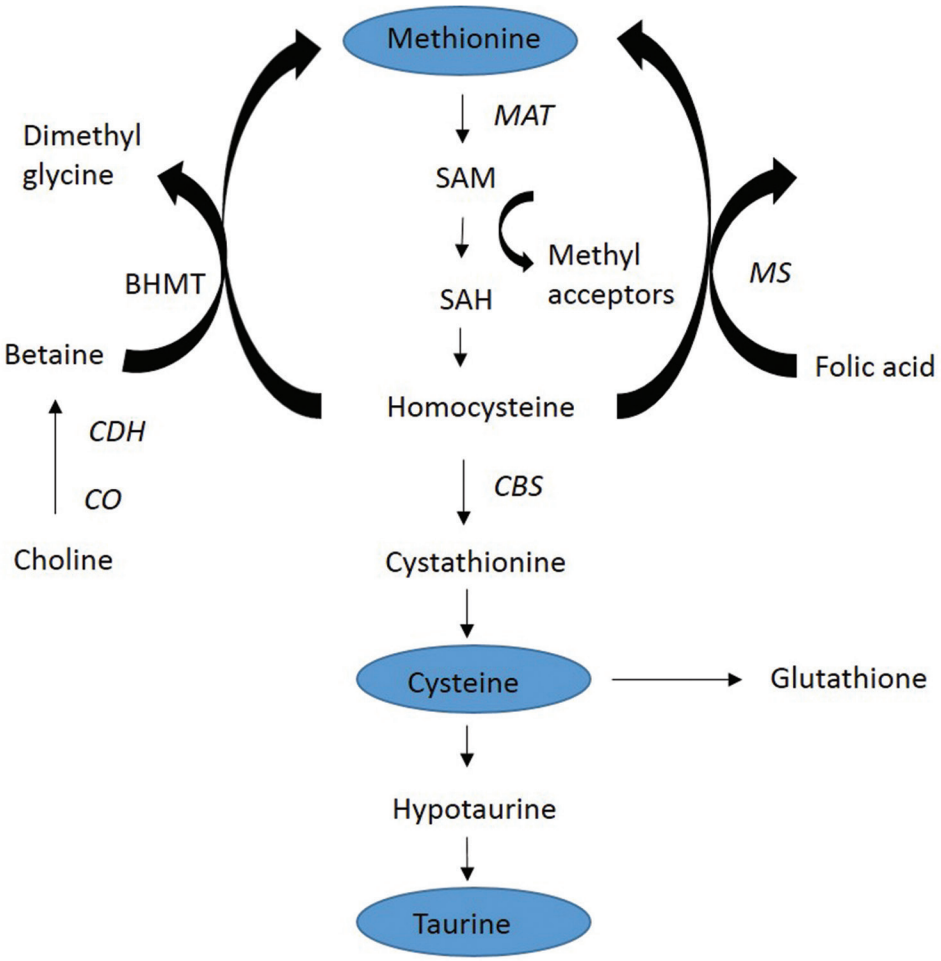

Figure 6. Schematic Figure of methionine pathways producing cysteine and taurine appearing in salmonids. MAT – methionine adenosyl transferase, SAM- s-adenosyl methionine, SAH – s-adenosyl homocysteine, MS – methionine synthetase, BHMT – betaine homocysteine transferase, CBS – cysthathionine beta-synthase, CO – choline oxygenase, CDH – choline dehydrogenase.

and spermidine (Figure 1, section 3.1.). SAM also donates methyl groups to phosphatidyl ethanolamine methyltransferase (PEMT) in the endogenous synthesis of phosphatidylcholine (PC), a major constituent of very low density lipoprotein (VLDL) assembling and thus transport of lipids from liver to peripheral organs. It also donates methyl groups for the synthesis of carnitine necessary in CPT-1 synthesis (135). SAM also is the methyl donor in the synthesis of creatine (135) and used in DNA and RNA methylation that may have significant effects on metabolism and phenotypes (136). Excess methionine is transsulfurated to cystathionine and cysteine, the precursors for taurine and glutathionine or degraded to propionyl-CoA entering the Krebs cycle, or excreted in the urine as taurine. Considering the number of metabolic pathways requiring methyl groups, it is not surprising that methionine availability have the potential to affect the metabolic health in animals, fish included, both when present in surplus or deficient concentrations (137,138).

During the last two decades most of the animal protein used in formulated aquaculture diets have been replaced with plant proteins of which may affect the requirement for precursor AA for other metabolite synthesis especially SAA as taurine is low or absent in plant proteins.

In animal models, AA directly stimulate protein synthesis in an insulin independent manner (reviewed by L. J. C. van Loon (139)). Likewise, salmon juveniles fed methionine deficient diets had reduced protein gain and growth (140,141) opposite to what was observed in adult Atlantic salmon where growth was unaffected by methionine intake (142). Additionally, methionine limitation reduced concentrations of PA in both liver and muscle tissues that may contribute to the reduced growth and protein accretion (140). Thus, it seems like during shorter periods of time the juvenile salmon were able to keep their liver sulfur metabolites in a physiological normal concentration, but seemingly started to deplete

their muscle stores and reduce the lean protein growth. During longer periods this may be detrimental to the liver sulfur metabolism and health of the fish. In rainbow trout methionine deficiency was reported to affect protein synthesis through its deactivation of mTOR signaling cascades (143-145) in a similar way as described in mammalian species, while muscle proteasome related genes increase. Similarly, juvenile Atlantic salmon reduced muscle protein deposition and IGF-1 gene expression after fed a low methionine diet for eight weeks (141). However, surplus methionine supplementation had no effect on protein turnover in rainbow trout (143).

In adult rainbow trout it was reported that methionine supplementation above established requirement reduced the relative weight of viscera (146), but growth was un-affected, while taurine supplementation had no impact on viscera mass. Juvenile salmon fed diets supplemented with taurine did not improve growth, but reduced the total body lipid content (147) and as such taurine may have a beneficial effect on health. The differences obtained in taurine supplementation might be due to the higher diet concentrations used by Gaylord *et al* (146) as compared to Espe *et al* (147).

Deficiencies of SAA, choline and betaine have been associated with metabolic stress in salmonids. When Atlantic salmon are fed low methionine diets, the PC was reduced in liver, but supplementation of choline to these diets increase the concentration of phospholipids in liver of salmonids (148). Opposite to this adult salmon fed low methionine diets, without choline supplementation, accumulated liver TAG, which was restored to normal values when adequate methionine levels were added to the diet (142,149). However, liver PC was not affected in the adult salmon. Liver lipid accumulation is associated with increased metabolic stress, energy depletion, cytokine activation and inflammation in rodent models and human beings (150,151). Development of fatty liver may also be detrimental to fish health. Juvenile salmon fed low methionine diets had increased gene expression of the cytokine TNF-alpha, but gene expression of pro-inflammatory interleukins were not affected (140,148). It is believed that liver TAG accumulation following low methionine and choline diets is due to reduced availability of PC for assembly of VLDL and thus transport of TAG from the liver to peripheral organs like the muscle (152). Therefore, deficiency of methionine during longer periods of time may be detrimental to fish health, but so far no one has proved that salmons actually develop the metabolic syndrome, even though the increased liver lipid accumulation (149) and increased viscera mass is reported also in salmonids (140,142,147,149). Salmon accumulate lipids in viscera when fed high plant oil and protein diets (153) as compared to groups fed diets that are based on marine oils and proteins. The increased visceral fat mass may increase inflammation and contribute to a reduced synthesis of lean protein in salmon as observed in mammalian models (154,155). Taurine administration in plant protein based diets fed to Atlantic salmon not only reduced the whole body lipid to protein ratio but also increased the concentration of PA (147). Likewise, taurine administration reduced the TAG accumulation in obese rodents (156). Recently it was reported that taurine administration reduced genes associated with inflammation and lipogenesis and increased the lipolytic genes in liver contributing to reduced severity of non-alcoholic fatty liver disease (NAFLD) in mice models (155). Opposite to this, methionine restrictions reduced liver de novo lipid and cholesterol synthesis in rainbow trout fed high carbohydrate diets (157).

Methionine derived metabolites as taurine and glutathione function as antioxidants within the body. In mammals, the transsulfuration, and thus taurine and glutathione status, have significant impact on oxidative and inflammatory status. Methionine limitations did however not affect the amount of total glutathione in juvenile salmon (140). In liver cells isolated from salmon, taurine supplementation improved viability and decrease activation of kinases cascade protein signaling cell death (158). These observations are probably linked to the anti-oxidative effects of taurine (reviewed by G. Atmaca (159). Taurine and especially its halides reduced pro-inflammatory cytokines and interleukins in adipose tissues (138) and as such has the capacity to modulate the inflammatory response. Taurine depletion may induce oxidative and inflammatory stress, which is closely associated with the metabolic syndrome, and may reduce cell viability (158). Similar cell models with low, adequate and surplus methionine supplementation showed that methionine limitation reduced viability, but surplus methionine has no beneficial effect on viability. Supplementation of betaine to these media had no beneficial effects on cell survival (160). In adipose tissues, taurine administration improves the inflammatory responses through decreased production of pro-inflammatory cytokines in diet induced obese mice (161). Likewise, taurine administration decreased inflammatory markers and lipid peroxidation in obese women (162), implying that taurine may have beneficial effects improving the metabolic health in obese models. During the later years more and more of the marine ingredients have been replaced by plant ingredients concomitantly the salmon has grown more obese with increased liver TAG and plasma lipids (153,163). Thus probably research on this will increase our knowledge of how AA delivery, and especially so SAA delivery might improve health status by decreasing this central lipid accumulation and thus reducing the pro-inflammatory signaling cascades as affected by SAA will increase significantly in the years to come. Chronic liver disease such as cirrhosis results in increased plasma methionine due to impaired conversion to SAM and most often is associated with decreased cysteine and taurine as well as the impaired synthesis of glutathione (164). Methionine deficiency increased

liver SAM in juveniles (140) and decreased liver SAM in adult salmon (142,149) thus altered methylation capacity may affect health and robustness of the fish. Interactions between SAA and other AA should be addressed in fish models, as inflammatory and oxidative stress may be detrimental. As circulating free AA are known to be affected in fish exposed to external stress (7,70) this will probably also be the case during metabolically induced stress.

Methylation of the DNA accept methyl groups from SAM. This may affect the expression of genes, and modulate the phenotypes, although this has been studied in different mammalian models (reviewed by Wang *et al* (136)) little information in salmonids exist. Both hypo- and hyper-methylation of DNA is linked to development of obesity and cancer in several models (136), but this has to our best knowledge not been addressed in either salmon or rainbow trout. In conclusion, deficiency and distribution of methionine and sulfur metabolites have a significant impact on metabolism of which resembles the mechanisms described in mammalian models. When low in SAA, both growth and type of growth especially protein growth is affected and so is inflammation and oxidation. SAA restrictions induce metabolic changes similar to those described in the metabolic syndrome described in mammalian. Until today, several of the mechanisms behind these metabolic changes are still largely unknown in mammalian models, and even less is known in fish.

3.6. Branched chain amino acids

Leucine, isoleucine and valine constitute the BCAA. As in mammalian species, all three of these are EAA for fish. BCAA are essential for protein synthesis, but also an important regulator of protein degradation. Unlike the degradation of the other AA, degradation of BCAA starts in extra hepatic tissues producing glutamate and branched chain keto-acids before being transported to the liver for further degradation. These AA and their degradation metabolites are associated with maintenance of the lean body mass during prolonged exercise in both man and mammalian models (reviewed by Molfino *et al* (165)). Also in salmon the BCAA are linked to maintenance of lean body mass during exercise as for example forced swimming (166). In man, nutritional disturbance in BCAA are associated with liver diseases. In chronic liver diseases as cirrhosis, serum BCAA concentration is low concomitantly the aromatic AA (AAA) are elevated (167). In mammalian models, BCAA supplementation is linked to life span, mitochondrial biogenesis and defense against ROS (168). This is linked to activation of sirtuins, a family of deacetylation proteins that regulates key metabolic pathways by regulating PGC1-alpha, FoxO, p53, AMPK, eNOS and several other enzymes. Thus, BCAA availability is linked to metabolic health and welfare in mammalian models. To our knowledge, no work has been published about sirtuins in fish models. BCAA supplementation during liver diseases are beneficial in mammalian models as they reduce muscle proteolysis, increase glutamine production and protein (169)synthesis, but their beneficial effects strongly depends on the type of liver disease as reviewed elsewhere (170). As most literature on fish deals with requirement of BCAA to maximize growth and accretion (171,172), the following will focus on BCAA and their effects on metabolism in animals in general and link this to fish metabolism improving metabolic health.

BCAA and primarily leucine increase pathways involved in muscle protein synthesis through both insulin dependent as well as insulin independent pathways. Leucine directly activate the mTOR in skeletal muscle and stimulate downstream phosphorylation of p70S6 kinase and 4E-BP1 signaling RNA translation and protein synthesis, although this signaling is transient (164). Furthermore, BCAA attenuates muscle wasting through interactions with ubiquitine proteasome pathway (164,173) of which may involve the protein kinase Akt/PKB known to phosphorylate the transcription factor forkhead box class-O (FoxO) that signals downstream to two major ubiquitine ligases the atrogin-1 and muscle RING finger protein (MuRF-1) (164). These pathways also regulates protein synthesis in salmonids (144,145). Recently Lansard *et al* (174) reported that leucine participated in activation of the mTOR pathway in rainbow trout hepatocytes. There still is little literature available on protein turnover as affected by BCAA in fish. As BCAA and especially leucine supplementation is linked to lean growth, future studies might bring more insight into the possibility of BCAA to maximize lean growth in fish.

Recently using a metabolomics approach it was found that BCAA and their metabolites are more strongly associated with obesity and type 2 diabetes than are fatty acids (FA) and FA derived metabolites (reviewed by C. B. Newgard (175)). However, BCAA requires the background of a high fat diet to promote insulin resistance (IR) associated with the metabolic syndrome in mammalian models (175). Metabolome studies has proved that BCAA and AAA also had the strongest association with diabetes development in obese men (136). Adipose tissue metabolism seems heavily involved in the development of metabolic syndrome in mammalian models. Adipose metabolism release BCAA and catabolic metabolites thereof (175). Adipose metabolism probably will be focused in the years to come and make us able to understand the metabolic cross talks between lipids and AA's in development of metabolic dysfunction and development of the metabolic syndrome in different models fish included. The International Study of Macro/Micronutrients and blood pressure demonstrated that higher BCAA intake was associated with a reduced prevalence of obesity in middle-aged individual from East Asian and Western countries (176). Alterations in the AA profile is associated with obesity,

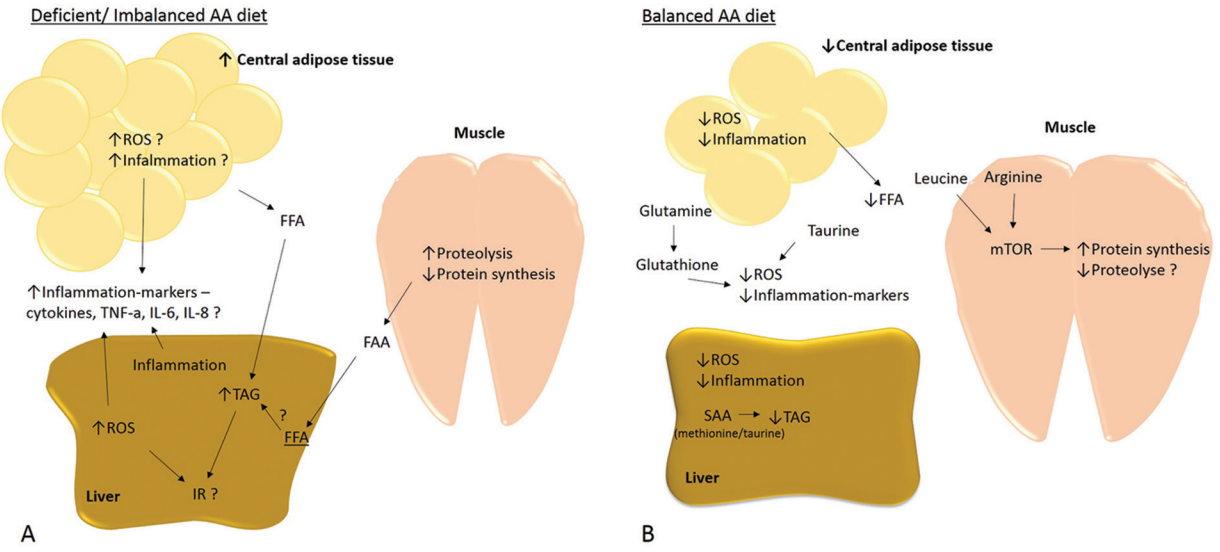

Figure 7. A **-** Effects of an unbalanced AA composition on fish metabolism, growth and inflammation. An unbalanced diet increases central deposition of lipids and increase TAG accumulation in liver and relative liver weight. This has been associated with increased expression of inflammation markers and increased oxidative stress. This has the potential to induce oxidative stress in mammals. A diet marginal in methionine will increase proteolysis and/ or reduce protein accretion in the muscle in order to supply the liver with sufficient AA. Thus, an unbalanced diet will favor visceral growth rather than muscle growth. B – A balanced diet will limit inflammation and oxidative stress. Sulfur amino acids (SAA) inhibit TAG formation in the liver, while arginine and leucine stimulate protein synthesis in the muscle.

and especially BCAA are linked with obesity and IR in rodent models (177). Today no clear mechanism has emerged explaining the role of BCAA in obesity, but there is a consensus that the altered BCAA signature is a risk factor for developing IR and diabetes 2 in these models. Rats fed high fat diets supplemented with BCAA reduced their mesenteric lipid depot and had reduced plasma and liver TAG (178). Indicating that BCAA administration may modulate metabolic disorders as describe in the metabolic syndrome and obesity in these models (167,179). In fish the effects of BCAA on reducing liver lipid accumulation and improve health status has to our best knowledge not been addressed. The concentration of circulating free AA, BCAA included, are affected by stress in fish (7,70) and these alterations may contribute to reduced lipid and increased protein accretion, but any beneficial effects for the health and welfare of the salmonids still needs to be proven.

Administration of beta-hydroxyl-beta-methyl-butyrate (HMB), a metabolite of leucine catabolism, enhanced the immune response and survival in fish following bacterial infections (180,181). Beneficial effects of BCAA administration are not focused in fish nutrition as most articles in salmonid nutrition and BCAA describes the requirement, not actually looking into possible functional properties of BCAA supplementation. Given that BCAA influence growth, health and metabolism significantly in mammalian models, it is likely that this area of research will increase in the years to come in fish nutrition also.

4. METABOLIC PERSPECTIVES

In humans, deficiencies of SAA, choline or betaine or surplus BCAA have been associated with metabolic stress commonly referred to as the metabolic syndrome (175). The metabolic syndrome is associated with obesity, central fat accumulation, inflammation, fatty liver (NAFLD) and IR, and is often the cause of prolonged excess energy intake (136). In fish, negative health effects of high-energy consumption have not been reported. However, AA deficiency or imbalance has showed negative health effects similar to those observed with metabolic syndrome in humans. When the diet is limited in one AA, deposition patterns of protein, lipids and glycogen are affected in Atlantic salmon (182). Similarly, when the AA composition is unbalanced, AA and nutrient uptake, growth and deposition patterns are altered (6,36) (Figure 7A). Methionine deficiency causes increased hepatic TAG accumulation in Atlantic salmon (142,149). Further methionine may protect against oxidative stress by being the precursor for the antioxidants taurine and gluthationine. Thus, deficiency may increase ROS. Methionine is also needed for the production of PA, through the aminopropyl donor dcSAM, and in Atlantic salmon methionine deficiency had greater impact on PA production than did arginine (42,140). Arginine supplementation have shown promising to reduce adipose tissue, reduce plasma glucose and improve insulin sensitivity in mammals. While arginine stimulates insulin secretion and reduces plasma glucose in salmonids (50,51), the exact effects on lipid metabolism

is not established. Generally, dietary AA composition has minor effect on liver AA, indicating an importance of maintaining liver AA homeostasis. When the diet is insufficient of one or more AA, increased proteolysis is observed in muscle, in order to supply the liver and likely the heart and brain (143). The muscle might act as a storage of AA for more vital organs, and will then be the first tissue affected when fed an imbalanced AA diet.

Inflammation in liver and adipose tissue is associated with metabolic syndrome in humans, with increased secretion of cytokines, TNF-alpha expression and ROS production. Similar signs are observed in fish fed diets unbalanced in AA. In Atlantic salmon fed marginal methionine, hepatic TNF-a expression increased and PA metabolism was affected in liver and muscle indicating increased inflammation and ROS production (140). Both taurine and glutathione function as antioxidants, and taurine protected against apoptosis (158) while glutamine protected against ROS (63) in fish models. Arginine increases abundance of anti-inflammatory markers (35) and stimulates NO synthesis (34) and methionine is required to avoid TNF-alpha activation (140), demonstrating the necessity for a balanced AA diet to avoid inflammation and oxidative stress. Further, arginine stimulates insulin release (50) and both arginine and tryptophan inhibits cortisol secretion (32,84), thus directing the cell towards energy uptake and storage rather than glycogenolysis and proteolysis (Figure 7B).

By balancing the AA composition of the diet, also avoiding deficiencies, metabolic disturbances may be avoided. Once the liver receives sufficient AA to maintain optimal metabolism, protein synthesis is stimulated in the muscle, favoring growth of muscle tissue rather than adipose tissue. Especially the AA arginine and leucine is known to stimulate protein synthesis through TOR activation (45,171). Supplementation of taurine to plant protein diets decreased lipid gain (147). Whether the metabolic syndrome is relevant to fish needs further study, especially regarding carbohydrates and IR, since salmonid species have long been regarded as glucose intolerant.

5. SUMMARY

Overall, several important functions for AA in fish health and welfare have already been described in fish. Many of functions is similar to what have been described in mammalian models, though many functions still has to be proven in fish models. Several AA also affect the same pathways and may affect metabolism of each other. Hence, interactions between multiple amino acids has to be studied to fully understand consequences of an unbalanced diet on metabolic health. Both external and metabolic stress may affect the requirements of AA to counteract the increased metabolic burden. More research is needed to understand the underlining mechanisms and signaling between body compartments in order to fully take advantage of this information when designing fish diets and optimizing fish health and welfare.

6. ACKNOWLEDGEMENT

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Abbreviations: AA, amino acids, EAA, essential amino acids, FAA, functional amino acids, SAA, sulfur amino acids, BCAA, branched chain amino acids, NO, nitric oxide, NOS, NO synthase, GS, glutamine synthase, PA, polyamines, ODC, ornithine decarboxylase, SSAT, spermidine/spermine-N1-acetyltransferase, GABA, gamma-aminobutyrate, ROS, reactive oxygen species, 5-HT, serotonin, SAM, S-adenosylmethionine, SAH, S-adenosylhomocysteine, BHMT, betaine homocysteine methyltransferase, PC, phosphatidylcholine, PEMT, phosphatidyl ethanolamine methyltransferase, LCFA, long chain fatty acids, IR, insulin resistance, CPT-1, carnitine palmitoyl transferase-1, VLDL, very low density lipoprotein, TAG, triacylglycerol, mTOR, mammalian target of rapamycin, HMB, beta-hydroxyl-beta-methyl-butyrate, NAH, N-acetyl histidine

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Send correspondence to: Marit Espe, NIFES, PO BOX 2029 Nordnes, 5817 Bergen, Norway, Tel: 47- 41463903, Fax: 47-55905299, E-mail: mes@nifes.no