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REVIEW

REVIEWS IN Aquaculture

Creatine: A valuable supplement in aquafeeds?

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Abstract

Creatine is an amino acid derivate commonly found in vertebrate muscle tissue. Creatine facilitates the recycling of adenosine triphosphate and thus contributes to the energy supply of the muscles as well as the brain. Creatine is used as a supplement for several reasons and its effects in humans, particularly in sports medicine, have been studied excessively. Also, creatine supplementation has been studied for its functions and benefits in terrestrial farm animals. Up to date, little is known about the use of creatine as a supplement in fish nutrition. Yet, due to its many physiological functions, creatine may serve as a valuable supplement in aquafeeds of farmed aquaculture species. Indeed, creatine plays a pivotal role in the fish's muscle and may help to enhance performance of fish reared in aquaculture systems. With regard to swimming exercise, creatine may even amplify its metabolic effects. Upon supplementation, creatine stimulates muscle growth increasing body mass and it has the potential to improve feed utilisation particularly of plant-based diets. Also, creatine plays a part in osmoregulation when fish adapt to changes in salinity. Furthermore, it may improve product quality upon slaughter. Here, we compile what is known about the many functions of creatine as well as its physiological effects in fish in comparison to mammals. We also highlight its potential beneficial effects as a supplement in aquaculture and infer why creatine can help increase the sustainability of fish feeds.

KEYWORDS
carbohydrate, exercise, fish meal, guanidinoacetate, supplementation, sustainability, swimming enhanced growth

1 | INTRODUCTION

Creatine is a dietary supplement intensively used in sports to enhance endurance and strength in athletes.[1–5] It is available in the diet through the consumption of milk, red and white meat, fish, molluscs and crustaceans (Table 1). On average, a 70-kg man has a creatine pool of 120–140 g (1.7–2 g kg^{-1}).[1,10] Approximately, 95% of the creatine pool is located in skeletal muscle. The remaining 5% is stored in the brain, liver and kidney.[11] In fish, creatine content per body weight is up to five times higher which is also attributed to a higher ratio of the skeletal muscle to body weight in fish compared with terrestrial animals. Naturally, creatine concentrations in fish muscle range between 2 and 7 g kg^{-1} (Table 1).

Creatine has pleiotropic effects which are mostly based on the functions of creatine kinase converting creatine to phosphocreatine.[10,12,13] In combination, creatine kinase and phosphocreatine mainly function as an immediately available temporal energy buffer, a spatial energy buffer or intracellular energy transport system and act as a metabolic regulator.[10,12] Creatine provides energy before glycolysis and respiration start delivering energy for metabolic processes.[14–16] The aim of creatine supplementation is thus to increase resting phosphocreatine as well as free creatine in the tissue,

in most cases muscle tissue, with the ultimate goal to increase muscular performance. Indeed, cells with high energy demand rely on the creatine-phosphocreatine energy buffer system to recycle adenosine triphosphate (ATP) from adenosine diphosphate (ADP).[14] For example, muscle cells store sufficient phosphocreatine and ATP for 10 s of high-intensity activity.[1] Thereafter and when these reserves are exhausted, recycling of phosphocreatine from creatine is required which in turn phosphorylates ADP to ATP.[14]

The basic unit of muscle is the myofiber which is derived from stem cell myoblasts. Normally, protein biosynthesis and degradation rates of myofibers are in a relatively balanced state.[17,18] When protein synthesis is higher than degradation, muscle mass increases.[18] This equilibrium may be modulated by shifts in gross nutrient composition, nutritional regulation, exercise or nutrient interventions such as creatine supplementation.[17] Muscle growth may result from both hyperplasia and hypertrophy.[19–21]

TABLE 1 Content of creatine in raw meat and animal products

Food source	g kg^{-1} mass	Reference
Herring, fillet (*Clupea harengus*)	6.5–10.0	6, 7
Yellowtail (*Seriola quinqueradiata*)	5	8
Salmon (*Salmo salar*)	4.5	6, 7
Tuna	2.7–6.5	6, 7
Hake (*Merluccius merluccius*)	5	9
Cod (*Gadus morhua*)	3–4.4	6, 7
Alaska pollock (*Gadus chalcogrammus*)	4.7	9
Blue whiting (*Micromesistius poutassou*)	3.7	9
Saithe (*Pollachius virens*)	3.4	9
Plaice (*Pleuronectes platessa*)	2	6, 7
Shrimp (*Penaeus* sp.)	0.7	9
Blue mussel (*Mytilus edulis*)	0.08	9
Cockle (*Cerastoderma edule*)	0.05	9
Pork	5	6, 7
Beef	4.5	6, 7
Milk	0.1	6, 7

Among those genes that regulate the development of skeletal muscle, the myogenic regulatory factors (MRFs) play a key role. The MRFs include four transcription factors, myogenic determining factor (MyoD), myogenic factor5 (Myf5), muscle regulatory factor-4 (Mrf4) and myogenin (Myog) (see Table 2). Key factors in the development of skeletal muscle have been shown to be stimulated by creatine supplementation.[22–24]

While the biosynthesis of creatine, its application, dosage and the effects of creatine supplementation were intensively studied in humans, different model organisms and several terrestrial livestock species, our understanding of creatine in fish is very limited. Only a small number of studies investigated the effects of creatine supplementation as well as supplementation with its precursor guanidinoacetate (GAA) in fish so far (see Tables 3 and 4). Based on the effects observed in humans and several terrestrial species, creatine supplementation in fish is a promising research field.[25,27,32] Indeed, considering that the aquatic environment challenges organisms differently than the terrestrial habitat, creatine might play a prominent role as metabolic regulator which is not yet discovered. The study of creatine in fish might provide new insights into the various functions of creatine in aquatic animal species. Creatine supplementation might turn out to be beneficial for the cultivation of aquatic species in aquaculture, particularly with regard to the utilisation of carbohydrates and the adaptation of reared fish to modern husbandry systems and production cycles. Via its potential to upgrade aquafeeds for carnivorous species or supplement deficient diets, creatine might also help in reducing the limited meat- and fish-meal resources in aquaculture feeds and improve sustainability of fish nutrition.[12,27,32,34]

2 | BIOSYNTHESIS

In fish, the concentration of creatine in muscle tissue is much higher than in mammals.[8,9,41] In mammals, reptiles and birds (Figure 1), there is only a negligible biosynthesis of creatine in the muscle.[10,42–44] Instead, arginine and glycine are transformed to GAA by the enzyme arginine:glycine amidinotransferase (AGAT, EC:2.1.4.1). This biosynthesis occurs predominantly in the kidney and liver at approximately

TABLE 2 Key genes involved in muscle growth (for references refer to text)

	Gene		Function
Myogenic regulatory factors (MRFs)	MyoD	Myogenic determining factor	Differentiation myogenic cells
	Myf5	Myogenic factor5	Differentiation myogenic cells
	Mrf4	Muscle regulatory factor-4	Differentiation myoblasts
	Myog	Myogenin	Differentiation myoblasts
Transforming growth factors	Mstn	Myostatin	Promotion protein degradation, inhibition protein synthesis
	Mef2	Myocyte enhancer factor 2	Differentiation myoblasts
Growth hormones	Gh	Growth hormone	Myocyte proliferation, stimulation of protein synthesis, hypertrophy, hyperplasia
	Igf1	Insulin-like growth factor	Myocyte proliferation, hypertrophy, hyperplasia, proliferation muscle satellite cells, stimulation of glucose uptake

TABLE 3 Creatine supplementation in aquaculture fish species

Species	Optimal dosage [range assessed]	Duration (days)	Effect	Reference
Clarias gariepinus	40 g kg^{-1} [0, 10, 20, 30, 40 g kg^{-1}]	84	Growth ↑ FCR → Moisture ↓ Protein ↑ Lipid ↓	25
Dicentrarchus labrax	[0, 20, 50, 80 g kg^{-1}]	91	Growth → FCR → Protein → Lipid →	26
Ictalurus punctatus (fry)	[0, 20 g kg^{-1}]	56	Growth ↑ FCR → Moisture → Protein → Lipid →	27
	[0, 20 g kg^{-1}]	70	Growth → FCR → Moisture → Protein → Lipid →	27
Morone chrysops × *Morone saxatilis*	20, 40 g kg^{-1} [0, 10, 20, 40 g kg^{-1}]	84	Growth ↑ FCR → Moisture → Protein → Lipid →	28
Oreochromis sp.	0.4 g kg^{-1}	56	Growth → FCR → Moisture → Protein ↑ Lipid →	29
	0.4 g kg^{-1} [0.4, 0.8, 1.2 g kg^{-1}]	56	Growth ↑ FCR ↓ Moisture ↓ Protein ↑ Lipid ↓	30
	1.2 g kg^{-1} [0, 0.8, 1.2 g kg^{-1}]	84	Growth ↑ FCR ↓ Moisture → Protein ↑ Lipid →	31
Sciaenops ocellatus	40 g kg^{-1} [0, 5, 10, 15, 20, 40 g kg^{-1}]	49	Growth ↑ FE ↑ Moisture → Protein → Lipid →	32
	20 g kg^{-1} [0, 20 g kg^{-1}]	56	Growth ↑ FE →	33

TABLE 3 (Continued)

Species	Optimal dosage [range assessed]	Duration (days)	Effect	Reference
			Moisture → Protein → Lipid →	
	40 g kg^{-1} [0, 5, 10, 15, 20, 40 g kg^{-1} (5 ppm salinity)]	56	Growth ↑ FE ↑ Moisture → Protein → Lipid →	27
	20 g kg^{-1} [0, 20 g kg^{-1} (3–15 ppm salinity)]	84	Growth ↑ FE ↑ Moisture → Protein ↑ Lipid →	27
Sparus aurata	50, 80 g kg^{-1} [0, 20, 50, 80 g kg^{-1}]	69	Growth → Muscle A ↑	23
	[0, 20, 50, 80 g kg^{-1}]	69	Growth → FCR →	34
Litpoenaeus vannamei	5.12, 8.28 g kg^{-1} [0, 1.23, 2.58, 5.12, 8.28, 14.12, 24.49 g kg^{-1}]	46	Growth → FCR → Moisture → Protein ↑ Lipid →	35

Abbreviations: FCR, feed conversion ratio; FE, feed efficiency.

1 g day^{-1}, corresponding to 1.5%–2% of the creatine pool.[10,42,45] Glycine can be synthesised in fish while it is conditionally indispensable in mammals which can synthesise arginine that is conditionally indispensable in fish.[46,47] It is known that creatine supplementation down-regulates AGAT, both at the transcriptional and activity level in animals.[10] Therefore, supplementation of creatine slows down endogenous creatine synthesis. Recently, it has been suggested that the bacterial flora in a healthy gut may accelerate the synthesis of GAA by secreting the enzyme guanidinoacetase, interlinking the synthesis of GAA and the microorganisms of the gastrointestinal tract.[17,45,48]

In a second step, GAA is methylated by guanidinoacetate N-methyltransferase (GAMT, EC:2.1.1.2) using an activated methyl donor, S-adenosyl methionine (SAM).[17] In mammals, creatine is released into the plasma, transported to the muscle and taken up via the creatine-specific transporter Slc6a8.[49,50] In contrast, in trout and several other fish species (Figure 1), gene expression levels of both enzymes AGAT and GAMT are predominantly found in muscle tissue, suggesting that the complete creatine synthesis is mainly localised to the muscle.[16,44] The presence of creatine transporters in fish muscle, however, confirms that creatine is also imported from the feed. Considerable differences of Slc6a8 expression between muscle, liver and kidney were found between fish species.[16,44] To date, there is still a discussion on the creatine-phosphocreatine system in the brain as there are indications that, at least to some extent, there is a creatine biosynthesis in the central nervous system and that some independence of creatine import exists.[51,52] Finally, creatine as well as phosphocreatine are spontaneously converted to creatinine which is excreted via the kidneys.[17,53] By injecting ^{14}C-creatine Danulat and Hochachka documented that overall creatine turnover is relatively slow in fish muscle as compared with mammals,[54] which furthermore suggests a great potential of creatine supplementation in fish.

3 | APPLICATION FORM OF CREATINE

In addition to the endogenous biosynthesis, creatine can also be obtained exogenously, for example from the feed. In human applications, creatine monohydrate powder (CAS No.: 6020-87-7) is the most commonly used form since the early 1990s.[23,49,55] Other formulations include creatine hydrochloride and, recently, a co-amorphous formulation of creatine and citric acid, which has a better solubility in water.[56] In fish as well as humans, creatine anhydrous (CAS No.: 57-00-1) has equally been used.[1] Other forms such as creatine salts, creatine complexed with other nutrients, creatine dipeptides, creatine nitrate or creatine ethyl esters have been claimed to be more effective, but data-based evidence is often missing.[55] Recently, increased bioavailability has been documented for a colloidal creatine formulation in humans.[57]

TABLE 4 Guanidinoacetate (GAA) supplementation in aquaculture fish species

Species	Optimal dosage [range assessed]	Duration (days)	Effect	Reference
Ctenopharyngodon idella	0.3 g kg^{-1} [0, 0.15, 0.3, 0.45, 0.6 g kg^{-1}]	60	Growth ↑ FE ↑ Moisture ↓ Protein ↑ Lipid ↑	36
Cyprinus carpio	0.25, 0.5, 1 g kg^{-1}		Growth → Feed intake → Moisture → Protein → Lipid →	37
Oreochromis niloticus	1.8 g kg^{-1} [0, 0.6, 1.2, 1.8 g kg^{-1}]	60	Growth ↑ FCR → Moisture → Protein → Lipid ↑	38
	[0, 0.6, 1.2, 1.8 g kg^{-1}]	60	Growth → FCR → Moisture → Protein → Lipid →	39
	1.2 g kg^{-1} [0, 0.8, 1.2 g kg^{-1}]	84	Growth ↑ FCR ↓ Moisture → Protein ↑ Lipid ↓	31
	0.6 g kg^{-1}, −50 kcal [0, 0.6 g kg^{-1}] (decreasing energy)	60	Growth ↑ FCR →	40
Sciaenops ocellatus	[0, 5, 10 g kg^{-1}]	56	Growth → FCR → Moisture → Protein → Lipid →	33

Abbreviations: FCR, feed conversion ratio; FE, feed efficiency.

The origin of the creatine is utmost important as alternative synthesis methods exist and may lead to contamination with up to 5.4% dicyandiamide, 0.09% dihydrotriazine, 1.3% creatinine, dimethylsulphate, thiourea and/or concentrations of heavy metals like mercury.[58] To exclude adverse effects of contaminants, a German-sourced creatine has been recommended for research in sports medicine[55] and should congruently also be used in animal nutrition research to avoid charge-specific effects and improve reproducibility. Creatine monohydrate powder shows no degradation into creatinine over years, even upon storage at high temperatures.[59] Still, in solution, creatine degrades fast into creatinine, particularly at low pH and higher temperatures, which needs to be considered in dietary supplementation in fish. In the intestine, at very low (<2.5) or very high pH (>12.1), degradation is substantially reduced or haltered.[59] Therefore, with regard to the moderate pH in the digestive tract offish, particularly in herbivores, degradation needs to be considered higher than in mammals.

Alternatively, driven by lower prices and better stability in water,[22,45,60] GAA as a creatine precursor has also become a popular supplement used today.[49] Still, its administration is rather unexplored. More importantly, an increase in serum homocysteine after GAA administration is regarded critically and needs to be prevented. It has been shown that co-administration with methyl donors such as vitamin B12, choline or serine helps control hyperhomocysteinaemia and is hence strongly recommended.[61,62] Hyperhomocysteinaemia increases risks such as cardiovascular malfunction,[63–65] neurodegenerative disorders[65,66] and osteoporosis in humans.[67] Still, GAA as a food supplement has been used

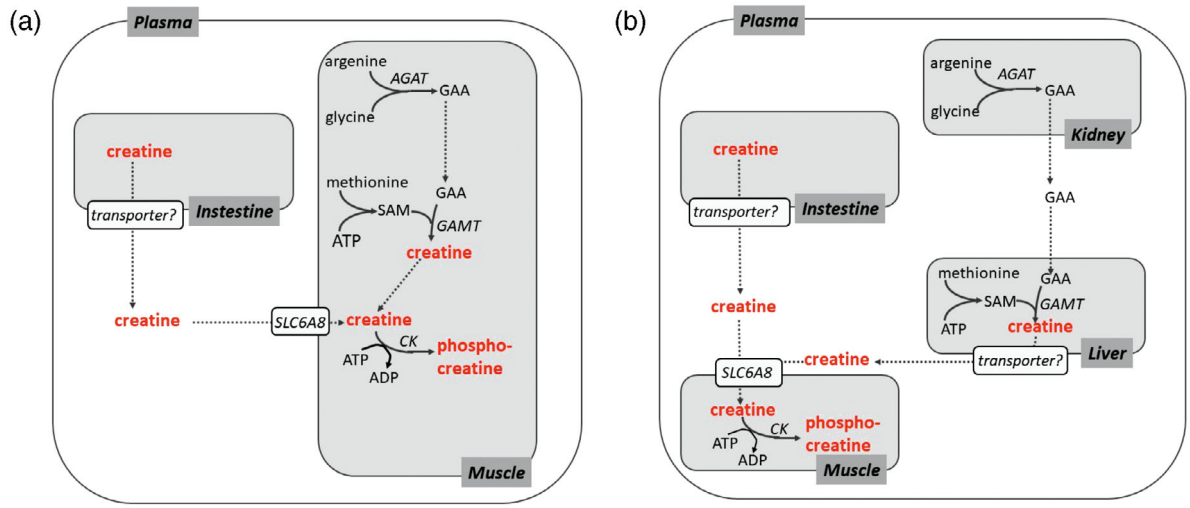

FIGURE 1 Creatine biosynthesis in trout (a) and mammals (b). AGAT, arginine:glycine amidinotransferase; CK, creatine kinase; GAA, guanidinoacetate; GAMT, guanidinoacetate methyltransferase; SAM, S-adenosyl-methionine, SLC6A8 creatine transporter (solute carrier family 6 member 8)

for chickens and pigs to improve growth, breast meat yield and feed conversion ratio.[68–71] In Nile tilapia (*Oreochromis niloticus* [Linnaeus]), GAA increased growth rates at 0.6–1.8 g kg^{-1} after 60 days of supplementation.[38] Nevertheless, in a similar study at the same concentrations, GAA did not increase growth performance or feed efficiency in this species.[39] Stites et al.[33] even reported decreased weight gain, increased whole body lipid and protein as well as feed efficiency at much higher concentrations (5–20 g kg^{-1}) in Red drum (*Sciaenops ocellatus* [Linnaeus]). Still, GAA supplementation increased weight gain, feed intake and feed efficiency already at concentrations between 0.15 and 0.6 g kg^{-1} in Grass carp (*Ctenopharygodon Idella* [Valenciennes]) over a 60-day period.[36] Taken as a whole, effects of GAA are rather controversial. In fish, methyl donors to prevent hyperhomocysteinaemia have rarely been used.

4 | DOSAGE

In general, dietary supplementation with creatine increases muscle creatine content.[28] Still, in fish, the process until maximum concentrations are realised is generally slower than in humans and requires higher dosages. The ideal dosing of creatine supplementation has been subject to controversial discussion. Indeed, there are two opposing strategies, established for the supplementation in sports. The first approach favours a high dosage, a short time loading phase (20–25 g day^{-1} or 0.3 g kg^{-1} day^{-1} for 5–7 days in athletes) followed by a maintenance phase at a lower dose for a prolonged period (3–5 g day^{-1} for usually 4 weeks), whereas the second approach favours a continuous, lower dosing strategy up to 12 weeks.[1,55] Green et al. found that creatine supplementation and combined ingestion of a simple carbohydrate improved creatine accumulation in the muscle.[72] This was attributed to a stimulatory effect of insulin release on muscle creatine transport. In a recent review, Hall and Trojian recommend 0.03 g kg^{-1} day^{-1} as a maintenance dose for 4–6 weeks.[4] In fish (Tables 3 and 4), supplementation is usually carried out at higher dosages of 20–80 g kg^{-1} dry feed (mostly 20–40 g kg^{-1}) in a continuous manner.[23,25,32] Due to a slower saturation, prolonged supplementation of up to 12 weeks have been carried out[25,32] and minor effects have been reported in cases, where application was relatively short (<12 weeks).[23,33] Indeed, Ramos-Pinto did not observe significant growth effects during a relative short feeding trial of 69 days, although high creatine concentrations (80 g kg^{-1}) were administered. Similarly, in Red drum, Stites et al.[33] observed minor improvement of weight gain and feed efficiency at creatine supplementation of 20 g kg^{-1} over a period of 8 weeks. Consequently, longer exposure times should be considered when supplementing fish with creatine.

At present, little is known about the content of creatine in animal-source feedstuffs.[73] In common aquaculture-related feeding trials, the creatine content of the diet is rarely considered or analysed.[74–76] Different sources of feedstuff show a wide variation in their creatine content ranging from 1 ± 0.03 mg kg^{-1} in feather meal to 127 ± 2.9 mg kg^{-1} in fish meal.[73] Still, in fish meal derived from different species, variations by a factor >9 may occur.[73] As creatine is highly soluble in water, fish meal processing techniques might affect the creatine content in the final product further. Creatine in control diets (without creatine supplementation) has been determined in very few studies,[25,27,28,32,35] and most studies present nominal concentrations. From the data available creatine ranges at approximately 1 mg kg^{-1} at 120–290 mg kg^{-1} fishmeal.

5 | EFFECTS OF CREATINE AND GAA SUPPLEMENTATION

5.1 | Growth performance

One of the observed effects of creatine supplementation is increased body mass. A meta-analysis revealed that approximately 60% of

studies noted a statistically significant increase in body mass or body composition in humans upon creatine supplementation.[2] Without participation in an exercise programme, increases in body mass are suggested to be a result of increased intracellular water due to the osmotic activity of creatine. In conjunction with an exercise programme, greater increases were observed, though. Congruently, in fish, several studies reported an enhanced growth performance upon feeding creatine supplemented diets (Tables 3 and 4). Other studies found creatine to enhance growth when used to supplement in plant-based diets.[25,26] Still, some reports do not reflect such effects,[26,34] others are controversial.[39] In hybrid Striped bass (*Morone chrysops* × *Morone saxatilis*) reared in freshwater, no growth promotion or improved feed efficiency was observed, but, in fish transferred to brackish water, Burns and Gatlin reported slightly increased weight gain but no effect on feed efficiency or fillet ratio.[28] When Red drum was reared in hypoosmotic and isosmotic water, creatine supplementation improved weight gain.[27] In Pacific white shrimp (*Litopenaeus vannamei* [Boone]) reared in fresh water, creatine supplementation at levels from 1.23 to 24.49 g kg^{-1} did not affect growth.[35]

Improved growth may furthermore be a result of increased appetite, palatability[25] and, ultimately, food consumption whereas some studies reveal an improved feed conversion upon supplementation. Often, this growth is expressed in an increased muscle growth and fillet ratio as a result of increases in muscle protein.[25,27] Correlated decreases in lipid content have been assigned to a protein-sparing effect of dietary creatine.[30,77,78] When comparing studies, it does not seem that the effects of creatine supplementation on lean muscle mass are strictly dose-dependent.[1,3] In addition, when creatine is supplemented, methionine, arginine and leucine, required for creatine synthesis, could subsequently be used for protein synthesis and growth.[12,78] Due to the high creatine content in fish muscle, this sparing effect might be significant.

Several endocrine growth promotors and modulators have been upregulated upon creatine supplementation (Table 2), including IGF1[22,30] and growth hormone (Gh),[23] which directly stimulates protein synthesis in the muscle via the Gh receptor in athletes.[79] Also, creatine has been shown to increase muscle-related genes such as myogenic differentiation 1 (Myod1),[23] calpain genes (capn1, capn1a, capn3),[23] Type I and II myosin heavy chain (mhc),[80] and, most importantly MRFs (Myo-D, Myog, Mfr-4, Myf5).[22,24] The primary MRFs MyoD and Myf-5 are required for myogenic determination, whereas Myog and myf-4 are downstream transcription factors involved in muscle differentiation.[81] Since muscle fibre recruitment continues into the adult stages, creatine supplementation is a promising way to enhance muscular growth.[81] Also, creatine may reduce myostatin and thereby prevent muscle atrophy.

5.2 | Aerobic endurance and anaerobic sprint

Sustained swimming in fish is driven by red muscle and characterised by aerobic processes fuelled by glucose.[82] Burst activity and exercise to exhaustion, in contrast, are primarily driven by white muscle resulting in the rapid anaerobic consumption of energy stores such as phosphocreatine and ATP.[82–84] Phosphocreatine and ATP stores are rapidly exhausted when white muscles are mobilised.[85] In Rainbow trout *Oncorhynchus mykiss* (Walbaum), a single tail-flip is sufficient to decrease the phosphocreatine content of white muscle by 50%.[21,85] When fish are exercised to exhaustion for 5 min, muscle phosphocreatine concentration decrease between 50% to near depletion.[86–88] Recovery, however, may be rapid and replenishment was observed within minutes or less than 1 h post-exercise.[86,87] When exercised juvenile Rainbow trout were supplemented with creatine, endurance in a fixed velocity test increased.[89] Exercise itself can increase the amount of phosphocreatine in the muscles as well. Solstorm et al.[90] observed an increase in phosphocreatine when Atlantic salmon (*Salmo salar* [Linnaeus]) post-smolt were reared at water velocities of 1.5 BL s^{-1} over a 6-week trial period. This was referred to as an up-regulation of the phosphocreatine shuttle, compensating for an increased demand for ATP transport from the mitochondria, as observed in humans.[91]

Studies at the beginning of the last century revealed the importance of carbohydrate as a fuel during exercise in humans.[92,93] Since, the importance of muscle glycogen has been confirmed in numerous studies. It is also recognised that glycogen is more than a store, but acts as a regulator of many signalling pathways. In Rainbow trout, 90% of the musculature is white muscle,[94] which is characterised by poor vascularisation,[95] low mitochondrial density[94] and low myoglobin content.[96] It is fuelled, primarily, by anaerobic glycolysis derived from glycogen stores in the muscle. The link between glycogen depletion and impaired muscle function during fatigue is not well understood. Each glycogen granule has its own metabolic machinery with glycolytic enzymes and regulating proteins. Indeed, the ability of the muscle is compromised when glycogen is reduced to low levels, which impairs ATP regeneration. Following prolonged glycogen-depleting exercise, decreases in phosphocreatine and increases in ADP are observed. This energy deficiency theory is challenged since even after recovery periods where ATP levels are restored, decreased muscle function is observed.[92] Here, the relationship between muscle glycogen content and the Ca^{2+} release rate seems responsible for prolonged fatigue.[92] In muscle fibres, where global ATP can be kept high and constant, low glycogen levels are associated with an irreversible force depression.[97,98]

There is accumulating evidence that commencing endurance exercise with low muscle glycogen content enhances the transcription rate of several genes involved in the training adaptation such as heat shock protein Hsp72, interleukin Il6, pyruvate dehydrogenase kinase Pdk4 and uncoupling protein Ucp3.[99–101] This is probably because several transcription factors contain glycogen-binding domains, and when muscle glycogen is low, these factors are released and associate with different target proteins involved in glycogen synthesis.[102] It has also been shown that creatine reduces myostatin, which inhibits muscle growth and protein biosynthesis required for muscle fibres. Myostatin is equally decreased within hours following endurance or resistance training.[103,104] Myostatin seems to be a key target for understanding myofiber growth by creatine. Creatine

supplementation increased glycogen stores in humans by 20%.[105] Upon endurance training over 7 days, glycogen increases by 30% in the muscle of Rainbow trout have been reported.[89]

During endurance training creatine kinase activity increases, documenting a higher need for phosphocreatine.[106] At the same time, hydroxyacyl CoA dehydrogenase activities in white and red muscle increased, indicating an enhanced capacity for fatty acid catabolism with training. Indeed, there is a crosstalk between glycogen and lipid metabolism. Feeding a high-fat diet increases hepatic glycogen stores due to increased expression of the glycogenic scaffolding protein Ptg/r5 via the mTORC1/SREBP1 pathway.[107] Ptg overexpression dramatically increases glycogen stores which in turn shifts energy stores to lipogenesis by induction of lipogenic genes.[107]

5.3 | Osmoregulation

Safdar et al. showed that short-term supplementation for 10 days increased the expression of several genes involved in osmotic regulation in young men.[108] Creatine also promoted muscle water retention, inducing changes in cell osmolarity.[108] In African catfish (*Clarias gariepinus* [Burchell]) creatine supplementation resulted in a decrease in moisture,[25] indicating the osmoregulatory activity of the creatine molecule in the plasma. Increased intracellular osmolarity subsequently induces cellular swelling, which activates cell-volume sensitive signalling cascades. For example, cell swelling is a potent stimulus of glycogen synthesis in muscle and liver. This may explain the effect of creatine on glycogen stores. In hybrid Striped bass transferred to higher salinity, increases in growth performance have been reported, suggesting that creatine supplementation energetically assisted in osmoregulation.[28] Remarkably, effective dose of creatine (4% of dry food, 5%–7% feeding ratio, corresponding approximately to 2–2.8 g kg^{-1} body weight) was very high compared to the dosage used for application in humans. The phosphocreatine/creatine kinase shuttle seems to coordinate the extra energy demand of the Na^+-K^+-ATPase to pump out excess ions under hyperosmotic conditions.[109] During times of osmotic stress, creatine kinase activity increases, suggesting that creatine supplementation has ergogenic benefits.[109–111] With this in mind, creatine supplementation could be of great benefit in specialised feeds administered before or during the critical periods of the transition between freshwater and seawater, such as smoltification. During smoltification, the fish experience hypo-osmotic stress[112] which is being compensated by Na^+-K^+-ATPase activity.[113–115] A temporary switch to creatine supplemented feed might assist the adaptation of the fish, enhancing osmoregulatory capacities when there are foreseen or unforeseen fluctuations in salinity, for example due to changes in external conditions or planned measures. However, no research has been done so far.

5.4 | Product quality

Improving meat and overall product quality is an important task of a sustainable agriculture production, in particular in high-priced products such as fish and seafood. Creatine supplementation stimulates protein synthesis and may thus lead to an improved product quality for the consumer. Some studies addressed the possibility to manipulate parvalbumin as major fish allergen (95% of cases reported,[116]) by creatine supplementation.[26,34] Nevertheless, no effects on the protein level have been detected.[26,34] In pork, creatine supplementation prior to slaughter seems to affect post-mortem muscle metabolism (decrease in pH) and improve overall meat quality.[117] Creatine supplementation affecting post-mortem muscle pH was also observed in Sea bass (*Dicentrarchus labrax* [Linnaeus]).[26] In freshwater reared Pacific white shrimp creatine supplementation decreased myofiber diameter and increased myofiber density overall leading to a more favourable product quality in terms of muscular hardness and chewiness.[35] In contrast, increases in muscle fibre diameter may also have a negative impact on the flesh texture.[23] In Gilthead seabream (*Sparus aurta* [Linnaeus]), creatine increased calpains capn1 and capn2 which play a role in myoblast fusion and affect flesh texture.[23] Indeed, this group of cysteine proteases has been suggested as potential markers of flesh quality.[118] Furthermore, creatine is a carninutrient which is only available via animal foodstuff (Table 1). Since creatine needs to be supplemented via the food, creatine content of the fish is a product quality criterion. In Grass carp, GAA supplementation resulted in an increase in muscle flavour nucleotide, flavour-related fatty acid and amino acid contents.[36] Also, GAA increased water-holding capacity and firmness of the fillet.

5.5 | Carbohydrate utilisation

In animal and human studies, creatine supplementation together with exercise training revealed beneficial effects on glucose metabolism. This is particularly important in carnivorous fish species, which have a low carbohydrate utilisation and a low ability to control hyperglycaemia. Therefore, creatine in combination with exercise may boost glucose metabolism and help to explore new aquafeeds in aquaculture. Carbohydrates are an excellent source of energy in feed formulations, also in terms of low price. To illustrate this, the unit cost of carbohydrates is approximately three to five-fold less than that of lipids or proteins. Furthermore, optimal inclusion of carbohydrates improves retention of proteins, reduces nitrogen emissions, helps pellet binding and increases floatability as well as stability of the pellet. In addition, increased palatability is often observed.[119,120] Taken as a whole, carbohydrates are often underrated ingredients in fish feeds and increasing carbohydrate contents is highly desirable from a feed formulators perspective.

With regard to the replacement of fishmeal by plant-based protein sources, increasing amounts of carbohydrates are inevitably observed in the feed.[121–124] Among the carbohydrates abundant in plant ingredients, only glucose and starch have a nutritive value for fish nutrition.[125,126] Although fish including carnivorous species have the biological machinery such as metabolic enzymes, endocrine regulation, glucose sensing components and glucose transporters, there are remarkable differences between fish and other livestock animals. Particularly carnivorous fish have a low intestinal glucose uptake and

a slow clearance rate of glucose from the blood and are thus less tolerant to carbohydrate-rich diets. In salmonids for example, maximum inclusion is approximately 15%–25% of carbohydrates in the diet, whereas up to 50% may be included in the feed of herbivorous species.[119,120]

Creatine along with carbohydrates improves muscle creatine retention compared to creatine alone.[72] Furthermore, creatine stimulates insulin secretion,[29,127] increases muscle glycogen stores (see above) and ameliorates hyperglycaemia. In addition, exercise induces numerous metabolic benefits, including insulin-independent muscle glucose uptake and insulin sensitivity.[128] Therefore, it has been suggested that creatine supplementation and exercise training synergistically improve glucose metabolism. The possible mechanism underlying the effects of combined exercise and creatine supplementation is an enhanced glucose transport into muscle cells by the glucose transporter Glut4 translocation to the sarcolemma. Four modes of action for the creatine-exercise induced effects on glucose homeostasis have been described.[128,129] First, creatine modestly increases insulin secretion.[30,127,130] Second, creatine induces Glut4 expression increasing the cellular uptake of glucose.[30] Third, creatine stimulates the energy sensor Ampk that induces a modulation of glucose and fatty acid oxidation. Amp-activated protein kinase is a key enzyme that mediates between cellular energy status and diverse effector proteins. By phosphorylation of transcriptional regulators, enzymes and proteins related to cellular structure, it can modulate energy metabolism.[131] Finally, an exercise-mediated effect has been suggested that synergistically increases Ampk and Glut4.[129] In pig, feeding creatine in combination with a high glycaemic carbohydrate increased loin muscle area.[132] Furthermore, creatine supplementation tended to increase the intramuscular fat content.[133–135] Accelerated glycolysis during exercise will supply acetyl-CoA molecules required for lipogenesis.[136] Taken as a whole, creatine seems to promote the inclusion of carbohydrates particularly in carnivorous species.

5.6 | Brain functioning

Although most of the body's creatine is found in skeletal muscle, the brain is also very active metabolically, accounting for up to 20% of human energy consumption. A brain-specific isoform of the creatine kinase (BB-CK) is expressed in brain neurons, suggesting that creatine is relevant for energy storage and provision to the central nervous system. In fact, creatine depletion leads to major mental disorders such as mental retardation, learning delays, autism and seizures in humans.[52] While muscle relies on dietary ingestion and endogenous synthesis from the liver, kidneys and pancreas in mammals and zebrafish, the brain can synthesise creatine. Indeed, the enzymatic machinery required for the endogenous creatine synthesis is found in the nervous system and creatine transporters are detected at the blood–brain barrier, neurons and oligodendrocytes, suggesting that brain creatine may be relatively independent.[52,137] Still, if brain synthesis is limited due to a disfunction, dietary provision can establish normal concentrations.[51,138] Up to date the optimal supplementation strategy to establish regular brain creatine concentrations is unknown,[52] but should be more prolonged than those strategies typically used to increase muscle creatine.[51] Since brain creatine relies less on exogenous creatine than muscle, a down-regulation of brain creatine synthesis upon supplementation may occur.[52] Also, limitations in the creatine transporter may turn supplementation less effective in the brain.[139] Interestingly, equimolar supplementation with guanidinoacetic acid (GAA) was more efficient than creatine in increasing brain creatine content, suggesting that dietary GAA could be imported to the brain through additional transporters and delivery routes. Nevertheless, effects on the cognitive performance remain controversial.[52] The role of creatine system in the brain might be particularly important in conservation aquaculture when fish are produced for conservation purposes such as restocking or stock enhancement.

6 | SAFETY CONCERNS

In humans, the International Society of Sports Nutrition states that 'there is no scientific evidence that the short- or long-term use of creatine monohydrate has any detrimental effects on otherwise healthy individuals'. Nevertheless, contamination of creatine supplements has been recorded, including heavy metals and toxic organic contaminants.[58] Also, if GAA as creatine precursor is used as supplement (see above), methyl donors such as vitamin B12, choline or serine should be added to avoid hyperhomocysteinaemia. Interestingly, in several studies in fish lacking a methyl donor, no effects on growth were reported.[36] This may already indicate malnutrition but has not been studied in detail.

It has been concluded that feed supplementation with creatine monohydrate at levels up to 50 g day^{-1} for 5 days prior to slaughter does not increase the level of heterocyclic aromatic amines detected as mutagenic activity formed upon frying of pork.[140] However, dietary creatine supplementation level at 24.49 g kg^{-1} increased muscular creatinine content and extensive retention of creatinine in the muscle may constitute important precursors of heterocyclic amines that formed on the surface of meat when cooked at high temperature,[141] suggesting that creatine supplementation should be in a proper level.[35]

7 | CONCLUSION

Creatine and GAA have been successfully supplemented in aquafeeds to improve growth performance, physical performance and in order to support osmoregulation. Still, experimental design, in particular dosage and duration vary substantially. Furthermore, effects on performance parameters (growth, feed efficiency, protein and lipid content) are often controversial, partly explained by species-specific responses to supplementation. In most cases, physiological mechanisms were not studied. With regard to an exploration of creatine and GAA in fish nutrition, increased utilisation of carbohydrates – particularly in carnivorous species – has a great potential, improving the overall sustainability of aquaculture production. Due to its instability and high costs,

future research should focus on GAA supplementation, but hyperhomocysteinaemia needs to be considered. Here, co-administration of a methyl-donor such as vitamin B may compensate adverse effects. In carnivorous species, fishmeal-based feed usually ranges between 1 and 2 g kg^{-1} feed and beneficial effects are observed at higher inclusions of up to 80 g kg^{-1}. In plant-based diets, creatine is not present and an inclusion seems advisable.

AUTHOR CONTRIBUTIONS

Sven Wuertz: Conceptualization; writing – original draft; writing – review and editing. **Stefan Reiser:** Conceptualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author.

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