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Functional Fish: Improving Nutrition for the Elderly

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Mini Review

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Abstract

Elder people need highly digestible foods that can also provide health benefits even to those suffering from chronic diseases. Furthermore, such foods should be palatable as well as familiar for elder consumers. Fish is a high-protein, low-fat food that potentially provides a range of health promoting effects which may be further improved with suitable approaches in the production systems. The present mini-review intends to report possible aquaculture interventions to enhance the positive impact of fish on elder health and to promote its function in terms of prevention and recovery of specific diseases. Some fish species during their lifespan experience periods of food restrictions that can be mimicked in aquaculture without affecting fish welfare. Under these circumstances fish can modify the fatty acid profile and increase the use of muscle proteins to fulfill their energy requirements, by activation of muscle endogenous proteases. Degradation of muscle proteins can enhance their digestibility and possibly the release of encrypted bioactive peptides, showing a plethora of biological actions, including the antihypertensive activity. The degree of myofibrillar protein degradation and the fatty acid profile of fish fillet can then be managed by suitable and sustainable feeding protocols in the context of farming conditions.

Keywords: Elderly; Health; Fish; Aquaculture; Digestibility; Bioactive Peptides

Eating Fish for Healthy Aging

According to current estimations, people in the world are living longer. Most of the evidence indicates that reduction in mortality is due to advances in health services, medicine, wealth and income, nutrition, behavior, and education [1]. However, longer lives do not mean healthier lives, since increasing age is the major risk factor for developing many major chronic diseases. Anyway, aging, which has been defined as a multidimensional process characterized by several physical, social and physiological alterations occurring in humans during the course of life [2], is not in itself a disease and does not inevitably result in a decline in health status or function [3]. In fact, the elderly are a very heterogeneous group with respect to health and functional status and many factors contribute to successful aging [3]. It has been shown that aging is malleable to specific types of genetic mutations, diet, and drugs, which can extend lifespan and improve health during aging [4]. In particular, diet seems to have a much more pervasive and prominent role than previously thought in modulating mechanisms of aging and its associated diseases [5]. Many studies have highlighted the association among low energy intake, inadequate intake of protein and vitamin D, and an increased risk of frailty development [6,7]. Thus, improvement in nutrition could provide health benefits to older people and many age-related diseases and conditions can be prevented, modulated or ameliorated [8].

In the elderly nutritional deficiencies can result from the physiological decline in food intake, which is very common among older people [2]. Hence, food for the elderly should be palatable as well as familiar for these consumers. Encouraging older adults to prepare meals can also increase appetite and food intake, and providing opportunities for older adults to eat a wide variety of foods, in the company of others, is a simple strategy to increase food intake [8].

Consuming food rich in nutrients and antioxidants has the potential to prevent the majority of the age-related disorders that impair the quality of life. In fact, recent evidences suggest that older adults need to intake more dietary proteins than do younger adults to support health, promote recovery from illness, and maintain functionality [7,9]. Concerns regarding health hazards related to high red meat intake have been increasing during recent decades, although restrictive recommendations should not be applied to subjects above 70 years of age [10]. Therefore, high-quality proteins from fish, containing essential amino acids in considerable amounts, can represent an important and healthier alternative to proteins from other animals. Interestingly, the dietary protein source appears to have specific protective properties in maintaining cardiovascular health [11]. This was demonstrated in a clinical trial where the intake of lean fish reduced cardiovascular lipid risk factors in healthy subjects more than the intake of non-seafood proteins [12]. The gastrointestinal digestion of fish proteins can also result in the release of bioactive compounds associated with health benefits [13]. Fish, for its components and properties, is hence a matter of interest for health in aging populations. Traditionally, the long chain polyunsaturated omega-3 fatty acids (n-3 PUFAs), including eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have been the primary components which may contribute to improving human health [14-16].

Evidence has been provided of potential benefits of n-3 PUFAs consumption in the protection against the risk of developing cardiovascular disease (CVD) [17], although the achievement of optimal performance requires an appropriate quantitative composition and the proper proportions of delivered fatty acids [18]. Considering the metabolic competition between n-6 and n-3 PUFAs and their opposing properties [19], an optimal ratio of n-6 to n-3 PUFAs should be recommended as its alteration has been associated with health risks [20]. Moreover, fish consumption has a protective and anti-inflammatory function on skeletal muscle and its biologically active compounds, such as n-3 PUFAs, proteins, vitamins D and E, magnesium and carnitine help to maintain good muscle performance, preventing sarcopenia [21]. The role of dietary n-3 PUFAs as a link between musculoskeletal and cardio-metabolic health in older adults has been recently shown [22].

The decline of physiological functions among the elderly can also cause eating problems, which includes poor digestion. Despite its claimed digestibility, fish proteolysis has been found highly affected by elderly gastrointestinal alterations, with an important decline in leucine release [23]. Intervention on fish microstructure could improve its physiological interactions within the human gastrointestinal tract, providing functionality to fish over and above simple nutrition [24]. In order to provide these and other health benefits to older consumers of fish it may be reasonable to implement suitable interventions in the production systems by adopting specific approaches tailored to the human nutritional needs. Accordingly, the aim of the present mini-review was the description of possible aquaculture interventions aimed to enhance positive impact of fish on elder health and to promote its protective effects against specific diseases.

Cultured Fish Could Contribute to Elderly Health

Aquaculture's rapid expansion and the decline of marine fishery resources highlight the relative contribution of farmed fish to the total fish consumption [25]. At present, farmed products are assuming greater significance as nutritious food in the context of low impact production systems [26], as predicted by the 2013 "Fish to 2030: Prospects for Fisheries and Aquaculture" [27]. Compared to extractive fishing, aquaculture enables influencing the characteristics of the final product by means of different management strategies [28]. In fact, the composition of the edible part of fish varies as a function of several factors, including species, strain, animal sexual maturity stage and size, as well as nutritional and environmental factors, such as oxygen availability, temperature, photoperiod, or pH [29-31]. Among them, food sources and nutrients are considered as primary contributors [32]. Novel technologies are now available to provide insight into the main underlying metabolic mechanisms of how fish adapt to feeds, and ultimately to characterize the influence of fish nutrition on fillet composition [33].

In the context of human health, the impact of fish feed on human nutrition is critical in that changes in feeding practices may affect the health-giving properties of cultured fish [34].

For example, a significant increase in vitamin D content of the salmon fillet was observed following feeding *Salmo salar* of additional vitamin D [35]. Based on the finding that altering feed composition changes the nutritional content of farmed products, which in turn impacts on human nutrition, Fry et al. [36] proposed a conceptual framework which described the potential links between the use of feed ingredients in aquaculture and human health. Following this approach, aquaculture could play a significant role in the transition to efficient, resilient and health-promoting food systems. Evidence has been further provided regarding the inclusion of cultured fish as "functional food", which can be specifically designed to deliver nutritionally valuable component(s) able to decrease the risk of certain diseases while improving health and wellness [37]. However, to achieve such outcomes, scientific research must effectively establish the bioavailability and efficacy of these compounds at levels that are physiologically achievable under typical dietary patterns [38]. On the other hand, an increasing amount of evidence suggests that these bioactive molecules might not act alone, but that their activity could be enhanced in the context of the natural food matrix, and needs to be characterized in such contexts [39]. As the nature of the food matrix can greatly affect the utilization of certain nutrients, the "whole food' approach is certainly the preferential choice. As an example, the health-promoting effects of fish consumption on the risk of CVD most likely results from the interaction between nutrients in fish, supporting the idea that consumption of whole fish would have a much greater impact on human health compared to fish oil supplements [40,41]. It is thus of importance to evaluate possible aquaculture interventions, including feeding practices, in order to enrich fish nutritional properties specifically directed towards elder health, while maintaining the "whole food" approach, which corresponds to the needs of this increasing population group.

A large extent of experimental data widely supports the observation that aquafeed is the main driver of the fatty acid (FA) profile in the edible tissues of farmed fish [42,43]. Consequently, FA profile of fish muscle can be manipulated by modifying FA composition of fish diets [44]. However, the recent trend in large scale substitution of fish oil with sustainable-alternative vegetable-origin lipid sources in aquafeeds has nearly halved the actual content of EPA and DHA in farmed fish [34]. In response to this evidence, in recent years several alternative raw materials have been identified for their inclusion in aquafeeds to optimize fish FA profile. Due to their minimal overall environmental impact, single cell-based dried biomasses have recently attracted the attention of the feed industry sector as a partial substitution for fish-derivatives [45-48]. Dried biomasses of certain marine microalgae, which are rich in n-3 PUFAs, have successfully shown to be effective as partial replacers for fish oil in feeding selected fish species [49-53]. A further advantage of microalgae feeding is their content in carotenoids [54]. The use of yeast biomass, engineered to produce high levels of EPA and treated to improve digestibility, could represent another strategy to increase the availability of n-3 PUFAs when fed to Atlantic salmon (*Salmon salar*) [55].

Adoption of alternative feeding procedures, including pre-slaughter starvation or reduced feed rations, is economically advantages to the fish industry as well as justifiable since many species of fish exhibit remarkable, although variable, resistance to starvation [56,57]. In fact, some wild fish species experience periods of fasting or food restriction for several reasons, such as the physiological state related to the reproductive season and difficulties in reaching the feed due to food availability and environmental limitations [58]. Therefore, manipulation of quality through different feeding management approaches can be achieved without compromising growth performance while addressing environmental issues [59]. As an example, a selective retention of n-3 PUFAs was observed in fillet of trout (*Oncorhynchus mykiss*), which had been subjected to a period of food deprivation prior to a fish oil finishing diet [60]. Final levels of n-3 PUFAs and FA preferences in energetic mobilization during feed restriction or deprivation varied among fish species, like sharpsnout seabream (*Diplodus puntazzo*) [61], black seabream (*Acanthopagrus schlegeli*) [62], dentex (*Dentex dentex*) [63], and Atlantic salmon (*Salmon salar*) [64]. The proportional contribution of the FA appeared also variable within the same species. Following changes in rainbow trout (*Oncorhynchus mykiss*) ration levels, SFAs were the most stable FA, while MUFA and n-3 PUFA were the most responsive ones [65]. In a more recent study, lower feeding rations resulted in rainbow trout (*Oncorhynchus mykiss*) muscle with lower relative amounts of SFA, while MUFA and PUFA were not affected [66].

As reported below in more detail, we reinforced the feeding management approach showing that seabream (*Sparus aurata*) food deprivation was an effective tool in obtaining good-quality fillets providing potential health benefits for hypertensive and gastroesophageal reflux disease (GERD) consumers [67].

Feeding Intervention and Fish Muscle Changes: Digestibility and Bioactive Peptides

During feed restriction, fish experience transition from anabolic to catabolic states and can undergo alterations in muscle metabolism. Hence, the fish nutritional status, through the enhanced expression of autophagy related genes, modulates the catabolic conditions and eventually some skeletal muscle protein mobilization [68]. When protein degradation exceeds protein synthesis as a consequence of feed deprivation, the muscle can undergo to atrophy [69,70]. Mechanisms of metabolic changes in skeletal muscle are different among fish species and also depend on the degree of feed restriction. In fact, specific proteins are preferentially deposited or mobilized in the muscle in response to varying feeding levels [71]. In rainbow trout (*Oncorhynchus mykiss*) the fasting-induced muscle degradation is associated with elevated expression of genes involved in the catheptic (cathepsin L, D and S) and collagenase proteolytic pathways, as well as with a marked depletion of muscle proteins, such as myosin heavy chain, and alpha-actin [72]. Similarly, an equally quite severe feed restriction (101 days of feed deprivation) allowed for the upregulation in Arctic charr (*Salvelinus alpinus*) muscle of mRNA transcripts regulating protein degradation via the autophagy pathway (cathepsin D and L) [73].

Partial restriction might overcome the undesirable effects associated with prolonged starvation, whilst maintaining some positive effects. In fact, moderate levels of restriction in feeding rates prior to slaughtering significantly decreased α-actinin in seabream (*Sparus aurata*), but no detrimental effects on the muscle firmness and structure, fillet yield, or nutritional quality were observed [28]. In rainbow trout (*Oncorhynchus mykiss*) feeding restriction, but not pre-slaughter starvation provided the early delocalization of troponin from myofilaments, the appearance of an actin 26 kDa-fragment and the detection of enigma protein, all being signs of impaired muscle integrity [74]. In seabream (*Sparus aurata*) a pre-slaughter starving regime (21 days of feed deprivation) resulted in the early degradation of myosin light chains (MLCs), whereas skeletal alpha-actin fragmentation was similar regardless of the pre-slaughter feeding system [67].

Muscle protein changes can therefore vary depending on a range of factors, and it is possible that some of them can be managed in order to modulate the catabolic conditions and eventually some skeletal muscle protein mobilization. Fish rearing conditions could represent a strategy for controlling the hydrolysis of specific proteins thus increasing their susceptibility to digestibility as well as the release of bioactive peptides with different potential health benefits for elderly.

Protein Digestibility

Human digestion of food proteins is a complex process that involves the concerted action of several digestive proteases, which are closely controlled by hormonal and neural regulatory mechanisms [75]. Protein digestion starts with a short food chewing step in the mouth. The sequential activity of pepsin in the stomach and of the pancreatic and intestinal proteases in the small duodenum results in the liberation of amino acids and peptides. Amino acids are absorbed by the villus enterocytes through several systems that vary in solute specificity and ionic-dependency [76], while di/ tripeptides are efficiently absorbed by the peptide transporter 1 (PepT1) complex [77]. It has been evaluated that the absorbed dietary proteins in the blood stream are represented by about 90% amino acids and 10% dipeptides and tripeptides [76].

A decline of certain gastrointestinal functions, including a reduced saliva production and mastication [78], alteration of gastric juice associated to atrophic gastritis [79], defects of pancreatic enzymes and bile [80] secretions, is not infrequent in the elderly, leading to maldigestion and malabsorption and typically resulting in protein deficiency. To counteract such conditions, a daily protein intake of 1.0-1.2 g per kg of body weight is recommended over 65 years [7]. Given the appropriate balance of essential and non-essential amino acids [81], fish is therefore well advisable for elderly.

In addition to gastrointestinal conditions, inherent characteristics of food can influence the protein's behavior during digestion. Because of the different protease specificity, the gastrointestinal transit time will determine the type of released peptides and their digestion/absorption [82]. Major factors are: i) the protein's structural properties, e.g. the presence of hydrophobic β-sheet structures makes protein digestion difficult [83]; ii) the food source, e.g. in *in vitro* digestion static protocol, the skeletal muscle actin did not seem to be completely degraded in the pork, beef, and chicken samples, but it was degraded in the silver carp (*Hypophthalmichthys molitrix*) samples [84]; iii) the use of domestic and/or industrial processing methods, e.g. marinated and cooked salmon showed a decreased proteolysis compared to the raw salmon under suboptimal intestinal conditions [85].

Human and animal models provide the most complete data on the digestion of proteins, but have ethical restrictions, high costs and a long duration. To overcome these limitations, several *in vitro* simulated gastric and small intestinal digestion models have been developed based on the use of bioreactors mimicking the chemical and enzymatic conditions of each digestive compartment (static models) [75,84]. Recently, an international consensus of digestion conditions was reached and a standardized method was proposed greatly improving inter-laboratory reproducibility [86]. Dynamic systems, either mono- or multi-compartmental are also available, but their cost is very high [82]. Both approaches have the advantages of mimicking digestive disorders by properly setting digestion conditions, such as those possibly occurring in elderly [23,87].

Interestingly, by using a static method, a recent study evaluated the protein digestibility of different cooked fishes (hake, seabass, salmon and sardine) subjected to elderly *in vitro* digestion model. As expected, proteolysis was markedly reduced in the elderly compared to healthy adults, but to a different extent depending on the fish species, salmon and seabass being the most affected ones (40 and 33%, respectively) associated with a markedly lower release of leucine [23].

Considering that raw products are growing in consumption popularity, we evaluated the impact of seabream *(Sparus aurata*) on the human gastric digestibility of raw fillets, using seabream starved for a period of 21 days before slaughtering [67]. The proteolysis was tested by the above-mentioned standardized method of *in vitro* simulated gastric digestion under conditions relevant to the treatment of GERD, whose prevalence is strongly associated with increasing age [88]. Remarkably, in an altered gastric milieu, i.e. at pH 4 that may occur in drug-treated patients, gastric digestibility of the main myofibrillar proteins from starved fish fillets was much higher than that from fed fish. As a more pronounced degradation of MLCs has been found in starved fish at slaughter, the higher fillet digestibility has been attributed, at least partially, to the early MLCs cleavage, which could have increased the susceptibility of the myosin complex in relation to pepsin digestion. Therefore, further nutritional benefits can occur for the increasing number of people undergoing acid- suppressant therapy for GERD, thanks to the enhanced gastric digestibility of the main myofibrillar proteins, ascribable to seabream pre-slaughter starvation.

Bioactive Peptides

Recent scientific evidence suggests that food proteins, including fish proteins, not only serve as nutrients, but can also modulate the body's physiological functions through bioactive peptides that are encrypted inside the native protein sequences, from which are freed by the action of proteases. Bioactive peptides usually contain 2-20 amino acids and can provide health benefits positively affecting the majority of body systems [89,90]. Their actions could therefore minimize the risks of chronic diseases during aging.

Farmed and wild fish species, including many carp, salmon, tilapia, sardine and tuna, have been studied for the discovery of new bioactive peptides, which have been mainly isolated from protein hydrolysates generally obtained by treatment with microbe-extracted enzymes (e.g., alcalase, neutrase, thermolysin) [90]. Several techniques have been developed for the extraction and purification of bioactive peptides, since these govern the structure of the final product and its mode of action on the target site [90,91]. Their identification and structural characterization have been made possible thanks to the impressive development of high-resolution mass spectrometry, despite of the lack of complete fish-specific protein sequence databases [92].

Many *in vitro* biochemical assays, cell models, and animal models have been applied to test the bioactivity and the kinetics of fish bioactive peptides and to evaluate their therapeutic potential. Fish peptide extracts showed interesting bioactivities, including antibacterial for several marine teleost fish [93], antiviral for the polar fish (*Pleunorectus americanus*) [94], antioxidant for Pacific hake (*Merluccius productus*), satiety enhancer for smooth-hound (*M. mustelus*), a-amylase inhibition and antidiabetic for Australian salmon (*Arripis trutta*), and antihypertensive activity (angiotensin-converting enzyme (ACE) inhibitor activity) for dried bonito, sardinelle (*Sardinella aurita*), Australian salmon, barracouta (*Thyrsites atun)*, and silver warehouse (*Seriolella punctata*) [95,96]. Indeed, the majority of bioactive peptides are not specific and can be found in many species.

Among these bioactivities, ACE inhibitors are considered to provide better life expectancy, due to the high prevalence of hypertension and closely related cardiovascular events in aging [90]. The discovery of ACE inhibitors from fish started in the early 1990s in dried bonito (Katsuobushi), a traditional Japanese seasoning made of bonito muscle [97]. When the most efficient peptide (LKPNM), previously identified by *in vitro* studies, was tested in spontaneous hypertensive rats, it appeared that the parent peptide had to be hydrolyzed (LKP) to exert a health beneficial effect [98], highlighting that *in vivo* studies are crucial to evaluating the bio-efficacy of bioactive peptides. It is now widely accepted that the presence of a hydrophobic amino acid at either of the peptides' terminals is vital for ACE-inhibition activity of a peptide, namely, phenylalanine, proline at C terminal and leucine, isoleucine, valine at N-terminal [90].

In spite of these promising findings, few studies have evaluated the digestion behavior of fish bioactive peptides in humans and the metabolic processes that occur before peptides reach their site of action [82]. Indeed, peptides need to resist the action of digestive enzymes during their transit through the gastrointestinal tract and need to cross the intestinal epithelial barrier to reach the target organs intact. Interestingly, to date, four different routes of peptides absorption have been described: paracellular diffusion, transcellular passive diffusion, transcytosis, and carrier-mediated transport by the PepT1 complex [82,99]. The hydrophobicity/hydrophilicity properties and molecular mass of the bioactive peptides govern the route of absorption, e. g. PepT1 preferentially binds short-chain bioactive peptides, such as antihypertensive biopeptides [100,101], and the peptides transported by PepT1 show a higher bioavailability than those transported through the other routes [102].

The new peptidomics approaches permitted the investigation of fish muscle endogenous peptides by eliminating the need for purification of protein hydrolyzates [92]. The activity of endogenous proteases, especially calpains and cathepsins, has been associated with the hydrolysis of myofibrillar proteins during *post-mortem* storage, resulting in the softening of fish texture and the production of bioactive peptides [95]. In seabass (*Dicentrarchus labrax*), a recent study identified 119 peptide sequences with 17 of them having post-translational modifications typical of *ante-mortem* signalling pathways. Peptides were 2-16 amino acid long and mostly hydrophilic and basic. Interestingly, the peptide mixture mainly contained anti-hypertensive (ACE inhibitors) and hypoglycemic (*Dipeptidyl peptidase-4* inhibitors) sequences [92], and many of them have been previously found in other species [103].

In seabream *(Sparus aurata*), we recently found [67] two anti-ACE, gastric-resistant dipeptides, which were previously isolated from a sardine muscle hydrolysate and acted as ACE competitive inhibitors [104]. One of them (VF) seemed to appear in larger amount in fish that experienced a 21 days starvation period before slaughtering, where a marked and selective degradation of MLCs was observed at slaughter. Consistently, a bioinformatic analysis by BIOPEP search engine showed that this dipeptide can potentially originate from MLCs. We calculated that, given the IC_{50} and the released amount of the dipeptide VF during simulated digestion, and assuming a complete absorption in the blood stream, a normal fillet portion from starved fish should provide a VF amount able to inhibit at least few point percentages of ACE activity [67]. Moreover, several other ACE peptides potentially present in fish fillets [92] could synergistically contribute to controlling the blood pressure.

All together, these findings highlight that fish can provide health benefits especially to elderly people suffering from hypertension. Moreover, in the concept of prevention and treatment of hypertension anti-ACE fish biopeptides, similar to other bioactive peptides, have shown to produce their effect with negligible cytotoxicity, making them potentially superior to synthetic drugs [90]. A better characterization of fish as source of health-promoting agents through new studies aimed at investigating the bio-accessibility and bioavailability of biopeptides in humans, would really contribute to greatly promoting its conscious consumption by the elderly.

Conclusions

The nutritional properties of fish as such are extremely important for the promotion of the health in the aging. Aquaculture fish, which is easily available and cheap, can therefore be a source of health benefits for this population. As documented, fish functionalization through interventions in primary production could improve its characteristics, giving rise to additional health functions. In this sense, future research would be desirable in order to provide tailored products able to delivery specific nutrients that would contribute to the prevention, modulation or amelioration of the main pathologies affecting the elderly population.

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Conflicts of Interest

The authors declare no conflicts of interest.

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