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Review

Fish Welfare in Aquaponic Systems: Its Relation to Water Quality with an Emphasis on Feed and Faeces—A Review

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Abstract: Aquaponics is the combination of aquaculture (fish) and hydroponic cultivation of plants. This review examines fish welfare in relation to rearing water quality, fish feed and fish waste and faeces to develop a sustainable aquaponic system where the co-cultured organisms, fish, bacteria in biofilters and plants, should be considered holistically in all aquaponics operations. Water quality parameters are the primary environmental consideration for optimizing aquaponic production and for directly impacting fish welfare/health issues and plant needs. In aquaponic systems, the uptake of nutrients should be maximised for the healthy production of the plant biomass but without neglecting the best welfare conditions for the fish in terms of water quality. Measures to reduce the risks of the introduction or spread of diseases or infection and to increase biosecurity in aquaponics are also important. In addition, the possible impacts of allelochemicals, i.e., chemicals released by the plants, should be taken into account. Moreover, the effect of diet digestibility, faeces particle size and settling ratio on water quality should be carefully considered. As available information is very limited, research should be undertaken to better elucidate the relationship between appropriate levels of minerals needed by plants, and fish metabolism, health and welfare. It remains to be investigated whether and to what extent the concentrations of suspended solids that can be found in aquaponic systems can compromise the health of fish. Water quality, which directly affects fish health and well-being, is the key factor to be considered in all aquaponic systems.

Keywords: fish welfare; water quality; suspended solids; micronutrients; aquaponics; sustainability

1. Introduction

Aquaponics, the combined production of fish in recirculated aquaculture systems and hydroponically grown plants, has gained increased interest over the last several years due to its sustainability. Aquacultural waste contains nitrogen (in the form of ammonia and nitrate) and phosphorus (mainly in the form of phosphate), which are essential nutrients for plant growth.

The plants filter dissolved waste products from the system by utilizing them as a nutrient source, thereby reducing the need for biological or chemical filtration for water changes and water quality management. The use of dissolved nutrients from fish excreta for plant production has been shown to increase plant biomass, and freshwater aquaponic systems can be used to reduce the environmental effects associated with food production on land [1]. Aquaponically grown products provide consumers with high quality, safe and nutritious freshwater fish and plants grown in a sustainable manner. As aquaponics is an integrated system that covers the co-production of plants and fish, the combination of fish and plants should be compatible with the characteristics of each production type to balance nutrient production from fish culture and nutrient uptake by plants. The selection of plant and fish species based on this fact is of critical importance in terms of suitability for aquaponics production. Due to their coherent features with respect to aquaponic production, tilapia, koi, goldfish, carp, catfish, barramundi, and various ornamental fish species are the main produced fish species and the lettuce, pak choi, kale, basil, mint, water cress, tomatoes, peppers, cucumbers, beans, peas, squash, broccoli, cauliflower and cabbage are the main produced plants in the present conditions. In aquaculture operations, including aquaponics systems, fish welfare is an important factor that plays a significant role in a successful business operation. Fish welfare in general is a comprehensive issue integrating physiological, behavioural and cognitive responses, indicating adaptive responses to stressful stimuli. Thus, Segner et al. [2] have stated that poor welfare may provide a strong indication for the existence of infectious and non-infectious stressors, leading to impairment in maintaining homeostasis. Fish welfare must be viewed as the interaction of multifactorial effects, such as stocking density, diet, feeding technique, and management procedures, possibly affecting stress levels, subsequent stress tolerance, health, and the presence of aggressive behaviour. It has been revealed that these effects are related with the welfare of farmed fish. To improve the quality of the product with increasing its market value and to achieve a positive public perception, weak fish must be avoided, and healthy animals must be raised in accordance with welfare standards [3].

Fish welfare in aquaponic systems can be an important criterion for the consumer. The conditions in aquaponic systems should promote fish growth and health, commonly regarded as indicators of fish welfare, to deliver high quality products to consumers. In addition, it is well established that optimal growing conditions are necessary to fulfil the essential physiological requirements to promote fish health and welfare. However, the study of fish welfare is still lacking for the species cultured in aquaponics. The aim of this paper is to review the potential factors resulting from fish nutrition and water quality parameters on fish well-being under aquaponic culture conditions.

2. Water Quality

Water quality parameters are of major concern in hazard identification for the welfare risk assessment of various aquaculture operations; hence, aquaponic systems are not distinct from aquaculture. Fish raised in aquaponic systems require good water quality conditions which mean that parameters such as dissolved oxygen, carbon dioxide, ammonia, nitrate, nitrite, pH must be within acceptable species-specific limits. Sudden changes in the fish stocking density, growth rate, feeding rate or water volume can elicit rapid changes in water quality; hence, regular measurement of those critical water quality parameters is essential. The deterioration of water quality parameters affects fish physiology, growth rate, and feed efficiency, leading to pathological changes and even mortality under extreme conditions [4,5]. In terms of aquaponic systems and considering the fish welfare issues, carrying capacity is of major concern for maintaining the balance between plant and fish requirements in a co-culture medium. Carrying capacity expresses the maximum biomass of fish in the system with acceptable water quality limits. The carrying capacity of a given amount of water is determined by the oxygen consumption rate of the fish and their responses to ammonia, CO_2 and other potentially toxic metabolic wastes that are produced [5]. Stress involves a series of physiological and behavioural reactions that help fish resist death or adapt to changing conditions. When stress is severe or prolonged, the capacity of the fish to re-establish homeostatic norms can be inadequate. The ultimate result may include impaired immune function or death [6]. The stress responses of fish to environmental stressors are complex; therefore, evaluating the environmental conditions and understanding their potential deleterious effects on fish are of utmost importance in any system that holds fish in captivity.

For the welfare of fish in an aquaponic system, water quality is the primary environmental consideration with a potential to markedly affect fish health. Fish exist in intimate contact with the water through the huge surface area of the gills and skin, and it is widely acknowledged that fish are vulnerable to inappropriate water quality. Water provides fish with the oxygen required to survive, dilutes and removes potentially toxic metabolites, and provides support against gravity. Inappropriate levels of water quality parameters affect physiology, growth rate and feed efficiency (biomass increase/feed fed) and may cause negative stimuli. From the perspective of aquaponics, the stabilization of the chemical composition of water normally requires some time depending on the temperature and a range of other factors such as stocking density. The stability of water characteristics in aquaponic systems may set the biological limits for sustainable production. In other words, a production format for co-cultured species (aquatic organisms and plants) impairing the biological capacity of the fish may be caused by unstable water conditions, which affect the welfare conditions through complex interactions between water quality parameters.

Dissolved oxygen (DO) is the primary water quality consideration for aquaponic systems as in other aquaculture units. Fish extract oxygen from the water by passive diffusion through the gills. An adequate concentration of DO in the water is required to facilitate passive diffusion down a concentration gradient from the water into the blood [7]. If DO concentrations fall below the requirements of the fish, then fish cannot convert energy as efficiently into a usable form, resulting in reduced growth rate, feed efficiency and swimming ability [8]. The immediate response of fish to decreased DO concentrations is to increase the opercular ventilation rate and show a gasping response [9].

In aquaponics, the minimum oxygen levels to promote good health and physiological conditions in the fish stock can change based on fish species and fish size. The solubility of oxygen in water decreases as the temperature increases. Colt and Tomasso [7] contributed the basic points in aquaculture practise that are of importance in aquaponic systems when considering the allowable DO levels, i.e.,

- elevated temperature increases the metabolism, respiration and oxygen demand of fish;
- fish increase their oxygen uptake after feeding due to the oxygen demand required for feed processing, called specific dynamic action;
- oxygen consumption is proportional to the size and number of fish in a given system;
- smaller fish use more oxygen per unit weight than larger fish;
- stressful conditions such as impaired gill function, exposure to stressors and decrease in oxygen-carrying capacity lead to the increase in oxygen demand of fish.

In general, the recommended limit for DO levels in fish culture is 6 ppm for coldwater fish and 4 ppm for warmwater fish to protect the health [9].

In aquaponics, the nitrogen cycle is a critical factor. The cycle begins with the introduction of protein in fish feed, which is ingested by fish and then excreted to the aqueous phase in the form of total ammonia nitrogen (TAN, i.e., NH_3 and NH_4). Ammonia is first oxidized to nitrite (NO_2) by ammonia-oxidizing bacteria (mainly *Nitrosomonas* spp.) in a biofilter and then converted to nitrate (NO_3) by nitrite-oxidizing bacteria (mainly *Nitrobacter* spp.). In the aquatic environment, ammonia exists in two forms in equilibrium: un-ionised ammonia and ionised ammonium. Thus, the total ammonia concentration is the sum of the concentrations of un-ionised ammonia and ionised ammonium. The equilibrium between the NH_3 and NH_4^+ varies in relation to the various factors, most significantly the concentration of hydrogen ions (i.e., pH) and temperature.

In an aquaponic system, most of the ammonia found in a fish farm is produced by the fish as in aquaculture systems. Ammonia is the primary waste metabolite produced by fish from the catabolism of protein contained within the feed. Most biological membranes are permeable to un-ionised ammonia and relatively impermeable to ionised ammonium. Therefore, in fish, ammonia in the external environment either induces retention of endogenous ammonia in the fish or enters via the gills by passive diffusion down a concentration gradient. The ammonia is excreted from the fish via the gills [10]. Ammonia toxicity is dependent primarily on the concentration of ammonia and the pH of the environment. Randall and Tsui [11] reported that acute ammonia toxicity affects the central nervous system of fish and manifests as a neurological disorder. Ammonia interferes with physiological processes that eventually result in the death of cells in the brain; however, ammonia toxicity and its exact nature are not understood in fish. High concentrations of ammonia decrease survival, inhibit growth, and cause a variety of physiological dysfunctions. The high level of ammonia in water acts as a stressor in that it stimulates the release of corticosteroid hormones into circulation, affecting the welfare of the fish. Masser et al. [12] stated that un-ionized ammonia nitrogen concentrations as low as 0.02–0.07 ppm have been shown to slow growth and cause tissue damage in several species of warm water fish. However, tilapia tolerate high un-ionized ammonia concentrations and seldom display toxic effects in well-buffered recirculating systems. However, in sensitive species such as rainbow trout (*Oncorhynchus mykiss*), the recommended level of un-ionised ammonia is $<0.02\ mg\cdot L^{-1}$ [9]. In the management of recirculating systems, ammonia should be monitored daily. If the total ammonia concentrations start to increase, the biofilter may not be working properly or the feeding rate/ammonia nitrogen production is higher than the design capacity of the biofilter.

Nitrite becomes toxic even at low concentrations for many fish species [13]. The degree of toxicity to nitrite varies with species. In freshwater fish, nitrite enters through the gills. Nitrite ions are actively taken up through the chloride cells, and they can be pumped in against a concentration gradient [14]. Blood plasma concentrations of nitrite can accumulate up to ten times greater than the ambient water concentration [15]. In fish, blood is the main target of nitrite action. Nitrite diffuses from the blood plasma into red blood cells, where it oxidises the Fe^{2+} in haemoglobin (Hb) to the Fe^{3+} oxidation state, converting haemoglobin into methaemoglobin (metHb). MetHb reduces the total oxygen-carrying capacity of the blood [16]. Nitrite exposure resulted in a reduction in haemoglobin and haematocrit in tilapia (*Oreochromis niloticus*) with mild methaemoglobinaemia following exposure to 0.50 and 1.38 $mg\cdot L^{-1}$ NO_2^--N for 48-h static tests [17]. Nitrite exposure in the range of 0.50 and 1.38 $mg\cdot L^{-1}$ NO_2^--N caused an increase in methaemoglobin levels. Methaemoglobin concentrations in excess of 50% are considered threatening to fish [18]. The physiological disturbances may be primarily rooted in the hypoxia caused by methaemoglobin accumulation. Thus, it is predictable that the oxygen starvation induces hyperventilation. Because the gills are directly in contact with the aquatic habitat, the morphological and physiological alterations in the gill tissue are of major concern. Svobodova et al. [19] reported that hyperplasia, vacuolisation and elevated numbers of chloride cells were the main histological lesions that occurred in the gills of nitrite-treated carp (*Cyprinus carpio*). Nitrite can reach high concentrations in recirculating aquaculture systems in which high densities of fish are kept. Scaled fish species are generally more tolerant to high nitrite concentrations than species such as catfish, which are very sensitive to nitrite [12]. Low concentrations of dissolved oxygen affect the toxicity of nitrite. Because nitrite affects the ability of blood to transport oxygen, a reduction in ambient water DO concentrations increases the effect of toxicity. Although there are many studies about acute and sublethal effects of nitrite on fish in the literature [14,16,20–22], comprehensive studies on the chronic effects of nitrite on different fish species under aquaponic conditions will be necessary considering the interaction of plant roots and the efficiency of related bacteria in the system. In brief, the related bacteria that may be involved in aquaponic systems are the following: ammonia-oxidizing bacteria (AOB), including bacteria of the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio*, and nitrite-oxidizing bacteria (NOB), including bacteria of the genera *Nitrobacter*, *Nitrococcus*, *Nitrospira*, and *Nitrospina* [23].

In general, information on chronic nitrate toxicity in cultured fish species across various life stages is limited. Nitrate, the end product of nitrification, is relatively non-toxic except at very high concentrations (over 300 ppm [12]). However, Davidson et al. [24] reported that cases where recirculating aquaculture systems (RAS) were operated with very low water exchange nitrate caused chronic toxicity to various fish species. Thus, the study of Davidson et al. [24] underlines how relatively low nitrate levels (80–100 $mg \cdot L^{-1}$ NO_3-N) were related to chronic health issues in and welfare impacts to juvenile rainbow trout under RAS conditions. Changes in swimming behaviour, slightly decreased survival and reduced total biomass were the main findings of impaired welfare conditions in a RAS system with rainbow trout. Nevertheless, in the case of aquaponics, nitrogen compounds should always be evaluated with the vegetation factor. Nitrate and ammonium are the most common forms of nitrogen taken up by the plants. The removal of nutrients is affected by the plant species and cropping method, as reported by Buzby and Lin [25]. The potential adverse effects of ammonium or nitrate on the fish well-being are expected to be relieved with the removal of these compounds via plant uptake in the aquaponic system. However, the designing of aquaponic systems and the techniques used are the factors affecting nitrogen compounds concentrations in the system and their possible stress effects on fish as well as stress type.

A disruption of the balance between fish, filters and plants at any time may also result in ammonia or nitrite that could be fatal to the fish and certainly reduce performance and increase the susceptibility to diseases. Such disruptions may arise from a significant decrease in plant or fish biomass, a significant increase in feed input, a decrease in filter capacity as a result of sloughing of accumulated bacteria/organic matter, a cleaning of the system, or a sudden change in pH. Temperature is a vitally important physical property of the water in aquaponic systems. The amount of dissolved oxygen is directly linked with water temperature. Water temperature affects the rate of decomposition and photosynthesis, which will affect the oxygen demand in systems and the ionisation of ammonia [7]. Optimal temperatures for growth and spawning have been examined for many species that are important to aquaponics. Increasing the temperature has been found to increase the growth and infectiousness of many fish pathogens [4] as well as the toxicity of many dissolved contaminants [9]. All of these factors have the capacity to compromise the health of fish under aquaponic conditions. Model studies on water temperature to maintain the well-being of fish are necessary to achieve optimal plant growth in aquaponic systems.

The management of pH is also necessary in aquaponic systems because pH will steadily decline as a result of the nitrification process, which increases H^+ and NO_3^- ions in the system. A crucial item in aquaponic systems is pH stabilization because it is critical to all living organisms within a cycling system that includes fish, plants and bacteria. Most plants require a pH value between 5.5 and 6.5 to enhance the uptake of nutrients. However, the optimal pH of three major bacteria has been stated as (1) *Nitrobacter*: 7.5; (2) *Nitrosomonas*: 7.0–7.5; and (3) *Nitrospira*: 8.0–8.3 [26]. However, in terms of the tolerance of fish to pH changes based on fish species and size, the recommended pH for aquaculture is 6.5–8.5 [27]. In integrating the hydroponic systems with aquaculture the pH value seems to be the drastic factor in order to concurrent maintenance of nutrient uptake by the plant and optimal pH value for fish and the bacteria in biofilter. pH values higher than 7.0 causes the reduced micronutrient and phosphorus solubility and plant uptake of certain nutrients is restricted in the aquaponic environment [28]. On the other hand, as acidic water is one of the stressors in aquaculture environments, low levels of water pH in aquaponic systems have the potential to affect negatively the welfare of fish. Another subject related with pH levels is the interaction between pH and phosphorus (P) availability and speciation. Cerozi and Fitzsimmons [29] reported that P availability decreased with high pH value of aquaponic nutrient solutions and insoluble calcium phosphate species formed. Hence high pH level seems to be a preclusion in the plant uptake of P in aquaponics. In terms of fish welfare, P in aquaculture is not classified as a water quality parameter that has the potential to impact on fish health/welfare. It is known that phosphorus can be toxic, but toxicity occurs rarely in nature and is generally not a concern except for the indirect effects of phosphorus.

It is always important to consider that sustainable aquaponic production requires balancing nutrient concentrations and pH for the optimal growth of three organisms: plants, fish, and nitrifying bacteria.

The primary source of carbon dioxide is fish metabolism in aquaculture. It is known that free carbon dioxide is toxic to fish. Fish cannot release endogenous carbon dioxide into water when ambient CO_2 concentrations are high, resulting in CO_2 increases in the blood, a condition described as hypercarbia. Due to decreases in blood pH along with acidosis in hypercarbia, the oxygen-carrying capacity of the blood declines (the Bohr effect). Carbon dioxide toxicity can be characterized by moribund fish, gaping mouths and bright red gill lamellae [30]. The link between CO_2 and water hardness was reported as the cause of nephrocalcinosis in rainbow trout, with the symptoms of the calcifying material manifesting in the ureters and kidney and as impaired food conversion efficiency [31]. Land-based, recirculating aquaculture systems as well as aquaponic systems can expose fish to higher-than-natural levels of aquatic hypercarbia. Oxygen is artificially supplied to these systems to increase fish production; however, an increase in biomass normally leads to an increase in CO_2 production.

In general, the tolerable water quality parameters for fish are within the same range with the plants except water temperature and pH in the aquaponic system (Table 1). When considering the well-being of fish species in aquaponic systems, fish species with a high tolerance to pH and water temperature should be taken into account. It is obvious that pH and temperature are the parameters that have an impact on optimization of aquaponic production both for fish welfare/health issues and for plant needs.

Table 1. General water quality tolerances for fish (warm or coldwater), hydroponic plants and nitrifying bacteria (from Somerville et al. [32]).

Organism Type	Temperature (°C)	pH	Ammonia (ppm)	Nitrite (ppm)	Nitrate (ppm)	Dissolved Oxygen (ppm)
Warmwater fishes	22–32	6–8.5	<3	<1	<400	4–6
Coldwater fishes	10–18	6–8.5	<1	<0.1	<400	6–8
Plants	16–30	5.5–7.5	<30	<1	-	>3
Bacteria	14–34	6–8.5	<3	<1	-	4–8

In aquaponic systems, special care should be taken when treating the fish to eradicate diseases or parasites. Some chemicals in an aquaponic environment will harm or destroy the plants, and it is well known that many chemicals and metals will also be absorbed by the plants [33,34], hindering their use for consumption. The use of antibiotics for treating bacterial infections is one option, but they may be absorbed by the plants [35], thus causing withdrawal periods and marketing problems; this same problem is also seen with the fish.

In aquaponic systems, special care should be taken when treating the fish to eradicate diseases, parasites or water mould. For example, formalin is a common chemical used to treat certain fish parasites and water mould, and it can also be used in recirculation systems relatively safely without compromising nitrification in the biofilter [36]. However, in an aquaponic system, formalin can be expected to cause severe damage to the plants as formalin added into the soil has been shown to drastically decrease plant cover and shoot dry weight [37].

In an aquaponic system, the plants may also induce some direct effects on the fish, at least in theory. Plants are known to produce chemicals that they excrete, e.g., through their roots [38], in order to make their environment more favourable to themselves. This phenomenon is called allelopathy, which very often refers to the detrimental effects of plants in the surrounding environment. However, the effects could be positive as well depending on the interacting organisms and the concentration of the chemical in question [39,40]. Allelopathic plants, i.e., plants that release allelochemicals, have been discovered in several phyla [33]. The concentration of allelochemicals released by a plant can also vary widely depending on the surrounding plant species [38], and this could potentially cause fish welfare problems in an aquaponic system if the plant species used produce chemicals to fight each other. However, to the best of our knowledge, negative effects of potential allelochemicals excreted by aquaponically grown plants on fish have not been reported.

On the other hand, conditions in hydroponic systems, and naturally in aquaponic systems as well, are often perfect for the growth of different kinds of algae [41]. It is known that also algae can release allelochemicals which can be harmful not only for the fish but also for the microbiota and the plants [42], e.g., cyanobacteria (blue-green algae) can excrete cyanotoxins, which can directly place fish health at risk, or the physical presence of these algae can harm the gills [43]. In addition, the algal spikes of diatoms (e.g., *Chaetoceros* sp.) have been reported to damage the gills at a concentration of five organisms per ml with the consequence of excess production of mucus and inhibition of oxygen uptake [44]. Despite the potential adverse effects of algae on fish we have not found reported evidence of such incidences in aquaponic systems.

Measures to reduce the risks of the introduction or spread of infection and to reduce the conditions enhancing susceptibility to infections are called biosecurity [45]. These measures should be of utmost importance in every aquaponic operation. The methods for increasing biosecurity include considerations such as the water source, sources of fish and eggs, preventative disinfection practices for staff and visitors and the quarantine of new arrivals as reported by Timmons and Ebeling [45].

3. Feeds and Micronutrients

The diet and its use for the fish represent the highest impact on the carrying capacity of aquaponic systems [46]. Currently, very little is known about specific fish diets for aquaponic culture; thus, current diets for aquaculture, mainly those for RAS culture conditions, are widely used for both purposes. Apart from an adequate nitrogen/energy balance and a correct combination of feed ingredients and particle size, the diet formulation for aquaponics should contain adequate immuno-ingredients or additives that promote the best welfare conditions to the fish during the growing cycle, thus avoiding any extra treatments into the system. Moreover, the effects of specific feeds on water quality through diet digestibility, faeces particle size and settling ratio are also important aspects. Water quality modifications from specific diets and selected feeding strategies have been related to fish behavioural changes [40], which are essential for fish nutritionists to interpret dietary effects on the system. Food-anticipatory activity can be a good welfare indicator [47], which may be used in formula feed studies. An important factor to study under aquaponic culture conditions would be the relationship between the whole fish excreta and the plant nutrient requirements. Aquaponic studies may implicate interdisciplinary research areas to achieve more adequate fish feed formulations and feeding strategies, not only focusing on fish growth and well-being, but also promoting the best plant growth and quality for the consumers.

Although the running of an aquaponic system could be improved through optimal fish and plant species selection [46], and the correct fish feed regime [48], most of the reported research shows extra nutrient inputs needed for the plants, which should be added to the growing medium or as a foliar spray to improve plant growth [49,50]. Although from literature N, Mg and some other plant nutrients appear to be adequate from fish excreta, this is not the case for P, K, Cu, Fe, Mn, Zn and S [51,52], which are normally included in the growing bed to be absorbed for the plants. In this sense it is important to note that no information has been reported regarding the possible impact of the surrounding water nutrients on fish wellbeing or welfare. It is well known that fish expend energy to respond to the changes in surrounding medium osmolality by regulating their body fluid volume and solute concentration through endocrine control, with marine and freshwater animals exhibiting different strategies to maintain their homeostasis (ionic and osmotic gradients between the body fluids and surrounding seawater) [53].

Nutrition and feeding influence fish growth, welfare and health and their response to physiological and environmental stressors and pathogens. The micronutrients P, K, Cu, Fe, Mn, Zn and S represent a very small percentage in fish feed, and information related to the requirements and effects of these micronutrients on fish aquaculture is relatively scarce. From published papers, micronutrient deficiencies are more extensively reported compared with studies where excess amounts have been tested, i.e., possible toxicity effects. During last years, the effects of dietary Cu, Mn, Fe and Zn, important micronutrients in fish metabolism, have attracted interest due to their limited availability in plant protein formulated feeds respect to the higher fish meal diets.

Copper (Cu) is an essential metal involved in several Cu-dependent enzymes, which mostly intervene in the defence against oxidation reactions and include the Cu/Zn superoxide dismutase (CuZnSOD), but Cu also participates in the production of energy at the cellular level, in neurotransmission, collagen synthesis and melanin production [54]. Low Cu levels may generate a reduction in feed efficiency and growth [55], while Cu toxicity produces gill damage and liver and kidney necrosis [54,56]. Iron (Fe) is involved in electron transport, oxygen transfer and cellular respiration, with a special importance in haemoglobin [56,57]. Fe can be partially absorbed via the gills; however, the majority is absorbed in the intestine. Fe deficiency causes anaemia, low haematocrit and reduced Fe in plasma, whereas excess uptake of Fe causes reduced growth, poor feed utilization, mortality and diarrhoea [54,56]. Manganese (Mn) is a transition metal essential for life, acting as a cofactor for essential metalloenzymes involved in the development of the organic matrix of bone [58]. Mn superoxide dismutase (MnSOD) intervenes in preventing the initiation of the radical chain reaction when an oxidation reaction occurs. Mn deficiency reduces MnSOD activity in fish tissues and the level of Mn, Ca and Na in the vertebrae [59]. Excess Mn may affect the integrity of intestinal immunity [60], which is essential mostly in marine fish to maintain correct ion regulation. Zinc (Zn) is an essential cofactor for several metabolic processes in fish and forms part of up to 20 metalloenzymes implicated in lipid, carbohydrate and protein metabolism. Zn is essential for structural components such as bone, skin and scales; plays an important role in regulating oxidative stress and immunity; and intervenes in reproductive processes. Zn is involved in bone formation and mineralization by activating osteoblastic cells and inhibiting osteoclastic bone resorption [61]. Thus, Zn deficiency in fish causes slower growth rates, cataracts, skin and fin erosion, and dwarfism [56]. Zinc can be absorbed via the gills and gut [62–64]. However, the presence of chelators or competitive substances may interact with zinc absorption. Calcium, phosphates, high water salinity and acidic pH are some of the factors that can alter zinc availability. Zinc deficiency may thus reduce production over the whole life cycle [54,56,65]. However, zinc toxicity has scarcely been studied, and most trials only reflect data concerning the survival in freshwater species exposed to high levels of waterborne zinc. Probably the most important toxic effect of zinc is related to the inhibition of calcium absorption. Calcium and zinc share transport channels, and at high levels of zinc, calcium uptake is severely reduced [66–69].

As a summary, it is important to note that in aquaculture, fish mineral requirements are still poorly reported, with a very low amount of information with respect to dietary mineral unbalance and excess on fish welfare. As fish may obtain minerals both from surrounding water and diets, research efforts should be made to understand the shared waterborne micronutrient compatibility and mutual benefits between plants and fish, and also to study the effects of higher dietary levels of the target plant minerals in the whole aquaponic production cycle, fish, and plants.

4. Wastes and Faeces

All materials which have been used but are not removed from the aquacultural system during harvesting can be regarded as waste [70]. The compounds contained in the feed are digested, absorbed and utilized in the metabolic processes and retained in different measures in the body of fish. One part of those compounds is excreted through the gills or as faeces. Only approximately 1/3 of the nutrients in the feed are removed in the harvest of the fish and 2/3 voided by fish during growth [71].

Uneaten feed, excreta, chemicals and therapeutics are retained in the waste produced by aquaculture. Waste originating from the feed includes dissolved components (such as phosphorus- and nitrogen-based nutrients) and suspended solids [72]. Fish food and faeces are known to be the main solid waste in intensive aquaculture. The faecal waste can be calculated by the proximate composition (reported on feed bag labels) and by the associated values of digestibility; indeed, the overall fraction of faeces produced per unit of feed consumed is obtained by summing the amount of all indigestible dietary components.

The production of feed wastes such as dust and uneaten food has been estimated to be 20% [73], whereas other authors [74,75] have proposed a range of 10% to 30% of waste represented by uneaten food alone from intensive aquaculture. Through a meta-analysis of published data from commercial producers in Brazil on expected feed intake, feed efficiency and other animal production indices and the body composition of tilapia (*O. niloticus*), Neto and Ostrensky [76] estimated that 18% of the feed given to the animals is not consumed and is lost in the aquatic environment and accumulated in the sludge.

According to Reid et al. [77], in the case of a common salmon feed, approximately 15% of consumed feed becomes dry faecal matter, while Butz and Vens-Cappell [78] affirmed that the faecal input is 260 g per kg of food on a dry weight basis.

The main phosphorus loss in fish is through faeces. High phosphorus loss through faeces was also found by Pettersson [79] in rainbow trout and by Johnsen et al. [80] in Atlantic salmon (*Salmo salar*). Hakanson et al. [81] calculated that of the total phosphorus and nitrogen fed to fish, 70% of the phosphorus and 15% of the nitrogen is lost through faeces, whereas Kristiansen and Hessen [82] reported loss values of 4.0% for phosphorus and 2.3% for nitrogen in Atlantic salmon, and 3.5% for phosphorus and 4.1% for nitrogen in noble crayfish (*Astacus astacus*) faeces. In a recent paper, Neto and Ostrensky [76] proposed that 17% of the feed input for nitrogen and 37% for phosphorus is lost as nutrients from the faeces in Nile tilapia reared in cages. Although the data of Neto and Ostrensky [76] were based on cage breeding systems, recently Goddek et al. [26] observed similar values in RAS. Certainly, the species affects the entity of these losses, which are affected also by the physiological stage within the species. Neto and Ostrensky [76] also found relevant variations in feeding losses in relation to the age of tilapia—30.9% for fry, 17.0% for juveniles, 17.6% for the growing phase and 16.7% at the slaughter stage.

Over the last several years, the progress in fish nutrition research and in feed manufacturing for the most important aquacultured species has contributed to a significant reduction of waste. The improved bio-availability of phosphorus and proteins in the diets has significantly reduced the faecal solids produced and improved the feed efficiency [70]. The addition of digestibility enhancers in the feed, such as phytase, has improved usability of phosphorus from plant proteins for fish and has helped to reduce the loss of nutrients as waste [83].

The labile form of phosphorus in the faeces, a form readily available to plants for their growth [78], ranges from 15% to 54% [79,84]. The quantity of P released by solid wastes is largely dependent on temperature (25 °C > 20 °C) and pH (higher at higher pH values) [84].

For the reduction of solid waste, the importance of feed processing and production should not be underestimated. Feed manufacturing processes aim to improve the nutritional quality of the diets but also to improve the stability and integrity of pellets, consequently reducing the pellet breakdown in water before consumption by the fish.

Through this double approach (diet formulation and feed manufacturing), feeding management practices have maximized the feed utilization by fish and, at the same time, minimized the uneaten quantity of feed, achieving a more economical process because the economy and efficiency of the production process are strictly connected [85,86].

The particle size affects the ratio of settleable to suspended solids [77]. Larger particles are less numerous but occupy a larger volume, whereas smaller particles are more numerous but occupy less volume overall. Buryniuk et al. [87] found a strict correlation between fish size and the faecal size classes: faeces from 1 kg Atlantic salmon were not captured on mesh openings over 4 mm, while some 10%–20% of faeces from 5 kg salmon could be captured on a 25 mm mesh. Variability in faecal particle sizes is positively correlated with fish size [87,88]. Magill et al. [89] found the mean size of particles of sea bream (*Pagrus major*) and sea bass (*Dicentrarchus labrax*) fed the same diet to range from 0.3 to 2.5 mm (1.4 mm mean) and from 0.3 to 6.2 mm (1.12 mm mean), respectively. Fish size and species affect potentially also other physical and rheological properties of faeces such as settling rates that can be affected by many different parameters not dependent on fish.

The ingredients utilized in feed formulation can have a strong impact on the quantity of faeces produced and their specific characteristics. Indeed, the factors affecting faecal properties are largely diet-dependent; in particular, the indigestible components of the diet have a strong effect. For example, in salmonids, the presence of indigestible dietary components has been demonstrated to affect the physical characteristics of the faeces, such as cohesiveness and stability [77].

Specific dietary ingredients appear to be largely responsible for the changes in physical faecal properties, and their effect could be apparently more relevant than the changes due to the proximate composition of the feed. This is the case for the feed pellet binders, which can significantly affect the physical properties of faeces. Binders include a variety of materials with the aim of preventing feed pellet disintegration in water but also reducing the formation of feed dust when transported and handled [90]. Many binders do not have any nutritional value [91] and are poorly digested. An important topic for future research is to develop a feed capable of producing intact and high-density faecal pellets [66]. A strategy for reducing solid faecal production is based on pelleting feeds by extruders. The process of cooking the carbohydrates of the feeds reduces the need for indigestible binders [66].

Information on the biophysical properties of fish faeces (such as nutrient content, digestibility, particle size and density, mass fractions and settling rate) is highly variable and disparate in the scientific literature currently available [89]. Strangely, even if the physical properties of feed pellets have less importance on the water quality than the faecal properties, there is more information available on the settling rates and related physical properties of feed pellets compared with those of the faeces [77].

The management of feeding has a very important effect on feed utilization by the fish. The feeding time and the location of feeders can have an influence on the quantity of solids produced and their distribution within a fish tank [70]. Also the feeding frequency has been found to affect the feed intake of fish, quantity of uneaten feed, feed efficiency and, consequently, metabolites and excreta of fish and water quality [92].

It should be noted that the stability of faeces in water and the particle breakdown could be influenced by their intrinsic properties and by the interaction with water turbulence, mainly caused by fish motion or by the use of pumps [93].

Suspended solids (SS) have a direct impact on fish health, and this is particularly true when nearly closed environments of RAS are considered that are characterized by the accumulation of fine particulates [94].

The studies conducted, mainly on salmonids, highlight the negative effects produced by elevated levels of suspended solids on fish health, consisting of gill abrasion, decreased feeding, increased susceptibility to diseases and increased mortality [95–101]. Consequently, the acute exposure of salmonids to high concentrations of suspended solids subjects the fish to mechanical abrasion and clogging or coating of the gills, resulting in coughing, respiratory stress and mortality [97,98,102,103]. Hughes and Morgan [102] found a thickening of the gill epithelium in fish acutely exposed to high levels of suspended solids that was responsible for fusion of the adjacent lamella. In green grouper (*Epinephelus coioides*) exposed to various concentrations (0, 50, 100, 200, 1000 and 2000 $mg \cdot L^{-1}$) of suspended solids for 6 weeks, Au et al. [104] found nonlethal effects at environmentally realistic concentrations; however, they did find damage to the gill structure, such as epithelium lifting, hyperplasia in the pillar system, and reduction of epithelial volume. Clear signs of osmoregulatory stress have also been noted [102]. Pathological changes in the integrity of gill lamellae structures reduce the capacity for oxygen transfer and ammonia excretion, leading to respiratory stress and exposing fish to ammonia intoxication [105,106].

It is worth noting that tolerance to SS is a species-dependent factor. According to Wilber and Clarke [107], fish species with a 24-h lethal concentration 10% (LC_{10}) value at an SS concentration

<10,000 mg·L^{-1} are classified as sensitive. Unfortunately, as highlighted by Au et al. [104], very little information is available about the effects of high concentrations of suspended solids for extended periods on fish health, and the knowledge for non-salmonids or fish is until now very limited.

The gravity of the effects produced by the suspended solids is strictly dependent on the concentrations encountered. A concentration of SS from hundreds to hundreds of thousands mg·L^{-1} can be lethal, whereas concentrations in the range from tens to hundreds of mg·L^{-1} are typically sublethal [101,108]. As reported in the review by Wilber and Clarke [107], common sublethal responses to suspended solids are increases in red blood cell count, haematocrit and haemoglobin concentration. According to the findings of Ling and Chen [109], the degradation of suspended faecal particles can lead to an imbalance in the bacterial population with a consequent increase in the concentration of toxic compounds, such as ammonia and nitrite, impairing fish health [110].

The presence of waste in the water where fish are reared is stressful for the fish, and it can reduce their growth performance and expose them to pathological risks [111,112].

Suspended solids can harm fish directly by damaging or smothering gills but also indirectly by adding habitats for different kinds of pathogenic organisms [113,114]. As highlighted by Welch and Lindell [115], the decay of waste in water consumes oxygen and thus decreases the oxygen available for fish.

In natural environments, the concentration of suspended solids can show wide and sudden temporal and spatial variations, and the organisms living in aquatic environments are adapted to natural fluctuations in turbidity. It remains to be investigated whether and to what extent the concentrations of suspended solids that can be found in aquaponic systems can reach the threshold considered as critical to the health of fish.

The solid removal is a strategic point to maintain *in equilibrium* the systems based on water re-use, such as the RAS and the aquaponic plants. Another important point is that the solids should be removed by mechanical filters as soon as possible, to avoid or limit their breakdown in small particles, which are dangerous and also very difficult to collect. The biggest particles are the easiest to remove, regardless of the method chosen for particle removal. The water flow has a paradigmatic role in the management of the solid wastes, since low water velocity reduces the particle breaking, but a sufficient flow is needed to prevent particles settling. As highlighted by Lekang [116], in the case of the re-use systems the small particles will normally dominate, since the larger particles are easier to remove. The prevalence in water of the small particles is clearly demonstrated also by the brown colour of water in the high re-use systems.

According to Thorarinsdottir [117], the inadequacy of the solid waste removal is responsible for more than 85% of the failure of the aquaponic systems. Indeed, not only fish but also plants and substrates are negatively affected by the waste in water, due to the clogging effects on the roots for the former and to the increased oxygen demand and the consequent risk of hydrogen sulphide and methane production for the latter.

Different methods for water filtration can be utilised. Mechanical filtration (also called straining or micro screens), depth filtration (also called sand filtration or filtration) or settling can be chosen, whilst the other methods available, i.e., flotation, membrane filtration and ozonation, that are very effective in removing smaller particles. Unfortunately, these last methods are very expensive and their use in aquaculture is conceivable only in particular conditions.

Each system has its peculiarity in terms of the dimension of the particles removed. As example, the systems based on gravity are effective on particles of dimension >100 µm; the drum-filters mainly utilised have screens in the range of 40–80 µm, sand filters operate very efficiently on particles down to 5 µm, while protein skimmer or foam fractionators can efficiently remove fine solids (<30 µm) [117].

For the different kinds of filters, strengths and weaknesses can be highlighted, and are well summarized in Lekang [116]. The method specifically utilised for solid removal from the rearing unit in the aquaponic systems are recently reviewed in detail [118].

The sludge produced, comprising water and particles, should be removed from the system. The percentage of dry matter in the sludge ranges between 0.1% and 5%, depending on the kind of filters utilised. It is rich in organic nitrogen (3%–9% of dry matter, DM) and phosphorus (1%–4% of DM), with a concentration of heavy metals usually below the threshold limits [118]. To avoid the development of bacteria and the consequent loss of the nutrients, the sludge should be immediately stabilized after recovery. Its characteristics make the sludge useful as a fertilizer and this other output of the aquaponic production can be transformed into a valuable input in the cycle of the energy, nutrient and water, further contributing to make virtuous this example of integrated aquaculture.

In aquaponics, it is necessary to maximize the recycling rates of phosphorus and nitrogen and to fulfil the quality requirements of the resulting products such as plant biomass and effluent water, without forgetting the welfare of the fish. The decoupled aquaponic systems (DAPS), i.e., the systems where the fish and the plants are managed separately, can represent an optimal compromise to satisfy plants and fish needs.

5. Conclusions

The Farm Animal Welfare Committee in the U.K. issued a report in 2014 called 'Opinion on the Welfare of Farmed Fish' [119]. Based on this report, there is an increase in understanding of the most important factors regarding fish welfare, with water quality being of the highest importance. Water quality is a potpourri of components, including those necessary for survival but also harmful solutes as well as factors setting the limits for survival, such as pH and temperature. Many of these components are interdependent, with optimal ranges affecting each other. They are also species-specific, and optimal values or tolerance limits also vary depending on the ontogenetic stage of the fish. High fish stocking densities in aquaponic systems directly impact water quality as well as other welfare issues such as fin damage, disease transmission and social behaviour (e.g., competition).

Many factors important for welfare are also important for production, notably the avoidance of bacterial and viral diseases, parasites and physical skin and gill damage; thus, both welfare and production benefit from the control of these factors. However, some procedures that are detrimental to welfare are integral parts of the production process—for example, the crowding before and during transport and the handling of fish out of water. It is appropriate to find ways to reduce such impacts, even if they conflict with production priorities.

The principles of fish welfare and bio-security in responsible aquaculture can also be applied to aquaponic systems. It is important that the co-cultured organisms in aquaponics, fish, bacteria in biofilters and plants, be viewed in all applications. Further research to develop specific feeds for aquaponic conditions is needed. Fish health in aquaponic systems is one of the key factors for their sustainability in aquaculture. Nevertheless, specifically for the case of aquaponics, the optimization of water quality considering the characteristics of co-culture production of plants and fish is a vital subject for sustainability and success of aquaponics.

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