Feed Distribution Systems for Zebrafish

Subjects: Fisheries

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Zebrafish (*Danio rerio*) is a well-established animal model, used in a number of research areas. In the last decade, it has also emerged as a tool to evaluate the effects of diets and dietary components and to test novel paradigms in nutrigenomics, nutrigenetics, and nutritional physiology. The standardization of the zebrafish rearing conditions, including daily nutritional and good feed management practices, is not yet achieved. Researchers focus on some recent technological solutions provided by research groups and/or biotech companies in the field of facility design, with emphasis on automated feeding distribution systems.

feeding rearing systems zebrafish Automated distribution

1. Introduction

Zebrafish (*Danio rerio*) is a freshwater teleost (ord. Cypriniformes; fam. Danionidae) that has been used in home aquaria for many years; however, during the last three decades, it has become a key model in a variety of human-biology-related research areas, from biomedicine to toxicology [1][2], from human diseases to therapeutic drugs screening [3][4]. Its use back to fish biology as a tool for complementing research in aquaculture and commercial fish production processes [5][6] has enhanced and further extended its experimental relevance as an animal model. Zebrafish genome shares a high degree of synteny with both lower and higher vertebrate (from teleost fish to human) genomes [7][8]. Its sequence is fully accessible [9], a condition shared by many other teleost fishes, e.g., Japanese fugu (*Fugu rubripes*), green-spotted pufferfish (*Tetraodon nigroviridis*), medaka (*Oryzias latipes*), or three-spined stickleback (*Gasterosteus aculeatus*). Moreover, various established approaches in genetic manipulation make zebrafish transgenic lines available to date [10][11]. Among fish models, zebrafish is most likely the only one offering a very complete panel of experimental advantages, such as easy rearing and breeding in captivity, including very short generation time (≈3 months), large number of eggs (100–200 eggs/clutch), transparency during egg and larval period, and maturation of organogenesis in the larval stage (i.e., organs and systems are all functional making the larva physiologically comparable to the adult) [12]. The advantages of the experimental model go together with a community of zebrafish researchers spread worldwide and a robust and rather advanced technological support on the zebrafish-rearing aquaria systems. Recently, zebrafish has started to emerge as a model for evaluating the direct effects of administered dietary components on functional diet–gene interactions and for exploiting novel approaches in nutrigenomics, nutrigenetics, nutritional physiology, and immunity [13]. Despite the zebrafish use worldwide in the laboratory, the standardization of its rearing conditions, including daily nutritional requirements and good feed management practices, is still poorly studied [14]. To some extent, this is surprising when compared with what is available for other animal models, including terrestrial vertebrates such as rodents [15], or aquatic species such as tilapia [16], channel catfish (*Ictalurus punctatus*) [17], or common carp [18], among others. The reason for the lack of standardization lies perhaps in the fact that zebrafish is such an easy fish to keep in home aquaria that the optimization of standard conditions has never been evaluated as necessary, although it is obvious that parameters such temperature, feed composition, etc., will affect zebrafish like all other animals, regardless of its robustness.

2. Feeding Requirements

Dietary lipids in fish diets represent the main conventional energy source, especially in carnivorous species, although low efficiency rates and different growth performance, wellness, and body compositions among species are generally found [19]. In addition, fish diets do not require specific dietary carbohydrate levels [20]. Thus, proteins remain the most relevant dietary compounds in formulated diets. Notably, fish require higher levels of dietary proteins compared with terrestrial-farmed vertebrates, though this consideration needs to not be taken as absolute. In fact, fish and terrestrial vertebrates differ only in relative protein concentrations for achieving maximum growth rate, and such difference is explained by a lower basal energy needed for fish [19]. On these premises, fish reared under intensive aquaculture conditions are fed with common feedstuffs balanced to supply all the essential nutrients (protein, lipids and, carbohydrates, as well as minerals and trace elements) vital for growth, reproduction, overall wellness, and health [19].

2.1. Formulated Diets

Currently, several different formulated diets are available for zebrafish, including commercial dry feeds and live feed such as *Artemia* nauplii, rotifers (*Brachionus* sp.), *Paramecium caudatum*, and *Tetrahymena*. Among these, dry diets are generally assumed to be nutritionally complete, whereas live feed stimulates the associated predatory (fish–prey capture) behavior [21][22][23]. Zebrafish dry diets can be classified based on ingredient and nutrient composition: while some diets are used for specific nutrient requirements under determined experimental conditions, others have commercial applications and are designed for large-scale production [19]. At the time of the complete development of the gut (at approx. 5 days post-fertilization, dpf) [24][25], *Paremecium*, rotifers, and *Artemia* nauplii are usually administered as first feed because they are useful for increasing survival and early growth, as indicated by various authors [19][26]. After a period of early development (which generally spans from a few days to several weeks), artificial feeds are introduced in zebrafish larval diet [19]. The administered commercial feeds can be used in two different ways, which are as supplement to live diets or as the sole food source [27]. Currently, the most standard and widespread protocols for zebrafish nutrition include the administration of live feed combined with processed feed (usually as fish flake) or specific diets containing fish oil and fish meal, Despite a larger number of commercial dry feeds for zebrafish been commercialized in the last decades [28][29], the standardization of zebrafish feeding protocols has not yet occurred, and its development represents a great challenge. Compared with rodent diets, open formulations for zebrafish are not available, with the consequences that many nutrients (and nutrients) are not established, and the fidelity of many scientific experimentations is decreased. Moreover, the presence of many different diets commercially available leads to different and confounding results. The availability and use of multiple commercially available diets, each often characteristically used by a particular

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research laboratory, and across different life stages (larvae, juveniles, and adults), under scope the confusion that would inevitably result from the variance of results ascribed in the scientific literature [30].

2.2. Feeding Management

The lack of standardization of zebrafish feeding protocols not only concerns the great consideration for implementing factors affecting the daily nutritional requirements [27][31][32], but also includes feeding management practices, which are equally important and should be designed by taking into consideration the nutrient and physical properties of the diet. Many studies are used to report both feed amount and daily/weekly feeding regime (feed ratio and frequency), but each study follows its own personal scheme, and the direct effects of the various feed management criteria on specific outcomes have seldom been investigated [19]. Feeding ratio is the amount of diet administered per fish or per group of individuals, which is usually referred to as grams per individual or percentage of body weight [19]. Feeding frequency, on the other hand, is defined as the number of times feeding is provided (ratio per individual per unit time) [19]. Both practices are often determined by the availability and the economic resources of the operators [19], thus significantly affecting zebrafish nutrition, especially when using formulated diets [33][34][35]. Moreover, the feeding frequency should also be adjusted for different sizes and ages of zebrafish with the aim of reducing the suspension time in the water before ingestion [19]. Furthermore, the common practice in laboratories is to feed zebrafish *ad libitum* (i.e., the animals are offered as much food as they want), which can be followed by leaching from uneaten food and mixing of feeds with fecal material in the bottom of the tanks, thus reducing much of the ability to quantify feed intake—an essential practice in determining daily nutrient requirements—as well as water quality and, thus, fish welfare [19]. In addition to feeding frequency and ratio, feeding time (the time of day or night when the diet is provided) is also greatly affected by the operators [19]. For example, feeds can be administered any time of the day or night, depending on the operators' availability. Since feeding time can affect zebrafish behavior and feed intake [36], specific time(s) of feeding should be standardized and reported. Moreover, if multiple ratios per day are provided, the amount of each food ratio should be determined in order to optimize feed ingestion at specific times [19], since ingestion of a ratio may vary depending on the time per day (or night). To fully assess the potentiality of zebrafish as a model and its increasing use in research laboratories, facilities, and biotech companies (which invariably implies highly controlled fish rearing conditions), the need to combine novel standard diets which satisfies all nutrient requirements with a controlled and reproducible administration setup, far and independent from variables introduced by the operators, is increasingly urgent.

3. Automated feeding systems

In the last decades, both (from) single research groups and (to) biotech companies have developed automated systems for zebrafish including feeders. This has been accomplished to meet adequate rearing and nutrient conditions, improve zebrafish health and welfare, and implement new tools in the culture facilities to reach standardization and/or removal of human error factors. All the solutions are summerized in **Table 1**.

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Table 1. Summary of automated feeding systems for zebrafish.

Automated feeding system	Description
del Pozo et al. [37]	A self-feeder system with an infrared photocell acting as a food-demand sensor (high costs).
Argenton and Pivotti [38]	A small and practical pneumatic device delivering food (low costs).
Candelier et al. [39]	A semi-automatic dispenser for solid and liquid food (low costs).
Tangara et al. [40]	An open-source semi-automatic feeding system for dry and live food (low costs).
Yang et al. [41]	An automatic feeding system coupled with an EthoVision video-tracking system (high costs).
Doyle et al. [42]	An automatic feeder of precise amounts of foods (low costs).
Lange et al. [43]	A fully automated solution which provides standardized amounts of diets (high costs).
Brocca and Frangelli [44]	A robot able to deliver multiple dry and liquid diets (high costs).

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4. Conclusions and Future Challenges

When analyzing aspects of fish nutrition, such as those related to the effects of diets, nutrients, molecules, etc., it is not easy to generate highly significant datasets; this is often due to the absence of common and standardized rearing and feeding conditions for the raised animals. The intensive and increasing use of zebrafish as a well-established animal model in many different fields of the biological, biomedical, toxicological, and environmental research thus makes necessary the development and implementation of new automated systems that allow one to obtain as highly controlled rearing and feeding conditions as possible, and, in parallel, to reduce human labor and remove human errors. To date, the standardization of rearing and feeding protocols by adopting semi-automatic,

automatic, or even robotic, feed distribution solutions is a necessary goal to achieve in zebrafish nutritional research. The improvement of these technologies in conjunction with research laboratories and industry, combined with the thorough comprehension of the regulatory networks supporting the alimentary function(s), on one hand, and to the optimal formulation of experimental and commercial feeds, on the other, will significantly extend the potentialities of the zebrafish as a tool to evaluate the effects of diets, dietary components, ingredients or single nutrient molecules, and to test novel hypotheses in nutrigenomics, nutrigenetics, and nutritional physiology.[16]

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