ZEBRAFISH NUTRITION: PROMOTING FISH HEALTH AND WELFARE OF THE ANIMAL MODEL IN *HALAL* SCIENCE RESEARCH

RAJA IBRAH RAJA IDRIS[1], NORHIDAYAH MOHD TAUFEK[2], NOOR FAIZUL HADRY NORDIN[1], NURHIDAYU AL-SAARI[1*]

[1]International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur, Malaysia.*
[2]AquaNutri Biotech Research Laboratory, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia.

**Corresponding author: hidayusaari@iium.edu.my*

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ABSTRACT: Zebrafish (*Danio rerio*) is a popular animal model in scientific and even *halal* science research. As the use of zebrafish in research grows, so does the need to focus on their welfare to ensure that they live in normal-good health fit for research. Nutrition is an essential but often overlooked welfare factor in zebrafish, as it affects their development, health, reproduction, and response to stimuli. In many modern laboratory settings, zebrafish feeding is dependent on the researcher, with numerous types of feed and feeding regimens used without a rigorous assessment of the fish growth. This is mainly due to a lack of information about zebrafish nutritional requirements, which is the fundamental fault of any nutritional and growth study. A standard fish diet with an adequate amount of nutrition, including the appropriate ratio of protein, carbohydrate, lipids, vitamins, and minerals, are proposed to prevent the unplanned nutritional effect to the outcomes of such experiments. Protein is essential for the growth of the fish, while carbohydrates and lipid are the non-protein source of dietary energy for the fish diet. Therefore, this review addresses some nutritional requirements needed in zebrafish to be examined to produce a healthy zebrafish.

KEYWORDS: *Zebrafish; Danio rerio; Nutrition; Welfare; Halal science research*

1. INTRODUCTION

For more than a century, animal models (worms, flies, frogs, rodents, rabbits, rats, and guinea pigs) have been vital for biomedical and toxicological research. Over the last several decades, zebrafish has evolved as an animal model in the study of human health (Grunwald & Eisen, 2002), such as molecular biology, neurology including genetic research. Despite the zebrafish's growing importance in research, the protocols for effective management and husbandry, especially nutrition, are relatively poorly established as the zebrafish's daily nutritional requirements have yet to be defined. Researchers that utilise zebrafish as an animal model are interested in the paucity of knowledge on anticipated daily food nutritional requirements. In current practice, zebrafish were given live feed such as *paramecium*, *artemia* and *rotifers* as their first feeding. As they grow older, the zebrafish will then be fed with a formulated diet that is available commercially. However, the qualitative and quantitative composition in many formulated-commercial diets is unknown, (Fowler et al., 2019). There is a risk of unintended diseases from the live feed diets, which are equally unpredictable and unknown in the worst-case scenario. Research that uses zebrafish as animal models needs to consider the quantity and quality of specific nutrients to avoid mistakes that jeopardise the experimental results and cross-contaminate the rearing facilities. A range of commercial meals was used in zebrafish laboratories, including Otohime C1, Tetramin Tropical Flakes, Ziegler Larval AP100, and Gemma Micro 300, which have recently gained popularity.

It was believed that different diets would produce different effects according to their nutritional content. Nutrition studies show the importance of producing standardized feeding practices, appropriate reporting criteria, and nutritionally complete reference diets. A previous study conducted by Penglase, Moren, & Hamre (2012) mentioned that the standardization of diets for laboratory rodents in the 1970s minimized the nutritional effects to the outcome and made the result or research more reliable. There is a general lack of dietary nutrient information for zebrafish. Thus, this review will briefly explore and identify the type, criteria, and amount of critical nutrients required in laboratory zebrafish that should be considered to promote fish health and welfare, especially in *halal* science research.

2. ZEBRAFISH AS AN ANIMAL MODEL

Zebrafish or *Danio rerio* is a freshwater fish native to South Asia that can be found in a shallow pond, canals and slow flowing water. Zebrafish can survive in a wide variety of environmental circumstances, including water with a temperature range of 10°C to 40°C, water with a pH of 6–10 and clear or murky water, (Alestrom, 2020). Although zebrafish may live in a considerable variety of environments, many attempts have been made to provide suitable laboratory conditions, as zebrafish is an excellent model organism for many studies, such as molecular biology, neurobiology, genetic research aquaculture, and aquaculture even in *halal* science research. Historically, zebrafish have been used in research since the late 1960s, whereby George Streisinger is considered the founding father of zebrafish research. Over the years, scientists have better understood the biology of zebrafish, which led to an increasing number of advantages and applications being claimed in their research. Figure 1 depicts the number of scholarly papers mentioning ‘zebrafish’ published each year during the last decade, from 2011 to 2020. A total of 29,227 documents were retrieved from the Scopus database (www.scopus.com) using the keyword ‘zebrafish’ for publications that include research articles, review papers, book chapters, short survey, letter, book and data papers. It is clear from the data that the usage of zebrafish for research has increased, as has the number of zebrafish facilities around the world.

Zebrafish has gradually risen as one of the top animal models in both animal and human health research and more recently in aquaculture too due to their advantage characteristics such as short reproductive cycle, a high number of offspring due to high fecundity and rapid development that only require a few months to become adult, (Bournele & Beis, 2016). Figure 2 describes the short life cycle divided into three significant periods (fertilized embryo, larva and adult). The entire development of zebrafish from fertilized embryo to an adult only need 3 to 4 months, and its average lifespan is up to 3 to 4 years. Zebrafish embryos are translucent, allowing in vivo observation and real-time investigation of organs, tissues, and cells, (Modarresi Chahardehi et al., 2020). On the other hand, the availability of high level genomic sequences of zebrafish to humans (approximately 70% of human genes have at least one obvious zebrafish ortholog) (Choi et al., 2021) has also made them a suitable animal model for molecular, genetic and nutrigenomic studies.

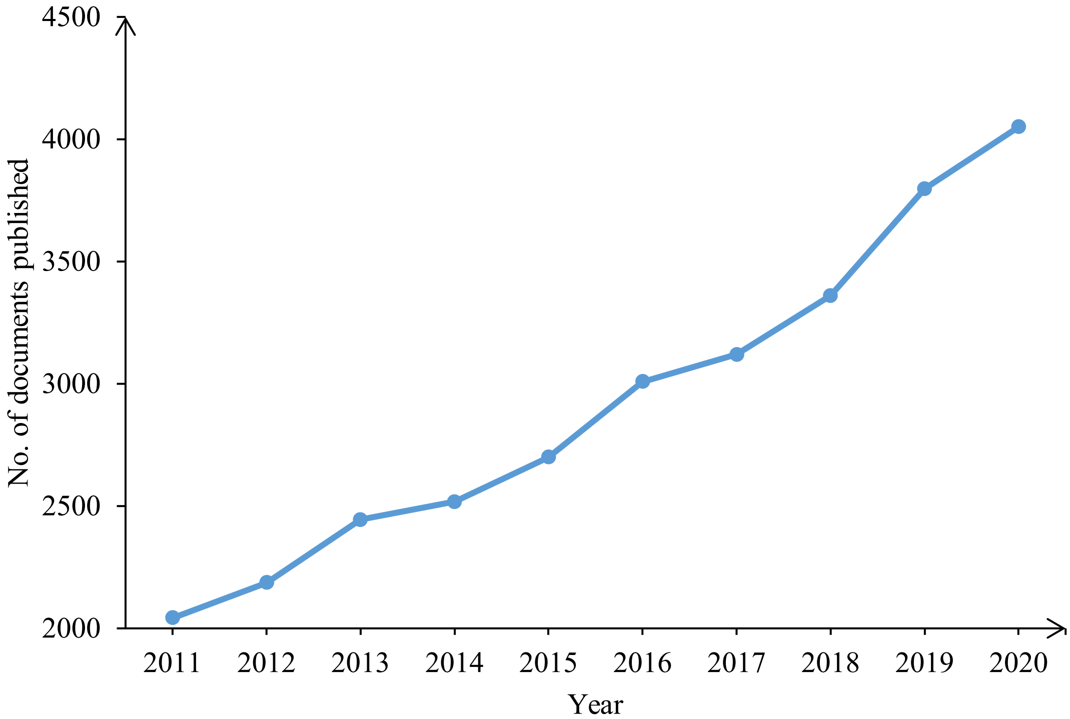

Fig. 1: The number of references found each year on the Scopus database (www.scopus.com) of Elsevier (Netherland), using the keyword 'zebrafish' for publication dated 2011-2020.

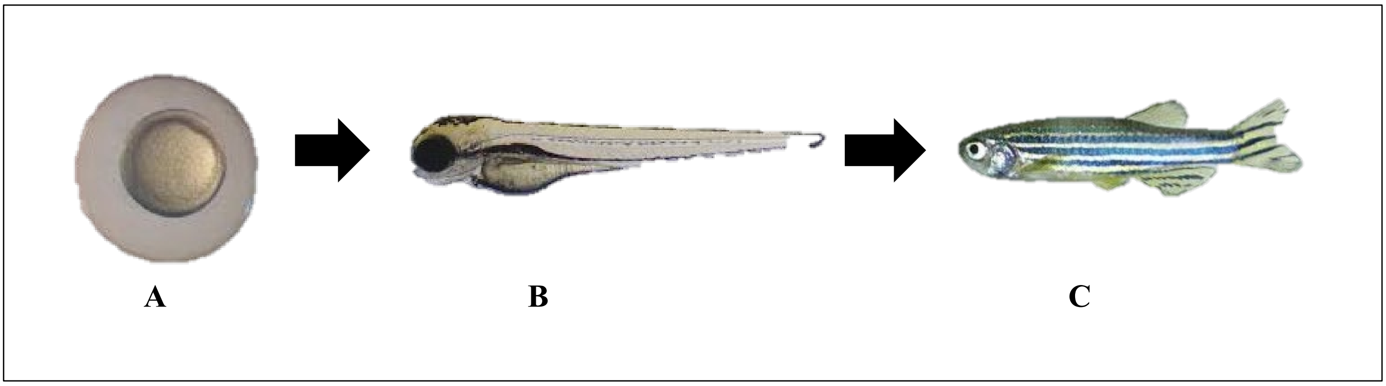

Fig. 2: Development of zebrafish from fertilized embryo to adult that takes only 3 months to become an adult and achieve sexual maturity A: Fertilized embryo (0-3 dpf), B: Larva zebrafish (4 dpf-30 dpf), C: Adult zebrafish (90 dpf onwards).
Dpf = Day post fertilization

3. FISH NUTRITION TOWARDS ANIMAL HEALTH AND WELFARE

In recent years, fish nutrition has evolved widely with developing a new and balanced fish commercial diet that is proper for fish to grow healthy and affordable. Usually, complete fish diets require all the ingredients such as carbohydrates, protein, lipids, vitamins and minerals for optimal growth and health of the zebrafish. The amount of nutrients required varies by species, which means that individual fish may require different nutrients, or even amounts of the same nutrients, during a particular stage of their lives. There is insufficient information about adequate nutrients such as protein, carbohydrate, lipid, mineral, and vitamins to achieve optimal zebrafish development and health. The following paragraphs provide an overview of some essential nutrients in fish diets and their functions and research updates on nutrients in zebrafish diets.

3.1 Protein

Protein is an essential nutrient required in a fish diet as it supplies amino acids (AA) for energy, growth and cell maintenance, (Andersen et al., 2016). Protein is the most expensive nutrient in fish diets; therefore, it is essential to find out the correct value or amount of protein requirement for each species that also depends on the life stages of fish. Proteins are composed of various amino acids that are either 'non-essential' or 'essential' (Gatlin, 2010), as each protein's composition reflects its distinct properties and functions. About 200 amino acids occur in nature, but only 20 amino acids are prevalent in fish. From these 20 amino acids, 10 are essential (indispensable) amino acids that cannot be produced by fish and must be supplied in fish feed (Craig et al., 2017), such as arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine. The other 10 are non-essential (dispensable) amino acids, for example, alanine, asparagine, aspartic acid, cystine, glutamic acid, glutamine, glycine, proline, serine, tyrosine, (Barnie, 2013). By definition, fish can synthesize an adequate amount of all the non-essential amino acids by themselves, (Li et al., 2009).

It is essential to determine and give zebrafish dietary protein and specific amino acid requirements to promote optimal growth and health. In general, the protein requirements are higher at the early life stage of fish, and it will decrease as fish grow larger. The dietary protein requirement also depends on the various environmental factors such as water temperature, water quality, dietary protein energy and feeding frequency of the fish, (Craig et al., 2017). This has also been explored in a prior study by Singh et al. (2016) revealed that body weight gain and hepatosomatic index (HSI) in Asian catfish, *Clarias batrachus* fry was influenced by the temperatures. This study evaluates the protein requirement of *C. batrachus* at two different water temperatures, 28°C and 32°C, with four diets containing different levels of protein which were 28%, 32%, 36%, and 40%. Diet with a 36% protein level showed the highest mean final body weight at 32°C. Dietary treatments and temperatures affected final body weight significantly ($p<0.05$). Diet with a 28% protein level at 28°C had higher feed efficiency (93.20%) than *C. batrachus* fry reared at 32°C (87.58%). Further, feed efficiency decreased with an increase in dietary protein levels. Furthermore, optimal daily protein intake showed higher daily weight gain at 32°C.

However, protein will not work alone; it will be utilized for fish growth if an adequate amount of other nutrients such as lipids and carbohydrates are present in fish feeds or diets. In this case, protein is quite expensive; the absence of lipid and carbohydrates will cause more protein to be used for energy and life support rather than growth, (Craig et al., 2017). Furthermore, based on Table 1, zebrafish must have ten indispensable or 'essential' acids (EAAs) to ensure protein synthesis. The inadequate daily requirement of a single EAA will decrease protein synthesis and then cause body protein to hydrolyse, leading to reduced muscle gain and, in extreme cases, loss of lean body mass. Dispensable or 'non-essential' amino acids (NEAAs) can be produced by transamination of dietary carbon skeletons that depends on the dietary protein intake and body protein hydrolysis. As mentioned earlier, NEAAs cannot be produced by fish, so the diet's inclusion of some NEAAs is essential to prevent the utilization of EAAs in the de novo synthesis of NEAAs, (Fowler et al., 2020). Deficiency and imbalance of functional amino acids can affect fish's homeostasis and body metabolism, (Andersen et al., 2016). The nitrogenous compounds such as hormones, vitamins, neurotransmitters, and other compounds require a specific amino acid precursor to regulate metabolic function, (Fowler et al., 2020). Recently, many researchers have shown a great interest in amino acid studies that can act as disease resistance, immune response, reproduction, behaviour and others. This has led to a boost of commercially available functional fish feeds that aim to optimize fish performance and quality of the product, (Andersen et al., 2016).

Presently, the dietary protein and indispensable amino acids for zebrafish are unknown. Zebrafish belongs to the *Cyprinidae* family, commonly called the minnow family, including carp and goldfish. Since the dietary protein nutrient for zebrafish is unknown, the diet can follow the other cyprinids members. This was successfully established as described by Harper & Lawrence (2016); the minimum requirement of protein for other cyprinids varies from 29% to 60%, which appears to support the growth of zebrafish and reproduction, depending on the setting. Besides, a previous study conducted by Fernandes et al. (2016) ran an experiment on the dietary protein requirement of juvenile zebrafish during the growth. According to the article, the estimated protein content in the zebrafish diet was between 37.6% and 44.8% for maximum weight gain and protein retention. The research study by O'Brine et al. (2015) also suggested that 32% of dietary protein is sufficient to meet the growing requirement for zebrafish. Usually, commercial diets consist of excessive protein that can be up to 60%, sometimes leading to a pollutant diet for the zebrafish. Amino acids over levels required are metabolized for energy, and the amine portion becomes ammonia that fish excrete and pollute the water. Furthermore, excessive protein also can cause a progressive decrease of protein efficiency ratio and protein retention in zebrafish, (Kaushik & Hemre, 2008).

3.2 Carbohydrate

Currently, there is limited data available for the dietary requirement for carbohydrates has been revealed in fish, (Hamre et al., 2002). However, carbohydrates are a vital energy and carbon source in feed formulations, (Kamalam & Panserat, 2016). Carbohydrates are the least expensive form of dietary energy for animals, and their utilization is varied according to the species. In addition, carbohydrate is one of the essential nutrient needed in the diet or food of fish. The absence or inappropriate carbohydrate level in diets can cause other nutrients such as protein and lipids to be catabolized for energy. It has been estimated that zebrafish display favourable growth feed conversion and weight gain rates when fed with a diet containing 25% of carbohydrate, (Harper & Lawrence, 2016). Fang et al. (2014) also emphasized that diets containing digestible carbohydrates can promote the rapid growth of fish. Furthermore, carbohydrates in fish will be stored as glycogen to satisfy energy demands later.

Furthermore, carbohydrate utilization is varied among the fish species depending on their feeding habit and digestive system. It has also been recognized that omnivorous and herbivorous fish have a higher ability to utilize dietary carbohydrates than omnivorous. It is also indicated that marked differences in intestinal mucosal enzymes' activity among the different fish species were observed corresponding to their feeding habit, (Wang et al., 2016).

The carbohydrate level needed for zebrafish is still unknown. However, Ma et al. (2020) had mentioned high carbohydrate diet (about 48%) could improve resistance to hypoxia stress for zebrafish. It is caused by the increased glycogen deposition and supply of glucose as fuel during an emergent state. Furthermore, the high carbohydrate diet enhanced the activation of an insulin signalling pathway involved in ATP production.

3.3 Lipid

Dietary lipids are also essential nutrients needed in fish feed for fish growth as it provides energy and supplies essential fatty acids (EFA). Presently, little information or data is available on dietary lipid requirements for zebrafish. High lipid diets have increasingly been used in aquaculture as it contains high energy nutrients that usually can be used as spare (substitute for) protein, (Craig et al., 2017). In general, lipid has double energy yield compared to protein and carbohydrates. In fish, the level of lipids in fish feed is approximately 7-15%, which can supply adequate essential fatty acids and serve as transporters for fat soluble vitamins, (Chatzifotis, 2010). A current trend in fish feed is to use higher levels of lipids in the diet for cost practical farming in aquaculture as lipid has a protein sparing effect and the protein source is quite expensive. However, high levels of lipids in fish feeds will affect the health of the fish, such as ectopic lipid accumulation in the tissue at liver and abdominal adipose tissue, metabolic disturbance and short shelf life of the final product, (Yan et al., 2015; Craig et al., 2017).

Lipid metabolism of the fish is similar to mammals mainly take place via the lipoproteins (LPs) process that undergoes two loops: an exogenous loop and an endogenous loop. Generally, the zebrafish zygote consists of a mass of yolks containing a lipid-rich structure. As the zygote turns into an embryo and develops into free feeding larva, the yolk contents will be decreased. Yolk lipids are packaged into lipoproteins in the yolk syncytial layer (YSL) before being exported to the body of the developing zebrafish, (Quinlivan & Farber, 2017).

3.4 Mineral

Minerals are typically divided into two groups which are macrominerals (sodium, chloride, potassium, calcium, phosphorus, magnesium, sulphur) and microminerals (iron, zinc, iodine, selenium, copper, manganese, fluoride, chromium, molybdenum), (Swain & Senapati, n.d). Minerals are non-organic substances necessary in the fish feed to maintain normal body functions. All aquatic animals require an adequate amount of minerals to support growth and metabolic physiology. Fish can absorb many minerals directly from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet, (Craig et al., 2017).

Macrominerals dietary are essential to regulate the osmotic balance, bone formation and integrity of the fish. Dietary deficiency of most macrominerals is challenging to detect because the gills' waterborne ions have been uptake. However, based on the previous literature, phosphorus is one of the macrominerals available only in water, (Banrie, 2013). Phosphorus is a significant constituent of hard tissues such as bone and scale. It is also present in various biochemicals. The deficiency of phosphorus in fish diet will affect the growth and feed efficiency and decrease tissue mineralisation and impaired skeletal formation for juvenile fish, (Fraser et al., 2019). Sodium, potassium, and chloride are electrolytes usually abundant in the water and fish diet, essential in osmoregulation and the acid base balance in the body. Besides, magnesium is usually found in the fish feed involved in intra and extracellular homeostasis in cellular respiration.

Microminerals, also known as trace minerals, are required in small amounts as components in enzyme and hormone systems. Iron, manganese, copper, selenium and zinc are the most critical supplement in fish diets as usually fish feed contains a low level of these trace minerals. In addition, the interactions of the microminerals with other ingredients may lower their bioavailability, (Banrie, 2013). Each of the trace minerals carries its essential functions to zebrafish. For instance, iron is an essential component in producing haemoglobin (Afifah, 2020) in red blood cells, essential for the transportation of oxygen throughout the body. Fishmeal, meat meal, coconut meal, and rice bran are dietary sources rich in iron. Manganese, another mineral, plays a vital role in vertebral development. In zebrafish, the regulation of manganese is not well understood, but insufficient manganese has been shown leading to the low body and vertebrate and dwarfism (short stature), (Prabhu et al., 2019). Copper and zinc function similarly on the oxidation-reduction enzyme mechanisms such as uricase, tyrosinase, amine oxidase and are also necessary to produce the pigment melanin. Rich dietary sources of copper such as soybean, corn gluten, linseed, and crab meals are essential components for metalloenzymes. Selenium, another example, is an active antioxidant that protects against reactive oxygen species. It is required to boost fish growth since it also functions as an antioxidant in stress responses, (Iqbal et al., 2020). Taken together, previous findings have shown the importance of all these minerals to zebrafish.

3.5 Vitamins

Fish needs vitamin to support their growth and health. Vitamins are not synthesized by the fish and must be provided in the fish diet. Vitamins are organic compounds that can be classified into two groups which are water soluble and fat soluble. Water-soluble vitamins include B vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, and cobalamins, inositol, choline) and vitamin C (ascorbic acid), (Craig et al., 2017). The most important vitamin is vitamin C because it has an excellent antioxidant activity to enhance fish's immune system. Fat-soluble vitamins include vitamins A (retinol, betacarotene), D (cholecalciferol), E (tocopherols), and K (phylloquinone).

Furthermore, among vitamins A, D, E and K, vitamin E gets the greatest attention because it carries a vital role as an antioxidant. Vitamin E and C as an additive can inhibit dietary lipid oxidation and help to improve shelf life, (Banrie, 2013). The most common symptom of any vitamin deficiency is a reduced growth rate. Scoliosis and dark colouration may result from ascorbic acid and folic acid deficiencies, respectively.

4. THE NEED FOR A STANDARD DIET FOR LABORATORY ZEBRAFISH

The value of the zebrafish model has been well established, and zebrafish possess several advantages over rodents. Therefore, the nutrition of this species should be approached to evaluate the nutrition then zebrafish can be defined as a normal healthy subject. The development of standardized zebrafish nutrition should start with understanding basic terminology associated with nutrition research, such as the importance of nutrients to animals' physiology (Watts et al., 2012). To date, the scale and sophistication of zebrafish research have even more expanded due to the wealth of genetic and genomic information available. It has been used in many nutrigenomic studies to describe dietary-induced health problems in humans such as obesity, gut inflammation and cardiovascular diseases, (Williams & Watts, 2019). As the use of zebrafish in many research grows, so does the need for standards and guidelines to support the maintenance of healthy fish for experiments. Moreover, little research has been done on the health implication of low or high protein and carbohydrate ratios on zebrafish. Nutrition is a significant, though long-overlooked, contributor to zebrafish welfare.

Besides, laboratories' lack of healthy control remains a concern relative to research inconsistencies. As observed in zebrafish, the diet's lack of nutrition or dietary ingredients can alter physiology, behaviour, and molecule pathway. It can also affect the interpretation or explanation of past and future research. The previous researcher also reported that healthy diets given to the adult zebrafish could produce a better growth development and healthy offspring, (Fowler et al., 2019).

Recently, various commercial diets have been utilized in different zebrafish laboratories. Fowler et al. (2014) had studied the proximate analysis of the commercial diets of zebrafish that were being highly utilized in laboratories according to the Zebrafish Husbandry Association (ZHA). Varied diets were thought to have different impacts depending on the nutritional composition of the diets and the fish itself. For instance, saponins and vegetal protein (components of plant-based meals) enhanced some fish's intestinal inflammation, especially in carnivorous fish. As an alternative protein source, soybean meal (SBM) has been used in fish feed production. Previous research conducted by Fuentes et al. (2014) reported no increase in the number of granulocytes associated with the digestive tract when zebrafish larvae fed a diet with a low concentration of soybean meal components. Table 1 summarises different studies on zebrafish's nutritional requirements at different life stages. Specific nutrients and dietary ingredients, or their absence, can potentially alter physiology, behaviour, and/or molecular pathways in zebrafish, as observed in mammals. Other studies have suggested that the nutrient content of adult zebrafish diets can even influence the development and health of their offspring, (Fowler et al., 2014). The evidence above emphasises the need for standardized feeding practice, appropriate reporting criteria and nutritionally complete reference diets for rearing laboratory zebrafish.

Table 1: A summary of research on different nutritional requirements of zebrafish.

No.	**Type of Nutrition**	**Age of Zebrafish**	**Main Conclusion**	**References**
1.	Protein level	Juvenile	This study indicated that the dietary protein requirement for juvenile zebrafish was 37.6% for maximum weight increase. A high protein content (up to 60%) can raise the ammonia level, resulting in water pollution. When fish consume an excessive amount of dietary protein, protein consumption decreases. Additionally, the protein level consumed affects the overall body composition, as feeding zebrafish a high protein diet results in a more extensive water and protein content with reduced energy content.	Fernandes et al., 2016
2.	Plant-based meal: Soybean components (saponins and soy proteins)	Larvae	In some fishes, fish feed containing plant-based foods such as saponins produced intestinal inflammation. The high saponin content of fish feed stimulated the formation of granulocytes and the expression of genes associated with the innate immune system in larvae zebrafish. A high concentration of saponins resulted in an anti-infection gene	Fuentes-Appelgren et al., 2014

			expression profile and elevated regulation of defence-related proteins such as *mpx* and *c3b*.	
3.	Protein: Indispensable amino acid (AA), lysine	Larvae	This study reported that inadequate lysine in fish feed reduced the growth rate and muscle proteins. Muscle protein such as myosin isoforms and α-tropomyosin declined when zebrafish were fed with a lysine deficient diet, as shown in whole body proteomic analysis.	Gómez-Requeni et al., 2011
4.	Unsaturated fatty acids	Adult female	This research examined the influence of highly unsaturated fatty acids on the tissue fatty acid profile and reproductive performance of zebrafish using squid oil and linseed oil. Zebrafish were fed three different experimental diets: SO (100 percent squid oil), SLO (50% squid oil, 50% linseed oil), and LO (50% squid oil, 50% linseed oil) (100% linseed oil). In summary, linseed oil affected the deposition of docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6), in all tissues, since the higher the linseed oil concentration, the more fatty acids were deposited. Additionally, zebrafish fed SLO produced the most embryos and had the highest hatching rate of eggs.	Jaya-Ram et al., 2008
5.	Artemia Flake feed Trout starter Mixture of artemia, flake feed and liver paste	Adult	Flake feed had the lowest number of eggs with the highest percentage of the hatched embryo. Zebrafish fed with trout starter produced the highest number of eggs but the lowest percentage of the hatched embryo. After a few days, the mean length of the newly hatched embryo was measured, fish fed with flake feed manifested the most extended length.	Markovich et al., 2007
6.	Vitamin E: (α-tocopherol)	Embryonic	Vitamin E: (α-tocopherol) is involved in the reproduction system of rodents as it is needed to prevent fetal resorption. Zebrafish	Miller et al., 2012

			have been used to be an animal model in this study. One old month zebrafish fed with a diet with sufficient vitamin E and low vitamin E for up to 1 year. Growth parameters for both groups showed similar trends. However, the zebrafish fed with inadequate vitamin E produced the embryos with higher mortality at 24 hrs post-fertilization and at 120 hrs post-fertilization had a combination of higher malformations and mortality of embryos compared to zebrafish fed with adequate vitamin E. In short, vitamin E is essential for normal zebrafish embryonic development.	
7.	Protein	Larvae to juvenile	Overall, zebrafish consumed diet with gluten protein had the shortest in length and lower body weight, but high percentage of body fat compared to other diets. Zebrafish fed with a casein protein diet also reduced weight gain and body length with a low percentage of body fat.	Smith Jr. et al., 2013
8.	Protein	Juvenile	This study evaluated the growth rate and growth related gene in the muscle of zebrafish after consuming two different diets that contained plant protein and fishmeal based diet. Zebrafish fed with fishmeal diets had significantly more significant body weight gain than plant protein diets. Moreover, a plant protein based diet influenced the expression of *Igf2a*, Myogenin, and *Mrf4* in the muscle of zebrafish males	Ulloa et al., 2013
9.	Commercial diet: Artemia Z12 Tetramin Gemma Otohime Zeigler	Larvae to adult	At the conclusion of the feeding trial, zebrafish fed with Otohime had the highest mean body weight and length and the highest percentage of successful spawning. Tetramin generated the zebrafish with the shortest mean length at the conclusion of the feeding trial. Additionally, the fish	Fowler et al., 2014

has the highest lipid content in their corpse. Additionally, fish fed the Ziegler diet resulted in the lowest lipid percentage of dry matter in the fish carcass. While the Gemma commercial food resulted in fish with the greatest mean body length at the end of the feeding trial, compared to those given Otohime C1, it also resulted in the fewest viable embryos. Finally, most zebrafish given artemia increased in weight and height throughout the first six weeks. Later in the feeding session, the rising rate was decreased, and the mean body weight eventually reached its lowest value. This diet has resulted in the fish having the highest rate of successful spawning.

5. THE CONCEPT OF ANIMAL WELFARE FROM *HALAL* SCIENCE RESEARCH

Animal welfare must be included both physical and mental well-being. One concept of good animal welfare is ensuring the animal feels well and functions properly. An adequate balanced diet can avoid the physical and psychological suffering from thirst and hunger. In addition, a properly balanced diet is crucial to maintain optimal performance and sustain optimal fitness. Generally, animals in farm or laboratory settings require energy, protein, carbohydrate, lipid, minerals, and vitamins supplied in the diet. Diet also should be palatable and free from biological and chemical contamination, (Hoitinga & Strubbe, 2007).

There are no past documents that specifically state the usage of zebrafish in *halal* science research. The Academy of Sciences Malaysia, in their 10-10 MySTIE framework, has recently defined the term *halal* science as "a scientific research pursuit aimed at supporting, expanding, and sustaining the *halal* industry globally. This encompasses the analysis and the use of new technology to create innovative products and services with great impact to society and environment". Therefore, we suggest some criteria to look for when searching for research articles related to *halal* science based on the definition of *halalan toyyiban*, which should contain an/all authors with some Islamic or Arabic name or having at least one affiliation from Islamic institution or country. In an analysis that follows that definition, this paper found that zebrafish are commonly used (but not limited to) in food safety research (Daddiouaissa et al., 2020), pollutants, and toxic compounds in the environment (Khan et al., 2019 & Rahman et al. 2020) an assessment on drug safety and toxicity, (Hayati et al. 2018 and Utami & Miladiyah, 2021). A previous study has revealed that a rising number of individuals, even non-Muslims, regard the *halal* label as a quality trait of credence, (Verbeke et al.,2013). Islam teaches zero-tolerance to all forms of animal abuse throughout the *halal* production supply chain, and although not many, there are a few demands on animal welfare as a purchase criterion, (Dudinskaya et al., 2021). Therefore, as far as *halal* science is concerned, care must be taken to ensure the protection and welfare of laboratory animals utilised in research.

6. EFFECTIVE MANAGEMENT OF ZEBRAFISH

This section addresses the suggestions of the effective management of zebrafish in a laboratory setting specifically for environmental conditions (temperature, dark-light cycle, water parameters) and feeding. The zebrafish is a Southeast Asian tropical fish that can live in a wide range of temperatures within 10°C to 40°C, but the optimal temperature for the best development speed is 28.5°C. It is essential to monitor the temperature of the water as it can affect the water chemistry and animal physiology, (Lawrence & Mason, 2012). The high temperature will drop the solubility of the oxygen in the water and cause the gasping behaviour at the water's surface to indicate the insufficient oxygen level. The recirculating water system is commonly used in a laboratory setting as it provides constant water motion that gives an adequate amount of oxygenation without any extra methods. Furthermore, the dark light cycle in nature varies depending on the seasons and weather, but in laboratory settings, the dark light usually provided to the fish is 10 hours dark and 14 hours light (Alestrom et al, 2020) that mimics the sunrise sunset. Applying the different setting will not affect the welfare of zebrafish, but zebrafish needs an entire dark period for better reproductive performance, (Villamizar et al., 2014).

Monitoring water parameters (chlorine, ammonia, nitrate, and nitrite) in aquarium or tanks are also essential to ensure the welfare of the zebrafish. Some laboratories depend on tap water, and some modern laboratories use reverse osmosis (RO) water for zebrafish. In tap water, chlorine needs to be removed as it is toxic to zebrafish. The safe level of chlorine for fish is 0.1 mg/l, the same for humans. Moreover, the recommended for ammonia, nitrate and nitrite are < 0.1 mg/l, < 25 mg/l and < 0.3 mg/l respectively, (Alestrom et al, 2020). Daily basis of water exchange in aquarium or tank typically 5% to 10% can keep the ammonia, nitrite and nitrate level low as high amount of the harmful pollutant can cause the fish cannot survive and sudden death without any physical abnormalities.

One of the main components of zebrafish management is the type of feed and feeding regime to zebrafish. Zebrafish have different life cycle stages, such as larvae and adult. In general, zebrafish accept a combination of both live and processed diets as both promote unique advantages such as growth at different stages of life. *Paramecium caudatum*, *rotifers* (*Brachionus* sp.), and *Artemia nauplii* are live organisms associated with fish prey-capture behaviour, whereas processed diets must be nutritionally completed, (Varga, 2016). At the larvae stage, zebrafish can consume the live feed with a smaller diameter, such as *Paramecium caudatum* and *rotifers* (*Brachionus* sp.). Naturally, live organisms can act as pathogen vectors; it should be managed appropriately to ensure pathogen free sources. Feeding frequency should be adjusted according to the zebrafish's developmental stages. Larvae multiply and should be fed two or three times a day with live feed or dry diets either by manual or automated feeding, (Lawrence et al., 2012).

7. CONCLUSION AND RECOMMENDATION

In conclusion, this review introduces the basic nutrients needed to understand producing fish diet, particularly for zebrafish. The essential ingredients such as protein, carbohydrate, lipid, minerals, and vitamins must be inadequate to ensure the fish grow at optimum level. The growing popularity of zebrafish as an animal model in many research fields, including molecular biology, neurobiology, genetic research, aquaculture, and others, has led the zebrafish community to seek a detailed understanding of nutrition in zebrafish. When using laboratory animal models to study human health, experimental variability must be minimized within and among research studies. Effective management of laboratory animals is essential when controlling for experimental variability, especially in nutrition, because there is little information available on a standardized diet for zebrafish. To this extend of knowledge, the nutrition requirement for zebrafish is indicated the same as other cyprinid species. The availability and utilization depend on the quantity and quality of a nutrient in a fish diet provided. Different quantities and quality potentially influence multiple outcomes in many research studies such as growth, reproduction, and disease development. It is hoped that this review has provided a perspective for the zebrafish nutrition studies to ensure the welfare of the zebrafish as one of the essential elements in animal welfare is diet. The animals need to eat proper nutrition in order for them to live in normal conditions without suffering from any disease and stress.

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