COMPARATIVE ANALYSIS OF ZEBRAFISH AND CHANNA PUNCTATUS WITH DIFFERENT DIET CONDITION

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ABSTRACT

Channa punctatus, a species of zebrafish, is used because of the zebrafish model's proven usefulness in studying this species' eating behaviors. Nonetheless, dietary differences across cultures grown in different labs continue to be a cause for worry. By creating standardized reference and open formulation diets, these researchers were able to reduce the nutritional heterogeneity seen in the majority of trials. Researchers working with zebrafish have been slow to adopt this strategy, as seen by the discrepancy between the results obtained using standard diets and those obtained using experimental diets. The absence of nutritional knowledge and uniformity in the zebrafish research community is discussed.

KEYWORDS:standard reference diet, zebrafish, process of production, nutrients, ingredients, testing

INTRODUCTION

When comparing the years 2001–2016, the average annual growth rate for global aquaculture output of farmed aquatic animals was 5.3%, although this rate slowed to 4.0% in 2015 and 3.2% in 2016. In 2016, aquaculture accounted for just 25.7% of global fish output; by 2015–16, that number had risen to 46.0%. (FAO, 2016). In order to increase production, feeds that include all the necessary nutrients for fish maintenance and growth must be developed. But, technological inputs like aquaculture also play a crucial role in the expansion of the aquaculture sector. Protein is a critical component of fish feed because it helps fuel vital metabolic processes and provides amino acids for development and protein synthesis. Protein quality, composition, and amount of critical amino acids all have an impact on fish development, feed consumption, and resistance to disease. Protein synthesis is severely impacted by a lack of any necessary amino acid, thus it's important to get all of the essential amino acids into your diet.

As the amino acid utilization coefficient varies depending on the components and the kind of fish being fed, knowing this information is crucial for creating diets that are both sustainable and adequate in amino acids. To guarantee the fish reach their full development potential, it is necessary to carefully determine the minimum required amino acid need while preparing refined meals that are both tasty and highly digestible. The diets utilized in this study are made from refined components like casein and gelatin that are both easy to digest and enjoyable to eat. To ensure that the fish got the same amount of amino acids from the casein and gelatin digestion as they did from the crystalline amino acids provided to their meals, the latter were coated with gelatin and casein before being finished off with CMC.

Wheat grain, mung bean, hydrolyzed feather, flesh and bone, soybean, maize, corn gluten, and fish silage are all examples of plant-based substances used in fish diets; nevertheless, tryptophan is a limiting amino acid in these formulations. Protein synthesis necessitates it, and it's also a precursor to things like the stress hormone melatonin and the anti-anxiety and immune-boosting neurotransmitter/neuromodulator serotonin (5-hydroxytryptamine) and the B vitamins kynurenic acid, quinolinic acid, and niacin.

The spotted *Channa punctatus*, is a freshwater fish that has historically been cultivated throughout South and Southeast Asia. The superior flavor and texture of its meat, its ability to heal wounds and internal injuries, its enhanced resistance to illness, and its high economic worth all contribute to its widespread popularity. As a result, there is a significant demand for this species throughout Asia.

During the 1970s, when diets for laboratory mice were standardized, the contribution of unwanted dietary effects to experimental findings has been reduced, allowing for more accurate comparisons between trials. While the zebrafish (Danio rerio) has been a very successful model organism, it is being given a variety of commercial diets the nutritional content of which is mostly unknown.

Despite the fact that zebrafish (Danio rerio) is widely used as a high-throughput development, toxicologic, and biomedical model, its daily food needs remain unknown.

Commercially available designed foods are sufficient for zebrafish of all ages. The combination of the two allows for adequate zebrafish development and reproduction. Unfortunately, it is difficult to assess the health of fish fed either live or commercial diets because of the lack of information on the contribution of certain nutrients. We would at least want to have the dietary control plan clearly specified in publications concerning such applications, even though the usage of unknown nutrition sources may not be of considerable relevance for them. Yet, the amount and quality of certain nutrients are crucial when using zebrafish as a model of human illness. Researchers working with zebrafish should learn from the blunders produced with mammalian models (mainly rats and mice) that used undefined meals. So, in investigations where illness initiation may be influenced or have a contributing nutrient, dietary interaction, or antinutritional substance, feeding the fish a chemically defined, nutritionally complete diet is crucial.

LITEARTURE AND REVIEW

Smita Sonawane et al (2012) Based on biochemical investigations conducted in KaigaonToka Dist. Aurangabad, this study details the diet and feeding behavior of Channa punctatus (Bloch, 1793). (M.S.). By analyzing the contents of their stomachs, researchers were able to learn more about the feeding habits and seasonal diet fluctuation of Channa punctatus from December 2007 to November 2009. Crustaceans, insects, mollusks, fish, and just a little amount of plant matter made up the diet, which reflected its carnivorous character and had clear seasonal variations. Glycosidases, proteases, and lipases, all digestive enzymes, have been found in distinct parts of the gut in biochemical research.

A. Surjya Narayan Datta, et al (2013) Channa punctata development under various feeding regimens was compared. The by-products of the agricultural and food processing industries were used to create six iso-proteinous diets. Specific growth rate of 0.408 was achieved with a diet composed of 66.75 percent rice bran, 11.50 percent mustard cake, 23.1 percent groundnut cake, 5 percent molasses, 1.5 percent vitamin-mineral combination, and 0.5 percent salt. The value of exponent 'b' was measured to be between 2.7675 to 4.3922 in the experimental fish. All of the fish in the experiments had condition factors ('K') over 1.0 (1.094-1.235), suggesting that they were healthy and robust.

According to C. P. Singh et al (2013) Channa punctatus' diet and feeding behavior were compared between its natural pond habitat and a reservoir in the Tarai area of Uttarakhand. Quantitative and qualitative analyses of gastrointestinal contents in addition to body size and dining habits were recorded. The ratio of body length to stomach length for the naturally occurring fish population in the reservoir was rather high, at 1:2.1. Crustaceans, insects, mollusks, tiny fish, and partially digested material make up the bulk of the gut's composition. The percentage of incidence of the food items was different between reservoir- and pond-reared wild stock ($p < 0.01$). The research found that the biological demands of the same fish species in terms of food and eating pattern are affected by the seasonal fluctuation of natural food items in various environments and their biological variety.

Pilar E. Ulloa et al (2014) To create a more environmentally friendly and sustainable aquaculture production system, the aquaculture sector has been pushing for increased variety in aquafeed components. Costing a lot of money and taking a long time to conduct, dietary trial evaluations on several aquaculture species is a hassle. Nevertheless, zebrafish (Danio rerio) can overcome these limitations as an experimental model, making it a superior creature for doing initial dietary research. The sequenced genome of the zebrafish makes it possible to make effective use of cutting-edge technologies like RNA-sequencing and genotyping platforms to investigate the molecular bases for the fish's nutritional response. The biotechnological techniques necessary to assess the immune response in vivo, such as transgenic lines with fluorescently tagged neutrophils, are widely accessible in this species. For this reason, zebrafish is an appealing platform for evaluating several chemicals to determine which ones will have the greatest chance of success in aquaculture. Some of the ways in which zebrafish might help advance the fields of nutritional genomics and nutritional immunology are described in this viewpoint piece.

By standardizing laboratory mouse diets in the 1970s, **Sam Penglase et al. (2012)** found that "unintended nutritional impacts on experimental results were reduced, and comparisons across trials were more trustworthy" (Nature 491, 31–33; 2012). While the zebrafish (Danio rerio) has been a very successful model organism, it is being given a variety of commercial diets the nutritional content of which is mostly unknown. Using the vast knowledge of fish nutrition from aquaculture, it is time to design a standard formula food for zebrafish in the lab. Four commercial zebrafish diets were analyzed for their iron level, and we discovered that the range was from 0.6 to 4.6 milligrams per kilogram (mg kg1) of dry feed. These greater amounts may be hazardous since fish only need 0.2 g kg1 dry diet of iron. For instance, A. Goksyr et al. (1994) in the Canadian Journal of Fisheries and Aquatic Sciences found that dietary iron levels in salmon influenced the activity of the cytochrome P450 detoxifying system. It has been shown that a lack of vitamin C in one zebrafish diet may modify cellular redox state, which in turn can affect research parameters like illness progression. Results from an experiment with zebrafish fed two different diets that is otherwise similar may not be reliable if these factors are not controlled for.

BASIC CONSIDERATIONS IN ZEBRAFISH NUTRITION RESEARCH

Knowledge of Zebrafish Biology is Essential

Whether you're studying it for nutrition or another subject, knowing something about an organism's biology is essential to devising effective management measures. It is crucial for nutrition studies to get a basic comprehension of the elements that impact feed intake and absorption. The good news is that zebrafish have been the subject of much reporting on population studies and observations of behavior in wild populations. These findings support the creation of diets and feed management systems that work with the organism's inherent biology. Zebrafish are common in shallow water environments like flood plains and areas where rice is farmed. They populate a wide variety of habitats, including open water and plant communities. While often mistaken for riverine species, zebrafish are really more common on sandy or silty soils. They prefer calmer waters (currents of 0.01-0.10 m/sec) and stay away from places with strong hydrodynamic activity.

This makes them suitable for cultivation in a variety of aquarium setups with restricted water circulation and exchange. Glass, fiberglass, or premium plastics may all be used to construct these aquariums. High conductivity metal surfaces are undesirable because they emit metal ions into the water, the toxicity of which is poorly understood for cultured zebrafish. As cultivated zebrafish often feed on the surface of their containers, an abundance of food and the formation of biofilms would hinder dietary studies. Several researchers depend on static renewal, recirculating, or flow-through systems since zebrafish are eurythermal and may survive at temperatures as low as 6 - 38^{o}C. Culture may be carried out across a wide temperature range. Changes in temperature during nutritional trials will have effects on zebrafish eating behavior, food intake, digestion, and reproduction even though these fish can tolerate a broad temperature range. Temperature is likely to have an effect on the quantity of gamete formation, which in turn will change the way energy is distributed. A number of sources recommend maintaining a temperature of 28 degrees Celsius for raising zebrafish.

Nutritional considerations have not been applied to many of the parameters listed in Table 1, even though they have been taken into account in ecological assessments. Each unquestionably would have an impact on both nutritional uptake and output. Most abiotic variables are amenable to manipulation in nutrition research, and studies assessing the significance of each component would add much to our knowledge of zebrafish biology and nutrition. One of the most crucial metrics to examine is water quality. Waste generation and those ions and the chemical environment thought to be best for life, development, and osmoregulation are also factors in water quality. Even anecdotal evidence should be documented, since biotic variables might be difficult to manage at times. Sexually-related interactions will be examined in detail. The findings of nutritional studies might be influenced by whether the study subjects are of both sexes or just one.

The importance of dissolved organic matter in zebrafish diet is another factor to think about. It is unclear how much dissolved organic matter contributes to overall creation, but even a negligible amount might have major implications for things like chemical attractants and signal molecules.

Table 1

Abiotic and biotic factors presumed potentially important in nutrition research

Abiotic	Biotic
Temperature	Life stage
Salinity or conductivity	Size
Photoperiod	Sex
Light intensity	Disturbance
Oxygen	Dominance and aggression
Water flow	Strain or phenotype
Water quality (e.g., ammonia, nitrite, nitrate, pH)	Disease
Ions	Symbioses or specific pathogen free

Culture System Designs

Studies on nutrition are conducted in "blue water," or sterile, artificial freshwater settings. The specifics might change depending on the goals of the research. Conditions of water quality and other abiotic and biotic parameters should be well established in order to estimate precise nutritional needs. In order to make meaningful comparisons across experiments, it is crucial that all culture conditions be accurately described in published papers. There are a plethora of specialized zebrafish culture systems, each of which has been adopted by a different research group. They may be as basic as a couple of glass aquaria or as complex as the rack systems offered by a number of companies today. The majority of culture systems are derived from methods used for cultivating other aquatic species. The properties of the water supply and the filters will serve as the primary criteria for defining culture systems. Common names for these setups include flow-through, recirculating, semi-recirculating, and static renewal. No particular aquarium, tank, or rack design will be discussed; nonetheless, the number of zebrafish that may be housed in a given space is directly proportional to the volume of that space.

DIET DEVELOPMENT

Purified, Semipurified, and Practical Ingredients

Empirical investigations of meals with chemically specified nutrients and components are necessary to have a complete knowledge of zebrafish nutritional needs and the variables that impact them. We suggest developing diets after a careful step-by-step analysis of daily nutritional needs and the nutritional worth of individual products. Identifying the needs for one set of nutrients necessitates a constant reevaluation of individual nutrients due to the large number of nutrient interactions. The biological impact of nutrients on development, reproduction, and other physiologic and molecular processes may be assessed via diet design. Several types of diets may be identified by the foods they include and the nutrients they provide (Table 2).

Research diets are employed in controlled laboratory settings to ascertain the optimal intake of individual nutrients. Some (called "practical diets") are developed for commercial usage and are meant for mass manufacturing and consumption; their constituent composition is determined by the lowest possible cost. Because of this, commercial manufacturers may alter their recipes at any time. Cost shouldn't be a factor when choosing a zebrafish diet, since they are often only utilized in scientific studies.

Table 2

Classification of feeds

Type	Application	Content	Relative cost
Purified	Experimental feeds, used to evaluate macro- and micronutrient requirements	Purified, chemically defined ingredients, usually with defined lot numbers and specifications	High
Semipurified	Experimental feeds, used to evaluate macro- and some micronutrient requirements	Both purified and practical ingredients, not completely defined	Moderate
Practical	Commercial production feeds, produced in mass quantities for aquaculture operations, cannot be used to evaluate nutrient requirements	Practical ingredients, not chemically defined	Low

Physical and Chemical Characteristics of Feed and Storage

Nutritional studies rely heavily on dietary intake data for conclusive conclusions. Yet, a meal must be consumed before its nutritional composition and associated biological consequences may be evaluated. For this reason, it is important to take into account a number of dietary physical and chemical properties while designing a diet. A feed pellet or flake's physical features include its dimensions, composition, feel, hardness, stability, and handling. Most pellets made through commercial extrusion or pelleting techniques are either spherical or cylindrical in form. Nevertheless, most commercially made pellets are too big for zebrafish to eat (the extruder's size range typically starts at 400 µm). Because to this fact, flake diets and pelleted "crumbles" are the most popular commercial sources of zebrafish food. Due to a lack of extruders in most nutrition research facilities, meals must be processed without the use of heat (cold processing) and pulverized to very fine particle sizes using very modest mills or grinders.

Basic Nutrient Requirements

This is because many species' metabolic processes are similar, making for a general similarity in their dietary needs. You may get a quick overview of the necessary diet nutrients in Table 3. Proteins, carbohydrates, neutral lipids, and polar lipids are all examples of nearby nutrients whose quality and amount will vary depending on their point of origin (e.g., menhaden, soybean, casein, starch). While most of these nutrients have not been tested on zebrafish, it is probable that they will have nutritional needs for many of them, and that high intakes of others would be poisonous and lead to stunted development or even death. Protein needs in zebrafish have only been thoroughly examined in a few number of studies. There is little information on the quality of the protein and most of the data on protein needs is for other cyprinids, which have recommended protein levels of 30-53%. There needs to be more study put into the importance of meeting protein (amino acid) needs. While the precise amount of carbohydrate needed has not been established, Robison and colleagues found that low carbohydrate diets slowed development and altered gene expression.

Table 3

Classes of nutrients and sources suggested to be important in zebrafish nutrition

Nutrient Class	Description
Proximate nutrients	Protein (nitrogen); essential and nonessential amino acids, dipeptides, tripeptides, hydrolysates, intact protein
	Carbohydrate (digestible); monosaccharides, oligosaccharides, polysaccharides
	Lipids; essential fatty acids, polyunsaturated fatty acids, long chain polyunsaturated fatty acids, n3 and n6, phospholipids, cholesterol
	Ash (non-nutrient, e.g., diatomaceous earth)
	Fiber (insoluble and soluble, nondigestible)
Macrominerals	Calcium, magnesium, phosphate
Microminerals	Iron, zinc, manganese, copper, selenium, cobalt, sodium, chloride, potassium, boron
Vitamins	Ascorbic acid, tocopherols, calciferols, naphthoquinones, retinols, thiamine, ribofl avin, pyridoxine, niacin, pantothenic acid, biotin, folic acid, choline, inositol, etc.
Carotenoids	Carotene and xanthophylls

In the College Aquafarm, we stimulated spawning of brood stock (with 0.4 mL ovaprim/kg.bw) in order to harvest snake head larvae measuring 4.62 + 0.08 mm and weighing 1.25 mg.

There was no statistically significant variation in dissolved oxygen concentration (DO) or pH (pH 7.5-8.2) or water temperature (29.1°C) between the groups in the experiment. Table 4 displays the development rates of *C. punctatus* larvae. Larvae fed plankton with fish paste and (72.8±4.0 mg) in fish paste alone had considerably ($p<0.05$) greater weight than those fed plankton with chicken intestinal paste (weight = 83.8±3.2 mg), but both groups showed poor development (weight = 69.6±1.5 mg).

Table 4:Growth performance of *Channa punctatus* fed with different diets

Diet types	Initial weight (mg)	Final weight (mg)	SGR (%)	Weight gain (%)	Survival rate (%)
Plankton soup	1.25 ± 0.06^{a}	76.3 ± 3.5^{bc}	6.376 ± 0.056^{bc}	6004 ± 280^{bc}	74.16 ± 6^{c}
Chicken intestine paste	1.25 ± 0.06^{a}	79.8 ± 4.0^{ab}	6.445 ± 0.082^{ab}	6284 ± 320^{ab}	36.6 ± 1.44^{d}
Fish paste	1.25 ± 0.06^{a}	72.6 ± 3.0^{c}	6.299 ± 0.053^{c}	5708 ± 240^{c}	34.16 ± 1.44^{d}
Plankton with fish paste	1.25 ± 0.06^{a}	69.6 ± 1.5^{c}	6.276 ± 0.07^{c}	5628 ± 386^{c}	82.50 ± 2.5^{b}
Plankton with Chicken intestine paste	1.25 ± 0.06^{a}	83.8 ± 3.2^{a}	6.522 ± 0.13^{a}	6604 ± 240^{a}	86.6 ± 1.44^{a}

In the same column, values with the same mean and the same number of superscripts are equivalent.

Fish given plankton supplemented with chicken intestine had a much greater survival rate (86.61±1.44%) than those fed any of the other diets. Fish fed just chicken intestine (34.16±1.44%) and fish paste (36.6±1.44%) had a dismal survival rate.

The current findings provide support for the idea that C. punctatus larvae could benefit from being fed either live zooplankton or a mix of live and solid food. Nonetheless, the fish's development and survival may be enhanced by feeding them a mix of live zooplankton and a solid meal.

CONCLUSION

According to these experts and others, one of the most significant causes of environmental variability may be managed via dietary standardization. Funding agencies that support this paradigm for biomedical research will soon push back against the existing and inevitable variances originating from the use of commercial or other live and prepared diets among zebrafish researchers. Establishing stringent nutrition regimens for zebrafish populations will likely be necessary for future investigations to provide the groundwork for similar data sets.

REFERENCE:

1. Smita Sonawane, Ajit Gedam, Sunil Anand and Sandhya Pawa (2012) Food and feeding habits of Channa punctatus from KaigaonToka Dist. Aurangabad (M.S.) in relation to biochemical studies. Journal of Experimental Sciences 2012, 3(8): 07-13 ISSN: 2218-1768 Available Online: http://jexpsciences.com/

2. Datta, S.N., Kaur, V.I., Dhawan, A. *et al*. Estimation of length-weight relationship and condition factor of spotted snakehead *Channa punctata* (Bloch) under different feeding regimes. *SpringerPlus* **2**, 436 (2013). https://doi.org/10.1186/2193-1801-2-436

3. Singh, C & Ram, R. & Singh, R. (2013). Food and feeding pattern of Channa punctatus in two different habitats at Tarai region of Uttarakhand. J Environ Biol. 34. 789-92.

4. Ulloa PE, Medrano JF and Feijoo CG (2014) Zebrafish as animal model for aquaculture nutrition research. Front. Genet. 5:313. doi: 10.3389/fgene.2014.00313

5. Penglase, S., Moren, M. & Hamre, K. Standardize the diet for zebrafish model. *Nature* **491**, 333 (2012). https://doi.org/10.1038/491333a

6. Ulloa, P. E., Iturra, P., Neira, R., and Araneda, C. (2011). Zebrafish as a model organism for nutrition and growth: towards comparative studies of nutritional genomics applied to aquacultured fishes. Rev. Fish Biol. Fish. 21, 649–666. doi: 10.1007/s11160-011-9203-0

7. Ulloa, P. E., Peña, A., Lizama, C. D., Araneda, C., Iturra, P., Neira, R.,et al. (2013). Growth response and expression of muscle growth–related candidate genes in adult zebrafish fed plant and fishmeal protein–based diets. Zebrafish 1, 99–109. doi: 10.1089/zeb.2012.0823

8. Wickramasinghe, S., Cánovas, A., Rincón, G., and Medrano, J. F. (2014). RNA-sequencing: a tool to explore new frontiers in animal genetics. Livest. Sci. 166, 206–216. doi: 10.1016/j.livsci.2014.06.015i

9. Xu, H., Lam, S. H., Shen, Y., and Gong, Z. (2013). Genome-wide identification of molecular pathways and biomarkers in response to arsenic exposure in zebrafish liver. PLoS ONE 8:e68737. doi: 10.1371/journal.pone.0068737

10. Qian, X., Ba, Y., Zhuang, Q., and Zhong, G. (2014). RNA-seq technology and its application in fish transcriptomics. OMICS: J. Integr. Biol. 18, 98–110. doi: 10.1089/omi.2013.0110

11. Oyarbide, U., Rainieri, S., and Pardo, M. (2012). Zebrafish (Danio rerio) larvae as a system to test the efficacy of polysaccharides as immunostimulants. Zebrafish 9, 74–84. doi: 10.1089/zeb.2011.0724

12. Ouyang, W., Rutz, S., Crellin, N. K., Valdez, P. A., and Hymowitz, S. G. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu. Rev. Immunol. 29, 71–109. doi: 10.1146/annurev-immunol-031210-101312

13. Oliveira, S., Reyes-Aldasoro, C. C., Candel, S., Renshaw, S. A., Mulero, V., and Calado, A. (2013). Cxcl8 (IL-8) mediates neutrophil recruitment and behavior in the zebrafish inflammatory response. J. Immunol. 190, 4349–4359. doi: 10.4049/jimmunol.1203266

14. Morais, S., Pratoomyot, J., Taggart, J., Bron, J., Guy, D., Bell, J.,et al. (2011). Genotype-specific responses in Atlantic salmon (Salmo salar) subject to dietary. BMC Genomics 12:255. doi: 10.1186/1471-2164-12-255

15. Long, Y., Song, G., Yan, J., He, X., Li, Q., and Cui, Z. (2013). Transcriptomic characterization of cold acclimation in larval zebrafish. BMC Genomics 14:612. doi: 10.1186/1471-2164-14-612