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THE EFFECT OF FOOD TYPE (NATURAL VS. FORMULATED DIET) ON GROWTH PERFORMANCE AND COLORATION OF JUVENILE JAPANESE ORNAMENTAL CARP (Koi, *Cyprinus carpio* L.)

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Abstract

Fish shape, size and coloration are important quality indicators for determining the market value of ornamental fish species. Therefore, ornamental carp rearing technologies must target proper values of these quality indicators, while maximizing fish growth performance and profitability. The aim of present study is to evaluate the influence of administrated food type (natural diet - V1 8.8% protein vs. formulated diet - V2 55% protein) on growth performance and coloration of Japanese ornamental carp, while maintain the feed operational cost. The average specific growth rate indicates a superior fish production at V2 (formulated diet), compared to V1 (natural diet) experimental variant, while the average food conversion ratio (FCR) indicates better values for V2 (1 g feed/g biomass gain), compared to V_1 (4.5 g feed/g biomass gain). The protein efficiency ratio (PER) registered higher values at V1 (2.52), compared to V_2 (1.8), most probably due to protein input limited by feed operational cost restriction. The administration of the live food indicates a better fish coloration, compared with V_2, where artificial feed was administrated. Therefore, natural diet can improve coloration of juvenile ornamental carp.

Key words*: ornamental carp, live food, pellets, rearing, recirculation system.*

INTRODUCTION

Globally, the ornamental fish sector is growing and their production and trade is a profitable activity in aquaculture industry. Over 1 billion ornamental fish are traded globally each year (Hana et al., 2014).

Koi carp (*Cyprinus carpio* koi) is a popular and economically valuable ornamental fish, as koi industry has spread worldwide (Hongjian et al., 2015). Within the past few decades, the commercial production of koi has emerged as a major segment of the fish industry and koi carp (especially high-quality individuals) trade plays a major role in meeting a growing worldwide demand (Feng et al., 2019).

Koi (*Cyprinus carpio* L.) is a subspecies cultivated as an expensive, beautiful, and colourful pet fish for personal pleasure or competitive show, especially in Japan but also worldwide (Ping et al., 2018). The koi carp is characterized as an economically important species of ornamental freshwater fish (Raj et al., 2015).

Colour of ornamental fishes is an essential prerequisite for the quality as they fetch a higher price in the commercial market and is considered as an important quality attribute of the fish for consumer acceptability. Ornamental carp (koi) are characterized by a wide diversity of colours and colour patterns. More than 100 different types of coloration have been developed for these fish species, which are valued as pets (Xiangjun et al., 2012). Colour production in fish is due mostly to food (Elsah et al., 2018).

There are different source of carotenoids such as natural and synthetic origin which have been used to enhance the coloration of the fishes. The different synthetic carotenoids (β-carotenoids, canthaxanthin, zeaxanthin and astaxanthin) and natural carotenoids (such as plant materials, bacteria, algae, crustaceans, microalgae etc.) are used as colour enhancer. However, the high price of synthetic carotenoid forces the researcher to explore the natural sources and their application as colour enhancer (Manas et al., 2017).

Feed is the highest cost of production, having the greatest influence on profit, which is why the correct choice of feed is of crucial importance. Therefore, the aim of present study is to evaluate the influence of administrated food type (natural diet vs. formulated diet) on growth performance and coloration of Japanese ornamental carp, while maintain the feed operational cost.

MATERIALS AND METHODS

The experiment was conducted at the aquaculture pilot station of "Dunarea de Jos" University of Galati, Faculty of Food Science and Engineering, in four rearing units, equipped with individual water conditioning modules.

Thus, for biological, chemical and mechanical filtration, each rearing unit was connected to a Hagen AquaClear power filter, while for maintaining the oxygen concentration of technological water within optimum limits, a Resun Air Pump (1.6 L/min) was used. A daily water exchange rate of 40% was applied in order to assure optimum growth conditions for the biological material.

The biological material consists in 240 exemplars of koi carp, with mean body weight of 2.14 ± 0.015 g and mean body length of 5.02 ± 0.08 cm, acclimated for 10 days before the beginning of the experimental period. The exemplars were equally distributed in four rearing units, in order to assure two experimental variants, in duplicate (V1 - fish feed with natural diet, respectively V2 - fish feed with formulated diet).

The exemplars from V2 were adapted from natural to formulated diet during the acclimatization period (10 days before the beginning of the experimental period).

In V_1 experimental variant, the natural fish diet consists in *Tubifex tubifex*. The biochemical composition of *T. tubifex* carcass (% w/w basis), according to the producer, is presented in Table1.

Also, similar results of *T. tubifex* biochemical composition are reported by Voican and Radulescu, in 1979, and Mandall et al., in 2018 (Table 1). A daily feeding rate of 7% from total fish body weight (BW) was applied.

The feed was administrated two times per day, in the morning and late in the afternoon.

Table 1. Biochemical composition of *T. tubifex*

Parameter	Units	Values reported by the producer, for natural feed used in V1	Values reported by Mandal et al., 2018	Values reported by Voican and Radulescu, 1979
Protein	% w/w basis	8.8	4.02-6.38	8.8
Crude lipid	% w/w basis	2.8	0.85-3.02	3
Ash	% w/w basis	4.34	2.43-2.98	1

The evaluation of biomass gain was performed both at the end and at the middle of the 30 days experimental period, in order to o adjust the diet.

In establishing the feeding rates and respectively, the total feed protein input, the economical aspect was considered.

Thus, the desideratum of maintain the same feed operational cost for both experimental variants resulted in different protein input by natural, respectively by formulated administrated feed quantity.

At the end of feeding trial, the fish biomass growth performance was evaluated by considering the main parameters: weight gain percentage, specific growth rate (SGR), survival rate, feed conversion ratio (FCR) and protein efficiency ratio.

In V2 experimental variant, the fish were manually fed, four times / day, with formulated diet. The biochemical composition of the formulated diet is presented (Table 2). A daily feeding rate of 7% was applied.

Table 2. Biochemical composition of pellets

Parameter	Units	Value
Crude protein	%	55
Crude fat	%	16
Ash	%	10
Fiber	%	0.6
Phosphor	%	1.45
Vit. A	UI	14000
Vit D_3	UI	2300
Vit E	Mg	250
Vit C	Mg	500

The growth performance parameters were calculated according to Nuwansi et al. (2019), as follows:

(1) SGR (% day^{-1}) = (log$_e$ Final weight - log$_e$ Initial weight)/no. of days) * 100
(2) FCR = Feed Given (Dry Weight in g)/Net weight gain (Wet Weight in g)
(3) PER = Net Weight Gain (Wet Weight in g)/Protein Feed (g)
The temperature, pH and dissolved oxygen were monitored daily. The following equipment was used to measure the water quality parameters: oxygen concentration and temperature were measured by using the WTW Oxi 315 I, while the pH meter WTW, model pH 340 determined the pH value.
The evaluation of koi carp color is made by comparing the photo with a color ranking which varies in from 1 to 7 points (1 is the lowest color intensity). Color was judged by test panels of 10 persons, randomly recruited from the students. The treatments were not revealed to the individuals who were asked to rank the fish according to intensity of color. Score were subjected to statistical analysis (ANOVA test).

RESULTS AND DISCUSSIONS

The monitored water quality parameters were within the tolerable limits for ornamental carp rearing. Water temperature ranged from 23ºC to 26ºC at both experimental variants, with no statistically significant difference (p>0.05); pH ranged between 7.54-7.98 at V1 and between 6.57-7.72 at V2 experimental variant (Figure 1); dissolved oxygen (DO) ranged between 4.1-5.3 mg/L at V_1 and 4.1-5.1 mg/L at V2 experimental variant (Figures 1 and 2).

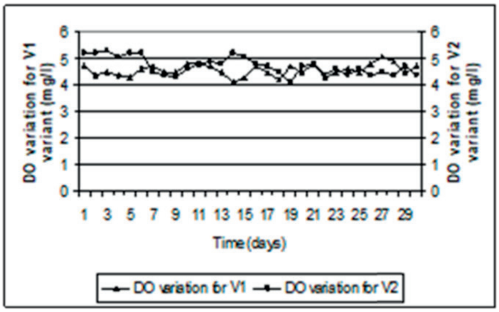

Figure 1. The dynamics of do for both experimental variants

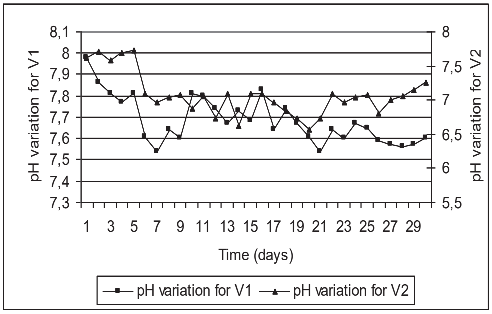

Figure 2. The dynamics of ph for both experimental variants

Colour is one of the most important quality criteria which determine the market value of koi carp. The ornamental carp colours are determinate by the distribution of specifically cells named chromatophores that are situated in the epidermis and in the dermal superior tissue. They are rich in pigments and can be situated in the skin layer or immediately below the scales. A chromatophore is a branched cell, within which the color pigment can be moved. The pigment spreads throughout the entire cell (which gives the koi exemplar the color of the cell) or it is concentrated in one small spot in the center (resulting in the background color, usually pale or dark).
They vary in size, density and superimposition of these cells or the complete absence of one or more basic colours can produce colours or patterns that characterize koi carp or other fish. Dark pigment (melanophore) and red-orange pigment (xanthophore) combine, in order to produce dark and bright colours in carp. The distribution of these pigments is affected by a number of different factors including: water quality, background color, administrated diet or temperature.
Ornamental carp (koi) are characterized by a wide diversity of colours and colour patterns. More than 100 different types of coloration have been developed for these fish, which are valued as pets.
However, colour production in fish is due mostly to food. In conditions of captivity, the type of food is restricted, while various types of food are used in aquaculture, from processed dry food to small aquatic animals. Also, fish growth is affected by several environmental factor such as temperature (Sarvendra et al.,

2018), photoperiod and food availability, technological factors and also, genetical factors.
Regarding colors maintenance, in the case of present experiment, after only 30 experimental days, it has observed an evident decrease of color for koi carp exemplars which were fed only with formulated diet, fact that can indicate a degeneration of pigments who gives the specifically colors. The colors were more vivant in variant which were fed with natural diet, formed with *T. tubifex*. The conclusions are based on the registered results, after processing the scores resulted after ranking the fish exemplars from both experimental variants according to the intensity of their tegument color. Thus, the exemplars reared in V1 experimental variant registered an average score of 7±1.05 points, while the exemplars from V2 registered an average color intensity ranking score of 3.3±0.94 points. The results indicates are statistically significant ($p<0.05$) and confirms the importance of natural feed administration for maintaining and improving koi carp color of tegument.
Growth performance and feed utilization of the experimental biomass of koi carp, fed with different experimental diets are presented in Table 3.

Table 3. The growth parameters for both variants

Parameters/Variant	V1 - natural feed	V2 - formulated feed
Mean initial weight (g)	2.14	2.3
Mean final weight (g)	3.13	7.26
Weight gain (g)	0.99	4.96
Weight gain (%)	46	316
Survival (%)	100	100
GR	0.03	0.16
SGR (% BW/day)	1.26	3.83
FCR	4.5	1
PER	2.52	1.8

Thus, it can be observed that fish biomass reared by applying the feeding regime and the specific diet administrated in case of V2 assures better growth parameters, compared to V1 experimental variant. The average specific growth rate indicates a superior fish production at V2 (3.83 %BW/day), compared to V1 (1.26 % BW/day) experimental variant. Also, from the perspective of feeding strategy efficiency, the average food conversion ratio (FCR) indicates significantly better values for V2 experimental variant (1 g feed/g biomass gain), compared to V1 (4.5 g feed/g biomass gain). However, the protein efficiency ratio (PER) registered higher values at V1 experimental variant (2.52), compared to V2 (1.8), most probably due to feeding diet and feeding regime applied, revealing the ability of fish organism to utilize better the proteins provided by natural feed, compared to those provided by formulated feed input.
In V1 variant, food restriction, due to the purpose of maintaining a similar feed operational cost between both variants, had significant effects on growth performance of ornamental carp. This can explain the inferior results registered in term of growth performance for fish biomass fed with natural diet formed by *T. tubifex*.
Also, referring to technological water quality parameters, it can be observed that the water treatment units were less performed at V1, compared to V2. Thus, lower values of water pH and DO are recorded in V1 experimental variant, compared to V2, fact which may indicate a possible higher organic load for the experimental variant where natural diet was administrated, compared to the variant where formulated diet was used. However, the production system performs well in case of both variants, fact which reveals its ability to support more intense feeding regimes than those applied in present experiment. It has been revealed that the excess feeding increases the organic load, principally carbon and nitrogen (mainly as total ammonia nitrogen). As aerobic conditions need to be maintained for fish growth and welfare, a higher organic load is expected to cause enhanced oxygen demand for its treatment (oxidation). The increasing oxygen demand leads to higher energy consumption for aeration, increasing system operational cost and a larger environmental footprint (Uri et al., 2020). Thus, in order to achieve high economical performance, it is recommended to use aquaculture production systems close to their production capacity limits.
The registered survival rate of 100% confirms that both feeding regime, as well as the production system, performed properly for

assuring optimal conditions for rearing koi crap in the tested development stage.

Optimization of dietary protein level in the diets of *Cyprinus carpio* is important factor because the cost of feed, which is considered to have a large share in relation to total variable costs of the aquaculture economic activity, is largely influenced by source, quality and level of protein. Optimization of dietary protein available for somatic growth is necessary for an efficient cost-effective use of feed. Generally, an increase of protein level in fish diet improves fish production, but proportionally increases feed cost (Lee & Sang-Min, 2005).

Feed and feeding activity are the most critical factors to be considered for profitable aquaculture. Fish farmer spends a significant amount of their total production cost on feed, which is considered as the most expensive item in aquaculture. For successful aquaculture practices, it is essential to determine the minimum level of protein that can assure the maximum growth of fish biomass, while achieving the best operational costs.

Rearing temperature and diets are considered the main growth limiting factors in fish. In the livestock industry, feed plays an important role as it accounts for approximately 60-80% of the production cost, depending on breed and growth and reproduction stage of the fish (Uyeh et al., 2018).

Generally, feed formulation is done by specifying the nutritional requirements as rigid constraints and an algorithm attempts to find a feasible cost-effective formulation.

Inadequate protein in feed causes reduced growth, but when excess is given in a diet, the additional protein is transformed into energy by direct oxidation of amino acids, which leads to increased production costs and additional nitrogen waste. Poor dietary protein and energy levels and their ratios will result in decreased fish performance, increased production cost and deterioration of water quality resulting from wasted food.

CONCLUSIONS

From an economic point of view, appropriate feeding strategy is fundamental for the success of ornamental carp culture.

The growth of the fish from V1 experimental variant was seriously affected by the economical constraints, related to the high cost of the *T. tubifex*, which limits the daily feeding ratio. It is essential to provide the best diet at the least possible cost in order to cut down operational costs and subsequently increase profit, while maintain proper growth rate for biological material.

Thus, the growth performance parameters indicate better results for the experimental variant where formulated feed was administrated. This can be related to lower cost of formulated feed, compared to natural feed formed by *T. tubifex*. This situation generated a higher protein input in V2 experimental variant, due to higher feed quantity administrated, while respecting the constrains related to the use of identical amount of feed operational cost for both tested variants.

However, by analyzing the PER value from both experimental variants, it can be concluded that the protein provided by natural food is better assimilated by koi carp biomass compared to the protein provided by formulated feed.

Also, since koi carp is considered an ornamental fish species and its aspect and color mainly reflects its market value, the color examination must be considered as an important indicator in choosing the proper diet from those tested in present experiment. Thus, better results in terms of color are registered when using natural diet, compared to formulated diet.

Therefore, for ornamental koi carp nutrition, in order to maintain and improve the color of the tegument throughout the entire production cycle, it is recommended to use natural diets. However, formulated diets which contains colors additives can have a precise and predictable effect, although constrains related to feed operational costs may occur.

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