



## FEEDING AND GROWTH EFFICIENCY OF COMMON CARP *CYPRINUS CARPIO* L. FRY FED FISH BIOSILAGE AS A PARTIAL ALTERNATIVE FOR FISH MEAL §

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### Abstract

This study was carried out to evaluate the partial replacement of fish meal by fish silage in feeds formulated for common carp *C. carpio* fry. Proximate composition of fish meal, fish silage and different experimental feeds were analyzed and several parameters of fish feeding and growth were studied. Biosilage was prepared by fermenting marine by-catch fish with date fruit residues, domestic vinegar and citric acid. The produced biosilage was incorporated in feeds to replace 0, 25, 50 or 75% of fish meal protein. Fish were fed for 10 weeks on isonitrogenous (42% protein) and isocaloric (4600 Kcal/kg) feeds and feeding and growth parameters were close in the four feed groups. Very few significant differences were observed between the experimental feeds as to specific growth rate, thermal growth coefficient, feed conversion ratio and protein efficiency ratio. Fish survival rates were very similar between the different feed groups during the experiment (94.4-96.7%). The study concluded that fish biosilage can be used successfully as fish meal alternative in feeds for common carp fry without adverse effects on feeding and growth efficiency.

**Keywords:** *Fish biosilage, carp fry, feeding, growth efficiency.*

§ This research is a part of doctoral thesis for the third author.

### 1. Introduction

With the continuous increasing in world population and demand for food resources especially animal protein, aquaculture still contributes substantially in global food security. The cost of feed represents an important proportion of aquaculture operational costs. Protein sources are the major contributors in aquaculture feed costs. Fish meal is the preferred dietary protein source for many farmed fish and shrimp species because of its amino acid balance, vitamin content, palatability and unidentified growth factors (Majumdar *et al.*, 2014). However, fluctuating supplies and thus significantly elevating prices during the last decade have encouraged the search for fish meal alternatives from different plant and animal sources. Plant materials suffer from low digestibility, high fibre content and antinutritional factors which limit their use effectively in aquaculture feeds (Lall and Anderson, 2005; Atanasoff, 2014). Animal protein concentrates like blood meal and meat and bone meal were banned due to the outbreaks of BSE disease (De Vos and Heres, 2009).

For all the above mentioned reasons fish biosilage became a viable alternative for fish meal in aquaculture. Fish biosilage is defined as a liquid product produced from the whole fish or parts of it, to which acids or lactic acid-producing bacteria are added, with the liquefaction of the mass provoked by the action of enzymes from the fish (Arruda *et al.*, 2007). It is characterized by similar or even better proximate composition in comparison with fish meal. The high quality content of fish oil rich in PUFA fatty acids makes fish silage an excellent source of essential fatty acids. It could be made easily even at farm level from different raw materials like by-catch fish or fish wastes without need for advanced technology (Ghaly *et al.*, 2013; Goosen *et al.*, 2014).

The common carp *Cyprinus carpio* L. is one of the most important culturable freshwater fish all around the world. Carp fry is a critical stage in the early life of this species. Fry are the transitional stage between the dependence on yolk sac then natural food for nutrition and the shift to artificial feeds. With their poorly developed digestive system, fry feeds must be formulated carefully to gain the maximum advantage of supplementary feeding. Therefore, the goal of feeding of fry is not limited with growth enhancement but also includes another crucial aspect which is fry survival rate and its implication on the final productivity and profitability of the culture process (Takeuchi *et al.*, 2002).

The current study was carried out to evaluate incorporating locally produced fish biosilage as a partial replacement for fish meal in common carp fry diets and its effects on feeding and growth performance as well as fry survival rate.

### 2. Materials and Methods

Fish biosilage was produced using marine by-catch fish obtained from marine shrimp fisheries at Al-Fao city southern Basrah. Ensiling fermentation process was carried out by adding date fruit residues (10%) as carbohydrate substrate, domestic vinegar (20%) as an acidulant and inoculant and citric acid as starting acidifying agent. Ensiling mixture was incubated at 35°C for 10 days with occasional agitation. Fish meal was produced by the standard method according to FAO, Fisheries Industries Division (1986) from the same fish sample for comparison purposes. Proximate composition analyses of fish biosilage, fish meal and experimental diets were carried out according to AOAC (2003). Gross energy was calculated using values of 9.1, 5.5 and 4.1 Kcal/g for lipid, protein and carbohydrates, respectively (New, 1987).

Common carp fry (average weight 0.68± 0.06 gm.) were obtained from outdoor ponds of fish farm at Marine Science Center, University of Basrah. Upon arrival to the Fish Hatchery at the Department of Fisheries and Marine Resources fish were distributed in culture system of 12 glass aquariums (60 x 30 x 42 cm) each containing about 57 liters

of dechlorinated tap water, acclimatized to the laboratory conditions for 3 days and to the experimental diets for 2 other days. Before the beginning of the experiment, weak and abnormal fish were excluded and the remaining fish redistributed on aquariums at 30 fry/ aquarium. The experiment included 4 treatments with 3 replicated aquariums for each. Feeds A, B, C and D were designed according to NRC (2011) criteria to be isonitrogenous (42% crude protein) and isocaloric (4600 Kcal/kg) replacing 0, 25, 50 and 75% of fish meal protein content by fish biosilage. Each aquarium was equipped with air flow and thermostat controlled heater fixed at  $28 \pm 1^\circ\text{C}$ . Fish were fed 10% of body weight daily twice daily (8 am and 2 pm) six days a week. About 30% of aquarium water was changed daily before morning feeding.

Fish were weighed biweekly and feed ration was adjusted accordingly. Specific growth rate (SGR), feed conversion efficiency (FCR) and protein efficiency ratio (PER) were calculated according to Hepher (1988) and thermal growth coefficient (TGC) according to Jobling (2003). Fish survival was monitored also during this experiment which lasted for 10 weeks from 28 April to 7 July 2013. Water quality parameters (temperature, oxygen, salinity, pH, nitrate and ammonia concentrations) were monitored on a daily basis and maintained within the suitable ranges for this species (Rahman *et al.* 2008; Markovic *et al.*, 2009). The data were analyzed by one-way analysis of variance (ANOVA, F test) and LSD for significantly different means at a significance level of 0.05 using IBM® SPSS® version 19.

### 3. Results and Discussion

#### 3.1. Proximate composition of feed ingredients and experimental feeds

The proximate compositions of fish meal and fish silage are fairly close except the higher lipid content in fish biosilage in comparison with fish meal (22.05 vs. 8.03%, respectively). As shown in table 1, protein contents (70.1 and 59.03% in fish meal and fish biosilage, respectively) are suitable for formulating fish feeds so as the comparable energy content. These results agree well with several previous studies on fish meal and fish biosilage proximate compositions (Fagbenro, 1994; Al-Faraje, 2000; El-Ajnaf, 2009).

**Table 1. Proximate composition (%  $\pm$  S.D.) of fishmeal and fish biosilage used in fry feed formulation**

Composition	Fishmeal	Fish silage
Moisture	6.90 $\pm$ 0.768	6.28 $\pm$ 1.01
Protein	70.10 $\pm$ 0.907	59.03 $\pm$ 0.83
Lipid	8.03 $\pm$ 0.840	22.05 $\pm$ 0.38
NFE	1.10 $\pm$ 0.061	0.41 $\pm$ 0.15
Fibres	-	1.26 $\pm$ 0.21
Ash	13.87 $\pm$ 1.371	10.17 $\pm$ 1.13
Gross energy (Kcal/kg)	4730.01 $\pm$ 30.29	4316.09 $\pm$ 56.35

Values are averages of at least three replicates.

The different experimental feeds were formulated using fish meal and fish biosilage as major protein sources (table 2). Fish meal was replaced partially by fish biosilage on per protein content basis because of different protein content in both ingredients. Vegetable oil was also added to equalize lipid and energy contents because of higher lipid content in biosilage.

**Table 2. Fry feed formulation and proximate composition using fish silage as a partial fish meal alternative**

Feedstuff, %	Feed formulation			
	A	B	C	D
Fishmeal	43	32.25	21.5	10.75
Fish silage	0	12.75	25.5	38.25
Soybean meal	17	17	17	17
Corn meal	15	15	15	15
Barley flour	10	10	10	10
Wheat bran	7	7	7	7
Corn oil	6	4	2	0
Premix*	2	2	2	2
Proximate composition, %				
Moisture	6.51 $\pm$ 0.99	6.83 $\pm$ 1.55	6.87 $\pm$ 1.23	7.07 $\pm$ 1.41
Protein	42.82 $\pm$ 3.68	43.09 $\pm$ 2.92	42.03 $\pm$ 3.13	42.77 $\pm$ 2.89
Lipid	12.71 $\pm$ 1.98	12.83 $\pm$ 1.66	12.91 $\pm$ 1.28	13.3 $\pm$ 2.02
NFE	27.37 $\pm$ 3.22	26.40 $\pm$ 2.98	27.25 $\pm$ 3.09	25.88 $\pm$ 2.83
Ash	10.59 $\pm$ 1.59	10.85 $\pm$ 1.87	10.94 $\pm$ 1.69	10.98 $\pm$ 1.44
Gross energy, Kcal/kg	4634 $\pm$ 91.9	4620 $\pm$ 313.9	4604 $\pm$ 355.7	4610 $\pm$ 366.6
P/E Ratio, mg/Kcal	92.41 $\pm$ 6.55	93.27 $\pm$ 7.31	91.30 $\pm$ 6.69	92.78 $\pm$ 7.81

A, 100% fish meal; B, 75% fish meal+ 25% fish silage; C, 50% fish meal+50% fish silage; D, 25% fish meal+75% fish biosilage calculated as per protein content. \*Vapcomix, VAPCO Veterinary and agricultural product manufacturing Co., Amman, Jordan.

The four different feeds have very close proximate composition and energy content which could satisfy the nutritional requirements of common carp fry especially from protein, energy and protein to energy ratio as proposed previously (Takeuchi *et al.*, 2002; NRC, 2011).

### 3.2. Feeding and growth efficiency of common carp fry

Table 3 present the various feeding and growth efficiency parameters of carp fry fed the four experimental feeds with different replacement levels of fish meal by fish biosilage (0, 25, 50 and 75% of fish meal protein). Weight gain was very similar between the experimental diets except one significant difference ( $p < 0.05$ ) between B and D feed groups which can be attributed to the higher initial weight of fish (0.750 vs. 0.611 gm, respectively). This was confirmed by specific growth rate SGR values which did not differ significantly ( $p > 0.05$ ) between the four experimental feeds (3.178-3.220, table 3). However, thermal growth coefficient TGC values showed rather different trend and varied between 0.474- 0.509 with significant differences ( $p < 0.05$ ) between both A and B feed groups from one side and the other two experimental feeds from another. Feed conversion ratio FCR values varied between 2.101- 2.151 with significant differences ( $p < 0.05$ ) between A feed group and treatments with higher fish silage contents C and D. Values of protein efficiency ratio PER ranged between 1.179 in feed group D to 1.466 in feed group B which in turn differed significantly ( $p < 0.05$ ) from the other three treatments. Fish survival rates were within 94.4- 96.7 % range with no significant differences ( $p > 0.05$ ) between the four experimental feed groups (table 3).

**Table 3. Feeding and growth performance of common carp *C. carpio* fry feed different experimental feeds**

Parameter	Experimental feed			
	A	B	C	D
Initial wt., gm	0.664 ± 0.05	0.750 ± 0.06	0.691 ± 0.10	0.611 ± 0.04
Final wt., gm	6.324 ± 0.75	7.005 ± 1.01	6.390 ± 0.88	5.682 ± 0.85
Weight gain, gm	5.660 ± 0.61 <sup>ab</sup>	6.255 ± 0.94 <sup>a</sup>	5.699 ± 0.55 <sup>ab</sup>	5.071 ± 0.69 <sup>b</sup>
SGR	3.220 ± 0.35 <sup>a</sup>	3.192 ± 0.44 <sup>a</sup>	3.178 ± 0.31 <sup>a</sup>	3.186 ± 0.32 <sup>a</sup>
TGU	0.495 ± 0.05 <sup>a</sup>	0.509 ± 0.06 <sup>b</sup>	0.492 ± 0.05 <sup>a</sup>	0.474 ± 0.04 <sup>c</sup>
FCR	2.101 ± 0.25 <sup>a</sup>	2.133 ± 0.30 <sup>ab</sup>	2.148 ± 0.28 <sup>b</sup>	2.151 ± 0.34 <sup>b</sup>
PER	1.347 ± 0.14 <sup>a</sup>	1.466 ± 0.11 <sup>b</sup>	1.327 ± 0.17 <sup>a</sup>	1.179 ± 0.12 <sup>a</sup>
Survival, %	96.7 ± 1.0 <sup>a</sup>	94.4 ± 0.58 <sup>a</sup>	95.6 ± 0.58 <sup>a</sup>	96.7 ± 1.0 <sup>a</sup>

SGR, specific growth rate; TGC, Thermal growth coefficient; FCR, feed conversion ratio; PER, protein efficiency ratio. Values in the same row which carry different superscript letters are significantly ( $p < 0.05$ ) different.

The four experimental feeds performed in a very close manner with very few significant differences in the studied growth parameters indicating the feasibility of using this fish biosilage as a partial replacement for fish meal in common carp fry diets. Several authors confirmed that fish silage could replace fish meal partially in fish feeds without any adverse effects on feeding, growth and survival of fish while reducing feed costs. They explained that by the close resemblance in major nutritional components between the two ingredients as they are produced from similar raw materials (Fagbenro and Jauncey, 1998; Soltan and El-Laithy, 2008; Ayoola, 2010).

Growth parameters of fish in this experiment compares similarly or favourably with some previous studies. Gumus *et al.* (2009) used tuna liver meal (TLM) to replace fish meal (FM) in diets for common carp fry. They fed fish with average weight of 0.32 gm on each of six isonitrogenous (42%), isolipidic (16%) and isoenergetic (18 KJ DE g<sup>-1</sup>, 4302 Kcal/kg) diets prepared to include 0, 10, 20, 30, 40 and 50% of FM protein being substituted by TLM. The control diet contained fish meal (17.14%) and soybean meal (46.9%) as the main sources of dietary protein. After 13 weeks of feeding, they reported SGR values of 1.49-2.24, FCR 1.53-3.52, PER 0.72-1.63 and survival rates 70-87.5% which are close or lower than those reported in the current study. They indicated that up to 20% of FM protein in fish diet can be replaced by TLM without adverse effects on fish growth, feed utilization and body composition. The differences with the results of the current study may be ascribed to the lower energy content in comparison with current study (4302 vs. 4600 Kcal/kg, respectively) and lower temperature of rearing water (25 vs. 28°C, respectively). Takeuchi *et al.* (2002) demonstrated that energy content, thus P/E ratio, in common carp diets are determinant factors that governs feeding and growth efficiency especially during the early life stages and diets must be designed carefully to satisfy the optimum levels of these components.

Desai and Singh (2009) indicated that common carp fry (Average weight 0.86 gm) showed better feeding and growth performance when they reared under temperature of 28°C in comparison with 32°C, considering it as an optimum for this life stage. Davies and Gouveia (2010) studied the response of common carp fry fed diets containing a pea seed meal (*Pisum sativum*) subjected to different thermal processing methods. Starting from initial weight of 7.50 gm in a 7 week trial on 40% CP diets, they reported SGR values of 2.51-2.84, FCR 1.58-1.82 and PER 1.38-1.58 which are relatively coincides with the results of the current study. They concluded that high grade protein pea concentrates (up to 60% CP after starch extraction) could replace about 20% of fish meal in common carp fry diets without significant alterations in feeding and growth performance. Rahman *et al.* (2012) reared mirror carp advanced fry in aquaria for seven weeks and fed it on different protein levels (25-45%). At the end of their experiment they recorded SGR values of 0.71-1.14, FCR values of 4.31-12.7 and survival rates of 70-93.33 %. These values are generally inferior in comparison with the results of the current study as shown in table 3. There are several potential reasons for these differences such as fish initial weight (2.29 vs. 0.68 gm in the current study), stocking density (1 fry/l. vs. 0.5 fry/l., in the present study) and feed protein source (10% from fish meal vs. about 30% from fish meal and fish silage in the current study). Several

researchers indicated that younger fish have higher growth rates, animal protein sources are better performed as feed ingredients than plant proteins and fish stocking density is correlates inversely with fish growth (Wilson, 2002; Jha and Barat, 2005; Dumas *et al.* 2010).

As to thermal growth unit coefficient TGC, three different formulae were observed in the literature which based on the same concept of weight gain, water temperature and duration of study. The only difference was in multiplication factor. The first formula was that of Cho (1992) which did not contained any multiplication factor; the second is that of Bureau *et al.* (2000) which multiplied by 100 and the last formula was that reported by Jobling (2003) which was multiplied by 1000. The latter was chosen for the current study because it offered the best fit with fish growth data. Values from researches used other formulae were converted accordingly for comparison purposes. However, TGC values which calculated in the current study (table 3) were very similar to those reported previously for common carp. Jafaryan *et al.* (2011) reported TGC values of 0.319-0.472 when they investigated the enhancement of growth parameters in common carp (*Cyprinus carpio*) larvae using probiotic in rearing tanks and feeding by various *Artemia* nauplii. They reared fish larvae (average weight 0.12 gm) at 24-26°C for 28 days and indicated that the addition of probiotic bacilli to rearing tanks had different effects on the growth parameters of common carp larvae when they were fed on different *Artemia* nauplii. Sahandi *et al.* (2012) reported identical TGC values (0.319-0.472) to that of Jafaryan *et al.* (2011) when they investigated the effect of probiotic bacilli on the growth rate of common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella* larvae (average weight 0.12 gm) which reared at 30°C for 28 days and fed on *Artemia* sp. They concluded that inoculation of rearing water with probiotic bacteria could improve larval growth performance under the favorable conditions by enhancing digestive activities within the alimentary canal of fish larvae. Al-Dubaikel *et al.* (2012, 2013) reported values of 0.40-0.49 at an average water temperature of 26°C and initial body weight 5.5-7.5 gm for common carp fingerlings fed either roquette oil (*Eruca sativa*) as an additive or different flavoring agents, respectively which are very close to the findings of the current study.

Jobling (2003) emphasized that the popularity of the TGC model for fish growth relates to its ease of use and flexibility; growth data collected for fish of given size at one temperature can be used to predict the growth of fish of a different size when held at other temperatures. The TGC has proven to be stable over a wide range of temperatures for several species and is also less affected by fish size and time interval between weightings than other growth rate estimates such as SGR, and thus offer a simple model for growth rate comparisons (Bureau *et al.*, 2000).

#### 4. Conclusions

Fish biosilage is a very satisfactory partial alternative for fish meal in feed formulated for common carp fry. Fish biosilage can be made easily from locally available and cheap raw materials. It can be included successfully into feed formulations intended for common carp fry without any adverse effects on feeding and growth performance as well as fish survival rate. From the economic view of point, fish biosilage is superior in addition to its environment friendly nature.

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