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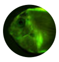
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Application of fish gelatin as diet binder in diets for the common carp *Cyprinus carpio* L. fingerlings

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Abstract:

Fish gelatin applied as an animal source binder and stabilizer in diets for the common carp *Cyprinus carpio* L. fingerlings in comparison with binder void diet. Experimental diets prepared from standard raw materials adding laboratory made fish gelatin at 1% as binder. Three types of gelatin applied (extracted from tilapia *Tilapia zilli* skins, common carp *Cyprinus carpio* L. gas bladder and barred needlefish *Ablennes hians* heads). Three diets containing each type of extracted gelatin were prepared to compare with gelatin void diet. Fish initial average weight 5.56 gm were fed at 5% body weight daily. Chemical and physical analyses performed on pellets thrown into water to feed fish as affected by added gelatin. The influences of gelatin monitored on pellet density, floating period, sinking velocity, water absorption rate and disintegrating rate as indicators for pellet quality and resistance into water. Feed intake by fish during research period studied also. Results showed that bladder gelatin diet gave the highest weight increase in comparison with other diets which reached 15.6gm. Physical measurements demonstrated that the highest density recorded in bladder gelatin diet (1.4 gm/cm^3) followed by head and skin gelatin diets (1.3 gm/cm^3) while the lowest density recorded in gelatin void diet (1.1 gm/cm^3). Floating time of 8.55 sec. and sinking velocity of 1.06 sec./cm recorded for bladder gelatin diet in comparison with 7.53, 7.31 and 5.30 sec. for floating time and 1.37, 1.43 and 2.47 sec./cm for sinking velocity of diets containing head , skin gelatin and control, respectively. Various treatment indicated impacts on growth where weight increase rated 10.26-15.6 g, specific growth rate 1.10-1.61 and relative growth rate 39.46-62.40 %. Bladder gelatin diet surpassed others in weight increase, specific and relative growth rates. Feed conversion ratios reached 3.156, 2.862, 3.597 and 4.306 for skin, bladder, head gelatin and control diets, respectively. As for feed intake, bladder gelatin treatment gave the highest values. Chemical composition did not affected by using binders.

Keywords: Gelatin, Common carp, Binder, Fish diet, Feed intake.

I. Introduction:

Many feeding stuffs added to fish diets for different purposes, some improve the functional properties of diet as stabilizers, binders, antioxidants and attractants which considered as non-nutritive additives or supplying diets with some nutritive additives such as essential nutrients included vitamins, minerals, cholesterol and phospholipids [1, 2].These, in turn, prepare diet in an acceptable state for fish to eat voraciously and be benefited [3].

Most artificial diets at the local markets are of inferior stability into water [4]. Winowiski (1995) indicated that fish need good quality pellets containing specific binders when it made by conventional pelleting method (6, 7]. The main purpose for use is to maintain the stability of diet into water and prevent wasting before consumption by fish [8]. New (1987) mentioned that any disintegration of diets accounted as waste because of use into water where pellet quality measured throw crash resistance. As fish feed slowly on introduced diets, dietary pellets should have good stability into water [6]. Huang (1990) demonstrated that binders categorized into three main sections: natural, cereal byproducts and artificial. In fish diets manufacture, many traditional binders used as starch, agar, gelatin, molasses, carboxy- methyl cellulose (CMC) and wheat meal which have various impacts on stability, floating and nutrient waste [10, 11, 12, 13]. Additionally, many non-conventional materials used as binders like cement kiln dust (cement manufacture byproduct) in rainbow trout diets [14]. Bentonite used also to rise pellet resistance and decrease disintegration which lead to reduce production costs by 20% of total costs and increase growth rate by 14% [15, 16, 17].

Fish gelatin considered of main protein sources and natural stabilizers which constitute in integration of pellets used in intensive and semi-intensive fish culture systems which depends on artificial feeding only because pellet physical properties affects considerably on fish production [18]. Thomas and van der Poel (1996) stated that different animal species require different physical properties for introduced feeds. Of the important characteristics of fish feeds are pellet sinking velocity, water absorbency and stability. Some researches approved that binder properties did not affected only by binder kind and concentration but also with diet composition, particle size, manufacture conditions [20] and protein source [13].

The aim of the present study was to investigate the effect of addition three sources of gelatin prepared in laboratory on the properties of pellets, feed intake and growth of common carp fingerling.

I. Materials and methods:

Common carp *Cyprinus carpio* fingerlings (average weight 5.56 gm) brought to the laboratory from the fish farm at Marine Science Centre, University of Basrah. Fish maintained into 30 L plastic aquaria supplied with ventilation. Aquaria disinfected with sodium hypochlorite solution (200 ppm) for one hour [21].

Fish acclimated to experimental conditions for 3 days during which it fed on the standard diet(27.89% protein). Fish divided at the beginning of experiment at 6 per aquarium, with two replicates for each treatment. Experimental diets made from standard raw materials (Table I) and adding laboratory prepared fish gelatin at 1% as binder. Three sources of gelatin (extracted from tilapia skins, common carp gas bladder and needlefish heads) used. Three diets manufactured each contain one type of gelatin in comparison with gelatin void diet (control). Fish fed at 5% body weight once a day at 9:00 am for 30 days. Fish weighed every 10 days and fed quantity adjusted accordingly. About one third of water quantity changed daily with siphoning of wasted feed and feces. Chemical composition studied along with the most important physical properties of dietary pellets. Gelatin influence monitored on pellet density (g/cm^3), floating period, sinking velocity (cm/sec), water absorbance (%) and disintegrating rate (%) as indicators for dietary pellet quality and resistance into water. In addition, fish feed intake studied during experiment period.

A. *Chemical composition of diets***:**

All chemical analyses carried out for manufactured diets with three replicates. These included moisture and ash percentages according to the methods of AOAC (1984). Lipid and total nitrogen estimated according to Egan *et al*. (1988).

B. *Physical properties of diets:*

1- Density of dietary pellets. To estimate the density of the prepared diets, method of Misra *et al.* (2002) was used. One gram of diet put into scaled cylinder containing known volume water. The rise in water level (volume change) because of adding diet, which represents diet volume, was recorde Then density calculated as:

Density (gm/cm^3) = Mass/ Volume (1)

2- Diet water absorbance. Water absorbance of diets calculated according to APHA (1992). One gram of dry diet moisturized by immersion into water for 1 minute then pellets took out of water and weighed. This process repeated for 3 and 5 minutes to determine the quantity of absorbed water as percentage of the original weight as:

% Absorbance= (Wt P / WtD) X100 (2)

3- Floating time. The potential floatability of pellets estimated according to Al-Habeeb (1996). Known weight of diet put into a glass aquarium filled with water. The time in which pellets remained floated on water surface monitored and recorded until pellet sinking down.

4- Sinking velocity (settlement). The method of Rout and Bandyopadhyay (1998) followed to estimate pellet sink ability. One gram of pellets put into glass cylinder filled of water

at height of 120 cm. The time for pellets to reach cylinder bottom from 40 cm mark and settle was recorded. Sinking velocity (Sv) calculated as:

Sv (cm/ sec)= 40 (cm)/ s (sec) (3)

5- Disintegration rate. The method of Egan *et al*. (1988) followed to measuring pellet disintegration rate. Five grams of pellets put into aluminum foil sheet, which folded 10 times. Then, sheet opened, disintegrated and solid portions isolated and weighed separately. The rate of disintegrated pellets calculated as percentage of the original weight.

Relative growth rate (RGR) and specific growth rate (SGR) calculated according to Jobling (1993) while feed conversion ratio (FCR) calculated depending on Hepher (1988) as:

RGR(%)=(Wti/ Wt1)X100 (4)

SGR = [(lnWt2- lnWt1)/D] X 100 (5)

FCR= Wtc / Wti (6)

Where:

WtP= Pellet weight after immersion.

WtD= Dry pellet weight after immersion.

Wt1= Initial weight

Wt2= Final weight.

Wti= Weight increment.

Wtc= Consumed feed weight.

Table. I: Composition of experimental diets for the common carp, using gelatin as binder.

Components (%)	Skin gelatin diet	Bladder gelatin diet	Head gelatin diet	Control
Fishmeal	25	25	25	25
Soybean meal	25	25	25	25
Wheat bran	19	19	19	19
Corn meal	14	14	14	14
Barley meal	14	14	14	14
Vitamins and minerals	2	2	2	2
Gelatin	1	1	1	0

II. Results:

Table II show the actual chemical composition of manufactured diets used for feeding the common carp fingerlings. Statistical analysis indicated non-significant differences (P>0.05) between treatments as to moisture, ash and lipid contents.

Protein content values in diets notable increase with adding gelatin in comparison with control diet. Bladder gelatin treatment reached the highest value of 32.20% while it declined to 27.89% in the control showing significant differences (P< 0.05) with other treatments. Skin and head gelatin diets did not differ significantly (protein values 30.18 and 32.09%, respectively). The same table indicates also significant differences (P<0.05) between head gelatin and control diets as to carbohydrate contents.

Table. II: Actual chemical composition of manufactured diets.

Treatment	Nutrient (%)					Caloric value, Kcal/100g
	Moisture	Ash	Lipid	Protein	Carbohydrates	
Skin gelatin diet	5.20 a	5.92 a	4.30 a	30.18 a	54.4 ab	446.90 a
Bladder gelatin diet	4.99 a	5.85 a	4.18 a	32.20 b	52.78 ab	449.43 a
Head gelatin diet	5.29 a	5.94 a	5.85 a	32.09 a	50.83 a	455.26 a
Control	5.29 a	6.20 a	4.29 a	27.89 c	56.33 b	443.13 a

Values in the same column with a common superscript are not significantly different (P>0.05).

Physical properties of manufactured diets are demonstrated in table III. Binder void diet attained the lowest density of 1.1 g/cm^3 while it ranged in gelatin containing diets between 1.3-1.4 g/cm^3. Statistical analysis showed non-significant differences between treatments (P>0.05). Bladder gelatin diet showed longest floating time of 8.55 sec. while it did not exceed 5 sec. in control diet with significant differences (P< 0.05) in comparison with other diets. However, no significant differences (P> 0.05) were shown between skin and head gelatin diets (floating times 7.31 and 7.53 sec., respectively). Pellet disintegration rate was 0% for all manufactured diets. This reflected on sinking velocities which were higher significantly (P< 0.05) in control diet (2.47 cm/sec.) comparing to other treatments. Skin, bladder and head gelatin diets recorded 1.43, 1.06, and 1.37 cm/sec., respectively.

Table. III: Physical properties of manufactured diets

Treatment	Density, g/cm^3	Floating time, sec	Sinking velocity, cm/sec	Disintegration, %
Skin gelatin diet	1.3 a	7.31 a	1.43 a	0 a
Bladder gelatin diet	1.4 a	8.55 b	1.06 b	0 a
Head gelatin diet	1.3 a	7.53 a	1.37 a	0 a
Control	1.1 a	5.30 c	2.47 c	0 a

Values in the same column with a common superscript are not significantly different (P>0.05).

The results in table IV indicated that gelatin addition affected significantly decreasing pellet water absorbability during various immersion times. The values start to increase for all treatments. Control diet attained highest value after 5 min. (210.17 %) which differed significantly (P< 0.05) with others. The remained diets maintained the same trend with values of 166.52, 161.0 and 164.08 % for skin, bladder and head gelatin treatments, respectively.

Table. IV: Diet water absorbability percentage.

Treatment	Water absorbance after		
	1 min.	3 min.	5 min.
Skin gelatin diet	141.40 a	155.79 a	166.52 a
Bladder gelatin diet	138.54 a	152.93 b	161.0 b
Head gelatin diet	140.97 a	156.45 a	164.08 c
Control	177.55 b	192.85 c	210.17 d

Values in the same column with a common superscript are not significantly different (P>0.05).

Table V demonstrated the biological parameters of feeding and growth. It showed that fish final weights did not differ significantly ($P > 0.05$) between skin gelatin and control diets (35.8 and 36.26 gm, respectively), while skin gelatin diet differed significantly with other diets. Additionally, results indicated significant differences between head gelatin diet with final weight of 44.4 gm and both skin gelatin and control diets, while not differed significantly with bladder gelatin diet of final weight 40.6 gm. Highest FCR values reached by control diet 4.306, which differed significantly ($P < 0.05$) with others. In contrast, bladder gelatin diet attained the lowest FCR value of 2.861. The latter differed significantly with head gelatin diet and non-significantly with skin gelatin diet. Values of RGR and SGR indicated the superiority of bladder gelatin diet on other diets (62.4% and 1.61 gm/d). As for absolute weight increment, bladder gelatin and control diets attained the highest and lowest values (15.6 and 10.26 gm, respectively).

Table. V: Initial and final weights, weight increment, relative, specific and daily growth rates, feed conversion
ratio and survival of fish fed on manufactured diets.

Treatment	Skin gelatin diet	Bladder gelatin diet	Head gelatin diet	Control
Fish No.	6	6	6	6
Initial Wt. , gm	23	25	30	26
Final Wt. , gm	35.8 a	40.6 bc	44.4 b	36.26 ac
Absolute increment	12.8	15.6	14.4	10.26
RGR, %	55.65	62.4	48	39.46
SGR, %/d	1.47	1.61	1.30	1.10
DGR, gm/fish/d	0.07	0.08	0.08	0.05
FCR	3.156	2.861	3.597	4.306

Values in the same row with a common superscript are not significantly different ($P>0.05$).

Fig. 1 illustrates fish feed intake. Bladder gelatin diet achieved significantly ($P < 0.01$) highest intake by fish in comparison with other diets. Intake decreased significantly in control diets as to other treatments. However, skin and head gelatin diets did not differ significantly ($P > 0.05$).

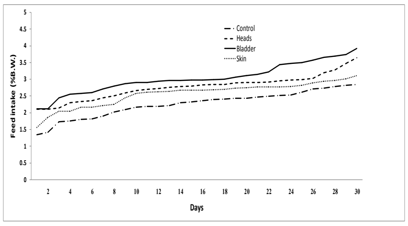

Fig.1: Feed intake as percentage of body weight(%B.W.) of common carp fingerlings at different days of experiment.

IV. Discussion:

The present study demonstrated that application of binder in diets improved its chemical and physical properties in addition to the role in promoting feed intake and growth parameters [8]. The suggested rate of binder usage in fish feeds is less than 2% [7]. It was found that gelatin addition to diets affected significantly its chemical composition especially elevating protein content. Gelatin could serve as a binder in the diet, beside its role as pure protein source [30]. Johnston-Banks (1990) indicated that gelatin is used to improve the nutritional quality for its high content of protein it also supports the stability, durability, and flexibility of foodstuffs in addition to improving the functional properties considerably. The results indicated the superiority of gelatin containing diets on control with the physical properties. Pellet density, floating time and sinking velocity are important for pellet stability into water because most fishes are surface feeders [7, 32].

Ward and Courts (1977) and Hudson (1994) stated that gelatin is a protein with gel forming ability between 30-35°C. It dissolves by heating into solutions and solidifies by cold in water, gelatin became semi solid with high viscosity showing clear solution [34]. The results indicated decreased water absorbability in gelatin containing diets comparing to control. This may be due to the stronger cohesion of pellets with fewer pores, which limit water permeation between components. As a result, water absorption decrease and resistance to disintegration elevate [35, 36].

Gelatin is an organic binder forms readily by hydrogen bonds with water because of the many available polar domains [35]. It swallows with water absorption to form a gel, which in turn compact and arrange into continuous molecules that became more configured and organized. Misra *et al*. (2002) stated that pellets water absorbability within known time could serve as an indicator for pellet quality of disintegration resistance into water, they indicated also that pellet quality could be evaluated through testing water absorbability and stability of pellets. From the current study, it was found that application of gelatin as binder affected positively the various growth parameters. Higher values attained by gelatin containing diets, this may be due to the improved diet quality and palatability [12]. And it was found that gelatins from dried fish skins had higher crude protein [37].

Glycine was the most abundant amino acid in both of gelatin sample and it was about 19-21% of total amino acids [38], the joint work of FAO/WHO/UNU (1985) demonstrated that gelatin produced from animal collagen of various origins contain high proportion of amino acids 25-50% which elevate its nutritional value. Gelatin contain also 98% protein with high levels of Glycine (26034%), Proline (10-18%), Hydroxyproline (7-15%) , Alanin (8-11), Arginine (8-9%), Glutamic acid (10-12%), Aspartic acid (6-7%) [39; 40;, 41]. This in turn improve feed conversion ration using the binder [42].

VI. Conclusion:

It was found that using gelatin from different source improve diet characteristics and growth of fish, also bladder based gelatin may be the best source compared to skin or head sources, but its yield were less than the other two sources for economic uses.

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