

Mucilage Extraction from Basil (*Ocimum basilicum*) and its Applications in the Diets of *Cyprinus carpio* Fingerlings

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Abstract: Mucilage extracted from the dried sweet basil *Ocimum basilicum* and used as a binder in the diets of *Cyprinus carpio* fingerlings as an alternative to some traditional binders. Three diets were tested, i.e. treatment T1 (control), treatment (T2) and treatment (T3), which contained 0, 1 and 2% from basil mucilage, respectively. Basil mucilage inclusion did not affect the chemical composition of the experimental diets. The results of the current experiment showed that treatment T2 was the best comparing to the remaining treatments in terms of food intake and diet acceptance by fishes. This is evident from physical and chemical tests in terms of buoyancy, stability, fragmentation, density and floating time. In summary, the study has concluded that the process of extracting this gel from basil is simple and cheap, in addition to being a natural polymer.

Keywords: Binder, Gum, Feed intake, Rihan, Physical properties

Introduction

Natural polymers obtained from plant sources are of high molecular weights that are molecules of monosaccharides bound with glucosidic bonds (Krishna et al., 2011).

Currently, there are many pharmaceutical and industrial extracts, most of which are of vegetable origin, some examples are starch, agar, alginate, carrageenan, guar gum, xanthan gum, gelatin, pectin, chitosan, acacia, cellulose, sugars, etc. These natural extracts find application in the pharmaceutical industry as binding agents, disassemblers, sustainable and protective agents, colloids, densities, gelling agents and stabilizers (Kolhe et al., 2014).

Food industries are aiming at the uses of new plant-based food hydrocolloids as gelling agents in product development (Hussain et al., 2019). Using of hydrocolloids in feed was the main purpose due to their ability to modify the rheology of the food systems by the long polymer chains of polysaccharides and proteins to develop gel-like characteristics (Salehi et al., 2015).

Mucilage is a gum, a thick, sticky substance produced by almost all plants for defensive purposes (Pawan et al., 2001). Also, in plants it has a role in the storage of food, water and seed germination, and besides thickening membranes, some microorganisms used it for adhesion (Mondal & Rahaman, 2018) and the proposed roles of extracellular mucilaginous material (ECMM) include attachment of spores or mycelium to various substrates and protection (Vesentini et al., 2007)

Basil, *Ocimum basilicum*, locally known as Rihan, belongs to the mint family Lamiaceae (ITIS, 2020), which have TSN (Taxonomic Serial No. 32627). Various parts of sweet basil have been generally utilized in conventional medication such as the leaves and blossoms that utilized in people medication as a tonic and vermifuge (Ahmad et al., 2015). Paton & Putievsky (1996) stated that the genus *Ocimum* is an important essential oil crop with around 100 ton produced throughout the world annually, half of this from *O. basilicum*.

Phytochemical (the bioactive nonnutrient plant compounds) investigation of whole or specific parts of basil have been done by many researchers and a number of active constituents have been identified and used in both the ancient Indian (Ayurveda) and traditional (Unani) systems of medicine (Muralidharan & Dhananjayan, 2004; Rubab et al., 2017). It is estimated that more than 5000 phytochemicals have been identified, but a large percentage still remain unknown (Shahidi & Naczk, 1995).

The extracted mucilage from basil contain about 55%, 25%, 2% and less than 1% of carbohydrate, fiber, protein and fat, respectively (Hussain et al., 2019).

Kolhe et al. (2014) mentioned that the synthetic polymers used to suffer from many defects such as high cost, toxicity and environmental pollution, while the advantages of natural vegetable polymers include low cost, free from side effects and considered as renewable sources.

As fish diets make up about 60-80% of the production costs in fish culture (Orire & Sadiku, 2014). Therefore, it should be stable with minimal ratios of loss and fragmentation, especially in species with slow feeding behavior (Volpe et al., 2012). The use of locally available materials encourages fish farmers to use sustainable sources due to the low cost (Oke et al., 2017).

Feed industries are focusing on using of new plant-based food hydrocolloids as gelling agents in product development with unique sensory qualities.

The purpose of the present study is to evaluate the specifications of basil *O. basilicum* extract as binders for fish diets, which are used as alternatives to other binders, and to examine the physical, chemical and biological specifications of the produced pellets and its application in the diet of the common carp *Cyprinus carpio* fingerlings.

Materials and Methods

Extraction of Mucilage

The extracting method of mucilage from *O. basilicum* was carried out according to Razavi et al. (2009) and Amid & Mirhosseini (2012), with modification. The

mucilage extraction condition started by drying the cleaned leaves at 60°C and pH 7 for 48 hours. After grinding, 100 mg was taken and diluted with 5 ml distilled water for a period of 24 hours. Ethyl alcohol (95%) was added to the extracted crude gum using 15 ml of alcohol to one volume of the extract and left for overnight. To recover the precipitate, a fine mesh has been used to allow the excess solvent to drain and the final mixture was filtered with filter paper and dried at 60°C to get rid of alcohol. The dried mucilage was then grounded and used in the diets.

Experimental Diets

Dried basil extract was added to the raw ingredients involved in the formulation of fish diets. The experiment was divided into three treatments:-

Diet 1 contains 0% basil mucilage (T1 control)

Diet 2 contains 1% basil mucilage (T2)

Diet 3 contains 2% basil mucilage (T3)

Table 1 shows the ingredients used in processing the diets used in the experiment.

Table 1: Ingredients of experimental diets for common carp fingerlings.

Ingredients	Inclusion in diets (%)		
	Diet T1	Diet T2	Diet T3
Fish meal	15	15	15
Wheat flour	30	30	30
Wheat bran	35	35	35
Soybean meal	17	16	15
Sunflower oil	2	2	2
Minerals and vitamins premix	1	1	1
Basil mucilage	0	1	2

Physical Tests of Diets

The physical tests were conducted for the prepared diets as mentioned by Rolfe et al. (2000), Sørensen (2007) and Efrizal et al. (2019).

Bulk Density (BD)

Samples from the diet were emptied into a 1000 ml cylinder and the weight of the 1000 ml diet was measured. Bulk density was then stated as g/cm³.

Relative Absorption Rate (RAR)

Relative absorption rate represents the measure of the volume of water absorbed by the pellets in relation to its initial weight and calculated as follows:

$$\text{RAR} = (M_2 - M_1) / M_1 \times 100$$

Where M₂ = Mass of wet pellets

M₁ = Initial mass of dry pellets

M₂ - M₁ = Weight gain after immersion in water.

Sinking Velocity (SV)

This test was accomplished by measuring the time required for pellets to move from the surface of the water to the bottom of the aquarium with a height of 20 cm from the upper surface of the water surface.

Floatability (F)

Pellets from each diet were tested by the appearances of 10 pellets (floated or sunk) noted after 10 minutes in 100 ml beakers after 10 minutes.

Disintegration Rate (DR)

The pellet hardness test was measured by using 5 gm of pellets and placed into an aluminum foil sheet, which folded 10 times. Then, the sheet was opened, disintegrated and solid portions were isolated and weighed separately. The rate of disintegrated pellets was calculated as a percentage of the original weight.

Feed Intake Experiment

The fingerlings of the common carp (6.15 ± 0.25 g) were brought to the laboratory from the fish farm at Marine Science Centre, University of Basrah. Saline solution (5% NaCl) for 5-10 minutes was used for fish sterilization after their arrival at the laboratory in the Department of Marine Vertebrates (Marine Science Center, University of Basrah) to eliminate fungi and/or other organisms that may be stuck on the skin or gills. Fishes were acclimated to experimental conditions for three days during which they were fed on the standard diet.

At the beginning of the experiment, fishes were distributed by placing 10 fishes in each aquarium ($18 \times 27 \times 34$ cm) with two replicates (six aquariums) for each treatment. Experimental diets were made from standard raw ingredients (Table 2). Fishes were fed at 5% body weight, once a day, at 9:00 am for 14 days. About one-third of water quantity was changed daily with siphoning to get rid of wasted feed and feces. Uneaten food was air-dried. The dry weight was measured and subtracted from the total food weight, to measure the weight of the food intake every day, the feed intake calculated as follows:

$$\% \text{ Feed intake} = \frac{\text{Diet weight (g)}}{\text{Fish weight(g)}} \times 100$$

Chemical Composition of Diets

All chemical analyses were performed for processed diets, with three replicates, according to the methods of AOAC (1984).

Statistical Analysis

Differences between the treatments were evaluated by using one way ANOVA and then Post hoc tests were applied by using LSD test. In all cases, the assumed significance level was 5% ($\alpha = 0.05$). All data were expressed as mean \pm standard

deviation (SD). This statistical analysis was completed with the computer software SPSS package.

Results

Water Quality

Water quality during the experiment was measured. Average water temperature was 27.6 ± 0.7 °C, dissolved oxygen concentration was 6.5 ± 0.5 mg/l, while the pH was 8.4 ± 0.17 and salinity was 0.79 ± 0.01 PSU.

Diets Composition

The chemical composition of the processed diets is demonstrated in Table 2.

Table 2: Chemical composition of processed diets.

Chemical composition (%)	Treatments		
	Diet T1	Diet T2	Diet T3
Moisture	$10.54^a \pm 0.42$	$10.49^a \pm 0.41$	$10.45^a \pm 0.41$
Protein	$24.04^a \pm 1.99$	$24.10^a \pm 1.97$	$24.16^a \pm 1.95$
Lipid	$5.15^a \pm 0.06$	$5.24^a \pm 0.06$	$5.33^a \pm 0.06$
Ash	$6.20^a \pm 0.97$	$6.30^a \pm 0.96$	$6.39^a \pm 0.95$
Fiber	$4.05^a \pm 0.64$	$4.12^a \pm 0.64$	$4.19^a \pm 0.63$
Carbohydrates	$50.02^a \pm 0.86$	$49.75^a \pm 0.85$	$49.49^a \pm 0.84$
Caloric value (MJ/kg)	$17.94^a \pm 0.23$	$17.88^a \pm 0.23$	$17.82^a \pm 0.23$

Values are in duplicate \pm standard deviation.

Different letters among rows are significantly different at $P < 0.05$.

Bulk Density (BD)

The bulk density mean values of fish feed pellets are shown in Table 3. These values were 1.17, 1.06 and 1.03 g/cm³ for T1, T2 and T3, respectively, with significant differences ($P < 0.05$) between them. The results indicated that the bulk density of pellets decreased with the increase in the basil mucilage inclusion.

Table 3: Physical properties of manufactured diets.

Physical properties	Treatments		
	Diet T1	Diet T2	Diet T3
Bulk density (BD g/cm ³)	$1.17^a \pm 0.01$	$1.06^b \pm 0.01$	$1.03^c \pm 0.00$
Floatability (F)	$3.00^a \pm 0.82$	$11.00^b \pm 0.82$	$14.00^c \pm 0.82$
Relative absorption rate (RAR %)	$141.43^a \pm 0.94$	$128.06^b \pm 0.88$	$135.97^c \pm 1.19$
Sinking velocity (SV cm/s)	$4.23^a \pm 0.39$	$1.90^b \pm 0.16$	$2.70^c \pm 0.33$
Disintegration rate (DR %)	$24.40^a \pm 0.71$	$11.40^b \pm 0.78$	$7.90^c \pm 1.10$

Values are in duplicate \pm standard deviation.

Different letter among rows are significantly different at $P < 0.05$.

Floatability (F)

Manufactured diets, fortified with basil mucilage (Table 3), had higher significant ($P<0.05$) floating time than control, about 73% and 79% mean floatability increased in 1% and 2% (11.00 and 14.00 F, respectively) diets containing basil relative to control (3.00 F).

Relative Absorption Rate (RAR)

The diets which contained 1% basil (T2) demonstrated the lowest water absorption (128.06%), which differs significantly ($P<0.05$) from the control (T1) and 2% diets (T3): 141.43 and 135.97%, respectively, as demonstrated in Table 3.

Sinking Velocity (SV)

Sinking velocity ranged between 1.90 cm/s in diet T2 to 4.23 cm/s in diet T1. Diet T3 had a medium value of 2.70 cm/s. All values were significantly ($P<0.05$) different.

Disintegration Rate (DR)

The results of pellets disintegration rate showed that there were significant differences ($P<0.05$) among the diets. The diet with basil 2% (T3) had the least percentage of disintegration (7.90%), while the control diet (T1) had the highest rate (24.40%).

The correlation coefficient between all physical parameters pointed out that six of these parameters were positively significantly correlated, while four of them were negative from the ten possible correlations (Table 4).

Table 4: Correlation coefficient between physical properties of the experimental diets.

Parameters	Bulk density (BD)	Floatability (F)	Relative absorption rate (RAR)	Sinking velocity (SV)	Disintegration rate (DR)
Bulk density (BD)	0	-1.00**	0.62*	0.80**	0.99**
Floatability (F)		0.00	-0.61*	-0.78**	-0.99**
Relative absorption rate (RAR)			0.00	0.90**	0.67**
Sinking velocity (SV)				0.00	0.82**
Disintegration rate (DR)					0.00

*Correlation coefficient (r) significant at $P<0.05$.

**Correlation coefficient (r) significant at $P<0.01$.

Feed Intake

No mortalities were recorded during the experiment period since water quality parameters did not vary significantly. No significant differences ($P>0.05$) were

found between treatments concerning the final weight (Table 5). However, T2 (1% basil) revealed significant differences ($P < 0.05$) in percentage weight increase with T1 (control) and T3 (2%), while food intake in T2 did not differ significantly ($P > 0.05$) with T3 but only with T1. However, T1 and T3 did not differ significantly ($P > 0.05$) in respect to the two parameters. The weight increase in T2 exhibited unexpected value reached 16.07%, while the other two treatments did not exceed half of this value.

Table 5: Performance of common carp fed the experimental diets.

Parameters	Treatments		
	T1	T2	T3
Initial wt. (g)	60.47 ^a ±3.20	62.46 ^a ±4.31	61.65 ^a ±2.43
Final wt. (g)	63.50 ^a ±2.77	72.47 ^a ±4.36	66.64 ^a ±1.68
% increase	8.12 ^a ±1.55	16.07 ^b ±1.04	5.05 ^a ±0.98
% food intake	2.17 ^a ±0.08	2.46 ^b ±0.54	2.03 ^{ab} ±0.11

Values are in duplicate ± standard deviation.

Different letters among rows are significantly different at $P < 0.05$.

Discussion

All the environmental factors in the present study were within the acceptable limits for the common carp growth and survival (Horváth et al., 2002).

The composition and manufacture of the diets is a process that combines scientific practice and scientific research (Hardy & Barrows, 2002). Table 3 shows the chemical composition of prepared diets used for feeding the common carp fingerlings. With respect to all contents, the inclusion of basil mucilage does not affect the chemical composition of the diets. Al-Dubakel et al. (2014) found the same result in adding fish skin gelatin as a binder for all contents except the protein, as well as Tiamiyu & Solomon (2012) when used different grain starches as binders in most diet contents. Ruscoe et al. (2005) stated that diets containing 5% binder retained significantly more dry matter than those with a 3% binder.

Proximate composition of sweet basil is shown in Table 6 on dry and fresh bases. Akah et al. (2017) revealed that dried *O. basilicum* may serve as enrichment material in products such as baked goods according to its chemical properties, while the study of Chaudhari et al. (2012) stated that the tablets produced by using basil as a binder gave comparable evaluation results to the tablets with starch as a binder.

Different studies showed different relations between binders and density. The lowest density was found in the control diet (Al-Dubakel et al., 2014), while Khater et al. (2014) noted that bulk density increases with increasing the pellet sizes and protein ratio. Oke et al. (2017) did not record significant differences in the bulk density of the nine feeds with okra and yeast as binders. Aquatic feed bulk density is one of the quality control measurements for extruding fish feed plants (Kaddour, 2018).

Table 6: Proximate composition of *Ocimum basilicum*.

Parameters	Contents	
	Dry (Shuaib et al., 2015)	Fresh (USDA, 2018)
Ash (%)	15.73±0.004	1.49
Crude lipid (%)	14.50±0.003	0.64
Moisture (%)	5.72±0.003	92.06
Crude fiber (%)	11.31±0.008	1.6
Crude protein (%)	30.00±0.000	3.15
Carbohydrate (%)	22.7±0.012	2.65
Caloric value (kcal)	281.5±0.022	23

It was reported that diets with yeast had a higher percentage of floatation than their corresponding counterparts without yeast (Momoh et al., 2016). Rokey & Plattner (2006) stated that pellets when immersed in water will quickly sink if their bulk density is more than 640 g/l (=0.64 g/cm³). However, pellets were considered very dense if their apparent bulk density ranging from 0.43 to 0.5 g/ml (Rolfe et al., 2000). Basil mucilage improves gels quality due to its high gel strength and low strain effects (Song & Kim, 2019). This indicates that gel enhances the floating characteristics.

Momoh et al. (2016) indicated that the inclusion of yeast influenced the relative absorption ratio which ranged from 39.77% to 70.56%. Oke et al. (2017) also revealed that the water absorption rate increased as yeast inclusion increased. The results of the present study were in line with the values obtained in gelatin addition which decreasing significantly pellet water absorbability (Al-Dubakel et al., 2014). Previous studies verified similar values with different binders (Al-Dubakel et al., 2014; Efrizal et al., 2019).

Oke et al. (2017) demonstrate that the crushing load test showed that good water stability did not certainly mean good hardness. Al-Dubakel et al. (2012) used roquette oil (*Eruca sativa*) as a food additive and found that all diets with and without addition had a 0% breakdown. Sinking velocities of the pellets were positively correlated with feed bulk densities. Obirikorang et al. (2015) reported similar results.

Diet characteristics, such as feed source whether living or non-living, particle size, texture, palatability and bulk density, all must be carefully considered for both species and size of fishes (Eriegha & Ekotoku, 2017).

Subramanian (2013) stated that physical characteristics such as excess of starch and fiber may determine fish feed intake because of the increase in density of the pellets. Basil also has low calorific value and high nutritional values (Dzida, 2010). The low food intake in diet contained 2% basil could be associated with odor. Efrizal et al. (2019) found that spinach extract inclusion in formulated diets had affected its textures and odor.

Feed intake is subjected to metabolic, endocrine, and neuronal control, which are closely related to the nutritional status of the animal (Lall & Tibbetts, 2009).

Conclusions

In the current study, the use of gel extracted from basil and using it in the diet of common carp fingerlings as a natural binder was demonstrated. Plants extractions is useful in fish culture as more natural and low-cost binders could replace the chemical ones. The present findings revealed that the physical properties of pellets were improved by the inclusion of gel (mucilage) extracted from the basil. Because the present findings could be applied in the diet of fishes, they are likely to be of great interest to the vision of researchers and fish culturists.

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