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Effect of dietary gelatinized starch on growth performance, feed utilization and body composition of Nile tilapia (*Oreochromis niloticus* L.)

[1] Maurice G Yeo, *[2] Célestin M Blé, [3] Soumaila Dabonne, [4] Yao L Alla, [5] Athanase K Otchoumou, [6] Assoi O Etchian

[1, 2,4] Département Aquaculture, Centre de Recherches Océanologiques (CRO), BP V 18 Abidjan, Côte d'Ivoire
[1, 3] Département des Sciences et technologies des aliments, Laboratoire de Biocatalyse et des Bioprocédés, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire
[5] Laboratoire de Biotechnologies, Filière Biochimie-Microbiologie, UFR Biosciences, Université Felix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire
[6] Laboratoire de Biologie et Cytologie Animales, UFR-SN, Université Nangui Abrogoua (Ex Université d'Abobo-Adjamé), 02 BP 801 Abidjan 02, Côte d'Ivoire

Abstract
A 5 week feeding trial was carried out to determine the effect of gelatinization temperature of starch on juvenile *Oreochromis niloticus* growth, feed utilization and body composition. Five isoproteic and isocaloric diets were formulated to contain different gelatinized starch (R0, R70, R85, and R100) and a control diet containing a maize flour. Growth performance and feed conversion ration responded in a positive manner with a R70 and R85, reflecting protein and lipid sparing effect. No further improvement in growth was observed with diet R100, but there is a protein sparing effect. Nitrogenous losses varied from 9.34 to 11.48 g Kg^{-1} day^{-1}, with the significantly lowest value was observed in fish fed with R70, the gelatinization reduced releases nitrogen in water. Whole body protein and lipid content were significantly affected by different gelatinized starches; the content is highest with R70 and R85. Under our experimental conditions, the best performances in terms of growth rate and nutrient utilization were observed with the starch gelatinized at 70 °C.

Keywords: gelatinization, protein-sparing, retrogration, *Oreochromis niloticus*, growth

1. Introduction

Starch is polysaccharid of vegetable origin (cereal grains and tubers), some fruits may also be rich in starch (Cheftel *et al*., 1977) [1]. The nutritional role of starches is important, they transformed into glucose after digestive hydrolysis, the principal source of calories in the human diet. Except human diet, the cereals are used in various fields such as animal feed (pisciculture, poultry farming, breeding of pig). The availability and accessibility of raw materials of vegetable origin are an advantage for substitution of fish meal in pisciculture (Soule and Gansari, 2010) [2].

The use of carbohydrates in fish diets is known for their capacity to provide energy in the form of digestible carbohydrates and to spare expensive proteins for fish growth (Oliva-Teles, 2002) [3]. Although lipids are also an important source of non-protein energy for fish, they are more expensive and less available compared to carbohydrates. The digestibility of carbohydrates depends to fish species, warm water fish species utilize carbohydrates more efficiently and at higher levels than cold water and marine fish species (Robinson & *al*., 2001; Stone, 2003) [4, 5]. Omnivorous species such as Nile tilapia can easily utilize dietary levels of carbohydrates (Amirkolaie & *al*., 2006) [6]. Technological treatment (extrusion, gelatinization) can improve the digestibility of carbohydrates (Svihus & *al*., 2005) [7]. Gelatinization is a hydrothermal treatment in which the texture of the starch grain is modified to improve the susceptibility of the starch degradation in the digestive tract (Svihus & *al*., 2005) [7]. Utilization of carbohydrates by fish also depends on the degree of gelatinization, the type of starch and dietary inclusion levels (Shiau, 1997) [8]. However, the dietary carbohydrates are known to cause a postprandial hyperglycaemia and metabolic disturbances associated with decreased fish growth rates (Enes & *al*., 2011, Panserat & *al*., 2013) [9, 10].

Tilapia *Oreochromis niloticus* is one of the most suitable species for aquaculture in sub Saharan African, particularly Ivory Coast (FAO, 2012) [11]. Because of its characteristics such as rapid growth, tolerance to various environments and good acceptance of commercial diet. This fish is a better asset for the evolution of fish production in Ivoiry Coast (FAO, 2012) [11]. In this study, three gelatinization temperatures (70 °C, 85 °C and 100 °C) are use to modify the structure of the corn starch grains and these gelatinized starches are used as ingredients in the feed of Tilapia *O. Niloticus*. The objective of this study is to determine the influence of the gelatinization temperature on the growth of tilapia.

2. Materiels and methods

2.1 Experimental fish

The experimental was carried out in the reproduction laboraty, at Center of Oceanological Research, Abidjan, Ivoiry Coast. The Tilapia *Oreochromis niloticus* (34.62 ± 2.03 g) used in this experiment was obtained from populations raised in the aquaculture ponds at layo (Dabou) experimental station.

Hundred and twenty were collected from the fish hatchery and acclimated to the experimental conditions for two weeks. During this period, fish received a commercial diet (35 % protein) twice daily (8 am and 4 pm). Fish were distributed over 15 glass aquaria stocking 8 fish per aquaria and 3 replicate glass aquaria assigned to each of 4 dietary treatments.

2.2 Experimental system

Glass aquaria (50 L containing 45 L tap water that has undergone a natural dechlorination of 24 h before adding fish) were in a closed water system. En electric motor pump ensured a constant flow (1.5 L/min) of well-aerated water. Water was filtered by setting at 30 % daily exchange. It was totally replaced weekly. Every day, at 7:30 am, before feeding, the water quality (temperature, pH and dissolved oxygen) was measured with a multi parameter (BANTE 900P) and a pH meter (WTW 330). Throughout the experimental period the water temperature, pH and dissolved oxygen varied between: 27.07 – 27.37 °C, 6.99 – 7.22 and 6.15 – 6.94 mg/l respectively and were considered favourable in tilapia culture according to Melard (2004) [12] and Kestmont (2004) [13].

2.3 Process of gelatinization of starch

Starch maize (10 % w/w 0.5 kg of starch in 5 kg of distilled water) were gelatinized at 70 °C (or 85 °C, 100 °C) for 30 min using a reactor with a paddle rotated at a fixed speed of 150 rpm. The starch slurry was heated from room to 70 °C (or 85 °C, 100 °C) and maintained at 70 °C (or 85 °C, 100 °C) for 30 min. Then, sample was freeze-dried (Biobase Model BK-FD 1 OP) for 24 h at -40 °C and 0,37 mbar vacuum pressure and heated up to 20 °C. The flour obtained was grounded and subdued with a sieve (ϕ = 1mm).

2.4 Diet preparation

Five isoproteic and isocaloric experimental diets were formulated with different gelatinized starch (0 °C; 70 °C; 85 °C; 100 °C) and a control containing maize flour (Table 2). Gelatinized starch was analyzed for their degree of gelatinization before the feed formulation. The degrees of gelatinization are at 70 °C (42.46%), at 85 °C (54.82%) and at 100 °C (71.89%). The protein in the diet was supplied by fish and soybean meal. Maize starch and wheat bran were used as a source of carbohydrates and crude fish oil was used as lipid source. The ingredients were purchased from local markets in Abidjan. The test diets were prepared as follows: all feed ingredients without digestive juice were ground to a suitable size and mixed in a commercial mixer (KENWOOD CHEF) for 15 min. Vitamin and mineral mixes were gradually added with continuous mixing. Distilled water was slowly added while mixing to achieve a consistency suitable for pellet production. Then, the 3 mm diameter pellets were formed through a kitchen meat grinder (Panasonic MK-G 1800P) and dried for 24 h at 37 °C. All diets was sealed in plastic bags and stored at 20 °C throughout the experiment.

Table 1: Proximate composition of ingredients (% dry weight)

Ingredients	Proteins	Lipids	Ash	Fiber
Fish meal	45.23	8.75	20.35	
Soybean meal	45.25	4.30	5.95	5.22
Wheat bran	18.81	3.89	5.86	10.16
Corn meal	9.17	3.20	1.08	6.29

Table 2: Formulation and composition of experimental diets (% dry weight).

			Diets		
Ingredient (g/100g)	**R0**	**R70**	**R85**	**R100**	**RC**
Fish meal	43	43	43	43	43
Soybean meal	32	32	32	32	32
Wheat bran	5	5	5	5	5
Corn meal	0	0	0	0	12
Starch native	12	0	0	0	0
Gelatinized starch	0	12	12	12	0
Cassava starch	2	2	2	2	2
Fish oil	4	4	4	4	4
Vitamin mixture[1]	1	1	1	1	1
Mineral mixture[2]	1	1	1	1	1
Total	100	100	100	100	100
Proximate analysis (% dry matter)					
Dry matter	90.13	90.48	90.73	90.45	90.11
Crude protein	35.04	35.04	35.04	35.04	36.17
Total fat	9,33	9,33	9,33	9,33	9,95
Ash	10,95	10,95	10,95	10,95	11,14
Crude fiber	2,18	2,18	2,18	2,18	2,57
Carbohydrate	36,1	36,1	36,1	36,1	33,99
DE (Kj/g) [3]	13,9	13,9	13,9	13,9	13,88
Protein/DE (mg/KJ) [4]	25,09	25,09	25,09	25,09	26,06

[1]Per kg premix: cobalt 20 mg, iron 17 600 mg, iodine 2 000 mg, copper 1 600 mg, zinc 60 000 mg, manganese 10 000 mg, selenium 40 mg

[2]Per kg premix: vitamin A1 760 000 IU, vitamin D3 880 000 IU, vitamin E 22 000 mg, vitamin B1 4 400 mg, vitamin B2 5 280 mg, vitamin B6 4 400 mg, vitamin B 1 236 mg, vitamin C 151 000 mg, vitamin K 4 400 mg, vitamin P 35 200 mg, folic acid 880 mg, choline chloride 220 000 mg, pantothenic acid D 14 080 mg.

[3]Digestible energy = 18.8 x protein + 37.7 x lipid content + 11.3 x carbohydrate content (Smith, 1971; Page et Andrews, 1973) [14, 15]

RC: diet containing corn meal; R0: diet containing starch native; R70: diet containing gelatinized starch at 70 °C; R85: diet containing gelatinized starch at 85 °C; R100: diet containing gelatinized starch at 100 °C.

2.5 Experimental condition

After the acclimation period, the fish were fed till apparent satiation with the experiment diets twice daily (8 am and 4 pm) for 35 days. To quantify the exact feed intake, refused feed was siphoned out immediately, dried and weighed. Once a week, five fish were randomly sampled in each glass aquaria to measure body weights and glass aquaria were cleaned. Dead fish were removed from glass aquaria per days. At the beginning the experiment, an initial sample of ten fishes were taken and frozen (-20 °C) for subsequent whole body proximate analysis. At the end of the experiment, the weight of each fish was recorded. Ten fish from each diet were stored at – 20 °C for whole-body composition analysis.

2.6 Chemical analysis

2.6.1 Starch gelatinization

The degree of gelatinization of different carbohydrate sources was estimated as follows (Guraya and Toledo, 1993) [16]. A known amount (0.2 g) of dried sample was mixed with 15 ml of 0.2 N potassium hydroxide followed by intermittent stirring for 30 min. The pH of the mixture was adjusted to 5.5 using 2 N phosphoric acid and the volume was made upto 100 ml with distilled water. Next, 100 µl of aliquot was transferred to a test tube and diluted to 5 ml with distilled water. Then 50 µl of standard iodine solution (4% KI, 1% I_2) was added and the absorbance of the solution was measured at 600 nm (A_1) against the reagent blank. Another aliquot was made by the same procedure by mixing 0.2 g of dried sample in 15 ml of 0.6 N potassium hydroxide and the absorbance was measured at 600 nm (A_2) as above. The degree of gelatinization was calculated as follows:

Gelatinization % = (A_1/A_2) 100

2.6.2 Proximate analysis

Proximate compositions of diets and fish were determined as followed standard methods (AOAC, 2000) [17]: Crude protein (N x 6.25) was determined by the Kjeldahl method after acid digestion. Crude lipid was determined by the ether extraction method (Soxtherm, Gerhardt, Germany). Dry matter was determined by oven-drying at 105 °C for 24 h. Ash and crude fiber were determined, respectively by combustion at 550 °C in a muffle furnace to a constant weight and by acid/alkali digestion. Gross energy contents were calculated, from fat and protein contents, using the equivalents of 38.9 KJ.g crude fats, 22.2 KJ.g^{-1} crude proteins and 17.2 KJ.g^{-1}carbohydrates (NFE) (Luquet and Moreau, 1989) [18].

2.7 Biological evaluation

Dailly weight gain (DWG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), protein gain (PG), lipid gain (LG), Nitrogen loss and gross energy (GE) metabolism, were calculated as followed:

- DWG = (W_2 – W_1) / t
- SGR = 100 (LnW_2 – LnW_1) / t; where W_1 is the initial body weight, W_2 is the final body weight and t is the duration of the experiment.
- FCR = Dry feed consumed / Wet weight.
- PER = Wet weight gain / Protein consumed.
- Protein gain or lipid gain = Final carcass nutrient content – Initial carcass nutrient
- GE = 22.2 x protein content + 38.9 x lipid content + 17.2 x carbohydrate content (Luquet and Moreau, 1989) [18].
- NL = nitrogen intake – nitrogen gain

2.8 Statistica analysis

Resultats were statistically analyzed with a one way analysis of variance (ANOVA) using Statistica version 7.1 software packages. Duncan's multiple range tests was used to compare difference between treatments means when significant F-values were observed. All percentage and ratio data were arc-sin transformed before analysis (Zar, 1984) [19]. The treatment effects were considered to be significant at $p < 0.05$.

3. Resultats & Discussion

The results of growth performance and nutrient utilization of *Oreochromis niloticus* fed with different diets at the end of 35 days feeding are reported in Table 3, the survival rate ranged from 83.33 to 91.67 %. Final body weight (FBW) and Specific growth rate (SGR) ranged from 55.55 to 61.9 g and 1.34 to 1.64 % d^{-1}, respectively with the highest values in fish fed with a diet containing gelatinized starch at 70 °C. Feed conversion ratio (FCR) ranged from 0.9 to 1.3, with highest values in fish fed with a diet containing gelatinized starch at 100°C, Protein efficiency ratio (PER) of fish fed with the diets containing gelatinized starch at 70 °C was significantly different from those of fish fed with the diets containing gelatinized starch at 0 °C, 85 °C, 100 °C and a control diet ($p < 0.05$). Nutrients utilization parameters were represented by Daily protein gain (PG), Daily lipid gain (LG) and nitrogenous losses. No significant differences were observed in Daily protein gain, furthermore, Daily lipid gain was highest in fish fed with the diets containing gelatinized starch at 70 °C and 85 °C ($p < 0.05$). However, nitrogenous losses varied from 9.34 to 11.48 g Kg^{-1} day^{-1}, with the significantly highest value was observed in fish fed with diet containing gelatinized starch at 100 °C ($p < 0.05$).

At the end of the experimental period, the whole body moisture, gross energy and ash content of fish did not differ significantly between the experimental diets (Table 3). However, the whole body protein and lipid content were significantly affected by different gelatinized starches.

Table 3: growth performance, nutrient utilization and Body composition of *Oreochromis niloticus* fed with a differnts diets for 35 days.

			Diets		
Parameters	**R0**	**R70**	**R85**	**R100**	**RC**
IBW (g)	34.82 ± 1.8	34.9 ± 1.42	34.62 ± 2.03	34.69 ± 1.28	34.85 ± 1.66
FBW (g)	55.87 ± 3.56^{a}	61.9 ± 2.43^{b}	58.78 ± 2.2^{ab}	55.55 ± 3.08^{a}	56.62 ± 3.25^{ab}
Survival (%)	87.5 ± 12.5^{a}	87.5± 12.5^{a}	83.33± 14.33^{a}	91.67 ± 7.22^{a}	83.34 ± 14.43^{a}
GWG (g d^{-1})	0.6 ± 0.05^{a}	0.77 ± 0.08^{b}	0.69 ± 0.05^{ab}	0.6 ± 0.05^{a}	0.62 ± 0.05^{a}
SGR (% d^{-1})	1.35 ± 0.05^{a}	1.64 ± 0.16^{b}	1.51 ± 0.16^{ab}	1.34 ± 0.06^{a}	1.39 ± 0.03^{a}
FCR	1.27 ± 0.11^{b}	0.9 ± 0.1^{a}	1.07 ± 0.09^{ab}	1.3 ± 0.11^{b}	1.18 ± 0.08^{b}
PER	2.28 ± 0.2^{a}	3.2 ± 0.36^{b}	2.68 ± 0.23^{ab}	2.21 ± 0.19^{a}	2.34 ± 0.15^{a}
PG (g. Kg^{-1} day^{-1})	4.28 ± 1.20^{a}	5.26 ± 0.04^{a}	5.94 ±1.44^{a}	4.05 ± 0.50^{a}	4.85 ± 1.40^{a}
LG (g. Kg^{-1} day^{-1})	1.96 ± 0.44^{ab}	2.37 ± 0.52^{b}	2.61 ± 0.55^{b}	1.57 ± 0.17^{ab}	2.33 ± 0.55^{b}
NL (g. Kg^{-1} day^{-1})	11.26 ± 0.95^{b}	9.34 ± 0.35^{a}	10.45 ± 0.28^{ab}	11.48 ± 0.89^{b}	11.18 ± 0.82^{b}
Body composition (%)					
Moisture	76,97 ± 1.23^{a}	75.3 ± 1.34^{a}	75.35 ± 0.84^{a}	75.55 ± 1.96^{a}	76.99 ± 0.35^{a}
Crude protein	13.8 ± 0.28^{a}	15.15 ± 0.21^{c}	14.81 ± 0.28^{bc}	14.3 ± 0.29^{ab}	13.8 ± 0.04^{a}
Total fat	4.81 ± 0.33^{b}	5.66 ± 0.34^{b}	5.27 ± 0.24^{b}	4.48 ± 0.67^{b}	5.03 ± 0.2^{5a}
Ash	3.77 ± 0.81^{a}	3.45 ± 0.67^{a}	3.25 ± 0.13^{a}	4.45 ± 0.18^{a}	3.46 ± 0.42^{a}
Gross energy (Kj g^{-1})	5.05 ± 0.16^{a}	5.64 ± 0.03^{a}	5.57 ± 0.19^{a}	5.13 ± 0.53^{a}	5.14 ± 0.19^{a}

Means in a row with different superscripts significantly ($p < 0.05$).

Initial composition of fish: moisture 79.03%, crude protein 13.39%, fat 3.32 %, Ash 3.58 %, gross energy 4.38 Kj g^{-1}

These results show the influence of the gelatinization treatment on the use of starch in the feeding of fish. There is an improve of growth performance and nutrient utilization is observed when fish are fed with a diet contained gelatinized starch at 70 ° C, with a degree of gelatinization of 42.46%. The growth performance of the fish is proportional to the temperature of gelatinization, when the temperature or the degree of gelatinization increases, the daily weight gains decreases. These results are in contradiction with those of Kotara and Fuchus (2001) [20] which a growth of the pigs when the degree of gelatinization of starch increases. Hongtrakut and *al*. (1998) [21] indicates that the results of our study are due to the effect of a phenomenon which occurs during gelatinization called retrogradation. The modified starch obtained by a thermal treatment approximately of 100 °C in the presence of a water excess are very unstable at ambient temperature. During cooling, the macromolecules reorganize, giving rise to the phenomenon of retrogradation. In our study, gelatinization was carried out in the presence of a water excess and above the temperature of gelatinization of corn starch (60 -72 °C.), which favored the retrogradation in the starches at 85 °C and higher at 100 °C. When the temperature of gelatinization was high, the rate of retrograded starch was significantly high during cooling. Therefore, although gelatinization of starch increases the digestibility of carbohydrates, retrogradation decreases digestibility of starch in the small intestine (Haralampu, 2000) [22]. However, fish fed with the diets containing native starch, gelatinized starch at 100 °C and maize flour did not show a difference in growth performance and dry feed consumed. The treatment at 100 °C did not improve the digestibility of the starch, at this temperature and with the process of gelatinization used; the modified starch obtained became less digestible than the native starch and the cereal flour. This result contradicts with those Wilson (1994) [23] and Krogdhal and *al*. (2005) [24] which consider that gelatinization improves digestibility of starch in fish. However, it is agree with those of Peres and Oliva-Teles (2002) [25] on *Dicentrarchus labrax*, which obtained a growth gain, specific growth rate and a low dry feed consumed in fish fed with the diets containing gelatinized starch compared to native starch.

At level the protein gain, there is no significant difference between the formulated diets, but the lipid gain was greater in fish fed with the diets containing gelatinized starch at 70 °C at 85 °C. This lipid gain indicates a deposition of lipids in carcass of fish and also a sparing lipid to provide energy to the body. The low nitrogen losses in fish fed with a diet containing gelatinized starch at 70 °C indicates that proteins have not been catabolized but spare for growth. This metabolism shows the high capacity of juveniles of *Oreochromis niloticus* used carbohydrates to provide the energy finally to spare the proteins. These results are in agreement with those of Qadri and Jameel (1989) [26] which obtained improve growth and use of diets in *O. niloticus* fed with a diet rich carbohydrate (40% carbohydrate). However, this sparing protein depends on the species and their temperature of rearing (Brauge & *al*., 1995, Enes & *al*., 2006) [27, 28].

The biochemical composition of *Oreochromis niloticus* at the end of the experiment is not affected by the gelatinization treatment at the level of ash, moisture and energy. It is a agree with Xiao and *al*., (2007)[29] which did not observed significant difference in the body composition of *Sparus latus* fed with the diets containing different ratios of native starch and gelatinized starch. However, protein body and body lipid content appears to be influenced by gelatinization. The use of diets rich in digestible carbohydrates stimulates lipogenesis in omnivorous species (Wilson, 1994) [23], which increase a deposition of lipids in the carcass of fish. The deposition of proteins in the carcass may result from protein sparing, which avoids catabolism of proteins to produce nitrogenous waste (Cahu, 2004) [30].

4. Conclusion

The heat treatment result shows that the efficiency of the gelatinized starch depends on the cooking temperature. Gelatinization has improved the growth performance of fish. The gelatinized starch may be used for partial substitution of fish meal in aquaculture feeds.

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