Contribution to the Identification of a Local and Available Food Source for Sustainable Production of Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1758) in the Democratic Republic of Congo

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Summary

This experiment was conducted in parallel with work in the Democratic Republic of the Congo (DR-Congo) with the objective to evaluate the growth performance and production costs of Nile tilapia fed diets formulated using local plant protein sources, and to compare them to those obtained with an optimized commercial fish feed. Ninety monosex male tilapia juveniles (Oreochromis niloticus; mean weight 17.3±0.2 g; mean length 9.6±0.1 cm) were reared in 9 acrylic aquaria supplied by closed water recirculation system. Three diets were tested in triplicate: 1) Rcongo, the test diet formulated by using local feed ingredients collected in the DR-Congo; 2) Rcanada, the diet formulated with the same ingredient composition as the first one, but sourced in Canada: and 3) Rcommercial, a fishmeal-based commercial control diet. In vivo feed digestibility and biochemical analysis of samples were carried out. The effects of diet and rearing time were measured on fish performance, including fish biomass, K coefficient, weight gain (WG), feed efficiency (FE), feed conversion rate (FCR), specific growth rate (SGR), protein efficiency ratio (PER), apparent digestibility coefficient (ADC) and on nutrient budget and fish biochemical composition. After four weeks, the fish achieved weight gains of 52% for Rcongo diet (17.2±0.4 to 26.2±2.6 g), 59% for Rcanada diet (17.4±0.1 to 27.6±3.2 g) and 153% for the commercial diet (17.3±0.2 to 43.8±2.0 g). The costbenefit analysis has indicated that the Rcongo diet was economically advantageous, reducing fish production cost up to 36% compared to commercial feed.

Résumé

Contribution à l'identification d'un aliment local et disponible pour une production durable du tilapia du Nil (*Oreochromis niloticus*, Linnaeus, 1758) en République Démocratique du Congo

Cette étude a été conduite en parallèle à des recherches menées en République Démocratique du Congo (RD-Congo) avec l'objectif d'évaluer les performances de croissance du tilapia du Nil et les coûts de production d'un aliment produit à partir de sources de protéines végétales locales et de les comparer à ceux obtenus avec un aliment commercial optimisé. Quatre-vingt-dix tilapias juvéniles monosexes mâles de Oreochromis niloticus (poids moyen 17,3±0,2 g; longueur moyenne 9,6±0,1 cm) ont été élevés dans 9 aquariums en recirculation d'eau fermée et distribués de manière aléatoire. Trois rations ont été testées en triplicata: 1) la ration Rcongo, principal aliment test formulé à partir d'ingrédients locaux en RD-Congo; 2) Rcanada, aliment de même formulation que Rcongo mais dont les ingrédients ont été acquis au Canada; et 3) Rcommercial moulée commerciale et aliment témoin. L'étude de digestibilité des aliments et les analyses biochimiques des échantillons ont été réalisées. Les effets des rations et de la période d'élevage ont été évalués sur les performances zootechniques (biomasse, coefficient K, gain de poids (GP), efficacité alimentaire (EA), taux de consommation alimentaire (TCA), taux de croissance spécifique (TCS), coefficient de l'efficacité protéique (CEP), coefficient de digestibilité apparente (CDA) et sur le flux des nutriments et la composition biochimique des poissons. Au terme de 4 semaines d'essai, les poissons ont réalisé des gains de poids de 52% (17,2±0,4 à 26,2±2,6 g) pour la ration Rcongo, 59% (17,4±0,1 à 27,6±3,2 g) pour la ration Rcanada et 153% (17,3±0,2 à 43,8±2,0 g) pour la ration Rcommercial. L'analyse du rapport coût/bénéfice a indiqué que la ration Rcongo a été économiquement meilleure, car elle a permis une réduction de 36% du coût de production rapport par à Rcommercial.

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Introduction

Nile tilapia (Oreochromis niloticus, Linnaeus, 1758) is the main farmed fish in Africa and constitutes a major source of animal protein and income in developing countries. It is recognized as the most suitable fish species for tropical aquaculture having advantages such as: relative rapid growth, disease resistance, ability to adapt to a variety of environmental conditions, ability to thrive under extensive and intensive farming, and good quality flesh highly appreciated by consumers (23, 39). Among the most produced fish around the world, Tilapia spp represent the second highest group with 3.49 million tons (Mt) after carp (24 Mt), followed by Clariidae with 2.97 Mt and Salmonidae with 2.36 Mt (8, 17). Thus, the improvement of tilapia farming productivity is a promising alternative as animal protein source for human consumption that can contribute to alleviate food insecurity in DR-Congo and in Africa as a whole, owing to the aforementioned advantages and its remarkable adaptation to various conditions (16, 23, 32).

However, increasing tilapia yield is highly dependent on feed ingredient quality, which also depends on diet cost and its inputs (9, 15, 16). Unfortunately, the use of optimized feeds based on fishmeal or animal byproducts at elevated levels, is one of the major constraints for fish farming, given their high costs and limited availability. Additionally. the use of compounded feeds represents 50-70% of production costs in intensive fish farming (15, 16, 37). Nevertheless, the use of local and readily-available ingredients, mainly identified as agricultural byproducts, could improve availability and reduce production costs. Currently, this is in agreement with many efforts identifying less expensive protein sources for total or partial replacement of fishmeal, which could contribute to optimize growth rates of fish fed these diets (16, 23, 27).

To date, a number of inexpensive by-products that can be sourced locally have been used as fish feed ingredients able to reduce production costs (15, 19, 39). Previous work has reported that the replacement of fishmeal by certain by-products from oilseeds or cereals such as wheat bran, rice bran, soybean meal (SBM), brewers grain, has supported adequate fish growth performance (7, 14, 19, 22). The use of these by-products can therefore be adapted in the DR-Congo particularly and the surrounding sub-region in general, with the ultimate aim of contributing to increasing aquaculture productivity and sustainably (3, 7, 15, 17, 22, 39).

Therefore, this study was aimed to measure digestibility, growth performance, and to evaluate nutrient budgets of tilapia fed typical agricultural by-products. Additionally, the production costs of fish and feed formulated from local vegetable protein sources were analyzed and compared to those obtained with a conventional commercial fish feed.

Material and methods

Fish and experimental conditions

This experiment was conducted at the Laboratoire de Recherche des Sciences Aquatiques (LARSA) at Université Laval (Québec, Canada). It was carried out in accordance with the requirements of the Animal Protection Committee of Université Laval. Ninety monosex male tilapia juveniles (Oreochromis niloticus; mean weight 17.3±0.2 g; mean length 9.6±0.1 cm) were obtained from Sand Plains Aquaculture (Ontario, Canada) and reared in nine acrylic tanks supplied by a recirculating aquaculture system. Upon arrival, fish were subjected to a twoweek acclimation period. All fish were weighed, measured for total (TL) and standard lengths (SL) and transferred to 9 rectangular aquaria randomly assigned, each having a 10 liters water volume. Each aguarium was stocked with 10 juveniles, giving a density of 17 g/L. During the acclimation period, the fish were fed to satiety with a commercial feed (Corey Optimum, 2 mm dia, Fredericton, NB, Canada). The physicochemical parameters of water were regularly verified and maintained within tolerable limits for tilapia O. niloticus (16, 35). Temperature (26±0.5 °C), dissolved oxygen (9.2±1.6 mg/L), and pH (7.1±0.4) were continuously monitored. Ammonia (NH₃ \leq 0.01 mg/L), nitrites (NO₂ \leq 0.01 mg/L), and dissolved CO₂ (\leq 0.01 mg/L) were measured weekly using a Hach® Spectrophotometer; conductivity (117.8±1.2 µs/cm), alkalinity (9.2±0.1 mg/L CaCO₃), and water hardness (424 mg/L CaCO₃) were also verified weekly. The photoperiod (14 h: 10 h light: dark) was maintained using incandescent lights. A water flow of 1 L/min, corresponding to 6 tank water renewals per hour, was verified and adjusted daily. A permanent supply of freshwater and demineralised water in every aquarium was ensured according to the ratio (75: 25 w:w) in order to maintain pH 7, tolerable for tilapia (16). The behavior of fish was observed twice a day; mortalities and all anomalies observed in aquariums were recorded (decreased feed intake, incidence of disease, water flows changes...). Every day before the morning feeding, the aquaria were cleaned and siphoned off to remove biofilm and feed and fecal waste.

Feeding and diet formulation

Diet composition and nutrient content are presented in Table 1. Three experimental diets were tested in triplicate:

- *Rcongo*, a feed formulated from local ingredients from the Democratic Republic of the Congo,
- *Rcanada*, a feed formulated with the same ingredients as the first one, but acquired in Canada and,
- *Rcommercial*, a fishmeal-based commercial feed (Corey Optimum 2 mm, Fredericton, NB, Canada).

Ingradiante and production costs	Experimental diets							
Ingredients and production costs	Rcongo	Rcanada	Rcommercial					
Wheat bran (%)	46,7	46,7	-					
Wheat rice (%)	5	5	-					
Corn (%)	5	5	-					
Brewers grain (%)	5	5	-					
Soybean meal (%)	23,3	23,3	-					
Blood meal (%)	10	10	-					
Bone meal (%)	2	2	-					
African palm oil (%)	2	2 2						
Sipernat™50 marker (%)	1	1	1					
Feed cost USD/kg diet	0,38	0,50	1					
Production costs USD/kg fish	1,45	1,81	2,28					
Biochemical analysis								
Dry matter (%)	89,6	89,1	90,4					
Crude protein (%)	32,2	32,2 32,0						
Lipid (%)	5,0	6,3	18,3					
Gross energy, Mj/kg (kcal/kg)	19,7 (4541)	19,9 (4541)	23 (5497)					
Phosphorus (%)	1,0	1,0	1,4					
Crude fiber (%)	9,7	9,3	1,2					
Ash (%)	9,0	7,5	11,7					

 Table 1

 Biochemical composition, production costs and analysis of experimental diets in Nile tilapia Oreochromis niloticus¹.

¹Values are means of three fish in triplicate.

An indigestible silicon dioxide marker (Sipernat[™]50[®] Evonik, Piscataway, NJ) was added to all three feeds (1% w: w).

These diets were formulated according to the methods and recommendations of nutritional requirements of Nile tilapia and the apparent digestibility of the ingredients for the species (18, 34, 35). To prepare the feed, every dry ingredient was finely ground (appx. 120 µm) using a grinding mill (Foss CT 193 Cyclotech[™], Sweden), then weighed and homogeneously mixed. Palm oil and Sipernat[™]50[®] were mixed together before being added to the dry ingredient mix. Distilled water was added to 45% in the final mixture to form a homogeneous dough. The commercial feed, which had been previously prepared, was milled as above and mixed (Retsch®, Düsseldorf, Germany) in order to be able to integrate the Sipernat. Using an extrusion machine with a helical screw, the dough was pressed through the 1.9 mm mesh of matrix # 9. The knife attached to the output of the granular produced 2 mm long granules. The resulting granules were spheronized to standardize the particle size, according to the Tilapia Nutritional Requirements guidelines (18). The feed produced was then dried overnight at room temperature (25 °C) under a fume hood, sieved and stored at -20 °C in sealed plastic bags until used. Feeds were allocated to three different randomly-selected aquaria. The fish were fed twice daily at 8:00 a.m. and 4:00 p.m.

In order to avoid any waste or loss of feed, the diet (4% of the body weight/day) was distributed in small meals that lasted one hour. The amount of ingested and uneaten feed was recorded daily.

Digestibility assays in Nile tilapia fish

The faeces collection started 3 days following initiation of feeding of experimental feed intake. This has been facilitated by self-cleaning aquariums connected to the plastic collection vessels, with a conical end facilitating collection of faeces in a timely manner. The faeces were collected twice a day before each meal in order to avoid any faeces contamination by feed. To harvest the faeces, bottles were removed from aquariums and suspended before being carefully siphoned and emptied of their contents. The faeces were stored at -20 °C, lyophilized, dried at room temperature and finely ground for biochemical analysis.

Experimental sampling

One day prior each sampling (T_0 : initial, T_1 : intermediate and T_2 : final), the fish were subjected to a 24-hour fast. The initial sampling (T^0) started on 15th day since reception of fish. All fish were anesthetized by 3-4 minute bath in tricaine methanesulfonate (MS-222) and sodium bicarbonate solution (100 mg of MS-222 + 200 mg of sodium bicarbonate/liter of water), according to the protocol described by Popovic *et al.* (38).

Anesthetized fish were counted, individually weighed (Mettler Toledo[®], Switzerland), measured for TL and SL and kept by groups of 10 randomly-sampled fish in each aquarium for experiment.

Fifteen additional fish served as an initial sample (T_0) for proximal analyses. Individual fish were weighed and measured for length. The intermediate sampling (T₁) carried out on 29th day and all fish were counted, weighed, measured for TL and SL and 3 fish/aquarium were randomly sampled for proximal analyses. Finally, the final harvest (T_2) carried out on 43th day; all fish were sampled counted, individually weighed, measured for length and 3 randomly sampled fish in each aquarium were retained for proximal analyses. The sampling data were used to calculate fish survival rate and growth parameters, such as, weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), feed conversion rate (FCR), protein efficiency ratio (PER), feed efficiency (FE), feed intake (FI) and K coefficient, as indicated in equations I, II, III, IV, V, VI, VII and VIII.

- Survival rate (%) = (final number of fish/initial number) x 100;
- DWG (g/d) = (mean final weight (g) mean initial weight (g)) / day;
 (II)
- WG (g/fish)= final body weight (g/fish) initial body weight (g/fish);
 (III)
- SGR (%/d)= [(In final weight In initial weight)/ time (d)] x100; (VI)
- FCR= (total quantity of dry feed ingested (g)/ dry weight gain of fish (g);
 (V)
- PER= weight gain (g) / protein ingested (g); (VI)
- FE= weight gain (g/fish) / feed intake (g/fish);
 (VII)
- K coefficient= (body weight/ body length³) x 100 (VIII)

After each sampling event $(T_0, T_1 \text{ and } T_2)$ and digestibility assays, sampled fish were euthanized by an overdose of MS-222 according to Popovic *et al.* (38) and stored at -20°C. Individuals were autoclaved at 100 °C for 1 h 30 min, and ground using a homogenizer (Turrax[®] or Tissumizer[®], Cologne, Germany) to obtain a homogeneous dough which was stored at -20 °C. Before use, the frozen dough was then lyophilized, dried at room temperature and finely ground to a homogeneous powder for biochemical analyses.

Biochemical and nutrient budget analysis

Biochemical analyses of diets and tilapia carcasses (% of dry matter) were carried out at the Laboratoire du Département des sciences animales at the Université Laval, according to the Official Methods of AOAC International developed by Thiex *et al.* (47). Crude protein (N x 6.25) was determined using the Kjeldahl method, using a titration with acid (H_2SO_4 95-98%) solution. Lipid and crude fiber contents were determined using the ANKOM technology (New York,

USA); lipid by extraction using diethyl ether at 90 °C for 120 minutes; and crude fiber by digestion with acid $(0.255N H_2SO_4)$ and base (0.313N NaOH) solutions. Gross energy was determined using the calorimetric bomb method (Parr 6300®, Illinois, USA). Moisture content was determined by drying to a constant weight overnight at 100 °C. The total ash content was determined by combustion overnight at 600 °C. The acid-insoluble ash (AIA) content was determined by using a digestion with hydrochloric (6N HCl) and nitric (HNO₃) acid (4, 5). The apparent digestibility coefficient (ADC) of diets was determined using the indirect method of NRC (34) and Cho et al. (11), using Sipernat[™]50 as an indigestible marker found in diets. Thus, the ADC for dry matter, ash, phosphorus, nitrogen, lipids, gross energy and crude fibers were calculated according to the equation IX.

$$ADC = [1-(F/D \times D/F_{i})] \times 100$$
 (IX)

Where:

- D= % nutrient or kJ/g gross energy of diet;
- F= % nutrient or kJ/g gross energy of faeces;
- *D_i*= % (digestion indicator) AIA of diet;
- F = % (digestion indicator) AIA of faeces

The budget or nutrient retention by fish (*NRF*) was estimated for nitrogen (g/kg), phosphorus (g/kg) and gross energy (Mj/kg) according to Ma *et al.* (29), by the equation X.

$$NRF (g/kg \text{ or } Mj/kg) = 100 \times [NFB \times WFB] - (NIB \times WIB)]/(NF \times FI)$$
(X)

Where:

- NFB= Nutrient content in final biomass (g/kg or Mj/kg);
- NIB= nutrient content in initial biomass (g/kg or Mj/kg);
- WFB= weight of final biomass (kg);
- WIB= weight of initial biomass (kg);
- NF= nutrient content in feed (g/kg or Mj/kg);
- FI= feed intake (g)

Evaluation of diet and fish production cost

In this study, the economic assessment of experimental diets was focused on a cost-benefit analysis (7, 15), based on the cost per kilogram of feed consumed by fish to produce one kilogram of final biomass. The calculation has only targeted the direct costs related to the acquisition of ingredients until a product or final food (transport and inputs costs). However, indirect costs (electricity, water, manpower) usually associated with fish production and diet manufacturing were not taken into account because of the experimental design. All monetary values were then converted into US dollars (USD).

- Diet cost (USD/kg)= amount of ingredient incorporated x ingredient cost per kg (USD); (XI)
- Fish production cost (USD/kg)= diet cost (USD/kg) / fish biomass (kg);
 (XII)
- Production cost reduction rate (%)= [100 x (control diet – test diet)] / control diet (XIII)

Statistical analysis

The software statistical package SPSS Statistics 21 was used to perform statistical analyses. The parameters measured, using а completely randomized design, were subjected to an analysis of variance (two-way ANOVA) according to the multivariate or univariate linear model. In the case of significant differences (P<0.05), the results were subjected to Tukey's multiple comparison test (P<0.05) to determine the differences between treatment means, and to determine the factor effects and their interactions.

Results

Evaluation of nutritional and growth performance in fish

The results of this experiment revealed a highly significant interaction (P<0.0001) between the feeding (P<0.0001) and the rearing time (P<0.0001) regarding fish biomass and lengths (TL and SL) (Table 2). At the end of the experiment, fish achieved a mean weight gain of 52% (17.2 ± 0.4 to 26.2 ± 2.6 g) for the Rcongo test group, 59% (17.4 ± 0.1 to 27.6 ± 3.2 g) for the Rcanada group and 153% (17.3 ± 0.2 to 43.8 ± 2.0 g) for the Rcommercial control group. Indeed, diet exerted a significant effect on the fish length-weight ratio (K coefficient) (P=0.019). However, the Tukey's multiple comparison test (P<0.05) did not reveal any significant difference between the Rcongo and Rcanada diets on the biomass and fish lengths. As illustrated in Table 3, the feeding (P=0.602) and the rearing time (P=0.810) did not have a significant effect on fish survival rate, because no difference was observed for this variable. However, the feeding alone influenced verv significantly all measured growth variables (P<0.0001), such as weight gain, feed intake, feed efficiency, FCR, SGR and PER. The commercial diet was significantly higher than the Rcongo and Rcanada test-diets for these parameters. Table 3 also showed that the rearing time resulted in a very significant effect on weight gain (P<0.0001) but slightly significant on SGR (P=0.036). The interaction between the feeding and rearing time influenced highly a fish weight gain (P < 0.0001), while it had a low impact on food consumption (P=0.055).

Apparent digestibility of nutrients in tilapia

The results of nutrient digestibility experiments are summarized in Table 4. The apparent digestibility coefficients (ADC) of nutrients analyzed (dry matter, ash, phosphorus, crude protein, lipids and energy) were significantly affected by the feeding (P<0.0001). Tilapia fed with Rcongo test-diet showed a higher phosphorus (P<0.0001) and protein (P=0.001) ADC than those of the Rcommercial control group. In contrast, the ADC of dry matter, ash, energy and lipids were higher in tilapia of control group compared to those of the test group (P<0.0001). The rearing time influenced only the digestibility of crude ash (P=0.003), phosphorus (P=0.011), proteins (P=0.033) and lipids (P=0.010). A positive interaction was observed only on the digestibility of crude ash and phosphorus (P=0.001). In addition, the Tukey test revealed significant effects of three diets on the digestibility of dry matter, phosphorus, energy and lipids (P<0.0001). However, no differences were recorded between the Rcongo and Rcommercial diets on protein digestibility (P=0.339).

Nutrient budget in tilapia

The present experiment showed a very significant effect of feeding on intake and nutrient excretion (Table 5), including nitrogen (P=0.001), phosphorus (P<0.0001) and energy (P<0.0001). Nutrient excretion was higher in the fish fed with both *Rcongo* and *Rcanada* test diets than control diet. However, the nitrogen (P=0.817) and phosphorus (P=0.139) retention was not affected by the diet, except for energy (P<0.004), which was significantly higher in the fish of control group compared to the test group. The interaction between the feeding and the rearing time did not influence the nutrient budget in fish.

Biochemical composition of fish

The results of the biochemical analyses performed at the initial (T_0) , intermediate (T_1) and final time (T_2) are shown in Table 6. Diet affected the biochemical composition of tilapia (P<0.001 to P=0.036). The crude ash and protein contents were higher in carcasses of the Rcongo test group than those of the Rcommercial control group (P=0.001). The inverse situation was observed with lipids (P<0.003) and energy (P<0.001), whose contents were higher in the carcasses of the control group than in the test batch. However, the interaction effect between the two factors did not generally affect the biochemical composition of tilapias, except for dry matter (P=0.042) and energy (P=0.024), which were slightly affected by rearing time. In general, for all variables measured in the present experiment, the Tukey test (P<0.05) did not reveal any significant difference between the Rcongo and Rcanada test diets, except when they were compared with the commercial diet.

 Table 2

 Effects of diets and rearing time (4 weeks) on the biomass, total and standard length and K coefficient in Nile tilapia¹.

				Expe	erimenta	ldiets				_		?	
Parameters	Rcongo			Rcanada			Rcommercial			SE	P value ²		
	Τ ₀	Т1	T_2	T_0	Τ ₁	T_2	T_0	T ₁	T_2	_	Diet	Time	DxT
Biomass (g)	17,2ª	21,2 ^b	26,2 ^c	17,4 ^a	21,7 ^b	27,6 ^c	17,3 ^a	26,9 ^c	43,8 ^d	1,6	<0,001	<0,001	<0,001
Total length (cm)	9,7ª	10,5 ^b	11,1 ^c	9,6 ^a	10,5 ^b	11,3 ^c	9,6 ^a	11,2 ^{bc}	13,0 ^d	0,2	<0,001	<0,001	<0,001
Standard length (cm)	8,1ª	8,8 ^b	9,4 ^c	8,1 ^a	8,9 ^b	9,4 ^c	8,1 ^a	9,4 ^{bc}	11,0 ^d	0,2	<0,001	<0,001	<0,001
K coeff. (kg/cm ³)	1,90 [⊳]	1,83ª	1,93 [∞]	1,95°	1,87 ^{ab}	1,89⁵	1,96°	1,96°	1,98°	0,1	0,019	0,128	0,480

 1 Values are means of three fish in triplicate; ²Two-way ANOVA (P<0.05); Values with the different letters within the same row are significantly different according to the Tukey's test (P<0.05); SE: Standard error. T₀: initial sampling; T₁: intermediate sampling; T₂: final sampling.

Table 3
Effects of diets and rearing time (4 weeks) on some nutritional and
zootechnical performances in Nile tilapia ¹ .

		Ex	perime	ental die		2					
Parameters	Rco	ngo	Rcanada Rcommercial					P value ²			
	T ₀ -T ₁	$T_1 - T_2$	$T_0 - T_1$	$T_1 - T_2$	$T_0 - T_1$	$T_1 - T_2$		Diet	Time	D x T	
DWG (g/d)	0,3 ^a	0,4 ^b	0,3 ^a	0,4 ^b	0,7 ^c	1,2 ^d	0,1	<0,001	0,002	0,025	
WG (g/fish)	4,0 ^a	5,5 ^b	4,3 ^a	6,4 ^c	9,6 ^d	15,8 ^e	1,0	<0,001	<0,001	<0,001	
FI (g/fish)	6,6 ^a	8,9 ^b	6,9 ^a	10,5 ^b	8,6 ^b	14 ^c	0,6	<0,001	<0,001	0,055	
FE	0,6 ^a	0,6 ^a	0,6 ^a	0,6 ^a	1,1 ^b	1,1 ^b	0,1	<0,001	0,574	0,723	
FCR	1,7 ^a	1,7 ^a	1,8 ^a	1,7 ^a	0,9 ^b	0,9 ^b	0,1	0,001	0,471	0,428	
SGR_(%/d)	1,5 ^a	1,7 ^b	1,3 ^a	1,9 ^b	3,2 ^d	3,2 ^d	0,2	<0,001	0,036	0,218	
PER	2,1 ^a	2,2 ^a	2,1 ^a	2,1 ^a	2,4 ^b	2,4 ^b	0,1	0,003	0,534	0,874	
Survival (%)	100	95,2	96,7	100	100	100	0,9	0,602	0,810	0,269	

¹Values are means of three fish in triplicate; ²Two-way ANOVA (P<0.05); Values with the different letters within the same row are significantly different according to the Tukey's test (P<0.05); SE: Standard error.T₀-T₁: rearing from initial to intermediate sampling; T₁-T₂: rearing from intermediate to final sampling. DWG: Daily weight gain; WG: Weight gain; FI: feed intake; FE: feed efficiency; FCR: feed conversion rate; SGR: specific growth rate; PER: protein efficiency ratio.

Table 4

Effects of diets and rearing time (4 weeks) on the apparent digestibility coefficient (ADC) of nutrients and energy in Nile tilapia¹.

		E	Experime	_							
Nutrients	Rcongo		Rcanada		Rcommercial		SE	P Value ²			
	T ₀ -T ₁	$T_1 - T_2$	$T_0\text{-}T_1$	$T_1 - T_2$	$T_0\text{-}T_1$	$T_1 - T_2$		Diet	Time	DхT	
Dry matter (%)	55,1 ^a	53,7 ^a	45,1 ^b	45,5 ^b	80,4 ^c	79,3 ^c	3,6	<0,001	0,578	0,816	
Ash (%)	40,1 ^{bc}	31,4 ^a	37,0 ^b	31,0 ^a	42,4 ^c	45,6 ^d	1,4	<0,001	0,003	0,001	
Phosphorus (%)	59,1 ^a	47,4 ^{de}	38,1 ^b	31,6 ^c	43,0 ^d	48,2 ^e	2,2	<0,001	0,011	0,001	
Crude protein (%)	90,8 ^a	88,9 ^b	87,2 ^c	87,2 ^c	89,7 ^a	88,6 ^b	0,4	0,001	0,033	0,186	
Energy (MJ/kg))	61,5 ^a	61,3 ^a	49,7 ^b	50,5 ^b	87,3 ^c	86,2 ^c	3,8	<0,001	0,876	0,809	
Lipid (%)	77,8 ^a	71,7 ^b	60,5 ^c	54,5 ^d	94,4 ^e	94,0 ^e	3,7	<0,001	0,010	0,193	

¹Values are means of three fish in triplicate; ²Two-way ANOVA (P<0.05); Values with the different letters within the same row are significantly different according to the Tukey's test (P<0.05); SE: Standard error.T₀-T₁: rearing from initial to intermediate sampling; T₁-T₂: rearing from intermediate to final sampling.

 Table 5

 Effects of diets and rearing time (4 weeks) on the nutrient budget (nitrogen, phosphorus) and energy in Nile tilapia¹.

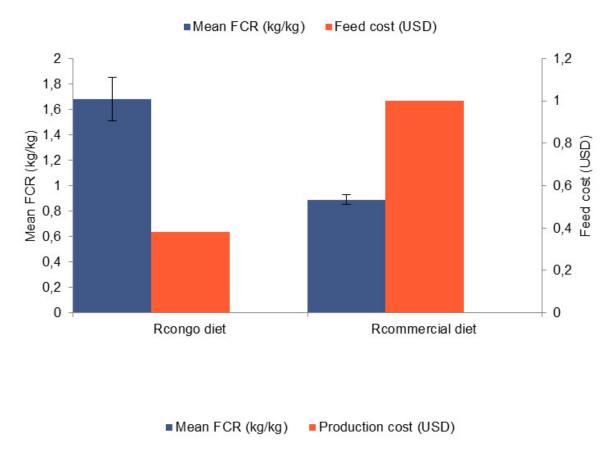
		E	Experime		_					
Nutrients	Rcongo		Rcar	Rcanada		Rcommercial		F	P Value ²	
	T ₀ -T ₁	$T_1 - T_2$	T ₀ -T ₁	$T_1 - T_2$	$T_0 - T_1$	T ₁ -T ₂		Diet	Time	D x T
Nitrogen (N) (g/kg)										
Intake N	76,3 ^a	74,4 ^a	75,4 ^a	75,5 ^a	66,9 ^b	65,9 ^b	1,2	0,001	0,585	0,878
Retained N	16,5	15,3	15,3	17,1	16,1	17,2	0,4	0,817	0,552	0,446
Rejected N	59,8 ^a	59,1 ^a	60,1 ^a	58,4 ^a	50,8 ^b	48,8 ^b	1,3	0,001	0,402	0,949
Phosphorus (P) (g/kg)										
Intake P	14,5 ^a	14,1 ^a	15,3 ^b	15,3 ^b	11,6 ^c	11,4 ^c	0,4	<0,001	0,587	0,902
Retained P	5,2	5,3	5,2	4,3	6	5,9	0,2	0,139	0,484	0,623
Rejected P	9,3 ^a	8,9 ^a	10,1 ^a	11,1 ^a	5,5 ^b	5,5 ^b	0,6	<0,001	0,740	0,573
Energy (E) (Mj/kg)										
Intake E	29,3 ^a	28,5 ^a	29,3 ^a	29,4 ^a	18,7 ^b	18,4 ^b	1,2	<0,001	0,589	0,867
Retained E	5,1 ^a	5,5 ^a	5,6 ^a	6,0 ^a	8,0 ^b	9,5 ^b	0,5	0,004	0,305	0,773
Rejected E	24,1 ^a	23,0 ^a	23,7 ^a	23,3 ^a	10,7 ^b	8,9 ^b	1,6	<0,001	0,225	0,806

¹Values are means of three fish in triplicate; ²Two-way ANOVA (P<0.05); Values with the different letters within the same row are significantly different according to the Tukey's test (P<0.05); SE: Standard error. T_0 - T_1 : rearing from initial to intermediate sampling; T_1 - T_2 : rearing from intermediate to final sampling.

Table 6								
Effects of diets and rearing time (4 weeks) on the biochemical composition (% DM) of								
Nile tilapia body ¹ .								

			E۶	cperime	ntal die							
Nutrients	T ₀ ²	Rcongo		Rcanada		Rcommercial		SE		P Value ³		
		T ₁	Τ2	T ₁	T ₂	T ₁	T_2		Diet	Time	DхT	
H ₂ O (%)	73,2 ^a	74,0 ^a	74,0 ^a	73,9 ^a	73,6 ^a	71,9 ^b	70,6 ^c	0,3	0,001	0,042	0,514	
Dry matter (%)	26,8 ^a	26,0 ^a	26,0 ^a	26,1 ^a	26,4 ^a	28,1 ^b	29,4 ^c	0,3	0,001	0,042	0,514	
Ash (%)	12,5 ^a	12,9 ^a	13,0 ^a	12,8 ^a	12,7 ^a	12,1 ^b	11,6 ^b	0,1	0,001	0,079	0,414	
Crude protein (%)	52,1 ^{ab}	54,8 ^a	54,1 ^a	53,7 ^a	54,6 ^a	51,2 ^b	50,1 ^b	0,5	0,001	0,404	0,519	
Lipid (%)	32,8 ^{ab}	30,3 ^a	31,4 ^a	30,9 ^a	31,0 ^a	34,3 ^b	36,2 ^b	0,6	0,003	0,179	0,748	
Energy (MJ/kg)	25,6 ^{ab}	25,1 ^a	25,4 ^a	25,3 ^a	25,2 ^a	25,9 ^b	26,5 ^c	0,1	<0,001	0,024	0,276	
Phosphorus (%)	2,1	2,2	2,2	2,2	2	2,1	2	0,1	0,036	0,060	0,381	

¹Values are means of three fish in triplicate. ²The initial sampling is unique for all three treatments. ³Two-way ANOVA (P<0.05); Values with the different letters within the same row are significantly different according to the Tukey's test (P<0.05); SE: Standard error. T_0 : initial sampling; T_1 : intermediate sampling; T_2 : intermediate sampling.



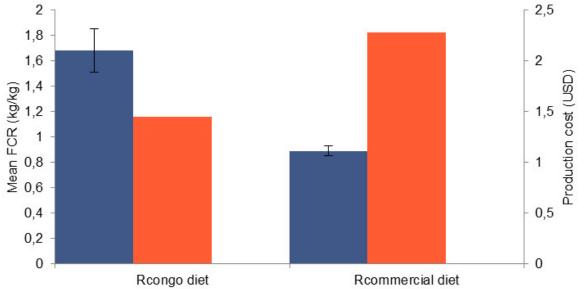


Figure 1: Comparison between food consumption (kg feed/kg fish) and costs of feeding (USD/kg feed) (A) and fish production (USD/kg fish) (B) of Nile tilapia rearing during 4 weeks. FCR: feed conversion rate in Nile tilapia *Oreochromis niloticus*

А

Diets and fish production costs

By considering separately the cost of each ingredient incorporated in diet formulation, the production cost of the *Rcongo* test diet was estimated at USD 0.38/kg of food, while the cost of acquiring the commercial feed was about USD 1/kg (Figure 1; Table 1). Thus, the cost/benefit ratio between feeding and fish biomass production allowed to estimate the production costs of fish at USD 1.45 and 2.28/kg, respectively, for the *Rcongo* test food and the commercial control feed. These data were used to calculate the cost reduction rates of feed and fish production in the test group, i.e., 62%= [100 x (USD 1 - USD 0.38)] / USD 1 for the feed, and 36% = [100 x (USD 2.28 - USD 1.45)] / USD 2.28 for fish.

Discussion

In the present study, the physico-chemical quality of tank water was maintained within limits tolerated by Nile tilapia, and the biochemical analyses of the ingredients used (Table 1) revealed adequate nutrient levels according to current recommendations. In terms of particle size and texture, the manufactured food pellets showed better cohesion of the particles (16, 35). The use of local ingredients as an alternative to reduce the cost of feeding did not affect fish survival. Throughout the experiment, fish showed no pathological signs, except for a few minor mortality cases (Table 3), likely associated with routine manipulations (16).

Globally, the best growth performance was recorded in fish fed with Rcommercial control diet compared to Rcongo and Rcanada diets. The K coefficient was higher in the fish of control group (1.97) than test group (1.87) (Table 2). According to Williams (49), as fish mass increases for a given length, K coefficient increased, implying that the fish in the control group demonstrated a superior condition factor (1), probably influenced by a high nutrient and energy density of the control diet. However, the lowest result observed in particular with the Rcongo test diet could be due to the nature and proportion of the plant ingredients used. It has been reported that an increase in the amount of plant by-products may negatively affect fish growth performance and feed conversion (6, 8, 24, 30). This explains the lowest SGR observed in the fish fed with the Rcongo test diet (1.6 vs. 3.2%/d for the control diet), and the highest FCR values induced by this diet, i.e., 1.7 vs. 0.9 for commercial diet (Table Similarly, numerous studies have 3). also demonstrated the link between plant ingredient type, their incorporation levels in diets and fish growth performance. For example, Azaza et al. (6) reported lower growth rates by incorporating 30% of tomato meal into the diet of monosex male tilapia juveniles (O. niloticus), with higher growth rates at 10 and 20% inclusion.

Additionally, the experiment of Richter *et al.* (41) showed that the inclusion of moringa meal at 10% in *O. niloticus* diet did not adversely affect fish growth, whereas by increasing up to 30%, the authors observed a growth decreased of 73% compared to control group.

The low performance expressed by the *Rcongo* test group fish could also be related to the requirements of the *Oreochromis niloticus* strain used in this study, which is a genetically improved fish tilapia (GIFT). Indeed, according to some previous studies, GIFT strains do not adequately express their production potential under conditions of low food inputs (agroindustrial by-products) (16, 23). Subsequently, differential results might be possible if the test diet based on local by-products had been tested on local tilapia strain, considered to be more resistant and easily adaptable to a wide range of adverse conditions.

this In experiment, the apparent digestibility coefficient (ADC) values of the nutrients for test diets are relatively low compared to commercial diet, especially for dry matter, energy, lipids (Table 4). These values are nevertheless within the limits observed by some authors, for example Wei et al. (48) obtained ADC values ranging from 85.7 to 94.4% for protein, 63.0 to 94.6% for energy and 33.0 to 83.4% for phosphorus, using six different sources of plant proteins in tilapia O. niloticus. Azaza et al. (6) observed similar values of protein ADC ranging from 73.7 to 90.1% in tilapia O. niloticus fed with diet based on different levels of tomato meal. It is also important to note that the high incorporation of wheat bran at about 50% in the Rcongo test diet could result in low ADC registered compared to the commercial diet. This corroborates the work of Wei et al. (48) who reported lower protein and energy ADC for wheat bran compared to wheat meal.

Regarding nutrient budgets (Table 5), the present results did not demonstrate an impact of diet on nitrogen and phosphorus retention in fish, except for energy, which was significantly higher in fish that consumed the control diet compared to those of the test group. This could be explained by the dietary protein content that affects nutrient utilization in fish (13). Indeed, Catacutan et Coloso (10) reported that when dietary protein exceeds the requirements of tilapia, the excess of these will result in oxidation for energy rather than for growth. This may justify higher values of retention (8.0 and 9.5 Mj/kg) and body content (25.9 and 26.5 Mi/kg) in energy registered in fish that ingested the commercial diet containing 51.5% protein, a level that was likely excessive for fish over 15 g (35). Similar observations were made by Ma et al. (29) by evaluating growth and nutrient retention in O. niloticus x O. aureus tilapias fed with different levels of dietary protein.

These authors recorded the peak of nutrient retention with a dietary protein level of 280 g/kg, while a decrease in nutrient retention was observed with a protein increase of 310 g/kg, this implies that the tilapias needs were satisfied with 280 g/kg of protein.

With regards to the present results, the limitations observed in the conversion and digestibility of the test feed could also explained by the effect of other nutritional and physiological parameters, including the high content of antinutritional factors (enzyme inhibitors, phytate, carbohydrates, fiber, etc.) contained in plant ingredients from some oilseeds and cereals, such as soybeans, wheat, maize (8, 27, 30, 31).

According to the aforementioned researchers, the predominance of these agro-industrial by-products (bran, cakes) in the diets exposes fish to antinutritional factors, which interfere with their growth and digestive capacity. Considering the dietary formulations (Table 1), these by-products constitute the major ingredients incorporated in the test diet with more than 87% of inclusion, whose wheat bran 47%, soybeans 23%, rice bran 5% and brewers grain 5%; these products are known for their high content of enzyme inhibitors and their deficiency in essential amino acids. The results of Nobah et al. (33) showed that by comparing to the control food, by-productbased feed did not induce better growth rates in hybrid fish from T. zillii (male) x T. guineensis (female). These authors found the values of 0.18 g/d for rice bran, 0.19 g/d for wheat bran and 0.20 g/d for corn bran vs. 0.56 g/d for the control food. These results, however, show a lower growth rate than those of the present experiment (on average 0.35 g/d for the test diet), although here the wheat bran has been incorporated at approximately 50% level. In addition, compared to corn bran, Bamba et al. (7) showed no better growth of fish with wheat and rice brans. El-Sayed (15) reported a significant decrease of growth and food efficiency in Nile tilapia due to a poor amino acid balance and the presence of trypsin inhibitors caused by replacement of fishmeal by soybean meal 30%. Recently a work of Ly et Ba (28) has supported that the inclusion of soybeans over 50% or 60% had reduced the growth performance and food digestibility in Nile perch (Lates niloticus). Thus, the results obtained by these different authors suggest that the poor growth and the low PER registered with the Rcongo test diet may be associated to trypsin inhibitors presence and the essential amino acid deficiency in by-products, which increases food catabolism and nitrogen looses in fish. However, the essential amino acid supplementation of these diets improves growth and dietary utilization, and the heat processing of these ingredients (e.g. soybeans) inactivates protease inhibitors (15, 30). Because of their very high levels in plant by-products, the antinutritional effects of carbohydrates on growth

reduction and nutrient digestibility have been reported generally in fish and particularly in tilapia and carp, although these are omnivorous (25). Indeed, carbohydrates are generally not digestible by monogastrics or fish, except starch which is poorly digested by high trophic-level fish due to limited activity of amylase (31, 46). Carbohydrate concentration increases with incorporation of plant ingredients to replace fishmeal, resulting in a decrease in crude ash in the food (12), as shown in Table 1. However, according to the aforementioned authors, protein digestibility can be improved by providing a supplementation of limiting amino acids in soybean meal-based diets.

The negative effects of food phytates may also be mentioned. Although in Table 5, phosphorus ADC values appear to be higher for the test diet than for the control (53.3 vs. 45.6%, respectively), the nutrient budget analysis (Table 6) reveals however that, the fecal excretion of phosphorus was higher in test group fish compared to those of the control group. This may imply the presumed effect of phytate on phosphorus digestibility and consequently on the digestive capacity of fish (8). Indeed, phytate is known for its lack of availability for fish due to the absence of endogenous or microbial phytase in the gastrointestinal tract. Phytate decreases the phosphorus bioavailability and the protein digestibility by forming an indigestible phytic acid-protein complex in monogastrics (42). It also forms complexes with bivalent minerals thus reducing their digestive utilization. However, supplementation of phytase in plant-based diets is indispensable for phytate digestion, as it increases the retention of dietary phosphorus in fish and improves intestinal assimilation (8, 42).

The fiber concentrated in plant ingredients also represents another factor that affects the digestive capacity of fish. Many authors have reported their negative effects on nutrient and energy digestibility (16, 31). Biochemical analysis of experimental diets (Table 1) show that the test feed contains a high crude fiber content compared to commercial feed, i.e., 9.7% vs. 1.2%, respectively. This could also justify the poor growth performance noted in fish belonging to the Rcongo test group. Similar observations have recently been made with related work as well as in other fish species. For example, Obirikorang et al. (36) observed a reduction in dry matter ADC in O. niloticus fed with two test diets based on coconut meal and palm kernel cake containing fiber content compared to the control diet, i.e., 9.5 and 11.7 vs. 3.4, respectively. Similarly, by substituting fishmeal at 51 and 60% by soybean meal in Nile perch juvenile, Ly et Ba (28) noted a reduction in growth and feed conversion with increasing fiber content, compared to the control diet, i.e., 7.56 and 7.64% vs. 3.80%, respectively.

Indeed, it is documented that the vegetable fiber is not digestible by the fish whatever the species. They can reduce protein and lipid digestibility by blocking them in complexes that prevent access of digestive enzymes to their substrate. A high inclusion of dietary fiber also accelerates gastrointestinal transit or peristalsis, reducing residence time and nutrient absorption, resulting in a decrease in digestible energy (31, 44). Thus, referring to the present results and to the aforementioned authors, it is important to stress the presumed consequence of food peristalsis acceleration in fish, which would expose them to a prolonged fast, because they have received a diet that corresponds to 4% of their weight, whereas an ad libitum feed intake would compensate nutrients losses associated to rapid feed transit due to high dietary fiber content.

Regarding the biochemical composition of fish carcasses, the present results show that the formulated diets had more or less an impact on the body nutrient contents (Table 6). Compared to the initial fish proximal analyses, dry matter contents decreased in the carcasses of the test groups and significantly increased in those of the control groups. The test diet induced higher carcass protein (54.5%) and crude ash (13.0%) compared to the control (50.7 and 11.9%, respectively) (Table 6). In contrast, the inverse situation was recorded with the fish in the control group, which showed higher contents of lipids (35.3%) and energy (26.2 Mj/kg) than those of the test group (30.9% and 25.3 Mi/kg, respectively). Analyses of these results suggests an imbalance between protein and body lipid content, which may be due to the phosphorus/energy (P/E) ratio in the feed, according to Coutinho et al. (13). Thus, the P/E ratio and dietary protein content are important factors affecting nutrient digestibility in fish. These aforementioned authors reported that the increase in P/E ratio results in a decrease in energy content than to that of dietary protein and finally leads to a reduction in body fat. Similarly, other studies have also reported the relationship between body protein content increase, body fat content decrease and protein content increase in feed (2, 29). Indeed, the present trends in fish body composition corroborate similar assays that reported the significant or nonsignificant effect of incorporating plant ingredients in fish diets. For example, Carvalho et al. (9) reported lower contents of body fat in Nile tilapia fed with diets rich in palm kernel cake and cassava leaves, while body protein content was correlated with that of feed ingested by fish. Ly et Ba (28) reported a decrease in body lipid content in Nile perch (Lates niloticus) fed with high incorporation of soybean meal. Similar results were still observed by Azaza et al. (6) which reported a decrease in lipid content in Nile tilapia carcasses fed with diets rich in plant-derived byproducts.

In contrast, by substituting fishmeal by different levels of rapeseed meal in *O. niloticus*, Luo *et al.* (27) did not observe the feed effect on the biochemical composition of fish carcasses.

Based on these data, the results recorded with the Rcongo test feed, corroborate most studies aiming at the similar objective, in the perspective of formulating a cost-effective feed by valorization of the local and available by-products. Including, Sousa et al. (45) observed a similar weight gain of 0.30 to 0.36 g/d in Nile tilapia fed with different levels of SBM. When comparing three populations of O. niloticus, Lazard (23) reported a mean FCR of 1.71±0.05, a value similar to that found in our experiment. The incorporation of agricultural by-products (soybean and cottonseed meal, corn bran, rice bran and millet bran) in the diets of O. niloticus fry enabled Bamba et al. (7) to record FCR (1.13 to 1.87) and PER (1.69 to 3.45), values similar to those of the present study. Luo et al. (27) observed the FCR ranging from 1.58 to 1.74 and PER from 1.83 to 2.04 in tilapia O. niloticus by replacing fishmeal with different levels of rapeseed meal. Furthermore, Azaza et al. (6) found the SGR values ranging from 2.01 to 2.46%/d, FCR from 1.48 to 2.11 and PER from 1.50 to 2.1 with O. niloticus fed tomato meal. However, compared to our results, Abdel-Tawwab et al. (2) reported in Nile tilapia a lower PER ranging from 0.99 to 1.53 in juveniles and 1.19 to 1.92 in fingerlings fed with SBM, wheat bran and corn bran-based diets. When substituting fishmeal by SBM in O. niloticus, Wu et al. (50) found values slightly lower than those of the present study (FCR 1.97 to 2.35 and PER 1.12 to 1.30), despite supplementation of synthetic amino acids and an animal-based protein meal. Ramos et al. (40) fed Nile tilapias with industrial by-products (cassava leaf, mesquite bean, cotton, cocoa, soursop and African oil palm kernel) and also obtained low CDA compared to our results, i.e., dry matter 26.0 to 57.6%, protein 17.9 to 80.1% and energy 14.1 to 65.8%. On the other hand, with the use of four diets based on cocoa meal, mesquite meal, palm kernel cake and manioc leaves in Nile tilapia Carvalho et al. (9) reported slightly higher values compared to our study, from 1.18 to 1.33 for FCR, 2.03 to 2.16%/day for SGR and from 2.78 to 3.14 for PER.

However, minor discrepancies observed in these experimental results with similar studies could be associated to variations in several parameters, including age, size and fish species; composition and feed processing; crop systems; types and nutritional value of ingredients and their contents of antinutritional factors (15, 27).

It should also be added that the lower growth performance observed in fish fed with the *Rcongo* test diet, could be considered as a response to mineral and vitamin deficiencies probably attributed to the lack of dietary supplementation (35, 43).

According to some authors, the deficiencies in vitamins (A, B2, B7, B9, C and E) and microelements (calcium, manganese) in Nile tilapia usually result in a syndrome of growth disorders and reduction in dietary consumption and efficacy. This can be observed in intensive fish farming where deficiencies (e.g. vitamin C) are sometimes due to inadequate formulation or prolonged storage of diets at very high temperatures (16, 18, 21, 26, 34). However, Jauncey (21) stated that vitamins and microelements deficiencies in tilapia are sometimes rare and difficult to evaluate under controlled farming conditions (fertilized ponds...), as most of these constituents exist in endogenous form in dietary ingredients and pond water. In addition, the vitamins contained in the natural foods of fertilized ponds and the microbial biosynthesis of some of them in the intestines contribute significantly to the vitamin requirements of Nile tilapia.

At the end of this study, the production cost of the Rcongo test-feed was USD 0.38/kg, while that of Rcommercial control-feed acquisition was USD 1/kg (Figure 1; Table 1). This comparison reveals that the Rcongo test feed costs 2.6 times less expensive than the commercial feed. According to the sustainable development approach targeted by this study, the cost of test feed meets the economic requirements of animal production in rural and peri-urban areas (3, 17, 20). For profitable production, these reports recommend that feed costs be less than USD 0.5/kg, as feeding represents the highest expenditure for monogastric livestock (pig, poultry, fish, etc.) (8, 15, 16). Thus, in the light of feed costs and final biomass, the fish production cost was estimated at USD 1.45/kg for the test feed and USD 2.28/kg for commercial feed (Figure 1). This implies that the use of Rcongo test-diet reduces feed cost at 62%, and cost at fish production 36%, compared to However, Rcommercial control-feed. this fish inversely proportional production cost is to experimental feeding duration and fish growth. It would appear more economical to extend the duration of this experiment, as several studies recommend 8

to 16 weeks to observe the significant effects of feeding on tilapias growth (16, 27, 29, 32). Finally, the economic evaluation of the present data demonstrated that, although the *Rcongo* test feed induced lower growth performance compared to the control feed, the cost-benefit analysis indicated that it was economically higher. This is in agreement with the objectives of many similar research mentioned above.

Conclusion

This study has showed a tendency regarding the growth performance and production cost of Nile tilapia. The main objectives have been achieved in terms of the valorization of plant ingredients and the reduction of fish production costs. The cost-benefit ratio indicated that the Rcongo test-feed is economically advantageous versus the commercial control feed. The identification of more available and less expensive feed is a key criterion for the development of freshwater fish farming. Thus, standardization of using these resources is necessary and should make them more applicable especially in the context of production in rural areas. Therefore, for future perspectives, it is imperative to evaluate different strategies for rational use of these resources and the control of antinutritional factors which limit their use.

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