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Different Protein Levels with an Equal Protein-Energy ratio, and Absolute Requirement in *Clarias gariepinus*

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ABSTRACT

Background. Due to the growing need to establish new farming methods that improve management and food efficiency of African catfish, 225 Clarias gariepinus fingerlings (10.1± 0.01 g, average starting weight), in order to evaluate three levels of CP (35.0, 40.0, and 45.0%), with an equal protein-energy ratio (20.0 g protein / MJ), and the same absolute requirement of CP (2.8 g of CP / kg of live weight), in the productive performance of these animals.

Methods. A simple classification method was utilized, with three treatments and three repetitions, and Duncan significance test with a 5% likelihood. The foods were added at 8.0, 7.0, and 6.2% of body weight, for 50 days.

Results. The food supplied did not differ among the treatments. However, the greatest final weights and the best food conversions were observed after raising the levels of diet protein. The economic analysis showed higher ration costs, but also better profits when using the diet with the highest crude protein levels.

Conclusions. Protein increase with equal absolute requirement (g CP/kg live weight) improves the productive performance of *Clarias gariepinus*.

Key words: fingerlings, catfish, digestible energy, nutrition (Source: *AGROVOC)*

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INTRODUCTION

African catfish (*Clarias gariepinus*) is a new species with a high potential for intensive aquaculture development in Cuba, due to management rusticity, high growth rate, and mastery of all its culturing stages (Toledo, Llanes, and Lazo de la Vega, 2011).

The growth rates, food conversion, and survival, are the main zootechnical indicators that influence on the productive and economic results in aquaculture (Michael, Onyia, and Kefas, 2015). Producing a specific species can only be cost-effective when all the qualitative and quantitative nutritional requirements are known. Therefore, it is important to search for nutritional strategies of African catfish in terms of protein demands, the most costly nutrient in the diet.

Protein efficiency depends on several factors, such as levels of lipids/carbohydrates that supply the energy in the ration. High energy in the diet may save protein, but cause excessive deposition of body fat, and low food consumption (Peres and Oliva-Teles, 2017), thus an optimum protein-energy ratio is fundamental to design any feeding strategy.

Vidotti, Carneiro, and Malheiros (2000) reported that the requirements of CP (crude protein) by *Clarias gariepinus* was 40% (maximum level evaluated), of which 50% must be from animal origin. These protein determinations are made to a diet based on growing levels of this nutrient, which provide different protein-energy ratios. Besides, the same food percentage is utilized, so animals are supplied different protein amounts of proteins/kilogram of live weight (absolute requirement).

In order to establish new exploitation methods that improve efficiency in nutrition and culture management, the aim of this study was to evaluate three levels of CP (35.0, 40.0, and 45.0%), using the same protein-energy ratio (20.0 g of protein/MJ), and the same absolute requirement of CP (2.8 g of CP/kg of live weight), for productive performance of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

This research was done at the Fish Nutrition and Foods Laboratory, Company for Development of Aquaculture Techniques, in Havana, Cuba.

Overall, 225 *Clarias gariepinus* fingerlings (10.1 ± 0.01 kg average starting weight), were distributed according to the simple classification model, in nine 68 L circular cement containers (three per treatment). Three experimental diets at different CP levels (35, 40, and 45%), and equal protein-energy ratios were tried (Table 1), which were added to 8.0, 7.0, and 6.2% of body weight/day so all the fish consumed 2.8 g of CP/live weight kg/day.

Table 1. Per cent and chemical composition of experimental diets (g/100 g)

Ingredients	**D-1 35 %**	**D-2 40 %**	**D-3 45 %**
Fish meal	20	20	20
Poultry by-product meal	14	23	32
Soybean meal	20.5	20	19
Corn meal	40.5	27	14
Soybean oil	4	9	14
Vitamin-mineral mix	1	1	1
Total	100	100	100
Dry matter	90.9	88.7	89.1
Crude protein	35.2	40.0	45.0
[1] Ratio AP/TP	0.64	0.71	0.76
Digestible energy (MJ/kg)	17.5	19.5	21.3
[2] CP/DE (g/MJ)	20.1	20.3	20.2

[1] Ratio animal protein/total protein
[2] Crude protein/digestible energy

The water flow at the experimental units was 0.1 L/min, for 24 h, and temperature and dissolved oxygen values were measured using a digital oximeter (HANNA, Rumania). The levels of ammonia and nitrite were measured weekly, using a colorimetric water quality monitoring kit (Aquamerck, Germany).

The meals were crushed with a non-conventional hammer mill at 250 µm, then it was mixed (HORBAT MC-600, Canada), for 10 min. Then oil was added to the the mix of vitamins, minerals, and water (30% of weight), and mixing was continued for 10 min. The pellets were made using a 3 mm-diameter meat grinder (JAVAR 32, Colombia), and it was dried in a stove (Selecta, Spain), at 600C, for 48 h.

The bromatological determinations were made according to the methods described by Latimer (2016), and digestible energy was estimated according to the caloric coefficients reported by Toledo, Llanes, and Romero (2015a).

The diets were offered in two rations (9:00 and 15:30 h), for 50 days. The rations were adjusted every 15 days, and at the end of the bio trial, each animal was weighed to calculate the following productive indicators: food supplied =amount of food supplied/number of final animals; protein supplied=amount of protein supplied/number of final animals; mean final weight; food conversion factor (FCF)=added food/weight gain; protein efficiency (PE)=weight gain/protein supplied; survival (s)=number of final animals/number of starting animals x 100.

Statistical analysis. Simple analysis of variance (ANOVA) was made using INFOSTAT, 2012 (Di Rienzo *et al*., 2012). When differences occurred (P<0.05), the means were compared using the Duncan test, with 5% significance.

Economic analysis. (Table 2) It was made according to the procedure recommended by Toledo, Llanes, and Romero (2015a). The cost of the experimental rations was calculated depending on the international prices of raw materials in February 2019 (www.fao.org/giews/pricetool), plus 40% due to additional expenses (transportation, manufacture, and others) for Cuba. These values were multiplied by the FCF obtained in this study, to know the costs of feeding, which considered 60% of total production costs. The value of production (USD $ 3 400.00/t) was supplied by the Economic Department of the Company for Development of Aquaculture Technologies.

Table 2. Prices of raw materials required to manufacture commercial foods

Raw materials	USD $ /t
Fish meal	1 472.28
Soybean meal	353.34
Poultry by-product meal	952.65
Corn meal	218.99
Soybean oil	772.82
Vitamin-mineral mix	1 975.11

RESULTS AND DISCUSSION

During the experimental period, temperature and the oxygen dissolved in water varied between 25.7 and 26.9º C, and between 3.1 and 5.0 mg/L, respectively. Ammonia and nitrite were monitored, and remained at 0.02 mg/L through the circulation of water. These values were within the environmental parameters required for proper species comfort (Toledo, Llanes, and Lazo de la Vega, 2011).

No significant differences were found in the food supplied (g/fish), though the feeding amounts were not the same (Table 3). On the contrary, the CP supplied (g/fish) differed when the diet protein per cent increased. This can be explained by the growth differences undergone by the fish under different treatments from the 30th day of culture on. It led to greater amounts of food supplied in the diets with the highest levels of CP, when the rations were adjusted every 15 days.

Table 3. Behavior of productive indicators using the experimental diets in *Clarias gariepinus* fingerlings

Indicators	D-1 35 %	D-2 40 %	D-3 45 %	±SE	*P*
Food supplied (g/fish)	44.4	44.9	45.1	0.45	0.845
Crude protein supplied (g/fish)	16,1^{a}	18.0^{b}	20.4^{c}	0.34	0.0003
Mean final weight (g)	35.5±1.49^{c}	42.8±1.49^{b}	54.1±1.52^{a}	-	0.0001
Food conversion	1.5^{c}	1.2^{b}	0.9^{a}	0.06	0.0021
Protein efficiency	1.9^{b}	2.1^{b}	2.4^{a}	0.07	0.0071

Survival (%)	100	100	96.0	1.05	0.000

Unequal letters on the same row differ statistically for P<0.05 (Duncan, 1955).

Importantly, poultry by-product meal was used to increase CP in the rations, which helped replace fish meal completely to feed *Clarias gariepinus* fingerlings (Toledo, Llanes, and Romero, 2015b). Consequently, the greatest inclusion of meal from by-products of birds elevated the animal protein/total protein ratio (Table 1), and led to an increase in the nutritional value (nitrogen content, balance of essential amino acids, and seeming digestibility of nutrients), and acceptability, which contributed to a more efficient use of energy, and better fish productive performance.

Moreover, although equal rates of crude protein/digestible energy (20 g of protein/MJ) were used, and the research started with the administration of equal protein grams/live weight kilogram (Table 3), the final weights of the treated animals D-3 (45% CP) were greater than D-2 (40% CP), both with significant differences from D-1 (35% CP).

These results did not match the results reported by Cho and Lowell (2002), who found the best weight using the diet with the lowest highest level of CP (28%), and attributed it to the fact that most of the energy in the diet with the highest CP (36%) was from lipids (fish oil), which produced high body fat levels compared to the ones that used corn. It is noteworthy that these authors used soybean to increase CP levels, which increased the plant protein/total protein ratio, and led to a decrease in the nutritional value of the ration, due to a rise in anti-nutritional factors (protease inhibitors, tannins, lactins, etc.), with an ensued less efficient use of energy.

Daniel (2016), reported that the inhibition of proteases is compensated due to an increase in the secretion of pancreatic enzymes, and though the digestive process might conclude well, the energetic cost of the fish might be high, as a result of additional enzyme synthesis. Hence, part of the diet energy would be unavailable for CP saving, which is needed for growth.

Li and Lowell (1992), found no differences among 26, 32, and 38% of CP, when American catfish Ictalurus punctatus were fed freely in tanks, but when the ration was cut down, the fish required 38% CP for maximum production, which coincides with this study, and demonstrates that catfish can feed on lower rations, provided that their CP levels per live weight kg are met.

Food conversion improved when the levels of diet protein were increased, maintaining the same absolute requirement (Table 3). A CP rise in the rations guaranteed a 300 g food reduction per every kg of live weight gain (Table 2). It coincides with other studies (Li and Lowell, 1992; Cho, and Lowell, 2002), who demonstrated that feeding efficiency improves when the food amounts are reduced, and the absolute consumption of phosphorous is kept (protein grams per live weight kilo), to not affect growth.

A similar behavior was observed in protein efficiency (Table 3), where the best values were achieved with the highest percentages of CP, which might indicate that the DE levels were in keeping with the tenors of CP, and protein was not used to meet the energy demands. Besides, these results can be backed up with better quality of CP, by having a higher proportion of animal protein, and an increased use of digestible energy (DE).

According to Toledo, Llanes, and Romero (2015a), when the food supply is not limited (*ad libitum*), it becomes the most important factor of growth and food conversion in fish. In practice, this feeding strategy is viable when the foods do not meet all the nutritional requirements of the animals, so growth cannot be affected, in spite of a deterioration observed in food conversion. On the contrary, in the same context, using restricted feeding (body weight per cent/day), growth is depressed, and food conversion is not affected.

Survival (Table 3) was high in all the treatments (greater than 95%); hence, a rise in CP concentrations in the diet, along with a reduction in the ration, did not cause to mortality, or size variations within the groups, which might lead to cannibalism.

Li and Lowell (1992); Cho and Lowell (2002), coincided in that the levels of diet protein did not influence on size variation of fish that met their feeding demands. However, the amount of food was a determining factor in size variations of American catfish in tanks, which coincides with the outcome of this study in African catfish, where size differences were caused by restricted feeding of fish.

Importantly, the size variations of animals can also take place due to the physical presentation of the food, when the pellet’s size is not appropriate for culture, especially seen in fingerlings. Whenever restricted feeding is implemented, it is important to have rations that provide a number of pellets proportional to the number of animals to be fed, and their sizes (Toledo, Llanes, and Romero, 2015a; Michael, Onyia, and Kefas, 2015).

Generally, the traditional CP level of commercial feedstuffs for catfish fattening is 32%. However, there are practical experiences that use more foods with lower CP tenors (25 and 28%), if the fish are fed until satiation (Li and Lowell, 1992). Nevertheless, feeding like this may be inconvenient and costly to most farms, since the fish are satiated, and food may be wasted despite the competence and experience of breeders. Besides, too much fecal material is generated, which can affect water quality.

On the contrary, more CP was supplied and the productive indicators were improved under the conditions of this experiment, using 45% of CP and a protein-energy ratio of 20 g of protein/MJ. This procedure is recommendable for culture intensification, which might include the supply of lower amounts of high-level protein included in lower amounts of foods, and consequently, less generation of organic matter, which would help improve water quality. However, the study of effluents is recommended (total suspended solids and nitrogenized and phosphorated compounds) in the tanks where this diet is supplied.

The economic analysis (Table 4) showed that the diets with higher CP levels were more costly, due to the high prices of conventional sources of proteins, especially from animals (fish meal and poultry by-product meal). Nevertheless, these produced the lowest feeding costs, with better food conversion, and therefore, they provided the best profits from one ton of whole fish.

Table 4 Economic analysis of production of *Clarias gariepinus* using the experimental diets (USD $/t)

Indicators	D-1 35 %	D-2 40 %	D-3 45 %
Cost of ration	895.44	1 025.72	1 155.05
Cost of feeding	1 343.16	1 230.86	1 039.54
Total production expense	2 238.60	2 051.43	1 732.57
Profits	1 161.40	1 348.57	1 667.43
Savings	-	187.17	318.86

Production value: $ USD 3 400.00/t of whole fish
Profits=Production value Total expense

Besides, it is important to consider other savings, such as less water use, labor force, electricity, lower risk of mortality thanks to little time consumed before the harvest time, as the high protein rations are supplied.

CONCLUSIONS

The increase of diet protein with equal protein-energy, and the same absolute requirement (protein grams/kg of live weight) improved the productive performance of *Clarias gariepinus* fingerlings, with a positive economic effect.

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