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Effect of different lipid and protein levels in the diet on the performance of angelfish juveniles *(Pterophyllum scalare)*

Efecto de diferentes niveles lipídicos y proteicos en la dieta sobre el desempeño productivo de juveniles del pez ángel o escalar *(Pterophyllum scalare)*

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ABSTRACT

Background: Currently, ornamental fish are an important element of the pisciculture in our country, with Angelfish (*Pterophyllum scalare*), being one of the most popular and desired cultured species in the fishkeeping field, given its beauty, majesty and stylized form. However, few authors have studied their nutritional requirements in terms of lipid and protein levels. **Objective:** This research evaluated six diets, formed by the combination of two levels of crude lipid (CL) 9 and 11% and three levels of crude protein (CP) 32, 34 and 36%, to determine its effect on productive performance and the survival of juvenile angelfish (P. scalare). **Methodology:** 324 juveniles of *P. scalare* with average weight of 753.5 ± 0.14 mg were randomized in 18 aquariums with volume of 105L, a density of 18 fish/aquarium and fed to 6% of the biomass. **Results:** We found that a 32% level of crude protein in the diet did not affect the survival, but affected the productive performance ($p < 0.05$), and that levels of 34 or 36% of CP improve it. It was also evidenced that there was no statistical difference ($P > 0.05$) between the two levels (9 and 11%) of the crude lipid used in the diets. **Conclusion:** It is concluded that diets for juveniles of this species, containing 32% CP can affect the productive performance in terms of low weight gain, high feed conversion and low specific growth rates and protein efficiency.

Keywords:

Fishkeeping
Nutritional requirement
Ornamental fish
Protein efficiency rate
Specific growth rate

RESUMEN

Antecedentes: Actualmente, en nuestro país los peces ornamentales son un segmento importante de la piscicultura nacional, siendo el pez ángel o escalar (*Pterophyllum scalare*), una de las especies cultivadas más popular y apetecida en el mundo de acuariofilia, gracias a su belleza, majestuosidad y forma estilizada. Sin embargo, son pocos los autores que han estudiado sus exigencias nutricionales en términos de niveles lipídicos y proteicos. **Objetivo:** La presente investigación evaluó seis dietas, conformadas por la combinación de dos niveles de extracto etéreo (EE) 9 y 11 % y de tres niveles de proteína bruta (PB) 32, 34 y 36 %, para determinar su efecto sobre el desempeño productivo y la sobrevivencia de juveniles de pez escalar (P. scalare). **Metodología:** Fueron usados 324 juveniles de P. scalare con peso promedio de 753,5 ± 0,14 mg, los cuales fueron distribuidos en 18 acuarios con volumen de 105L, a una densidad de 18 peces/acuario y alimentados al 6% de la biomasa. **Resultados:** los hallazgos mostraron que un nivel 32% de proteína bruta en la dieta no afecta la sobrevivencia, pero si el desempeño productivo ($p<0{,}05$), y que niveles de 34 ó 36% de PB lo mejoran. También se evidencio que no hubo diferencia estadística ($P>0{,}05$) entre los dos niveles (9 y 11%) de extracto etéreo utilizados en las dietas. **Conclusión:** Se concluye que dietas para juveniles de esta especie, que contengan 32% de PB pueden afectar el desempeño productivo en términos de baja ganancia de peso, alta conversión alimenticia y bajas tasas específicas de crecimiento y de eficiencia proteica.

Palabras Clave:

Acuariofilia
Pez ornamental
Requerimiento nutricional
Tasa específica de crecimiento
Tasa de eficiencia proteica

Introduction

The market for ornamental fish species is an important segment in global aquaculture and has shown great growth in recent years [1]. The global exports of this type of fish, from the year 2000 increased from US $ 177.7 million to a maximum of US $ 364.9 million in 2011, then decreased slightly to US $ 347.5 million in 2014 [2].

In 2014 fish harvested from aquaculture amounted to 73.8 million tonnes, which was estimated at a first sale value of 160.2 billion dollars, composed of 49.8 million tonnes of finfish, which generated a sale of 99.2 billion dollars [3].

Due to the great export potential and the possibility of increasing the income of rural fish farmers in various countries, the production and trade of ornamental fish is being increasingly encouraged [4].

In South America, exported freshwater ornamental fish mainly come from Colombia, Peru and Brazil [5]. In that sense, Colombia occupies the second place with the greatest biological diversity in the world, mainly in the Amazon region, which has a high number of ictic species with potential for the aquarium industry, which has led to the country's positioning in the international market of ornamental fish [6].

It is important to highlight that 98.7% of caught and exported fish are from the natural environment and only, the remaining 1.3% is produced in captivity [5]. This situation worries people who work in the preservation of natural resources, because the number of fish caught in a natural environment is very high and a small percentage arrives at the collection centers to be exported; unfortunately, there is no access to mortality data during the fishing and storage processes, which would better illustrate the serious problem of overexploitation to which these species have been subjected in recent years [7].

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The expansion of the production sector of ornamental fish and the consequent supply of internal and external markets depend on the intensification of the production systems and the generation of appropriate technologies; in addition, it is necessary to know the nutritional requirements of each species to formulate balanced rations [8].

As it has been mentioned, the angelfish *Pterophyllum scalare* is among the main species of ornamental fish. This fish has been captured, produced and marketed for many years in an empirical way, often causing high mortality, low fertility, incidence of diseases and slow growth [9]. The nutritional requirements in the diet of *P. scalare* and other cultivated aquatic species can be studied under five different nutrient groups: proteins, lipids, carbohydrates, vitamins and minerals. The science of fish nutrition and feeding is committed to the supply of these nutrients in the diet of fish [10]. The search to establish the nutritional requirements in fish to improve survival and productive performance in terms of growth, is associated fundamentally with the quantity and quality of the ingested food [11].

Of these nutrients, lipids and proteins are the most important in fish nutrition [12]. As ingredients in rations, lipids are low-cost nutrients and the ones with the higher energy content [13]. However, the maximum optimal levels of these nutrients are unknown in several ornamental species, as is the case of P. scalare; these findings are based on the research carried out by [8]. That concluded that diets containing 34% of crude protein and 5% of ether extract, fulfill the nutritional needs of *P. scalare* juveniles, so we decided to try in this study with higher levels of crude lipid, testing values of 9 and 11% and varying the level of crude protein 2% up and 2% down, 32 and 36% of CP, respectively; likewise, the inclusion of lipids in amounts of 1.8 and 2.2 times higher than those recommended by [8] was evaluated in this species.

Therefore, the goal of this study was to evaluate the effect of six experimental diets, composed of the mixture of two levels of crude lipid (9 and 11%) and three levels of crude protein (32, 34 and 36%), on the productive performance and survival of angelfish juveniles *(P. scalare)*.

Materials and methods

Fishes and experimental conditions For this research, 380 juveniles of angelfish (*P. scalare*) from the same spawning with an average weight of 170 ± 30 mg were used, which were acquired in the company Piscícola Agualinda (Cumaral, Meta, Colombia).

The specimens traveled 17 hours by land and once they arrived at the ornamental fish laboratory, located at the Elysian Fields Experimental Complex of the Francisco de Paula Santander University (UFPS as in Spanish), in the municipality of Los Patios, they were put in conditioned glass aquariums.

Before freely locating the fishes in the aquariums, the bags in which they came were placed inside them, to perform an acclimatization for a period of 45 minutes measuring temperature (using a HANNA® thermometer) and pH, which allowed to know and match the parameters in the water of the bags and the aquariums.

Later they were removed from the bags using a nylon net and distributed in 4 glass aquariums (6 mm thick), with dimensions of 47 cm high, 48 cm wide and 60 cm long, with a total capacity of approximately 135 L (105 L of useful volume), at a density of 1,106 L/fish (95 fish/aquarium).

After a 21-day period of adaptation to laboratory conditions, 56 juveniles of *P. scalare* were discarded because they did not present homogeneous weights. Thus, 324 juveniles of this species were finally used for the experiment.

The aquariums were maintained with continuous aeration and filtration (Resun® Filter HF-2001) suitable with wadding cartridge and activated carbon. The water used for the aquariums came from the aqueduct system of the municipality of Los Patios, department of Norte de Santander.

Experimental stage At the time of selecting the fish for the start of the experimental stage, 14.74% was discarded in order to homogenize the population; finally, 324 juveniles of *P. scalare* (85.26% of the initial population) were randomly distributed in 18 aquariums at a stocking density of 18 fish/aquarium (5.83 L/fish). The fish were fed according to the biomass calculation, five times a day during the entire experimental stage, which lasted 58 days.

The aquariums were daily siphoned for cleaning, removal of feces and renewal of 20% of the total volume of water (approx. 21 L). The fish were weighed by biweekly biometrics except for the last biometry that was performed with an interval of only 12 days (day 1, day 16, day 31, day 46 and day 58).

The biometrics were done in order to know the evolution of the weight to calculate the fish biomass and in this way adjust the amount of food to be supplied.

The weighings were performed on an analytical balance (Boeco® 0.0001 mg sensitivity). This procedure consisted in the individual weighing of each specimen, placing it on an absorbent towel to remove the excess of moisture and then in a plastic vessel previously tared on the analytical balance; moreover, the procedure never lasted more than 40 seconds in order to avoid stress of the fish.

During the 58 days of experimentation a natural photoperiod was maintained (12 h light: 12 h darkness), the average daily temperature of the laboratory was 30.1 ± 2.3 °C and the relative humidity of 68.6 ± 15.0%.

During the experiment, the following physicochemical parameters of the water were monitored daily: temperature (ºC) and pH using the HANNA® pH meter, and biweekly the dissolved oxygen (O2 mg/L), the non-ionized ammonium (NH3 mg/L), total hardness (CaCO3 mg/L), alkalinity (CaCO3 mg/L), carbon dioxide (CO2 mg/L), chlorine (Cl mg/L), sodium chloride (mg/L) and nitrite (mg/L NO2-) with the Hach® aquaculture water quality analysis kit Model FF-1[a].

Experimental diets and fish feeding The ingredients used in this experiment were obtained from national commercial suppliers. The proximate composition analyses (Table I) were carried out in the UFPS animal nutrition laboratory; the procedure was carried out for each of the ingredients, to be later used for the diet formulations.

Table I. Proximate composition of the ingredients included in the experimental diets1

Ingredient	Dry matter (%)	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Ash (%)	NFE (%)
Oatmeal	98,7	16,9	11,0	10,6	1,6	58,6
Cornmeal	93,0	7,8	0,5	1,7	0,7	82,3
Fishmeal	96,6	54,3	8,1	0,0	21,8	12,4
Soy flour	92,5	42,9	1,2	5,8	6,3	36,3
Banana flour	96,6	0,0	0,3	0,0	2,5	93,8
Wheat flour	92,4	11,0	0,2	2,3	0,7	78,2
Wheat bran	92,0	15,6	3,0	10,4	5,7	57,3
Cassava flour	92,8	2,4	0,1	5,4	0,2	84,7
Corn oil	----	----	99,0	-----	-----	

[1]**Average of two replicas. NFE** = Nitrogen-free extract

The ingredients of the diets were individually weighed and ground; then, they were combined dry and then corn oil and 40 % water were added. After obtaining the homogeneous mixture, the pellets were elaborated by using a meat grinder.

The pellets were spread on trays, drying them in an oven at 55 °C for 24 hours. The pellets were pelleted following the suggestion of [14], who recommended it for presentation in flour; their size was adjusted to the size of the mouth of the fish.

All six diets were stored in the refrigerator at 5 °C. The juveniles of *P. scalare* were fed during 58 consecutive days, with a frequency of supply of 5 times/day at 8:00, 10:00, 12:00, 14:00 and 16:00.

The amount of food offered daily was calculated based on the biomass of the fish set, following the recommendation of [15], which suggests a feeding level in proportion to 6 % live weight/day. The feed was weighed daily for each of the five rations, keeping records of these weights, to determine the daily and total consumption of live animals for each treatment.

Taking into account the nutritional composition of the available ingredients, six experimental diets were formulated: Diet 1 with 32% CP and 9% CL, diet 2 with 32% CP and 11% CL, diet 3 with 34% CP and 9% CL, diet 4 with 34% CP and 11% CL, diet 5 with 36% CP and 9% CL and diet 6 with 36% CP and 11% CL. The proximate composition of the experimental diets is shown in Table II.

Table II. Proximate composition of the experimental diets for juveniles of angelfish *(Pterophyllum scalare)* including two levels of crude lipid (9 and 11%) and three levels of protein (32, 34 and 36%)

Proximal composition (%)	32 % PB		34 % PB		36 % PB	
	9 % CL	11 % CL	9 % CL	11 % CL	9 % CL	11 % CL
Dry matter	97,36 ± 0,02	96,63± 0,06	96,90± 0,21	96,38± 0,69	96,88± 0,54	97,32± 0,11
Crude protein	31,96 ± 0,25	32,10 ± 0,03	34,15 ± 0,28	34,26 ± 0,14	35,94 ± 0,08	36,08 ± 0,14
Crude lipid	8,73 ± 0,26	9,96± 0,06	8,97± 0,16	10,84± 0,59	8,78± 0,13	11,93 ± 0,20
Crude fiber	1,00 ± 0,60	1,34 ± 0,11	0,96 ± 0,13	1,38 ± 0,13	0,88 ± 0,00	1,03 ± 0,11
Ash	16,03 ± 0,18	14,50 ± 0,06	15,55 ± 0,13	12,37 ± 0,10	14,59 ± 0,05	14,41 ± 0,66
Nitrogen-free extract [1]	39,64	38,73	37,27	37,53	36,69	33,87
Carbohydrates [2]	40,64	40,07	38,23	38,91	37,57	34,90
Gross energy[3] (kcal/kg)	4.300	4.399	4.348	4.556	4.405	4.597
Energy:Protein Ratio (kcal GE/g CP)	430:32	440:32	435:34	456:34	441:36	460:36

[1]**Nitrogen - free extract** = DM-(CP+EE+CF+MM),[2]Carbohydrates = (crude fiber + nitrogen-free extract), 3**Gross energy** = weighted arithmetically.

Bromatological analyses The chemical analyses of the ingredients and the experimental diets were carried out in the Animal Nutrition Laboratory, affiliated to the Department of Agricultural and Livestock Sciences of the Faculty of Agricultural Sciences and the Environment of the UFPS.

The analyses of the percentages of dry matter (DM), crude protein (CP), crude lipid (CL) and Ash were determined according to [16] and [17]. The dry matter (DM) content was determined by heating the samples at 105 ºC for 16 hours in a forced air oven.

The crude protein (CP) content was calculated by the amount of total nitrogen, determined by the Kjeldahl method and multiplied by the factor 6.25.

The crude lipid (CL) was analyzed after extraction in (Soxhlet), having as solvent the petroleum ether (boiling point 30 – 60 ºC), with continuous reflux through the sample, for 5 hours.

The concentration of Ash was determined by carbonizing the samples in muffle at 500ºC for 3 hours. The gross energy (GE) was weighted arithmetically, using the methodology used by [18].

Analysis of productive performance To evaluate the productive performance of juveniles of *P. scalare*, the following formulas were used:

Weight gain WG (g) = Final weight (g) – Initial weight (g)

Food conversion FC (g/g) = Individual food consumption (g/fish) / Weight gain (g)

Specific growth rate SGR (%/day) = 100 (ln final weight – ln initial weight / feeding period (days), where ln = natural logarithm

Protein efficiency ratio PER (g/g) = Weight gain (g) / consumed crude protein (g)

Survival rate (%) = 100 – (((# Initial fish – # Final fish) / # Initial fish) x 100)

Statistical analysis We used an experimental design with six (6) treatments in factorial arrangement 2 x 3, corresponding to two (2) levels of crude lipid (9 and 11%) and three (3) levels of crude protein (32, 34 and 36%). Each treatment used in the design had three aquariums as replicas (n = 3), for a total of 18 experimental units. All results were expressed as averages ± standard deviation.

The normality of the data [19], and the homogeneity of the variance [20], were performed before the application of the analysis of variance (ANOVA). The data expressed as a percentage (survival and specific growth rate) were previously transformed into: *arc sen (√(x/100))*.

When significant differences were found for the analyzed variables ($p < 0.05$), the averages of the treatments were compared with the Tukey-Kramer test. Statistical analyses were developed using SPSS 19.0.

Results and Discussion

Physicochemical conditions of water The values of the physicochemical parameters of water, determined in this study, remained within the ranges recommended for the culture of *P. scalare* [7]. The found values were homogeneous ($p > 0.05$) among the 18 aquariums where juvenile angelfish were kept.

The found average values (± standard deviation) were: temperature 28.60 ± 0.07 °C, pH 8.07 ± 0.01, alkalinity 85.50 ± 0.01 mg $CaCO_3$/L, hardness 149.15 ± 3.15 mg $CaCO_3$/L, sodium chloride 327.76 ± 17.04 mg NaCl/L, chlorine 202.22 ± 11.75 mg C_l/L, carbon dioxide 12.78 ± 0.52 mg CO_2/L, ionized ammonium 0.24 ± 0.01 mg NH /L, non-ionized ammonium 0.02 ± 0.00 NH_3 and nitrite 0.56 ± 0.05 mg NO_2^-/ L.

Productive performance and survival percentage The averages (± standard deviation) of the parameters of productive performance and survival in juveniles of angelfish or scalar *(P. scalare)* fed with two levels of lipids and three levels of protein, are shown in Table III. None of the evaluated variables had interaction between factors (lipid levels vs. protein levels) but analyzed separately, they showed significant differences ($p < 0.05$) for protein levels, but not for lipid levels.

The weight gain (WG), the specific growth rate (SGR) and the protein efficiency ratio (PER) presented significant differences ($p < 0.05$) in the diets containing a level of 32% of CP, with lower average values, comparing them with the levels of 34 and 36% of CP that did not differ among themselves, but had higher values (Table III).

The feed conversion (FC) presented significantly lower values (Table III), with levels of 34 and 36% of CP, which is advantageous from the economic point of view. However, a level of 32% of CP increased the values of the feed conversion, making spend more grams of food to gain a gram of weight.

The survival parameter was not altered ($p > 0.05$) in any of the diets, neither for the two lipid levels nor for the three protein levels.

Table III. Parameters of productive performance of juveniles of *P. scalare* (mean ± standard deviation) fed with two lipid levels and three protein levels

Levels	WG (g)	FC (g)	SGR (%día)	PER (%)	SURVIVAL (%)
Crude lipid(CL)					
9%	$3,84^{a}\pm0,64$	$1,86^{a}\pm0,43$	$3,10^{a}\pm0,26$	$1,68^{a}\pm0,32$	$98,15^{a}\pm3,93$
11%	$3,72^{a}\pm0,80$	$1,93^{a}\pm0,54$	$3,05^{a}\pm0,35$	$1,75^{a}\pm0,30$	$98,77^{a}\pm2,45$
Crude protein (PB)					
32%	$2,88^{b}\pm0,28$	$2,50^{a}\pm0,28$	$2,69^{b}\pm0,14$	$1,44^{b}\pm0,14$	$100,0^{a}\pm0,00$
34%	$4,10^{a}\pm0,21$	$1,63^{b}\pm0,12$	$3,24^{a}\pm0,08$	$1,92^{a}\pm0,10$	$96,30^{a}\pm4,54$
36%	$4,37^{a}\pm0,25$	$1,55^{b}\pm0,14$	$3,30^{a}\pm0,11$	$1,78^{a}\pm0,37$	$99,07^{a}\pm2,27$

Weight gain (WG), Food conversion (FC), Specific growth rate (SGR), Protein efficiency ratio (PER) and Survival (%). Different letters within the columns indicate statistical differences ($p < 0.05$) for the Tukey test.

According to the authors [21] fish use lipids as an energy source for maintenance, growth and reproduction, but not to maintain body temperature, as do mammals and birds. Thus, the main reason to supplement the diet of the grown fish with lipids, is to save the use of protein as an energy source [21].

In the case of this study, the high levels of lipids in the diet did not affect the survival and productive performance variables of juveniles of angelfish *(P. scalare)*; in this sense, the results agree with those of [11].

Where the authors did not observe significant divergences ($p > 0.05$) for the parameters of productive performance among the different sources of lipids evaluated in *P. scalare*; likewise, the mentioned authors, explain the absence of a significant effect of vegetable oil sources on the productive performance, which could possibly have occurred because the used treatment met the requirements of energy and essential fatty acids for this species.

Therefore, according to the results of this research, it was evident that it is possible to use levels of up to 11% lipids in diets for juveniles of *P. scalare*. Protein is one of the most important nutrients that affect the productive performance of fish, but it is also one of the most expensive components in the diet [22], which was reflected in this study, where the highest levels of crude protein (34 and 36%) favored greater weight gains, higher specific growth rates and higher protein efficiency ratios, as well as lower feed conversion rates.

In that sense, protein levels in the diet must ensure adequate amounts of amino acids, allowing the body to synthesize its own proteins for maintenance of proper growth and development [21]. This research shows the need to do future research with *P. scalare*, assessing the requirements of essential amino acids such as lysine and methionine, as well as the concept of ideal protein (exact amino acid balance), the most appropriate energy/protein ratio and the requirements of digestible energy in the diets for this species of ornamental fish.

Continuing the analysis, the weight gain (WG) was higher for juveniles of scalar, fed with diets containing 34 and 36% CP, evidenced possibly by the greater contribution of amino acids, compared to the lowest level (32% CP) that presented lower weight gains; in this respect [23]. Evaluated diets in scalar fish (*Pterophyllum scalare*) with protein content of 40 and 45% CP and reported weight gain results of 0.448 ± 0.02 g and 0.423 ± 0.33 g, respectively, data that are inferior to those presented in this study. Likewise, in this research the results were up to 6.70% higher than those reported by [23], who also used a diet of 32% CP and juveniles of *P. scalare* with initial weights of approx. 150 mg, with food supply for a period of 84 days. These differences may be a consequence that in [24], they used lower levels of crude lipid (4.48%), ash (7.34%) and gross energy (3,389 kcal/kg), as well as higher levels of carbohydrates (44.6%).

With respect to the feed conversion (FC), lower values were observed in juveniles of scalar fish (*P. scalare*) nourished with levels of 34 and 36% CP, results that are higher than those reported by [9], with levels of 34 to 46% CP. In this research, the 32% CP level presented the worst food conversion (highest values); however, this result is better than those reported by [13], which implemented a diet with 32% CP, evaluating feeding frequency and biomass rates in scalar fish (*P. scalare*) juveniles.

Such differences can be justified by the feeding frequency used in this experiment, since as it was shown in [13], the higher these frequencies are the better is the distribution of the contribution of nutrients and therefore the metabolism of animals is improved; in addition, it gives the possibility of restrictions when consuming the entire ration provided in a single time per day, basically depending on the digestive tract.

The specific growth rates in this investigation were higher than those observed in other studies conducted by [24], [14], [22], [25], and [26], which determines that the productive performance of the fish is not only influenced by feeding, but also by factors such as density; nevertheless, the live food was evaluated against dry food [12] and [27], obtaining a specific growth rate higher than the one reported in this study, since as these authors highlight, the live food indicates a greater use of the nutrients [12], because the fish, if possible, prefers it for its taste and/or aroma, which is reflected in its growth, representing a greater use of the nutritional components of this food, allowing more satisfactory results of growth and physical appearance [27].

Comparing the results with a fish of the same family *Cichlidae* [28] found with juveniles of red tilapia (*Oreochromis sp.*) that the specific growth rates (TEC) did not exceed 2.5% / day, this research shows that the growth potential of *P. scalare* is evident since even with the lowest protein level reached a TEC of 2.69% / day and with the highest protein level, a TEC of 3.30% /day. However, some studies have reported that very high levels of dietary protein cause low values of PER [24], leading to the use of part of the protein as an energy source, which agrees with that reported by [9], where a crude protein level of 46% presented a significantly lower PER than the levels of 34, 38 and 42%; in this case, survival was not affected by the different levels of protein, results also reported by other authors [24], [29], [14], [25], [12].

Conclusions

This research allowed to determine that levels of crude protein of 34 or 36% improve the productive performance of *Pterophyllum scalare* juveniles, in terms of greater weight gains, lower feed conversions and higher specific growth rates and protein efficiency; besides, a 32% level of crude protein degrade them without affecting survival.

It can also be evidenced that ether extract levels of 9 or 11% do not affect survival or productive performance. In this regard, diets containing 32% CP can affect the performance of the juveniles of this species.

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