REPLACEMENT OF FISHMEAL BY *Lupinus albus* AND INFERTILE EGG HATCHERY WASTE IN A TILAPIA (*Oreochromis niloticus*) RATION

Sustitución de harina de pescado por harinas de *Lupinus albus* y desecho de huevos infértiles en una ración para tilapia (*Oreochromis niloticus*)

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Abstract

Unconventional vegetable and animal materials represent attractive ingredients for the formulation of tilapia fish diets cultivated in intensive farms. In this study, we formulated an isoproteic, and isocaloric tilapia ration with *Lupinus albus* dehulled seeds and infertile eggs hatchery waste dry flours and compared its growth performance to that of a commercial feed (Nutripec) in an eight-week trial. We purchased tilapia fries from the Aquaculture Center of Tizapan Jalisco and reared them in six 300 L tanks (200 g of fish per tank) for a two weeks adaptation period. During this period, we fed the commercial ration (Nutripec), and in the third week, switched fries in three tanks to the experimental diet while the rest continue on the commercial diet. Fishes were fed twice daily with a quantity of food equal to 3.0 % of their live weight, divided into equal morning and afternoon portions. Every two weeks, we monitored the chemical water parameters and recorded the fishes' weight, length, and mortality. We calculated the specific growth (SG), defined as the days needed by fishes to increase 50% their average weight and compared the diet's performance using Student's two-tail t-test. The SG for the experimental diet was significantly higher (27 days) than for the Nutripec feed (24 days), $p < 0.05$. We found similar results when the increase in length was considered. The survival rate for both diets was >84 %, indicating that fishes adapted well to the diet and environmental growth conditions. Although additional studies are required, the two ingredients can potentially replace fish meal in the diet of tilapias cultivated in intensive farms.

Keywords: Specific growth, fish, intensive, farms

Resumen

Las fuentes de vegetales y animales no convencionales representan ingredientes atractivos en la formulación de dietas para peces cultivados en granjas intensivas. En este estudio, formulamos una ración isoproteica (37%) e isocalórica (~ 3520 Kcal/kg), con harinas de semillas descascaradas de *Lupinus albus* y desechos de huevos infértiles. El desempeño de esta ración se comparó con el de una dieta comercial (Nutripec) en una prueba de ocho semanas con tilapia en los estadios de crecimiento de alevín y juvenil. Se compraron suficientes tilapias en estadio de alevines en el Centro de Acuicultura de Tizapan, Jalisco y se sembraron aleatoriamente en seis tanques de fibra de vidrio de 300 L (200 g de peces en cada uno). Una vez sembrados, los peces se alimentaron por dos semanas, como periodo de adaptación, con la dieta comercial (Nutripec). En la tercera semana, la ración Nutripec se sustituyó por la dieta experimental en tres de los tanques, mientras que el resto de los peces continuaron con la dieta comercial. Los peces se alimentaron dos veces al día, durante ocho semanas, con una cantidad de alimento igual a 3.0% de su peso vivo. Cada dos semanas se midieron los parámetros químicos del agua y se registró el peso, la talla y la mortalidad de los peces. Se calculó el crecimiento específico (CE), definido como los días necesarios para que los peces aumenten su peso promedio 50%, y comparamos el desempeño de las raciones con una prueba de t de Student de dos colas. El CE de la dieta experimental fue significativamente mayor (27 días) que la dieta comercial (24 días), $p < 0.05$. Los resultados de la talla de los peces fue similar al del CE. La tasa de supervivencia para ambas dietas fue > 84%, lo que indica que los peces se adaptaron bien a la dieta y a las condiciones ambientales de crecimiento. Aunque se requieren estudios adicionales, los dos ingredientes podrían potencialmente reemplazar la harina de pescado en la dieta de tilapias cultivadas en granjas intensivas.

Palabras Claves: crecimiento especifico, granjas intensivas, fish

Introduction

Intensive inland aquaculture has become a vital human activity in some world regions. According to the FAO, worldwide production of fish and other aquatic organisms grew from 5.3% in 1970 to 32% in 2000 (FAO, 2016). Fish farming produces 30 billion more organisms than all farm and other animals used by humans (Lymbery and Oakesho, 2014). Between 2010 and 2019, the global production of farmed tilapia grew at a rate of 7.7 % and is expected to grow 4 % in 2020 (Ragnar et al., 2019). In 2017, Mexico was within the top 10 worldwide tilapia producers with an annual production of 117 000 MT (CONAPESCA, 2018). Besides, the state of Jalisco led the countries production for five consecutive years, reaching 34,000 MT in 2017 (Romo, 2018).

High quality and affordable feeds are essential to sustain the global expansion of the aquaculture industry. As an example, in 2003, Awity pointed out the challenges faced by Ghana’s aquaculture include farm management and the replacement of fish meal with high protein quality and inexpensive ingredients. Fish meal prices, along with the high demand for soybean by the livestock and food industries, drives the cost of aquafeeds (FAO, 2016). Furthermore, the lack of low cost and high protein quality ingredients could constrain the expansion of fish farming (Gatlin et al., 2007). This scenario has led investigators and producers to search for alternative ingredients to replace both fish meal and soybean. (Hasan et al., 2012; FAO, 2016; FAO, 2018). Hernandez et al. (2010) showed that poultry by-product meal-pet grade and a porcine meal effectively replaced fish meal in practical diets for fingerling Nile tilapia. Although other by-products of the meat and poultry industries, including hydrolyzed feathers meal (HFM) and bone and blood flours (FAO, 2016), can substitute fishmeal in tilapia diets there are few alternatives for soybean; nonetheless, some studies show promising results. Ngugi et al. (2017) reported that amaranth leaf protein concentrate could partially replace fish meal in Nile tilapia rations. In a study with tilapia fry, Abushweka (2018) found a feed conversion and protein efficiency ratio of 0.85 and 38.5 % for a pea seed protein concentrate, respectively. Although these results indicate that tilapia effectively utilized the protein source, feed consumption, and overall growth were low. Garcia-Lopez et al. (1990) reported the infertile eggs hatchery waste and *L albus* seeds could be a viable alternative for fish meal and soybean in fish feeds. *L albus* is a legume with a high protein content used in countries such as Australia, Russia, and Germany to feed humans, fish (aquaculture), and domestic animals, including pigs, poultry, beef, sheep, and goats (Von Baer et al., 2004). Nevertheless, as far as we know, in Mexico, the use of alternative ingredients in the formulation of diets for intensive and semi-intensive fish farming is limited. Therefore this study aimed to formulate a tilapia feed based on lupin meal (*L. albus*) and infertile eggs hatchery waste, as a protein source, and to compare its growth performance against that of a commercial feed (Nutripec) with tilapia in the fry to juvenile (*Oreochromis niloticus*) growth stages.

Materials and Methods

We conducted the study at Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) campus of the Universidad de Guadalajara (Zapopan, Jalisco México).

Rearing

Fish were reared in a closed recirculating aquaculture system housed in a greenhouse under ambient conditions. At the start of the study, we purchased tilapia (*O. niloticus x O. aureus*) fries from the Aquaculture Center of Tizapan Jalisco and reared them in six 300 L tanks (200 g of fish per tank) for a two weeks adaptation period. During this time, we fed fishes the commercial ration (Nutripec), and in the third week, switched fries in three tanks to the experimental diet while the rest continue on the commercial diet (25 – 45 fishes per tank). During the following eight weeks, fishes were fed their corresponding diet at a daily rate equal to 3% their body weight, divided into equal morning and afternoon portions. Every two weeks, we recorded the number of surviving fishes in each tank as well as the fishes’ length and weight. We kept water quality and temperature records and cleaned the tank once a week.

Diets

We formulated the experimental ration to match the protein (37 %) and energy content (~ 3500 Kcal/Kg) of Purina´s fish commercial diet Nutripec using lupin seeds and infertile egg hatchery waste, as a protein source (Table 1). A local supplier provided us with sufficient commercial feed for the experiment.

Table 1. Inclusion rate and nutrient content[3] of diet ingredients on as is percent basis.

Ingredient	Inclusion in Meal (%)	Dry Matter	Protein[2]	Ether Extract	Ash	Crude Fiber	NFE[1]
Inf ertile eggs	9.89	94.04	69.26	5.21	5.33	0.19	14.05
Lupinus albus	9.89	90.88	41.23	8.18	4.82	2.16	34.56
Soybean	34.87	93.00	48.10	0.60	5.50	1.59	35.89
Corn	34.87	91.50	9.10	1.16	1.52	2.11	76.61
Orange peel	3.34	93.15	5.79	1.83	3.53	14.08	67.92
Shrimp Shell	3.44	90.82	56.57	1.88	6.20	26.09	0
Fish Oil	3.12	100	-	100	-	-	-
Vitamin Mix	0.30	95.00	-	-	0.3	-	-
Mineral Mix	0.28	95.00	-	-	0.3	-	-

[1]Nitrogen free extract
[2]Calculated as % Nitrogen x 6.25
[3]Percent Wet Basis

Experimental Diet, Ingredients and Chemical Composition

L. albus seeds came from an agricultural experiment conducted during the 2016 winter season in Nextipac, Zapopan. We prepared fine lupin flour by milling dry dehulled seeds in a cyclonic mill with a 2 mm screen. Infertile eggs, collected at the end of a hatching cycle, were cooked for 15 min in 3.0% acetic acid at 100ºC and 15 lb/cm^2 of pressure. After cooking, the eggs were ground in a meat grinder, sun-dried, and re-ground in a cyclonic mill with a 2 mm screen (García-Lopez et al., 1990). Other ingredients included shrimp shell and orange peel flours, processed in the same manner as the infertile eggs, cornmeal, soybean meal as well as mineral and vitamin mixtures.

To prepared the experimental diet, we homogenized the ingredients in a mixer, pelletize it in a low-pressure extruder, and dried at 50°C in a forced-air oven. Both feeds were stored at 25 – 28º C during the study. Proximal chemical analysis of ingredients and experimental diet was carried out according to the AOAC methodology (AOAC, 1990), while the supplier provided the chemical composition of the Nutripec diet.

Total Energy (Caloric Value)

The energy content of the experimental diet was calculated using Atwaters' energy conversion factors, 4 Kcal/g for carbohydrates and protein, and 9 Kcal/g fat (Alsir et al., 2014).

Experimental Design, Data and Statistical Analysis

We used a completely randomized design with two treatments and three repetitions per treatment. The average fish weight and length were calculated based on the number of surviving fishes in each evaluation period and transformed into the $\log_{10}$ base. We then constructed a semi-log plot of the average weight vs. days, fitted the data to a linear function using Excel (Microsoft® Excel® 2016 MSO), and recorded its intersect and slope. Finally, we calculated the specific growth (SG), defined as the number of days required for the average fish weight to increase by 50 %, for each tank using the following formula:

SG = (Log (Weight at the intersect *1.5) – Log (Weight at the intersect))/Slope

We compared the SG of the experimental and Nutripec diets using a two-tail Students' t-test at $p \leq 0.05$ and the statistical software Minitab 17 (Minitab 17 Statistical Software (2010). [Minitab 17.1.0.0]. State College, PA: Minitab, Inc.). The fishes' average length data at day 56 was subjected to ANOVA using diet and tank as factors.

Results

Ingredients and Chemical Composition of Diets

The ingredients nutrient composition and its inclusion rate in the experimental diet are included in Table 1, while Table 2 contains the proximal composition of both feeds. It is fair to say that the Nutripec and experimental diets were iso-proteic since, on a dry basis, they contained 39.09 and 36.6 % protein, respectively. The crude fat and ash content were lower in the experimental diet; in contrast, the crude fiber and nitrogen-free extract were lower in the Nutripec diet.

Table 2. Nutripec and experimental diets nutrient composition.

Nutrient	**Percent (As is Basis)**	
	Nutripec	**Experimental**
Moisture	6.88	7.57
Dry matter	93.72	92.43
Protein (%N x 6.25)	36.64	33.83
Ether extract	6.25	3.97
Ash	7.34	6.43
Crude fiber	5.47	7.80
Nitrogen Free Extract	37.42	48.10

Specific Growth and Length

Fishes on the experimental diet required three additional days (27 vs. 24 days) to increase their average weight by 50 % (Table 3). The results of the t-test indicated that the SG for both diets was significantly different at $p = 0.032$.

Table 3. Specific Growth (SG) for fishes in Nutripec and experimental diets

Repetition	Specific Growth (days)	
	Nutripec Diet	Experimental Diet
1	23	27
2	24	29
3	25	27
Average	24 (1.3 SD[1])	27 (1.4 SD)

[1]Standard deviation

As can be seen in Table 4, the average fish length increased during the experimental period. After 56 days, the fishes' length in the Nutripec group increased 37.9 %, while in the experimental group increased 38.5 %. As with the specific growth, the diet had a significant effect on average length. Fishes on the Nutripec diet were 11 % (12.0 cm vs. 10.8 cm) larger in length than those in the experimental diet. Every 14 days, the average fishes' length increased 0.8 ± 0.3 and 0.7 ± 0.3 cm in the Nutripec and experimental diets, respectively.

Table 4. Average fish length (cm) during the experimental period.

Diet	Days				
	0	14	28	42	56
Nutripec	8.7 (1.3 SD[1])	9.8 (1.6 SD)	10.5 (1.8 SD)	11.4 (1.9 SD)	12.0 (2.1 SD)
Experimental	7.8 (0.9 SD)	8.9 (1.1 SD)	9.5 (1.3 SD)	10.3 (1.4 SD)	10.8 (1.6SD)

[1]Standard deviation

Discussion

In this work, we compared an experimental fish feed formulated with lupinus flour and infertile eggs hatchery waste, as a protein source, against a commercial feed (Nutripec) in tilapia in the fry to juvenile growth stages.

Even though the SG and length of fishes were smaller on the experimental diet than on the Nutripec group, the test diet (37% protein) contained sufficient protein to meet the requirements for juvenile tilapias. According to some authors, feeds with protein levels above 24-26 % satisfy juvenile tilapia growing requirements (Stickney, 1997; León, 2001). Similarly, Sumi et al. (2011) reported that meals containing 35 % protein were acceptable for cultivated tilapia. Although we could attribute the slower growth observed with the experimental diet to a deficiency of essential amino acids, the feed should have provided enough to meet tilapias' nutritional requirements. Despite that, lupin flour is limited in methionine and threonine; infertile eggs hatchery waste is rich in these two amino acids (García-Lopez et al., 1990; Starkute. et al., 2016). The difference in SG could also result from low feed consumption; unfortunately, we did not monitor the amount of feed consumed per day. Nevertheless, these results indicate that both lupin seeds and infertile eggs hatchery waste can replace, partially or totally, the fish meal in tilapias' diet.

Although there were differences in the content of few nutrients in the two diets, this is most likely because we formulated the experimental diet to match the protein and caloric content of the Nutripec feed using a limited number of ingredients. Therefore, it was not possible to match the crude fat, crude fiber, ash, and nitrogen-free extract content of the Nutripec diet. Other researchers have reported that it is possible to replace the fish meal with non-conventional vegetable and animal bio-products, including meat and blood flour, dry meat and bone, chicken hydrolyzed feathers and guts, mandioca, lupin, fungus, amaranth and algae (FAO, 2018; FAO,2016; Ngugi et al., 2017).

The survival rates for fishes on the Nutripec and experimental diets were similar (89.6 and 84.3 %, respectively) and agreed with those previously reported (Siddka et al., 2012; Chien and Chiu, 2003). The high survival rate observed also indicates that the fishes adapted well to both diets.

Notwithstanding that the SG for the experimental diet was slightly lower than for the Nutripec diet, our results indicate that it is possible to replace fish meal in tilapia diets with the two ingredients evaluated in this work. Although feed cost in critical in fish farming, we did not intend to formulate a minimal cost feed but only to assess the growth performance of lupin seeds and infertile eggs hatchery waste flours.

Conclusions

In summary, our study shows that both lupin seeds and infertile eggs hatchery waste can potentially replace fish meal in tilapia rations. Nonetheless, additional studies are needed to evaluate the performance of these two ingredients over tilapias complete growth cycle and to assess the formulation of competitively cost feeds.

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