


Original Research Articles

Replacement of fishmeal by common cricket (*Acheta domestica*) meal in diets for juvenile tilapia (*Oreochromis niloticus*)

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The present study was to substitute fishmeal with domestic cricket (*Acheta domestica*) meals in diets for tilapia (*Oreochromis niloticus*) farming. Productive performance was evaluated by two diets based on Pearson's square, the treatment T1 with 20% inclusion and treatment T2 with 35%, respectively, and a control T3 (commercial feed) performed in triplicate. Each treatment had ten organisms weighing 9+5 gr for 40 days. Initial biometry was performed, and after this, every five days until the end of the bioassay. The production and survival variables were evaluated; Finally, a proximal analysis of moisture, ash, lipids, and protein was carried out. According to the weight gain, the performance of the diets presented significant differences between the three diets. T2 diet results were similar to T3 (Control) due to tilapia being omnivorous and having excellent resistance and adaptability to different types of food. Therefore, the possible use of this food can be considered, favoring its economic impact on tilapia crops.

INTRODUCTION

World fish production reached 178 million metric tons in 2020; 16 million tons were destined for non-food uses, mainly for producing fishmeal and fish oil. In 2020, 86% of fishmeal was used in aquaculture.¹ Fishmeal is one of the main components of aquafeed; its highly digestible protein and good palatability make it an excellent component in aquaculture diets. However, the increase in fish production destined for human consumption, over-exploitation of wild stocks, and increasing costs of aquaculture production due to El Niño events generate significant instability in fishmeal production, which causes an increase in production costs.² This is why new sources of sustainable protein alternatives have been investigated, which provide a nutritional value similar to that of fishmeal for optimal fish growth.²

The nutritional value of insects in general and domestic cricket has long been recognized. In addition to providing a rich source of high-quality protein, crickets, and related insects offer several other advantages, such as food sources. They are short-lived, produce numerous offspring, are suitable for human consumption, and flourish under various environmental conditions. Cricket farming requires far fewer resources than conventional cattle, pig, or chicken

farming. Insects like crickets emit fewer greenhouse gases and less ammonia than cattle or pigs, requiring significantly less land and water than raising cattle.^{3,4} Most current research in fish feed aims to discover new protein sources so that insect meals can be a good substitute for fish meals. Domestic cricket meal has good characteristics to replace fishmeal. It has excellent potential as an alternative protein source that can help promote tilapia growth performance, as the amino acid composition has higher histidine, arginine, and threonine than fishmeal.⁵

Numerous experiments are related to using other insects in fish nutrition. Up to 30% of fishmeal could be replaced with *Tenebrio molitor*, *Hermetia illucens*, and *Musca domestica* meals in the diets of European sea bass.⁶ In sea bass (*Dicentrarchus labrax*), there was no preference for any meal in particular. No differences were observed in the structure and characteristics of the meat of the animals tested⁷; in Japanese carp (*Carassius auratus*) fed 100% with a meal of cockroach nymphs (*Periplaneta americana*), no differences were found between the fish fed with a modified diet and control diet (Hernández et al., 2008), in Nile tilapia (*Oreochromis niloticus*) a meal of *Tenebrio molitor* larvae was used as a substitute for fishmeal, reaching the conclusion that it would make it an insect and induced less oxidative stress,⁸ while the inclusion of 25–30 % of House fly worm

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meal did not affect growth performance and nutrient utilization of African catfish.⁹

On the other hand, up to 80% substitution of fishmeal for field cricket meal produced the highest biomass gains, apparent digestibility, and feed conversion efficiencies.¹⁰ Due to their high nutritional value, live crickets are now commercially available in pet stores, supplied as fish bait or supplemental food for ornamental fish and reptiles. A previous study by Taufek et al.¹¹ reported that cricket meals can replace up to 100% of fishmeal. It produced better growth performance than the control diet in the nutrition of African catfish. In this context, the data obtained made it possible to determine the effect of partially substituting fishmeal for cricket meals in tilapia (*O. aureus*) farming diets. This study aims to evaluate the potential of substituting fishmeal with common cricket meals in juvenile tilapia farming by assessing the cricket meal's chemical composition and the mortality, feed conversion rate, and feed conversion efficiency in the organisms cultivated.

MATERIALS AND METHODS

CRICKET FARMING

Cricket farming was carried out at the Yaqui Valley Technological Institute 27°24'50.4"N 110°07'56.2"W. A collection of 25 male and female crickets, was placed in 2 hatcheries made of polyethylene (PET) containers, 40 x 60 centimeters, equipped with drinkers (PET cut to 3 cm high) and nesting boxes (PET cut to 10 cm tall) with potting soil and cardboard as shelter.¹² Each container was identified and labeled according to its function in the development stage. The hatcheries, drinkers, feeders, nests, and other utensils were disinfected with diluted chlorine in a proportion of 5% and detergent. The crickets were fed dry food, mainly cat or dog croquettes and vegetable skins. The feeding frequency was done every three days. It was unnecessary to place temperature generators because the environmental temperature was 35°C, providing ideal conditions of 20°C and a maximum of 35°C for crickets to develop. The eggs were incubated in special containers for hatchlings with similar requirements to the hatcheries, for which the laying females were removed weekly and placed in said containers. Control cards were used to take daily notes on the status of each cage and each nest in use, indicating all activities within the location.¹²

PREPARATION OF CRICKET FLOUR AND PELLET PREPARATION

Once sufficient cricket biomass was obtained, they were slaughtered by freezing, then dried in a food dehydrator (NESCO American Harvest) for 24 hours at 35-60°C, followed by grinding with a coffee grinder (Hamilton Beach). Approximately 1200 gr of cricket flour was obtained for the proposed diets. Subsequently, the cricket flour, wheat flour, vitamins, and minerals were mixed with a blender (Vitamix), obtaining a 20% and 35% protein diet. Once the homogeneous mass was obtained, it was processed using a homemade meat grinder (Oster) and thus obtained the pellets.

Finally, the pellets were dried in a feed dehydrator (NESCO American Harvest) for 24 hours.

DIETS FORMULATION USING PEARSON SQUARE

The diets were formulated using the Simple Pearson Chart since it is one of the most used methods to formulate rations.¹³ The calculations for elaborating the diets require a proximal characterization of the cricket meal. The nutritional value of the common cricket is 62% crude protein, very similar to fishmeal, which is composed of 60% protein.^{14,15} Considering the protein content, it can be a potential substitute for this type of ingredient with high or medium protein content.

The treatments for this bioassay consisted of 2 combinations of common cricket and wheat flour, T1 (20% cricket meal + wheat flour) and T2 (35% cricket meal + wheat flour), each with three replications. Meanwhile, the control group of fish (3 replicates) was fed commercial pellets (Nutripec, Cargill). All feed samples were subjected to proximal analysis to determine crude protein (CP), moisture, and ash.

PROXIMAL CHEMICAL ANALYSIS

The quantitative parameters used the Association of Analytical Chemists (AOAC) standard procedures.

MOISTURE CONTENT

The determination of the total moisture content of cricket flour was carried out using the AOAC 950.46 (2006) method described by Ileleji et al.¹⁶ The cricket meal sample was placed in a previously weighed porcelain crucible. Subsequently, it was dried in a fan oven at 105°C for 4-5 hours to remove all moisture from the sample. Finally, the total moisture content was calculated using the following equation:

$$\text{Moisture (\%)} = \left[\frac{\text{Weight loss}}{\text{Initial weight}} \right] \times 100$$

TOTAL ASH

The procedure started with weighing nine crucibles carefully washed and dried in a 100°C oven. Once hot, they were marked with the numbers 1 to 12 and cooled in a desiccator before being weighed. Then, 3 g of the powdered sample was placed in each crucible and calcined in an oven at 550°C for 6 hours. After turning off the furnace and allowing the temperature to drop, the crucibles were removed, cooled in a desiccator, and reweighed. The ash percentage was calculated using the equation:

$$\text{Ash (\%)} = \left[\frac{W - Z}{N} \right] \times 100$$

Where W is the weight of the crucible and ash, Z is the weight of the empty crucible; and N is the weight of the sample.

CRUDE FAT ANALYSIS

The Soxhlet extraction method (AOAC Official Method 948.16 (2006))¹⁷ was used to determine the amount of crude fat in the powdered sample. The fat extraction was carried out using petroleum ether. Labeled thimbles were taken, and a 3.0 g of the powdered sample was placed in each thimble. In a 250 mL boiling flask, 100 ml of petroleum ether with a 40-60°C boiling point was placed. The extraction thimbles were plugged with absorbent cotton, and the Soxhlet apparatus was set up to conduct the reflux extraction for 24 hours. After extraction, the thimble was carefully removed, and the petroleum ether containing the fat was collected and drained into another container for reuse. The boiling flask was dried in a hot air oven until it was practically free of petroleum ether, then cooled in a desiccator and weighed to determine the amount of fat extracted. The ash percentage was calculated using the equation:

$$\text{Fat (\%)} = \left[\frac{\text{Weight of fat}}{\text{Weight of sample}} \right] \times 100$$

CRUDE PROTEIN ANALYSIS

The Kjeldahl method determined the crude protein content of the cricket flour powder sample (AOAC 981.1). In digestion, 0.15 g of flour was mixed with 2.5 ml of 98% sulfuric acid and 1 g of catalyst mixture in a 250 ml Kjeldahl flask. The resulting mixture was heated in the digestion chamber until it became clear and cooled before adding 7 mL of distilled water. The digested sample was then transferred to a Micro-Kjeldahl distiller with 10 mL of 30% (w/v) NaOH, and distillation was started. The released ammonia was trapped in 2% boric acid with 2 drops of methyl red, and a color change was observed in the indicator solution from red to green, indicating that all the ammonia had been captured. The solution in the receiving flask was titrated against 0.1 N hydrochloric acid until purple appeared. In addition, a blank test was performed along with the sample. After titration, the % nitrogen was calculated using the following equation:

$$\text{Protein (\%)} = \frac{(\text{HCl normality})(\text{mlHCl})(1.4 \text{ g/mol})(6.5)}{\text{gr Sample}}$$

EXPERIMENTAL DESIGN AND SELECTION OF ORGANISMS

The experimental design consisted of 400 fingerlings and juveniles with an average weight of 9.00 ± 3.10 g for treatment of 35%, 9.21 ± 2.67 g for treatment of 20% and 8.49 ± 1.35 g for treatment control, obtained from the Cajeme Fish Center in Esperanza, Sonora, and housed in 250-liter plastic tubs with continuous aeration. The initial density was 100 fish per tank, randomly placed in tubs with similar weights.

FEEDING AND PARAMETER MEASUREMENT

The fish were fed daily, and several biometric studies were carried out on the organisms to determine the portion of food per tube. This analysis was carried out every five days

till the end of the bioassay (40 days) to adjust the proportion of food according to biomass—the biometric analysis measured the weight and height of each organism.¹⁸ The ten organisms in each tank were weighed on a digital scale (Ohaus, CS Series) to calculate the biomass of each experimental unit. The size of each organism was measured with an ictiometer, measuring from the head to the caudal fin.

Temperature, pH (Ohaus pH meter ST10), and oxygen (Ysi Oximeter) were recorded twice a day (at 8:30 and 1:00 pm) to maintain the water in optimal conditions.¹⁸ Siphons were carried out twice a week to eliminate settleable particles. A 25% water change was carried out daily; every ten days, a 50% replacement was done by washing aeration stones.

Mortality was calculated, considering all dead animals throughout the three repetitions. It represents the ratio of the number of dead animals to the total number of animals,¹⁸ where:

$$\text{Mortality} = \left[\frac{\text{Amount of dead animals}}{\text{Amount of total animals}} \right] \times 100$$

The feed conversion factor (FCR) was determined according to the equation¹⁸:

$$\text{FCR} = \left[\frac{\text{Total feed consumed}}{\text{Total weight of product produced}} \right] \times 100$$

The total weight of the product produced = final weight of the product – starting weight. Finally, the Feed Conversion Efficiency (FCE) was determined with the following equation:

$$\text{FCE} = \left[\frac{1}{\text{FCR}} \right] \times 100$$

STATISTICAL ANALYSIS

Once the cricket flour was obtained, the diets were elaborated by elaborating the food with different diets formulated by Pearson's square. The elaborated diets consisted of a 20% and 35% substitution with cricket meal, and a 0% cricket meal diet was taken as a control. The feed evaluation consisted of a bioassay with blue tilapia. The design was completely randomized, with three repetitions in each treatment, including the control with 40 days.

RESULTS

PROXIMAL ANALYSIS OF TREATMENTS AND CONTROL TREATMENT

The result of the proximal analysis of the diets is shown in (Table 1). The protein content of T1 and T2 was 23.6% and 12.77, respectively. T2 and T3 have very similar protein content and do not differ significantly. On the other hand, differences were observed between the treatments concerning the percentage of lipids and ashes. However, the T2 treatment was the one that obtained the most significant similarity to the control treatment.

As seen in Table 1, the proximal content between the commercial feed and the feed made from cricket flour is very similar, which may mean that the content of essential amino acids is equivalent. The substitution levels applied

Table 1. Proximal analysis of the 2 treatments based on cricket flour (*Acheta domesticus*) and the control treatment

Diet Formulation	% Protein	% Fat	% Moisture	% Ashes
T1 (20%)	12.77±1.14 ^b	4.40±0.11 ^a	8.87±0.10 ^a	2.55±0.22 ^a
T2 (35%)	23.6±2.19 ^a	6.59±0.32 ^b	8.77±0.20 ^{ab}	13.08±0.11 ^b
T3 (Control)	23.62±0.52 ^a	8.97±0.98 ^c	9.22±0.23 ^c	9.07±0.14 ^c

a, b, c the different letters in the rows indicate that there are significant differences ($p < 0.05$) between the treatments.

*Different letters per column indicate significant differences.

Table 2. Effect of feed processing on the productive performance of Nile tilapia

% Productive parameters	Treatments		
	35%	20%	Control
Starting measurements			
Body weight (g)	9.00 ± 3.10	9.21±2.67	8.49 ± 1.35
Overall Length(cm)	7.87 ± 1.01	8.04 ± 0.78	7.84 ± 0.46
Final measurements			
Body weight (g)	658.6±23.60 ^a	492.3±21.64 ^b	684.9±21.45 ^a
Overall Length(cm)	31.64±5.32 ^a	29.50±3.45 ^a	31.36±2.26 ^a
Condition factor	0.00207±0.00013 ^a	0.00191±0.00012 ^b	0.00222±0.00015 ^a
Final biomass (kg/m ³)	10.972±1.12 ^a	8.201±0.76 ^b	10.269±1.25 ^a
Daily gain (g/fish)	4.857±0.5 ^a	2.701±0.3 ^b	5.378±0.9 ^c
Feed conversion factor	1.27±0.05 ^a	1.71±0.08 ^b	1.19±0.06 ^c
Mortality (%)	0	0	10

*Different letters per line indicate significant differences.

Table 3. Average costs of cricket and fish meal production

Description		Quantity	USD unit price	Total USD
Harvest	Hours	4	0.415	1.66
Food	Bag	1/6	1.52	0.25
Processing	Hours	2	0.207	0.20
Cricket meal		1000 g		2.11
Fishmeal		1000 g		3.3 - 4.5

in the present investigation did not show significant differences, T2 and T3; treatment T1 was below T2 of its lower protein content.

Regarding mortality, only 10% occurred due to causes external to the experiment (Table 2). In the bioassay, 1,376 kg of feed was used between the three treatments, and final biomass of 2,35±0.42 kg was obtained with feed conversion factors of 1.27. It indicated that the feed expenditure was within the established ranges. The tilapia needs to consume 1.27 kg of the food supplied to convert it into 1 kg of meat, which improves production at a lower cost (Table 3). Concerning feed conversion efficiency, treatment T2 (35%) presented 78.70% while treatment T1 (20%) did not exceed 59% efficiency (Table 4); this may be related to the protein requirement in the different stages of the fry and juvenile and the size and age of the fish.¹⁹

DISCUSSION

EFFECT OF FEED PROCESSING ON THE PRODUCTIVE PERFORMANCE OF NILE TILAPIA

Some authors report that protein levels above 24 or 26% always satisfy the growth requirements for juvenile tilapia.^{20, 21} Likewise, El-Wahab et al.²² suggested that diets with a protein content of around 34% are acceptable for tilapia farming. This result could be due to the content of amino acids present in the treatments since the amino acids required to improve tilapia performance are lysine, arginine, phenylalanine, isoleucine, leucine, methionine, threonine, and valine or a mixture of methionine, cystine, valine, and lysine.^{19,20}

The growth of aquatic organisms in aquaculture is influenced by external factors, among which the ambient tem-

Table 4. Feed Conversion Factor (FCR) and Feed Conversion Efficiency (FCE)

Treatment	FA (g)	FB (kg)	IB (kg)	ΔB (kg)	FCR	FCE (%)
T1 (20%)	369.15±58.14	1.64±0.3	0.9±0.31	0.85±0.06	1.71	58.54
T2 (35%)	493.8±44.20	2.76±0.41	0.92±0.26	1.82±0.18	1.27	78.70
T3 (Control)	513.6±69.16	2.57±0.32	0.84±0.13	1.72±0.31	1.19	83.78

Feed amount (FA), Biomass (final FB, initial IB, ΔB delta of Biomass), Feed Conversion Ratio (FCR) and Feed Conversion Efficiency (FCE) of *Nile tilapia* in the period from October to December 2020.

*Different letters per column indicate significant differences.

perature stands out. It is known that temperature changes govern different functions of the organism and are one of the factors that accelerate chemical reactions and metabolism in general, causing a greater consumption of O₂ by living beings²³; the environmental temperature was a minimum of 15°C, which affected the energy demand of the species.²⁴ Similarities between T1 and T2 may indicate that type of protein present in the cricket has similar assimilation to the protein present in the control diets; the common cricket contains 60% protein similar to that reported for fishmeal.²⁵ In recent studies, Orthoptera was reported to be 65% protein.^{26,27}

Feed conversions in tilapia farms range from 1.5 to 2.5,^{28,29} and for European sea bass conversions of 0.99 to 1.03^{6,30} were reported. The feed conversion factor can be affected by the stocking density, feed quality, and size of the specimen, also by the sudden mortality of the specimen in the cultivation phase, making it impossible to recover the biomass; this directly affects the FCR due to the variation in the density of organisms. However, 10% mortality is within the working parameters of fish farms.^{31,32} Mortality could be due to a combination of risk factors such as water source, temperature, salinity, and diseases; the latter are affected by excess feeding rates to achieve maximum weight gain to reach market size faster and are among the top stressors that favor disease outbreaks.³¹ Daily feeding is recommended to yield constant mass production.³³ It is known that the protein requirement of tilapia can be affected by the protein source, the ratio's energy content, the water's quality, and the culture conditions. On the other hand, some authors mention that the protein requirement differs at each stage of tilapia growth; for example, 28% of crude protein for the tilapia juveniles (10-60 g) and 22% for the biofloc (60-230 g).³⁴

PROXIMAL ANALYSIS OF TREATMENTS AND CONTROL TREATMENT

The protein requirement for tilapia fluctuates between 20-50% in the fry stages, which depends on size, protein quality, water salinity, feed availability, and handling.²¹ We can consider the treatments as iso-protein due to the protein content.

Although the nutrient content between the control and experimental treatments is different, the experimental diet was designed to match the protein and energy content of the commercial diet. Due to the reduced number of ingredients used in the formulation, it is impossible to match all

the nutrients in the control diet. However, it has been reported that it is possible to formulate this diet based on unconventional animal-origin ingredients. For example, some diets have included meat with blood, meat, and bone, hydrolyzed chicken feather meal, chicken and vegetable viscera meal, cassava, leucaena, lupines, mushrooms, and algae.³⁵ Vegetable products often show protein deficiencies or some essential amino acids for fish; they may even be deficient in minerals or have antinutrient factors. In addition, they generally have less digestible energy than other by-products of animal origin.^{36,37}

Some authors report a protein content of ~20 to 40%, depending on the phase of biological development.^{21,22} As stated before, lysine, arginine, phenylalanine, isoleucine, leucine, methionine, threonine, and valine are the amino acids linked to weight gain in tilapia feed with an insect-based meal.⁵ However, it is necessary to corroborate this information using an analysis of amino acids present in the two types of flour used since such confirmation was not carried out in this research. It has also been reported that grasshopper flour (*Acrida cinéa*) has an amino acid content similar to that of cricket flour and flour of fish containing values of methionine (2%), lysine (0.7%) and cysteine (3.8%) very similar, is that of the grasshopper meal with values of methionine of 2%, lysine of 0.7% and cysteine of 3.8%.³⁸ Other studies have shown that the substitution of insect meal in diets for fish does not show significant differences in weight and growth, as is the case with the use of cockroach nymphs (*Periplaneta americana*). A 30% substitution is used in Japanese carp (*Carassius auratus*).³⁹ In another study on Nile tilapia (*Oreochromis niloticus*), a meal of *Tenebrio molitor* was used as a protein alternative to fishmeal, concluding that the diet based on insect meal induces oxidative stress to a lesser extent.⁸

Finally, to evaluate the economic effect of replacing fishmeal with cricket meal in this study, the cost estimates for cricket meal production were based on the collection, establishment of the cricket farm, feeding, and processing for 80 days in the facilities of the Tecnológico Nacional de México Campus Valle del Yaqui. The methodology used was a replica of the processes carried out by Entomo Farms, dedicated to producing edible insects.

The cost calculations were made based on 1000g. of flour from approximately 6000 crickets. Stages such as collection, feeding, and processing are considered, and the cultivation period is around 75 to 80 days to obtain adult crickets.⁴⁰

CONCLUSION

The formulation of the diets was carried out in which only the protein was substituted. The protein content of the T2 diet based on cricket flour presented the same content as the extruded tilapia based on fishmeal; concerning the other inputs, vitamix was added to complement the vitamins and minerals required for the growth of tilapias. The productive parameters were not significantly affected by the levels of substitution applied, being T2 (35%) the most similar to the control because the percentage of protein is very similar between the two treatments. However, even though the type of processing used in the food preparation is different, as is the bioavailability of its nutrients, a gain in weight was obtained in all the treatments. Mortality was 10%, an indicator reflected by the control of physical-chemical parameters, which allowed stable animals during the bioassay. The breeding and reproduction of crickets is a viable strategy since the methodology is simple and the production is exponential. In addition, in the southern region of Sonora, this insect is considered a pest during the rainy season from June to September, which would take advantage of the collection of this insect for the production of cricket flour. Using the common cricket for the elaboration of flour as food for tilapia farming in the first stages of fingerling is considered feasible because it is a low-cost protein with a simple elaboration process; it does not have an ecological impact.

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