

Biological performance and feed utilization of carob moth (*Ectomeylois ceratoniae*) larvae as an alternative dietary meal on *Oreochromis niloticus*

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Received :11-07-2022; Accepted: 20-11-2022; Published: 19-01-2023

DOI: [10.21608/ejar.2022.135639.1230](https://doi.org/10.21608/ejar.2022.135639.1230)

ABSTRACT

Experiments using tilapia fish (*Oreochromis niloticus*) were used to assess the impact of dietary inclusion of *Ectomeylois ceratoniae* (EC) larval meal. A first growth trial was carried out on 360 Tilapia fish using three experimental diets, each of which had increasing quantities of EC meal inclusion. These diets were developed to contain EC meal inclusion at levels of 0 (EC0), 25% (EC1), 50% (EC2), and 100% (EC3). In 2021, our research was carried out at the department of agricultural sciences, Biskra University in Algeria. All fish given the test diets were examined for performance, as well as the fatty acid (FA) composition and proximal body profile. The addition of EC at 100% affects tilapia growth performance. When compared to EC1, EC2 and EC0, dietary EC3 improves tilapia growth and feed efficiency. Moreover, the impact on body weight, particularly protein and cinders, is significant, while EC3-fed fish had the lowest body lipid levels of all the treatments. Overall, the findings show that insect meal substrates will have an impact on fish performance; larvae are a good source of protein when used to substitute fishmeal. Therefore, more research is needed to optimize the EC harvest processing procedure. The nutritional composition of fish feed can also be improved to increase efficiency.

Keywords: Growth performance, insect meal, nutritional, Tilapia, *Ectomeylois ceratoniae*

INTRODUCTION

The fishing industry is one of the largest animal feed industries in the world. In the aquaculture industry, traditional nutrient components have been replaced by more cost-effective and environmentally friendly alternatives. Insects are a possible solution because they are very nutritious and have characteristics that promote a circular bioeconomy (Drillet *et al.* 2008). Understanding the trophic interactions of fish within a population requires insight into fish nutrition and feeding technique. (Blaber, 2000). Daily cycles alter food content (Carman *et al.*, 2006), fish size (Dinh *et al.*, 2017) and dwellings (Dinh *et al.*, 2020). A nutritional study carried out on specific tilapia diets was a significant advance during the same early growth stage. Fish by-products, by-products from vertebrates, and a range of vegetable matter were used as replacement sources of protein in tilapia meals, amounting to 25–28%. (Dong *et al.*, 1993; Chamberlain, 1993; Goldberg and Triplett, 1997). Unfortunately, it is no longer feasible economically or environmentally to provide this traditional protein source, even for the constantly expanding fish farming industry. (Boyd, 2015). In order to assess the utilization of substitutes for fish alimentation and fish oil in real-world diets, feeding tests were carried out.

Recent years have seen increased attention to insects, especially their juvenile stage, as a possible alternative to fishmeal and other usual protein sources in fish feed (Henry *et al.*, 2015). Insects are suggested for their durability since they require little space or energy to develop and reproduce and are very effective at bioconverting organic materials, according to FAO (2019). They build up significant amounts of proteins and lipids in their bodies throughout this process. (van Huis, 2013). Furthermore, both freshwater and marine fish consume them naturally. As a result, scientists have been more interested in using insect meal as an element in fish feed. (Rumpold and Schluter, 2013). Many comparative studies have looked up insect constitution, which has been researched in great detail. (Rumpold and Schluter, 2013; Barroso *et al.*, 2014; Makkar *et al.*, 2014 and Sanchez-Muros *et al.*, 2014). The exact composition of the insect, which varies based on its life stage, growing environment, and nutrition, must be established and evaluated to the requirements of a specific species of fish before any insect species is added to the diet of a types of fish. The dietary needs of fish, especially carnivorous fish, are high in comparison to the quantity and quality of protein in the diet. Fishmeal's high protein content and well-balanced amino acid profile have made it the finest useable protein supplement in feed composition for a long time. This study's objective was to determine whether three distinct insect meals of the carob moth, one of the most widely available and easily bred insects in the area, could replace fishmeal entirely or partially in aquaculture feed. Towards this give-up, nutrition and development tests on Tilapia fish species were carried out (*Oreochromis niloticus*).

MATERIAL AND METHODS

Carob moth larvae's mass rearing:

Our breeding was conducted with a strain of carob moth *Ectomeylois ceratoneae* which comes from the infested dates of the year 2021 harvested from palm groves of the region of Biskra which is the first producer of dates in Algeria. Infested dates were placed in breeding cages in a controlled environment (temperature: 28°C ±2, relative humidity: 65 percent and photoperiod: 16 hours of light / 8 hours of darkness). When the adults emerged, they were captured and placed in the mating jars without sex. After mating, the females lay eggs within the jars, which were then placed in boxes containing previously prepared breeding feed (date flour, wheat bran, and droplets of water). After that, the larvae will be allowed to complete their larval phases until they reach their maximum size, at which point they will be dried and mixed in with the other ingredients of the diet.

Fish feeding trial

Oreochromis niloticus fish weighing an average of 10.0 ±1.0 g was obtained from a commercial fish hatchery in Biskra City, Algeria (34.84038N, 5.75078E). Fish were brought to the department of agricultural sciences' experimental site at Biskra University in Algeria and placed in a 1000 L fiber glass tank. Later, the fish were randomly divided into three fiber glasspond, each with 30 fish, and allowed to acclimate for an additional 10 days. Each diet was assigned to the experimental groups in triplicate (pond). The steps for conducting this experiment were followed according to the Benha Faculty of Veterinary Medicine's Research Ethics Board (BUFVTM 02-08). Every day, the water's properties were examined.

All diet was supplied two times daily to three classes of 30 fish., seven days a week, until apparent satiation. In particular, the fish stopped eating, and the delivery of food was stopped, any pellets that had not been consumed were retrieved. The precise amount of feed supplied in each tank was recorded. After 15 days of acclimatization to the pond and foods, the study lasted 75 days and was monitored daily.

Considering the regular monthly increasing water temperatures in Biskra City, which went from 20 °C in February to 25 °C in April, in this work, immersion heaters were used to keep the water temperature around 30 °C. The dissolved oxygen concentration varied from 6.5 to 8.8 mg/L. pH (6.7-7.8), ammonia (<0.8 mg/l). After being stocked, the fish were fed experimental food and given four days to get used to the test conditions.

Diet formulation:

Three experimental diets (Table 1) were devised to meet the nutritional demands of Tilapia Fish. A diet that helps you stay on track (ECO). In the other three diets, the fishmeal was replaced with full-fat *Ectomeylois ceratoneae* larval food at 25 percent, 50 percent, and 100 percent (as fed basis) (EC1 EC2 and EC3, respectively). To keep the diets energetic, the quantities of the remaining ingredients in the combination (soybean, maize, and vegetable oil) were not modified. All nutritious components were crushed, fully mixed, and pelleted at a diameter of 3.5 mm in an industrial meat blender. After drying for two days at 40 °C, the pellets were filtered and kept at a low temperature.

Tables 1 and 2 include information on the ingredients in diets, their chemical components (ECO, EC1, EC2 and EC3), and their amino acid profiling.

Table 1. Chemical composition of diets substances

	Diet			
	ECO	EC1	EC2	EC3
Ingredient¹, g/kg as fed				
Fish meal	410	308	205	-
<i>E. ceratoniae</i> larvae meal	-	102	205	410
Soybean	350	350	350	350
Maize meal	140	140	140	140
vegetable Oil	80	80	80	80
Mineral mix ²	10	10	10	10
Vitamin mix ³	10	10	10	10
Chemical composition, % as fed				
Dry matter	81.57	82.68	82.91	84.74
Moisture	18.43	17.32	17.09	15.26
Crude protein	45.66	46.87	47.10	47.47
Ether extract	17.88	17.95	18.26	10.53
Total lipids	10.21	10.34	11.33	11.87
Ash	8.55	7.65	7.11	5.48
Crude fiber	1.27	1.85	2.33	4.95

¹The ingredients, used to formulate the experimental diets, were purchased from DZira Ponc SPA, Biskra, Algeria.

Table 2. Essential amino acid (%) profile of the test diets

	EC0	EC1	EC2	EC3
Lys	4.03	2.14	2.22	2.58
Val	4.12	1.51	1.55	1.69
Leu	4.44	2.66	1.74	1.84
His	1.33	0.66	0.69	0.77
Arg	3.25	2.01	2.11	2.27
Thr	2.11	1.32	1.37	1.41
Iso	2.21	1.05	1.10	1.25
Met	2.54	0.25	0.33	0.42
Ph	2.25	1.44	1.54	1.62

Estimation of growth indices:

When the fish arrived at the laboratory, they were independently weighed and measured to determine the standard length and maximum height. The length of the body was measured from the tip of the mouth to the end of the upper lobe of the caudal fin (total body length), and the height of the body, except for the fins, was determined vertically. Tilapia weight was calculated at the start of the trial and again to determine growth indices following fasting. (after 75 days). In each group, 30 fish were used for all measures. The parameter used to determine biological performance and feed conversion ratio were:

Specific Growth Rate: $SGR (\%/day) = 100 \cdot (\ln(\text{Mean final body weight}) - \ln(\text{Mean initial body weight})) / \text{time (days)}$

Body Weight Gain: $BWG (g/day) = ((\text{Mean final body weight}) - (\text{Mean initial body weight})) / \text{time (days)}$

Feed Conversion Ratio: $FCR = \text{Food fed} / \text{Live Weight Gain}$

Statistical analysis

After verifying the homogeneity and normality of all the data, one-way ANOVA was applied to all of them. Before analysis, all proportion data were transformed to arcsine. Duncan's multiple range tests were used to examine the group means when there was a significant difference. SPSS V. 22.0 was used to conduct the statistical analyses. With a 5% level of confidence (P0.05) and displayed as the mean standard error of the mean (SE).

RESULTS**Biological performance and body composition of fish:**

Table 3 shows data on growth performance and condition metrics, the mortality rates were not significantly different. (96.67 to 100%). All treatments ($P > 0.05$). BWG was unaffected by the diets (Table 3). In comparison to those given EC1, EC3, and control diets, fish on EC3 diets perform better in terms of FCR, SGR, and PER.

Table 4 shows the proximal fresh body components of Nile tilapia at the beginning and the final of the trial. Diet composition had no discernible effect on carcass composition. The protein composition of the carcasses did not change across the treatments. The water content of the carcass was considerably greater in fish given diet EC3 than in those fed the other diets, with the least rates seen in fish given a standard diet Body lipid levels were shown to fall when EC content in meals increased. Diet EC1-fed fish had much lower body lipid levels than the other diets. The amount of ash in the body did not differ considerably across treatments.

Table 3. Biological performance of *O. niloticus* experimented with different diets

Variables	Diets			
	EC0	EC1	EC2	EC3
IBW (g)	10.30 ± 0.02 ^a	10.20 ± 0.02 ^a	10.30 ± 0.02 ^a	10.10 ± 0.03 ^a
FBW (g)	105.75 ± 3.24 ^b	101.25 ± 3.24 ^b	102.75 ± 3.24 ^b	111.02 ± 3.24 ^b
SR (%)	100 ± 0.00 ^a	96.67 ± 1.23 ^a	100 ± 0.00 ^a	100 ± 0.00 ^a
BWG (g/day/ fish)	1.41 ± 0.23 ^b	1.35 ± 0.31 ^a	1.37 ± 0.33 ^a	1.48 ± 0.34 ^b
SGR (% /day)	1.12 ± 0.21 ^{ab}	1.73 ± 0.48 ^b	0.99 ± 0.15 ^a	1.13 ± 0.33 ^{ab}
FCR (g g ⁻¹)	2.61 ± 0.74 ^b	1.63 ± 0.46 ^a	2.83 ± 0.68 ^b	2.61 ± 0.74 ^b

Values are means ±SE of three replications (n=3 pond/meal); the same superscript letter in the same row indicate that the values are not considerably different. ($p > 0.05$). IBW, initial body weight; FBW, final body weight; SR, survival rates; DWG, daily weight gain; SGR, specific growth rate; FCR, feed conversion ratio.

Table 4. Body components of tilapia fed various regimens (%)

Body composition	EC0	EC1	EC2	EC3
Crude protein	78.07±0.57	69.83±1.26	73.07±0.22	82.85±0.55
Crude lipid	3.52±0.12 ^b	2.27±0.12 ^a	3.46±0.20 ^b	3.69±0.11 ^b
Moisture	8.41±0.74 ^b	7.74±0.42 ^a	8.84±0.62 ^{ab}	9.58±0.62 ^b
Ash	7.24±0.62	6.41±1.33	6.18±1.77	7.35±0.61

DISCUSSION

Several recent studies looked at the influence of using waste substrates from various sources on tilapia performance and waste reduction efficiency. On the other side, there are data on the effect of the substrate on fish performance. As a result, this research is being done as a pilot project to see how *E. ceratoniae* affects tilapia development, nutritional efficiency, and body composition. In the wild, juvenile and young tilapia fish are omnivores. They eat zooplankton and benthic animals mostly, although they also consume debris and graze on microalgae. When they reach about 6 cm in total length, tilapias become essentially herbivorous (Moriarty and Moriarty, 1973). Insect meal's possible application in fish diets has recently received a lot of interest. (Barroso *et al.* 2014; Henry *et al.* 2015). Insects are already a component of the traditional diet of fish. (Henry *et al.* 2015). As a result using insect pellets as a primary ingredient in fish diets seems reasonable. A recent analysis of a batch of tilapia feeding trials using various ratios of Black soldier fly larvae (BSFL) in place of fishmeal found that 50% of BSFL meal produced the maximum development. (Muin *et al.* 2017). According to our findings, the meals had no impact on the BWG (Table 3). In contrast, fish fed a diet high in EC3 perform better in terms of FCR, SGR, and PER than fish fed EC1, EC3, or control diets. In contrast to the research conducted by Muin *et al.* (2017). In this study, tilapia fed with EC meal performed better at transferring protein from food that contains standard diet to fish body mass. It is shown in lower FCR and greater PER.

Even if fishmeal were to be substituted, increasing dietary non-defatted dry silkworm pupa meal or oil resulted in a significant increase in lipid absorption that did not cause an increase in fat storage. (Nandeesh *et al.*, 1990, 1999). Additionally, Jayaram and Shetty (1980); Begun *et al.*, (1994) and Begun *et al.* (2001) found that adding defatted or non-defatted silkworm pupa meal to a cyprinid's diet resulted in very high digestibility in both tilapia and catfish (Hossain *et al.*, 1991; 1992; Boscolo *et al.*, 2001 and Borthakur and Sarma, 1998). The mealworm *Tenebrio molitor* is one source of proteins that may be used to replace fishmeal in fishmeals. *Tenebrio molitor* larvae and pupae are easy to raise and feed, and they are high in protein and lipids (Ghaly and Alkoai, 2009). Several experiments including mealworm in fish feed have yielded promising outcomes in terms of growth performance and nutrient absorption in *Clarias gariepinus* (Ng *et al.*, 2001), *Sparus aurata* juveniles (Piccolo *et al.*, 2017) and *Ameiurus melas* fingerlings (Roncarati *et al.*, 2015). Vargas-Abúndez *et al.*, 2019), crickets (Sen, 2019) and black soldier fly larvae (BSFL) (Caimi *et al.*, 2019; Terova *et al.*, 2019). Regarding oily fish and fishmeal, however, the fish feed industry has successfully developed methods to reduce its dependence on these limited resources of nature. The average fishmeal and fish oil yield of Norwegian salmon diets have decreased during 30 years, as indicated by the most recent of MOWI (2020), from a peak of 65 and 24 percent in 1990 to a low of 13 and 11 percent in 2019. The aquafeed manufacturing sector in Norway has become less reliant on fishmeal and fish oil increasing usage of both plant and animal resources for lipids and protein, dietary supplements that limit the intake of vital amino acids, essential fats, and micronutrients are also recommended. (Bandara 2018; Turchini *et al.* 2019; Aas *et al.* 2019; Hua *et al.* 2019; Boyd *et al.* 2020 and MOWI 2020). Bondari and Sheppard (1981) proved that the incorporation of insect meals into fishmeal might potentially affect the sensory characteristics of the fish, even if the evidence available to date on this subject has not revealed any harmful effects. For example, the findings of a panel test (aroma and texture) on catfish and tilapia given chopped HI larvae alone or in conjunction with conventional diets showed that the fish were rated and classified as comparable to reference diets. Dietary treatment did not affect crude protein levels in entire fish bodies in this investigation. At high EC integration levels, lipid accumulation was reduced, on the other hand (EC3). Previous research on Tilapia fish showed a reduction in carcass lipid with feeding diets that replaced soybean food combined with other plant-based sources of protein, like as *Cassia fistula* meal (Adebayo *et al.*, 2004); green algae *Ulva rigida* (Azaza *et al.*, 2008), and Roquette seed, *Eruca sativa* (Fagbenro, 2004). This was due to a decrease in lipid accumulation, which certainly had an impact on liver growth.

CONCLUSION

Finally, a sustainable food system ensures food and nutritional security for all people while also ensuring that the financial, social, and ecologic foundations for generating food production and nutrition for coming generations are not jeopardized, and that it is advantageous throughout, has broad-based social value, and has a slightly positive impact on the environment. It is obvious that a more comprehensive and coordinated response is necessary, and that these systems provide positive value across all three aspects of economic, social, and environmental consequences. As a protein supply, aquaculture can no longer rely solely on fishmeal. Other experiments, particularly oxidative stress response, should be conducted in the future to evaluate the antioxidant activity of fish fed various EC. *Ectomeylois ceratoniae* appears to be one of the most promising candidates for fishmeal replacement in fish diets.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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