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Research Article
Araştırma Makalesi

Growth Performance and Survival Rate of *Pontastacus leptodactylus* Juveniles Fed with Fresh Black Soldier Fly *Hermetia illucens* and Mealworm *Tenebrio molitor* Larvae

Asker Sineği *Hermetia illucens* ve Un Kurdu *Tenebrio molitor* Larvaları İle Beslenen *Pontastacus leptodactylus* Ergenlerinin Büyüme Performansı ve Yaşama Oranı

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Abstract: In this study, the survival rate and growth performance of crayfish *Pontastacus leptodactylus* juveniles were evaluated when fed with five different diets. These diets were CF: commercial feed, Hİ: Fresh *Hermetia illucens*, CF+Hİ; 50% commercial feed + 50% fresh *Hermetia illucens*, TM: Fresh *Tenebrio molitor*, CF+TM: 50% commercial feed + 50% fresh *Tenebrio molitor*. The experiments of the present study were carried out for 60 days. The results showed that the survival rate, final body weight (FBW), and weight gain (WG) were negatively correlated with fresh BSF and TM diets. All individuals fed only with BSF and TM diets died on approximately the 30th day of the experiment. The final weight, feed conversion ratio (FCR), molting rate and survival rate of crayfish fed with dietary treatment diets showed significant differences on the 30th day ($P < 0.05$). The crayfish fed with CF+BSFL and CF+TM diets had similar weight, FCR, molting rate, single cheliped injury with the control group on the 30th day and 60th day. However, the highest survival rate was observed in crayfish fed with CF diet followed by CF+BSFL and CF+TM groups on the 60th day. As a result, TM and BSF diets given fresh alone showed negative effects on growth and survival rates in crayfish larvae. Therefore using just fresh insects in the crayfish juvenile diets is not recommended. By decreasing 50% commercial feeds and using as supplementary feed of TM and BSF larvae can show similar growth as the control group. However, 50% TM supplemented with 50% commercial diets is not recommended due to reduced survival rates. On the other hand, fresh BSF can be given to *P. astacus* larvae after reducing the commercial feed by 50% without affecting the survival rate and growth.

Keywords
- Growth
- *Hermetia illucens*
- *Pontastacus leptodactylus*
- Survival rate
- *Tenebrio molitor*

Özet: Bu çalışmada *Pontastacus leptodactylus* juvenile kerevitinin beş farklı yemle beslenmesi durumunda yaşama oranı ve büyüme performansı değerlendirilmiştir. Deneme grupları; CF: ticari yem, HI: Taze *Hermetia illucens*, CF+Hİ; %50 ticari yem + %50 taze *Hermetia illucens*, TM: Taze *Tenebrio molitor*, CF+TM: %50 ticari yem + %50 taze *Tenebrio molitor*. Denemeler 60 gün sürmüştür. Sonuçlar yaşam oranı, nihai vücut ağırlığı ve ağırlık kazancının taze BSF ve TM beslemenin negatif ilişkili olduğunu göstermiştir. Sadece BSF ve TM ile beslenen tüm bireyler deneyin yaklaşık 30. gününde öldükleri gözlenmiştir. Kerevitlerin deneme sonu canlı ağırlığı, yemden yararlanma oranı, kabuk değiştirme ve yaşama oranı 30. günde gruplar arasında önemli farklılıklar göstermiştir ($P < 0,05$). CF+BSFL ve CF+TM ile beslenen kerevitlerin ağırlık, FCR, kabuk değiştirme oranı, tek çeliped yaralanması ve kontrol grubu ile 30. gün ve 60. günde benzer olduğu görülmüştür. Ancak en yüksek yaşama oranı CF ile beslenen kerevitlerde gözlenmiş, bu grubu 60.

Anahtar kelimeler
- Büyüme
- *Hermetia illucens*
- *Pontastacus leptodactylus*
- *Tenebrio molitor*
- Yaşama oranı

günde CF+BSFL ve CF+TM grupları takip etmiştir. Sonuç olarak, tek başına taze olarak verilen TM ve BSF besinleri kerevit larvalarında büyüme ve yaşama oranı üzerinde olumsuz sonuçlar göstermiştir. Ticari yemlerin %50 oranında azaltılması ve ek yem olarak kullanılmasıyla TM ve BSF larvaları kontrol grubu ile benzer büyüme gösterebilmektedir. Ancak yaşama oranlarının azalması nedeniyle ticari yemlerde %50 oranında TM eklenmesi önerilmemektedir. Taze BSF, *P. astacus* larvalarına, yaşama oranı ve büyümeyi etkilemeden ticari yemi %50 oranında azaltarak verilebilir.

1. INTRODUCTION

Fishmeal has long been a key component of aquafeeds, but its extensive use has strained natural fish stocks, posing threats to marine ecosystems (Lalander et al., 2015; Mousavi et al., 2020). In response, insects have emerged as a promising alternative protein source. Insects raised on organic waste offer sustainable technology for aquaculture, promoting natural nutrient recycling (Sánchez-Muros et al., 2020). Various insect larvae species boast rich nutritional profiles, delivering essential protein, amino acids, fats, carbohydrates, vitamins, and trace elements (Chen et al., 2009). Moreover, insect-based feeds are imbued with bioactive compounds and chitin, known for their antioxidant, antibacterial, and immunostimulatory properties, potentially bolstering the health and immunity of aquatic species (Li et al., 2017; Motte et al., 2019; Mousavi et al., 2020). Numerous studies have demonstrated the efficacy of black soldier fly (BSF) larvae in enhancing growth in aquaculture, showcasing their potential as a protein source for various aquatic species. Research by Zarantoniello et al. (2023), Chen et al. (2022), Richardson et al. (2021), Mastoraki et al. (2020), Herawati et al. (2019) has specifically highlighted the benefits of BSF larvae in fish farming. Similarly, studies conducted by Foysal et al. (2019), Foysal et al. (2021), Wang et al. (2022) have underscored their positive effects on shrimp growth. Additionally, BSF larvae have shown promise as a feed ingredient for crabs, though specific studies on this aspect may be limited.

P. leptodactylus is a native species of Europe. Populations of this species are at risk in many countries. In Turkiye, populations are also decreasing due to disease risk. Therefore, larval culture has become important. There is no formulated ration developed specifically for crayfish larval culture. Shrimp feeds, trout feeds, daphnia and chironomid larvae are commonly used in larval rearing. In this study, we aimed to investigate how reducing the rate of these feeds and supplementing the ration with fresh insects from the wild would affect growth and development.

2. MATERIALS AND METHODS

2.1. The brood stock and management

Egg-bearing females of *P. leptodactylus* weighing 49.59 ± 12.26 g and total length 12.23 ± 1.48 cm were collected by trapping (mesh size 1.5 cm) from Egirdir Lake in March 2020, Türkiye. The eggs were at Blastosphere Phase (Clada et al., 1987). Broodstock were transported to the laboratory of Egirdir Fisheries Faculty and stocked 25 individual / m^2 in tanks (bottom area 120 x 120cm and volume 720L) with freshwater flow (1.2 L min^{-1}). Pipes 8cm diameter and 14 cm length) were placed to provide shelter in each tank. Broodstock were fed ad-libitum with trout feed (45% protein, 20% lipid), fresh chara, fresh fish and potato once daily at 9 am. Leftover feed and feces were siphoned out every other day before commencement of the next feed. The mean water temperature for broodstock culture was 18 ± 1 ^oC.

2.2. Stage II juvenile culture

The eggs of broodstock hatched until early May 2020 and crayfish reached stage II at the end of May. In the culture of stage II juveniles, initial weight and total length of stage II juveniles were 0.038 ± 0.00 g and 10.60 ± 0.06 mm, respectively. The stage II juveniles were stocked randomly to 22 tanks as 75 individual / m^2 at an indoor facility with recirculation for the production of stage III juveniles. Each tank was 80x50x30 cm dimensions, water volume 88 L. The water flow-rate was adjusted to 0.9 L/min. The water in all tanks was provided through a recirculation system and changed systematically with 1 times fresh water and 9 times system water all day long. The system also had a biological filter. The water temperature of Stage II juvenile culture was constant at 18 ^oC. The stage II juveniles were fed ad libitum with dried chara, wheat bran, *Eisenia foetida*, *Hermetia illucens* larvae, *Tenebrio molitor* larvae, fish meal, trout feed throughout the period of 32 days. Juveniles were provided with food at the amount 4.3 mg / L-1 in terms of visibility of feed. However, juveniles consumed 50% of the feeds. Live H. *illucens*, T. *molitor* larvae and E. *foetida* were grounded with a blender to a size which they could eat (2-3 mm). All diets were fed once daily at 9 am. Leftover feed and feces were siphoned out every day. At the end of 1 month culture , survival rate for stage II juveniles was 73% with this feeding protocol (Table 1), while it was 12% for stage III juveniles, 88% for stage IV juveniles. The mean weights were 0.116 ± 0.010 g, and 0.066 ± 0.008 g respectively for the stage II and sage III juvenile groups.

Table 1. Feeding protocol of Stage II juvenile.

Stage II Culture days	**Dried Chara**	**Wheat bran**	***Eisenia foetida***	***Hermetia illucens* larvae**	***Tenebrio Molitor* larvae**	**Fish meal**	**Trout feed**
1st day	X	-	-	-	-	-	-
2st day	X	-	-	-	-	-	-
3st day	X	-	-	-	-	-	-
4st day	-	-	X	-	-	-	-
5st day	X	-	X	-	-	-	-
6st day	X	-	X	-		-	-
7st day	X	-	X	-	-	-	-
8st day	X	-	X	-	-	-	-
9st day	X	-	-	X	-	-	-
10st day	-		-	X	-	-	-
11st day	X		X		-	-	-
12st day	X	-	X	-	-	-	-
13st day	X	-	X	-	-	-	-
14st day	-	-	X	-	-	-	-
15st day	X	X	X	-	-	-	-
16st day	-	-	X	-	-	-	-
17st day	-	-	-	-	X	X	-
18st day	-	-	-	-	X	X	-
19st day	-	-	-	-	X	X	-
20st day	-	-	-	-	X	X	-
21st day	-	-	-	-	X	X	-
22st day	-	-	X	X	-	X	-
23st day	X	-	-	-	-	X	-
24st day	-	X	X	-	-	-	-
25st day	-	X	X		-	-	-
26st day	-	X	-	X	-	-	-
27st day	-	-	-	X	-	-	-
28st day	-	-	-	X	-	-	X
29st day	X	-	-	X	-	-	X
30st day	-	-	-	X	-	-	X
31st day	-	-	-	X	-	-	X
32st day	-	-	X	-	-	-	X

2.2. Stage III-IV Juvenile Culture

The effects of 5 different diets on *juveniles* of *P. leptodactylus* were researched in this study. The stage III juveniles were stocked randomly to the tanks as 40 individual /m^2 at the same indoor facility as described above in broodstock culture. The stock ratio in stage *III-IV* was reduced due to cannibalism and loss of cheliped in stage II. Live *H. illucens* and *T. molitor* larvae were grounded with a blender to a size which they could eat (3-4 mm). The commercial feed size was 1,5 mm. All diets were fed once daily at 9 am. The stage III juveniles fed 5% of their body weight. CF, BSFL, TM, 50:50 CF: BSFL, 50:50 CF:TM.

2.3. Preparation and Proximate Composition of *Hermetia illucens* and *Tenebrio Molitor* Larvae

Hermetia illucens larvae were obtained, and 0.5-1 cm size larvae were reared on kitchen (all kinds of meal and vegetable waste, bread) waste substrate, from Profatfood Food Feed Industry Co., Ltd. Afyonkarahisar/Türkiye. We fed larvae until they reached 1.5-2 cm in size with animal and vegetable kitchen waste in laboratory conditions. *Tenebrio molitor* larvae were purchased when they were 1-1.5 cm size larvae from Mira Live Animal İnsect, Agriculture Commerce Co., Ltd. Antalya/Türkiye. *T. molitor* larvae were fed with wheat bran, bread, corn, apple, carrot (for water need) until they reached 1.5-2 cm size laboratory conditions (Figure1).

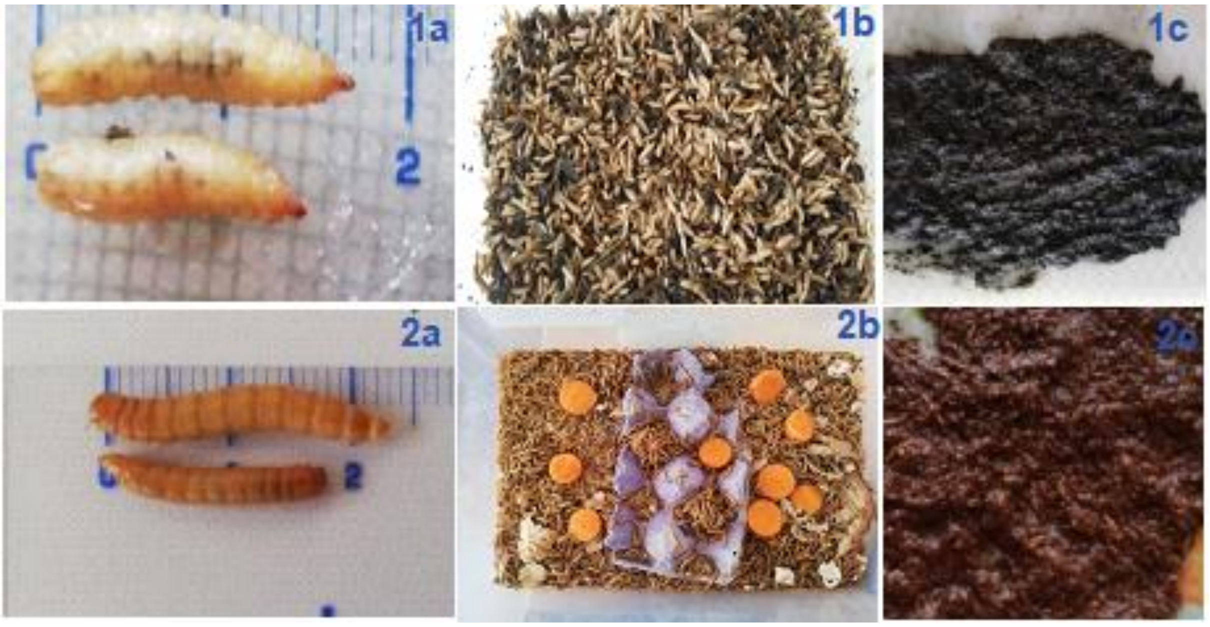

Figure 1. Fresh feeds, 1a; *H. illucens*, 1b; culture conditions of *H. illucens*, 1c; minced *H. illucens*, 2a; T. *molitor* 2b; culture condition of *T. molitor* 2c; minced T. *molitor*

The commercial and fresh feeds, used in this study, were given in the proximate composition as described in Table 2.

The analyses of experimental diets included the determination of dry matter, ash content, crude protein, ether extract according to AOAC (Association of Official Analytical Chemists) manual from 2004 in Egirdir Fisheries Faculty Laboratories. The commercial feed ingredients were fishmeal, fish oil, yeast products, wheat meal, wheat gluten, lecithin, monocalcium phosphate.

Table 2. Proximate compositions of experiment diets.

Based on dry weight (%)	CF	TM	BSFL
Crude protein	56.86	44.45	43.47
Crude lipid	15	33,19	37,73
Crude fiber	0.2	-	-
Ash	13.30	4.51	8.12
Moisture	9.33	38.84	43
Gross energy (Mj/kg)	21.9	26.67	26.94

CF: Commercial feed; TM: minced *T. molitor*; BSFL: minced *H. illucens*
Gross energy (Mj/kg)=(Protein x 0,236)+(Lipid x 0,395)+(Carbohydrate x 0,172)
Carbohydrates (%) = 100- (Protein + Lipid+ Ash)

2.3. Growth parameters

At the end of the experiment, a precision balance was used to measure the weights of crayfish subjects. A ruler was used for the measurement of the total length values. Growth parameters in the experiment were determined using the following formulas (Yu et al., 2020; Mazlum and Uzun, 2022).

Weight gain (WG) g = (final body weight (g) - initial body weight (g))

Specific growth rate (SGR) % = (ln final body weight - ln initial body weight) / experiment days x 100

Survival ratio (%) = (Nt/Nt-1) x 100

Nt = The number of crayfish at the end of the trial

Nt-1= The number of crayfish at the beginning of the trial

Single cheliped injury rate at the end of the trial (%) = 100 x (Single cheliped injury crayfish numbers / The number of crayfish)

Moulting rate (%) = 100 x Moulting number / total crayfish number)

Feed conversion ratio (FCR)= feed consumption (g) /body weight gain (g)

2.4. Statistical analysis

The significance of differences among the experimental groups was tested by one-way analysis of variance (ANOVA). All data were calculated using the IBM SPSS Statistics ver. 20 software (IBM. Armonk. NY. USA). Duncan's test was used to determine the growth differences among treatment means ($P < 0.05$).

3. RESULTS

3.1. Growth performance

The growth performance of crayfish fed with 5 different diets were given in Table 3. The final weight, FCR, molting rate and survival rate of crayfish fed with dietary treatment diets were determined to have significant differences on the 30th day ($P < 0.05$). The crayfish fed with TM and BSFL diets died at the end of 1 month. The crayfish fed with CF+BSFL and CF+TM diets had similar weight, FCR, molting rate and single cheliped injury, with the control group on the 30th day and 60th day. However, the highest survival rate was observed in crayfish fed with CF diet, CF+BSFL and CF+TM groups followed this group on the 60th day.

Table 3. Growth parameters of *juveniles* of *P. leptodactylus* fed with different experimental diets.

	CF	TM	CF+TM	BSF	CF+BSF
1th day Weight (g)	0.066 ± 0.008	0.066 ± 0.008	0.066 ± 0.008	0.066 ± 0.008	0.066 ± 0.008
30th day Length (cm)	2.08±0.03a	1.76±0.02c	2.05±0.03a	1.72±0.03c	1.91±0.04b
30th day Weight (g)	0.19±0.01a	0.12±0.00b	0.20±0.01a	0.13±0.01b	0.21±0.01a
30th day Survival rate (%)	96.88±1.80a	82.81±6.93ab	90.63±4.03ab	78.13±5.41b	92.19±5.92ab
30th day Single cheliped injury (%)	3.13±1.80	9.38±5.41	3.13±3.13	0.00±0.00	3.13±1.80
30th day Moulting rate (%)	15.50±2.50ab	9.00±2.12cb	16.50±1.66a	5.75±1.49c	15.50±2.53ab
30th day FCR	1.73±0.06a	3.83±0.24b	1.71±0.14a	4.23±0.19b	1.69±0.05a
60th day Length (cm)	2.64±0.04	-	2.61±0.06	-	2.55±0.06
60th day Weight (g)	0.36±0.02	-	0.35±0.02	-	0.35±0.02
60th day Survival rate (%)	91.67±4.17a	0	52.08±7.51b	0	70.83± 5.51ab
60th day Single cheliped injury (%)	30.05±10.00	-	17.04±11.92	-	11.19±5.65
60th day Moulting rate (%)	37.66±2.40	-	32.00±2.51	-	32.66±3.88
60th day FCR	1.33±0.17	-	1.47±0.17	-	1.37±0.12

Mean values with different superscripts in the same line are significantly different at $p < 0.05$.

4. DISCUSSION

At the end of this study, feeding fresh TM and BSF larvae to the crayfish suppressed the growth compared to other groups by the 30th day. By the end of the 60th day, there were no live crayfish left in these groups. The CF, CF+TM, and CF+BSF groups showed similar growth, but their survival rates decreased in the order of CF> CF+TM> CF+BSF. Similarly, He et al. (2022), found the best growth in *L.vannamei* fed with diet 75% commercial feed + 25% fresh BSFL. Muslimin et al. (2023), also reported that when cork fish (*Channa striata*) were fed with certain proportions of commercial feed and fresh BSFL meal, feeding more than 20% BSFL suppressed growth.

Similarly, M. Ordoñez et al. (2022) reported that *Colossoma macropomum* juvenile fed with fresh BSFL (50%) and commercial feed (50 %) had similar growth to that of fed with commercial fish feed. The worst growth was observed in the group fed with fresh BSFL. In crustaceans, He et al. (2022) determined that survival rate, final body weight, and weight gain were negatively affected when the amount of fresh BSFL was increased more than 50 % in the diet of L. *vannamei*. *To the best of our knowledge* there is no other reported study where BSFL were given freshly to crustaceans. We think that chitin containing diets negatively affect the growth of crayfish juveniles since their digestible system has not developed sufficiently to utilize chitin yet. Chitin on the outside of the insect larva, suppressing growth, is seen as a problem in fresh or processed products. Kroeckel et al. (2012), reported that chitin impaired the digestibility of crude protein. Bad smell was another disadvantage for fresh feeds.

Follow-up studies involved adding them to diets in flour form. Chen et al. (2022), reported a decreased growth performance of *Litopenaeus vannamei* fed with BSF meal diet with 30% replacement protein of fishmeal compared to the control (containing 25% fish meal). Richardson et al. (2021), found that 10.5% BSF in the diets of *L. vannemi* fry improved weight gain, feed conversion, and SGR (containing 15% fishmeal). Herawati et al. (2019), determined the best growth support in *L. vannamei* fry fed diet containing 16% BSF instead of fish meal (control contained 32% fish meal). Mastoraki et al. (2020), reported the best growth on fishmeal-based diet in shrimp *Palaemon adspersus* fed diets containing BSF+ soybeans and BSF+ Fishmeal (control contains 26% fishmeal). Foysal et al. (2019), observed similar growth between a diet composed of poultry byproducts + black soldier fly (BSF) larvae compared to a fishmeal diet in crayfish *Cherax cainii*. Wang et al. (2022), reported that the optimal nutritional status of FM for juvenile *Cherax quadricarinatus* was determined to be 21.9% and 17.1% of fish meal with TM and BSFL (black soldier fly larvae) meal. Replacing fishmeal with 50% TM in the diet of *P. leptodactylus* fry improved the comparative growth performance of the control diet containing 27% fishmeal (Mazlum et al., 2021). Similarly, Choi et al. (2018), stated that replacing fishmeal with 50% TM in the diet is optimal for growth support. Cai et al. (2022), reported that replacing 60 g/kg fish meal with TM reduced growth performance and nutrient utilization of *L. vannamei*. Feng et al. (2019), noted that the best growth was detected in *L. vannamei* fed with dietary inclusion of 12% TM among all groups.

Differences in studies may be due to different protein, lipid, and chitin levels of the BSFL and TM, also use of fresh or processed products. In addition, the feeding duration in the studies and fish meal levels in the control diets may affect the results.

As a result, TM and BSF diets given fresh alone showed negative results on growth and survival rate in crayfish larvae. Therefore using just fresh insects in the crayfish juvenile diets is not recommended. By decreasing 50% commercial feeds and using as a supplementary feed of TM and BSF larvae can show similar growth as the control group. However, supplemented 50 % rate TM to commercial diets cannot be recommended due to reduced survival rates. Fresh BSFL can be given to *P. astacus* larvae by reducing commercial feed by 50% without affecting the survival rate and growth.

FUNDING

No financial support was received in the conduct of this study.

CONFLİCT OF INTEREST

The authors declare that they have no financial interests or personal relationships that could influence this work.

ETHİCAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental invertebrate animals were used in this study.

DATA AVAİLABİLİTY STATEMENT

The data used in this study are available from the corresponding author upon reasonable request.

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