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The Effects of Using Different Levels of Calcium Carbonate (CaCO3) on Growth, Survival, Molting Frequency and Body Composition of Freshwater Crayfish Juvenile, *Pontastacus leptodactylus* (Eschscholtz, 1823)

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ABSTRACT
The present study was conducted to determine different calcium carbonate ($CaCO_3$) level effects on growth, survival, molting frequency and body composition of freshwater crayfish juvenile, *Pontastacus leptodactylus* (Eschscholtz, 1823). Initial mean weight and length juvenile crayfish were measured to be 0.53±0.01 g and 22.0±0.05 mm, respectively. Crayfish were stocked randomly in twelve aquariums in area of 0.2 m^2 at the rate of 50 crayfish/m^2. The different level of $CaCO_3$ was added into a commercial trout diet (Control), 3%, 6% and 12% for feeding the juvenile crayfish. After 90-day study, supplementation of $CaCO_3$ significantly ($P < 0.05$) did affect growth rate, specific growth rate, feed intake, molting frequency, biomass, but reduced survival of crayfish at level of 6% diet as compared to the other groups. The proximate composition of tail meat of crayfish was unaffected by the diet. These results indicated that the calcium carbonate addition is required for crayfish. At the end of the experiment, 6% of calcium carbonate was suggested to be used in formulated diets for crayfish juvenile.

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Farklı Seviyelerde Kalsiyum Karbonat (CaCO3) Kullanımının Yavru Tatlısu İstakozu Pontastacus leptodactylus (Eschscholtz, 1823)'larının Büyümesi, Hayatta Kalması, Kabuk Değişimi ve Vücut Kompozisyonları Üzerine Etkileri

ÖZET
Bu çalışmada, farklı seviyelerde kalsiyum karbonat kullanımının yavru tatlısu istakozu *Pontastacus leptodactylus* (Eschscholtz, 1823)'lerinin büyümesi, hayatta kalması, kabuk değişimi ve vücut kompozisyonları üzerine etkileri incelenmiştir. *P. leptodactylus* yavrularının başlangıç ortalama ağırlıkları ve uzunlukları sırasıyla 0,53±0,01 g ve 22,0±0,05 mm olarak hesaplanmıştır. Kerevitler, rastgele metrekareye 50 adet 0,2 m^2 alana sahip on iki akvaryuma stoklanmıştır. Yavru kerevitleri beslemek için farklı seviyelerde $CaCO_3$ ticari alabalık yemine (Kontrol), % 3, % 6 ve % 12 olacak şekilde ilave edilmiştir. 90 günlük çalışmadan sonra, yemlere %6 oranında $CaCO_3$'ın ilave edilmesi diğer gruplarla kıyaslandığında, büyüme hızını, spesifik büyüme hızını, yem alımını, kabuk değişim sıklığını, biyoması önemli ölçüde ($P<0.05$) etkilemiş, ancak kerevitlerin hayatta kalma oranının azalttığını göstermiştir. Kerevitlerin kuyruk etinden alınan örnekler sonucunda, protein, yağ, nem ve kül içeriklerinin muamele grupları arasında istatistiki olarak farklı olmadığı gözlenmiştir. Bu sonuçlar, diyet kalsiyum karbonat takviyesinin kerevit için gerekli olduğunu göstermiştir. Bu nedenle, deneyin sonunda tatlı su istakoz yavruları için formüle edilmiş diyetlerde % 6 kalsiyum karbonat kullanılması önerilir.

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INTRODUCTION

Astacid crayfish have a wide distribution and production potential in Europe. *Astacus leptodactylus* is naturally found in our country and in Europe and is of commercial importance (Mazlum and Yılmaz, 2012). Recent studies have reported that this species is genetically named *Pontastacus leptodactylus* (Akhan et al., 2014; Crandall and De Grave, 2017). *P. leptodactylus* is a cold water species like other European crayfish. Crayfish has played a significant role as export products in Turkey until 1984.

After 1986, however, the production of crayfish decreased significantly from 5000 tons to 200 tons in most lakes and dams reservoirs due to crayfish plague *Aphanomyces astaci* Schikora, 1903, and through overhunting, agricultural irrigation and water pollution. In 2016, the amount of crayfish production decreased to 544 tons (TUIK, 2017). Although plague is still observed in some lakes in Turkey, additional stocks in natural lakes or dams did not an increase in the amount of *P. leptodactylus* (Diler and Bolat, 2001). The total amount of crayfish obtained by hunting was about 1030, 609.6 and 544 tons for 2010, 2011, and in 2016, respectively (TUIK, 2017). When the fluctuations in crayfish production are examined in recent years, it is obvious that the amount of crayfish production continues to decrease. Until now, there were no crayfish aquaculture production facilities in Turkey (Mazlum, 2007). However, in 2015, crayfish hatchery was built in Egirdir Institute which is the first and only closed recirculating system. In Turkey crayfish production is based on only hunting, it is not implemented any alternative methods to increase its production. However, the different strategies such as monoculture and rotational systems were used in the world to increase crayfish production (Mazlum and Yılmaz, 2012).

Crayfish are invertebrates and have polytrophic feeding habits. Crayfish are classified as herbivorous, detritivorous, omnivorous and sometimes mandatory carnivores (Momot, 1995; Correia, 2003; Nystrom, 2002; Mazlum and Yilmaz, 2012). They are capable of living in many habitats in terms of physiological, morphological and behavioral characteristics. Crayfish are abundant and predominantly found among all invertebrates. This organizm play an important role in the freshwater food chain by feeding on the residues and detritus of thousands of animals, from living and rotten plants, cereals, algae and vertebrates to smaller vertebrates such as small fish species (Matsuzaki et al., 2009; Lodge et al., 2012; Twardochleb et al., 2013). Cannibalism is one of the most important problems in its culture. As the exoskeleton in crayfish normally limits growth, crayfish must molt and throw an outer shell to grow. Crayfish are immobile and vulnerable during shell changes. While crayfish prepare for shell change, they stop feeding and decrease their activity (Romano and Zeng, 2017; Marshall et al., 2005; Reynolds, 2002).

In aquatic organisms, calcium (Ca) is considered one of the most important minerals (Liang et al., 2018) and is required for normal growth, skeletal development and various physiological processes in aquatic species (NRC, 2011; Lall, 2002). The main sources of calcium in crustaceans and crayfish are water and nutrients containing ionic calcium in water (Zanotto and Wheatly, 2002; Hessen et al., 1991). Therefore, calcium is a critical macro element for crusted ecophysiological activities (Roy et al., 2009; Tavabe et al., 2013). All aquatic organisms require inorganic elements or minerals for their normal life processes (Chanda et al., 2015). Calcium is the primary inorganic component present in the shell and exoskeleton of crayfish (Luquet et al., 2016; Greenaway, 1993). Calcium concentrations vary commonly in aquatic ecosystems (Greenaway, 1993). That is, low calcium concentrations can limit growth and production (Hessen et al., 1991). Therefore, adequate calcium is required after each shell change (Rukke, 2002). Incomplete calcification reasons elongated soft external skeletons, and then freshwater crustaceans cause carnivore organisms (Stein, 1977), cannibalism (Dick, 1995) and external injuries (France, 1987). For example, the outer skeleton of red swamp crayfish, *P. clarkii*, contains 25 to 30% calcium. They need calcium every 7 to 10 days to change the shell of the crayfish. Therefore, calcification of the exoskeleton is necessary for all crustaceans. Crayfish and other crustaceans can use ionic calcium during mineralization of the new exoskeleton by obtaining water or body reserves at the time of shell change. The storage sites of calcium that can be used in the early stages of the mineralization of the new exoskeleton include gastroliths (gastric stones) and the midgut gland (hepatopancreas). Adequate calcium in water or diet will prevent both cannibalism and injury of crayfish. The use of calcium carbonate in the diet of *P. leptodactylus* is important not only for growth but also for the molting frequency and survival.

Therefore, in this study, we determined the effects of the use of different calcium carbonate ($CaCO_3$) levels in diets on the growth, survival, molting frequency and body composition of freshwater crayfish.

MATERIAL and METHOD

Ovigerious female *P. leptodactylus* were obtained natural crayfish stocks from Seydişehir Suğla Dam and in Konya. Freshwater crayfish were placed in the box and transferred to the Aquaculture Research Facilities at Mustafa Kemal University where spawning took place. The crayfish were kept in circular fiberglass tanks provided with shelter (onion sacks and pieces of polyvinyl chloride pipe), and well-aerated water was used in the flow-through system. Crayfish were fed with commercial trout diet to develop and shed eggs.

In this experiment, 12 aquariums with dimensions of 80 x 40 x 25 cm (length x width x height) and 0.2 m^2 surface area were used. The bottom of each tank was provided plastic pipes of different diameters and mesh pieces for shelter. The temperature of the water used in the experiment was sustained 13.9-24.3^{o}C, the oxygen content was between 4.3-7.8 mg /l and salinity was determined as 1%. During the experiment, water temperature (℃) and dissolved oxygen content (mg / l) in water were measured daily (08:00) daily using YSI 55 model oxygen meter. pH (YSI 60 model pH meter) values were measured weekly. Nitrite, nitrate, and ammonia was measured monthly and samples was read on the spectrophotometer in the central laboratory. Water salinity (YSI 85 model salinometer) was measured monthly.

The mean length and weight of juvenile crayfish were measured (22.0 ± 0.05 mm length and 0.53 ± 0.01 g) and randomly stocked into twelve aquariums without statistical difference with a stock density of 50 pieces / m^2 crayfish. Diets were prepared by adding control (0%), 3%, 6% and 12% $CaCO_3$ based on a commercial trout larvae diet containing 55% protein and 12% lipid. Each diet group was planned to consist of 3 replications. Different levels of $CaCO_3$ were blended in a food processor for homogeneous mixing and then adequate water was added to the mixture to form a soft dough. The dough was passed through a mincing machine with a pellet mold of 2 mm diameter. The pellets were air-dried at 40 ° C in an oven and stored at 4 ° C during the experiment. Crayfish were fed two times a day at a rate of 5% of the bodyweight for 90 days.

The experiment was conducted for 90 days and the growth, survival, molting frequency and feed conversion ratio were evaluated for each treatment group on the 30th, 60th and 90th days of the experiment. In each measurement, the crayfish in the aquarium were counted and their length and weight were measured. The length of the crayfish were measured with a 0.1 mm from the tip of the rostrum to the tip of the telson. The weight of the crayfish was measured by means of digital scales with a sensitivity of 0.01 g.

At the end of the study, biological parameters used to estimate growth, survival, molting frequency and feed conversion rate (FCR) were calculated as follows:In the study as growth parameters;

Growth was calculated at 30-day intervals over the 90-day study period. At the end of the growth trial, crayfish were counted and weighed. Average growth per day or specific growth rate (SGR) was calculated using the formula:

$$\text{Survival rate (SR)(\%)} = \frac{\text{Total number of crayfish harvested}}{\text{Total number of fish stocked}} x100$$

$$\text{Feed convertion ratio (FCR)} = \frac{\text{Quantity of feed supplied (g)}}{\text{crayfish yield (g)}}$$

$$\text{Harvest rate (HR) (g)} = \frac{(\text{Harvest weight (g)} - \text{Stocked weight (g)})}{\text{Rearing (90 days)}}$$

$$\text{Specific growth rate (SGR)} = \frac{(\ln \text{final mass} - \ln \text{initial mass})}{\text{Rearing (90 days)}} x100$$

$$\text{Daily growth rate (DGR)(g/crayfish/day)} = \frac{(\text{average final mass(g)} - \text{average stock mass(g)})}{\text{Rearing (90 days)}}$$

$$\text{Molting frequency (MF)} = \frac{(\text{Number of molt})}{\text{total crayfish}} x100$$

At the end of the experiment, all crayfish collected and weight. Subsamples (n=12 crayfish) were taken and kept for analysis of crude protein, crude lipid, moisture, and crude ash. The final length, SGR, FCR, molting frequency, survival rate, moisture content and proximate composition of *P. leptodactylus* were statistically analyzed with one-way analysis of variance (ANOVA) to determine significant differences among the dietary treatments by using the SPSS software (Version 16.0; SPSS; Chicago, IL, USA). A post hoc Duncan's multiple range test was used to determine the differences among the treatments. Differences were considered significant at the 95% confidence level (P<0.05). All means were presented with ± standard deviation (SD).

RESULTS

After a 90-day study, the effects of calcium carbonate (CaCO3) levels on the growth, survival, molting frequency and body composition of juvenile freshwater crayfish *P. leptodactylus* (Eschscholtz, 1823) were determined. Water quality parameter values used in the experiment were found to be acceptable for crayfish culture (Köksal, 1988; Mazlum, 2007). No statistical differences were observed in the mean water temperature, dissolved oxygen, pH and NO_3-N (P>0.05). The temperature of the water used during the experiment was averaged 19.9 ℃ (19.77-20.30℃) and the dissolved oxygen level varied between 4.8-8.6 mg / l depending on the water temperature during the 90-day experimental period. As a result of the weekly measurements, it was observed that the pH value was changed between 8.61-8.68, while the (NO_3-N) averaged 0.017mg/l, and ammonia averaged 0.06 mg/l during the experiment.

Average final wet weight of the freshwater crayfish fed with calcium carbonate was between 1.20 ± 0.21–2.47 ± 0.65 g and average length measurements were 33.3 ± 0.81–44.7 ± 1.31 mm. we observed that there was significant differences between in length and weight in the experimental groups. The average final length and weight fed with 6% calcium carbonate group was found to be different than the other groups (P <0.05) (Table 1). At the end of the experiment, the best growth (weight and length) was achieved in the group fed with 6% calcium carbonate.

Table 1. Mean ± SD values of some growth parameters of *P. leptodactylus* during the experiment period of 90 days.
Çizelge 1. *90 günlük deneme süresi boyunca P. leptodactylus'un bazı büyüme parametrelerinin ortalama ± standart sapma değerleri.*

	Treatments			
Growth parameters	**Control**	**3% $CaCO_3$**	**6% $CaCO_3$**	**12% $CaCO_3$**
Initial wet weight(g)	0.53±0.01	0.53±0.01	0.53±0.01	0.53±0.01
Final wet weight (g)	1.33±0.20^a	1.20±0.21^a	2.47±0.65^b	1.22±0.21^a
Initial total length (mm)	22.0±0.05^a	22.0±0.01^a	22.0±0.05^a	22.0±0.05^a
Final total length (mm)	35.6±0.80^a	33.3±0.81^a	44.7±1.31^b	34.7±0.80^a
SGR(% per day)	0.53±0.80^a	0.45±0.81^a	0.78±1.31^b	0.50±0.80^a
Final wet mass (g)	11.64±1.49^a	9.48±1.19^a	19.47±3.51^b	10.54±1.42^a
SR (%)	92.45±5.34	87.47±7.26	85.00±8.66	91.62±5.19
MF (%)	57.6±0.11^a	61.3±0.14^a	73.6±0.13^b	58.3±0.10^a
FCR	1.17±0.33	1.33±0.31	0.89±0.40	1.50±0.51

Mean values with the same superscript letters in the same row are not significantly different (p>0.05)
Aynı satırda aynı harflere sahip olan ortalama değerler belirgin ölçüde farklı değildir (p> 0,05)
SGR:.Specific growth rate, SR: Survival rate, MF: Molting frequency, FCR: Feed conversion ratio , $CaCO_3$: Kalsiyum karbonat
SGR: Spesifik büyüme oranı, SR: Hayatta kalma oranı, MF: Kabuk değişim sıklığı, FCR: Yem değerlendirme oranı, CaCO3: Kalsiyum karbonat

Survival rate of juvenile crayfish ranged between 85-92.45%. No statistically significant difference was found in survival rates among the treatments groups (P> 0.05) (Table 1). At the end of the experiment, the highest survival rate according to the measurement periods was observed in the control group with 96.66% on the 60 th day.

The molting frequency was 73.6% in the group fed with 6% calcium carbonate supplemented feed, 61.3% in the group fed with 3% calcium carbonate supplemented feed, 58.3% in the group fed with 12% calcium carbonate supplemented feed and control 57.6% in the group (Table 1). The group fed with 6% calcium carbonate supplementation was statistically different from the others (P <0.05).

At the end of the experiment, the highest specific growth rate was calculated in the group fed with 6% calcium carbonate supplemented feed with 0.78 value and the lowest specific growth rate was calculated in the group fed with 3% calcium carbonate supplemented feed with 0.45 value. Specific growth rates, the group fed with 6% calcium carbonate supplementation was different from the other groups (P <0.05) (Table 1). According to the sampling periods, the highest specific growth rate was obtained from the feed fed group with 6% calcium carbonate added at 1.25 day 60. This was followed by control, feeding with 12% and 3% calcium carbonate supplemented feed with 0.85, 0.77 and 0.76 values in the same period, respectively.

At the end of the experiment, feed conversion ratio in the groups fed with control, 3%, 6%, and 12% calcium carbonate supplemented feed were found to be 1.17, 1.33, 0.89 and 1.50 respectively. In terms of feed conversion ratio, it was found that although the group fed with 6% calcium carbonate supplemented feed had a better feed conversion ratio, but it was not found different from other groups (P> 0.05) (Table 1).

There was a significant difference among the groups in terms of final wet mass (P <0.05). At the end of the experiment, the highest product amount was obtained as 19.47 g from the feed fed the group with 6% calcium carbonate addition, the group fed with control was 11.64 g, the group fed with 12% 10.54 g and the group 9 fed with 3% 48 g (Table 1).

Table 2. Mean ± SD of the proximate composition (%) of the crayfish (*P. leptodactylus*) at the end of the experimental period of 90 days.
Çizelge 2. *90 günlük deneme periyodu sonunda P. leptodactylus istakozunun proximate kompozisyonunun (%) ortalama ± standart sapması.*

Parameters	Treatments			
	Control	3 % $CaCO_3$	6% $CaCO_3$	12% $CaCO_3$
Moisture (%)	79.74±0.22	80.69±0.42	80.63±0.51	81.59±0.28
Protein (%)	17.94±0.27	16.64±1.01	16.21±0.07	16.48±0.42
Lipid (%)	0.99±0.42	1.52±0.27	1.89±0.51	0.89±0.45
Total ash (%)	1.33±0.03	1.15±0.11	1.27±0.04	1.04±0.08

Mean values with the same superscript letters in the same row are not significantly different (P>0.05).
Aynı satırda aynı harflere sahip olan ortalama değerler belirgin ölçüde farklı değildir (p> 0,05)
$CaCO_3$: Kalsiyum karbonat

The body composition values of the different calcium carbonate added feeds are given in Table 2. As a result of the samples taken from tail meat of crayfish, it was observed that protein, fat, moisture and ash contents were not statistically different among the treatment groups.

DISCUSSION

Environmental factors (light transmittance, photoperiod and stock density) water quality parameters (water temperature, dissolved oxygen, pH, calcium, and magnesium) play an important role in the growth and survival of crayfish. (Nyström, 1994; Jover et al., 1999; Paglianti and Gherardi, 2004; Ramalho et al., 2008). In particular, calcium contains a wide range of biological functions (growth and molting) and has an important structural role in crayfish (Muyssen et al., 2009). In other words, crayfish need calcium to be able to harden the new shell after molting and they have to supply calcium from either water or feed (Hammond et al., 2006). Hammond et al. (2006) showed that increases in calcium concentration in water reduces cannibalism in crayfish. The effects of different concentrations of calcium on the survival, growth and molt of crayfish have been shown in previous studies (Türel and Berber, 2016; Sirin and Mazlum, 2017; Holdich, 2002). However, studies with calcium have been limited so far. Therefore, calcium carbonate was added to feeds in order to minimize losses due to cannibalism in the larval stage. Sirin and Mazlum (2017) indicated that when crayfish fed with calcium carbonate supplemented feeds had more gastroliths than fed with with calcium chloride in stomachs. They also observed that new molt and soft crayfish had larger and heavier gastroliths. Because, after the molting crayfish can not use gastroliths. At that time calcium carbonate is used for the formation of mouth and shell.

In this study, we investigated the effects of calcium carbonate on juvenile freshwater crayfish growth, survival and molting frequency. Crayfish with an average length of 22 mm and weight of 0.53 g were used. At the end of the experiment, different concentrations of calcium carbonate significantly influenced the survival of crayfish. Thus, the highest survival rate was observed in 12% calcium carbonate supplemented feed groups, followed by control, 3%, and 6%, respectively. These results are in parallel with the other studies. For example, Fotedar et al. (1999) found that the survival rate was 80%. In this study, the survival rate of crayfish was found to be 93.6%. Similarly, previous studies have shown that the survival of crayfish is proportional to the increase in calcium concentration in water, but is partly reduced by death due to molting (Taugbol et al., 1996). Again, Hammond et al. (2006) in a study on *Paranephrops zealandicus* crayfish survival in the amount of calcium in the water (up to 10 mg / L) is proportional to the increase in the amount of water, but the increase in certain amounts of calcium (eg 80 mg / L) have observed that the growth rate does not change. Moreover, some studies have shown that neither high nor low calcium concentration has a positive effect on growth.

The best growth was found to be 2.47 g in crayfish fed with 6% calcium carbonate supplemented feeds. Winkler (1986) in his study indicated that the lack of calcium in water affects crayfish molt, metabolic activity, and growth, although the high concentration of calcium in the water can be make toxic effects on crayfish. In this study, the frequency of molting was found to be highest in groups containing 6% calcium carbonate (73.6%). This value was found to be different from other groups. Similar studies *on Penaeus/Litopenaeus vannamei* showed that when Ca / Mg was 1: 3, survival and growth rates increased with increasing calcium and magnesium ions, but when calcium concentration reached 30 mg / L, growth and survival rates decreased with increasing calcium and magnesium ions. (Yue et al., 2009).

The calcium level in aquatic environments has an important role in the growth of freshwater crayfish (France, 1987). Calcium accumulates in the outer crust of many crustaceans and, as in freshwater crayfish, a sufficient amount of calcium is needed at each molt (Rukke, 2002). In the decency of calcium, molting activity progresses more slowly. Therefore, the soft shell period lasts longer and during this period, crayfish are vulnerable to predator attacks because they are vulnerable (Stein, 1977). Cannibalism also increases in this period (France, 1987). Rapid growth, frequent molt increases cannibalism and predators more (Lutz, 1983). No significant difference was observed our study groups.

Zahmetkesh et al. (2007), in their experiment, they studied the growth and survival rates of crayfish fed with different amounts of calcium supplementary feed (0, 1, 2, 3, and 4). As a result of the research, the best weight and length increase was observed in the feed group with 3-4% calcium content, but no significant difference was found in our experiment groups. The highest growth (11.65 g) was observed in the group fed with 4% calcium feed. The lowest survival rate (30%) was seen in 2% calcium fed group. In this study, it was determined that there was a significant increase in growth due to different concentrations of calcium carbonate. And the best growth and molting occurred in the group fed with 6% calcium carbonate supplemented feeds. The best temperature of growth activity of this species was determined as 21.8 ℃ (Nyström, 2002). *A. leptodactylus* species to grow under culture conditions, the most suitable water temperature values between 20-25 ℃ have been determined (Köksal, 1988). It is not correct to link these results to calcium compounds only. Because the temperature of water molting, survival, feeding, and growth is the first-degree factor in crayfish (Lowery, 1988; Whitledge and Rabeni, 2003). Generally, low temperatures are recommended for the survival of juvenile crayfish and high temperatures are recommended for rapid growth. Different researchers working with astacids and other family crayfish have reported an increase in survival rates in crayfish maintained at temperatures of 15 °C or lower, although temperatures of 20 °C or higher provide the best results for growth of crayfish (Westman et al., 1993; Gydemo and Westin, 1989; Mazlum, 2007). During this study, the water temperature was 19.9 ˚C. Another factor effective in the growth of freshwater crayfish such as pH level (Bradford et al., 1998). The acidity of lakes or waters can lead to significant calcification and reduced hardness of the outer shell. In acidic waters, calcium is almost negligible (Seiler and Turner, 2004). Even in this case, the crayfish can survive and reproduce. The pH of the water also affects the oxygen demand of the water. Optimal 6.5-8 pH levels have been reported to be suitable for *A. leptodactylus* (Köksal, 1988). In this study, pH values ranged between 8-8.7. Low dissolved oxygen concentration and sudden rise and fall, freshwater crayfish is one of the main problems seen in natural and growing environments (Huner, 1988). Oxygen content is a limiting factor in the growth of freshwater crayfish. In general, crayfish exposed to constant low oxygen levels are slowing growth and feeding (Chien and Avault, 1983).

In aquaculture, freshwater crayfish are stressed at oxygen levels below 3 mg / L. It has been determined that *P. leptodactylus*, which is naturally distributed in our country, can tolerate a minimum of 3.97 mg / L, and optimally needs oxygen concentration higher than 6 mg / L (Nyström, 2002; Wingfield, 2000). Oxygen values observed during the research were at the desired level and did not constitute a problem during the trial period.

At the end of the experiment, it was observed that the protein, fat, moisture and ash contents of the crayfish fed by adding different levels of calcium carbonate to the feed were not different among the treatment groups. Previous studies indicated that the moisture content of crayfish was 81% (Yıldırım et al., 1997) and 79-79.7% (Gürel and Patır, 2001). The results obtained in this study showed similar with other studies. Protein value of crayfish in the study of Yıldırım et al., (1997) was 14.2%, Gurel and Patir (2001) was 16.4-17.1%, Huner (1995) 16.79% and Mazlum (2007) found to be 16.5%. The values obtained in this study support these studies. In the study of Yıldırım et al. (1997) fat values between 1.26-1.51%, Gurel and Patir (2001) found the oil values between 0.40-0.50 %. These values are in comparable with our study. Although the meat yield of crayfish is low, the crude protein content is high. Meat yield and protein contents of crayfish were changed seasonally. Oil ratio of crayfish varies significantly depending on the reproduction period and nutrient multiplicity (Eversole and Türker, 1999; Eversole et al.,2002).

Crayfish have gastroliths called stomach stones. They are located on both sides of the cardiac stomach and contain calcium carbonate. During the molt, some of the calcium carbonates in the old shell accumulate in gastroliths. As a result of this experiment, the highest gastrolith accumulation was observed in crayfish in groups fed with 12% feed. Thus, calcium or its compounds in water or feed have a significant effect on the hardening of the shell of crayfish. Taugbol et al. (1996) stated that 10-20% calcium is required in the hardening of new crust and storage of gastric stone. Another result obtained from this study was that gastroliths were found to be larger and heavier in freshly shelled softshell crayfish. One of the most important reasons for this is that the newly changed individuals have not been able to use gastrolith. Because during the shell change calcium carbonate in the stomach is brought back into dissolved form and used in the formation of the mouth and outer shell. 1/3 of the amount of calcium carbonate required for the development of new crust is provided from the stomach, hepatopancreas, and blood (Taugbol et al., 1996; Holdich, 2002). Cerenius and Söderhall (1984), found that *Aphanomyces astacii*, the causative agent of crayfish plague, has an antagonistic effect of calcium and magnesium content in the water on zoospore production. Therefore, the calcium content of the waters to be cultured in crayfish should be at a certain level, otherwise, it has an encouraging effect on the high production of zoospores.

As a result, it was observed that calcium carbonate supplemented feeds had significant effects on survival, growth and molting frequency of juvenile crayfish. At the end of the 90-day trial period, the best growth (weight and length) was achieved in the feed fed group with 6% calcium carbonate supplementation. At the end of the experiment, the highest survival rate was obtained from only 12% group with 93.33%, while the lowest survival rate was found in the group of 81.11% and 6%. When specific growth rates are evaluated the highest value was 1.39 g in the group fed with 6% calcium carbonate supplementation and the lowest value was in the group fed with 0.84 g with 3% calcium carbonate supplemented feed.

In the 90-day period, it was found that the group fed with 6% calcium carbonate added feed had a better feed conversion rate and did not differ from the other groups. Among the treatment groups, the frequency of crust change was 80.6% in the group fed with 6% calcium carbonate supplemented feed, 48.6% in the control group, 49.0% in the group fed with 3% calcium carbonate supplemented feed and 12% in the group fed with calcium carbonate 43.3%, respectively. The highest amount of product was obtained from the group fed with 6% calcium carbonate supplemented feed with 20.26 g, the control group 11.64 g, the group fed with 12% calcium carbonate added to feed and the group fed with 11.54 g and 3% calcium carbonate was 9.62g, respectively. As a result of the samples taken from tail meat of crayfish, it was observed that protein, fat, moisture and ash contents were not statistically different among the treatment groups.

CONCLUTION

After 90-day study, supplementation of $CaCO_3$ significantly affected growth rate, specific growth rate, feed intake, molting frequency and biomass. However, survival of crayfish at level of 6% diet group was found lower when compared to the other treatment groups.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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