Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1215263

JAES
Year: 8, No: 1, 2023 (62-68)
AÇEH
Yıl: 8, Sayı: 1, 2023 (62-68)

ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The effect of prolonged cold storage period and starvation on total lipid content and adult cannibalism of *Tenebrio molitor* (Coleoptera: Tenebrionidae)

Evrim SÖNMEZ[1]* Necati Alp ERİLLİ[2]
[1]*Sinop University, Faculty of Education, Department of Mathematics and Science Education, Sinop, Türkiye*
[2]*Cumhuriyet University, Faculty of Economics and Administrative Sciences, Sivas, Türkiye*

Received: *06.12.2022* ***Accepted:*** *08.02.2023* ***Puplished:*** *31.03.2023*

How to cite: **Sönmez, E. & Erilli, N.A. (2023).** The effect of prolonged cold storage period and starvation on total lipid content and adult cannibalism of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *J. Anatolian Env. and Anim. Sciences*, ***8***(1), 62-68. https://doi.org/10.35229/jaes.1215263
Atıf yapmak için: **Sönmez, E. & Erilli, N.A. (2023).** Soğukta bekletme süresinin uzamasının ve açlığın *Tenebrio molitor*'un toplam lipid miktarına ve ergin yamyamlığına etkisi. *Anadolu Çev. ve Hay. Dergisi*, ***8***(1), 62-68. https://doi.org/10.35229/jaes.1215263

*: https://orcid.org/0000-0002-5412-5728
: https://orcid.org/0000-0001-6948-0880

***Corresponding author's:**
Evrim Sönmez
Sinop University, Faculty of Education, Osmaniye Mahallesi, Üniversite Caddesi No: 52B, 57000, Sinop, Türkiye.
✉: esonmez@sinop.edu.tr

Abstract: With the discovery that the larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) have a high protein and lipid content in recent years, they are grown in mass as additive feed or live feed. However, one of the most common problems faced by producers in production facilities is cannibalism, which occurs as a result of population density. For this reason, especially when the population is very dense, producers separate the insects that are in the larval or pupa stage from the culture and keep them in coolers such as refrigerators.Then, when needed, they take the insects out of the refrigerator and use them. However, because insects are ectotherm organisms, their life cycles are extremely dependent on temperature. Although the cold storage method extends the shelf life of insects, exposing them to low temperatures for long periods can both damage their life cycle and significantly affect their lipid and protein content. In this study, the effects of cold storage on total lipid content, total lipid percentage and cannibalism rate of *T. molitor* larvae, pupae, and adults were evaluated. The study was carried out in two stages. In the first stage of the study, the larvae were fed until they weighed 100-190 mg (larval stages 12-17). Afterwards, they were randomly selected from the insect culture and exposed to cold for 10, 20, and 30 days. No food was given to the larvae after their exposure to cold was over. In the second stage of the study, the larvae randomly selected from the culture were exposed to cold for 10, 20, and 30 days after pupation. Then, they were allowed to complete their development under normal laboratory conditions. Eventually, their lipid analysis and cannibalism rates were studied. Some pupae were observed to be adults here. These adults were also not given food. As a result, as the duration of exposure to cold increased, the total lipid content and percentages decreased in the larvae of the unfed control group, while it increased or remained constant in the unfed and cold-exposed group. In addition, cannibalism was observed in *T. molitor* adults when they were not fed, that is, in cases of hunger and thirst.

Keywords: Developmental stages, feeding, flour worm, temperature, total lipid.

Soğukta bekletme süresinin uzamasının ve açlığın *Tenebrio molitor*'un toplam lipid miktarına ve ergin yamyamlığına etkisi

***Sorumlu yazar:**
Evrim Sönmez
Sinop Üniversitesi, Eğitim Fakültesi, Osmaniye Mahallesi, Üniversite Caddesi No: 52B, 57000, Sinop, Türkiye.
✉: esonmez@sinop.edu.tr

Öz: *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvalarının son yıllarda protein ve lipit içeriğinin yüksek olduğunun keşfedilmesi ile katkı yemi veya canlı yem olarak kitlesel olarak yetiştirilmektedir. Fakat üretim tesislerinde üreticilerin en sık karşılaştığı sorunlardan biri popülasyon yoğunluğu sonucu ortaya çıkan kanibalizmdir. Bu nedenle, özellikle popülasyon çok yoğun olduğu dönemlerde üreticiler larva ya da pupa aşamasında olan böcekleri kültürden ayırarak buzdolabı gibi soğutuculara bekletmektedirler. Daha sonra, ihtiyaç olduğunda, böcekleri buzdolabından çıkarıp kullanmaktadırlar. Fakat böcekler ektoterm organizmalar olduklarından, yaşam döngüleri aşırı derecede sıcaklığa bağımlıdır. Soğuk depolama yöntemi böceklerin raf ömrünü uzatmakla birlikte uzun süreler düşük sıcaklığa maruz bırakmak onların yaşam döngülerine hem zarar verebilir hem de içerdikleri yağ ve protein oranını önemli derecede etkileyebilir. Bu çalışmada, soğuk depolamanın *T. molitor* larva, pupa ve erginlerinin toplam yağ miktarı, toplam yağ yüzdesi ve kanibalizm oranına etkileri değerlendirildi. Çalışma iki aşamada gerçekleştirilmiştir. Çalışmanın ilk aşamasında, larvalar 100-190 mg (12-17. larval aşamadakiler) ağırlığa gelene kadar beslendi. Sonrasında, rastgele seçilerek 10, 20 ve 30 gün boyunca soğuğa maruz bırakıldı. Soğuğa maruz bırakılma süreleri bittikten sonra larvalara besin verilmedi. Çalışmanın ikinci aşamasında, böcek kültüründen rastgele seçilen larvalar pupa olduktan sonra 10, 20 ve 30 gün boyunca soğuğa maruz bırakıldı. Sonra, normal laboratuvar şartlarına alınarak gelişimlerinin tamamlanması beklendi. Yağ analizleri yapılarak, kanibalizm oranlarına bakıldı. Burada bazı pupaların ergin olduğu görüldü. Bu erginlere de besin verilmedi. Sonuç olarak, soğuğa maruz kalma süresi uzadıkça beslenmemiş kontrol grubu larvalarda toplam yağ miktarı ve yüzdeleri düşmüş, beslenmemiş ve soğuğa maruz kalmış grubun ise artmış veya sabit kalmıştır. Ayrıca, *T. molitor* erginlerinde beslenmedikleri zaman yani açlık ve susuzluk durumlarında kanibalizm görülmüştür.

Anahtar kelimeler: Gelişim aşamaları, beslenme, unkurdu, sıcaklık, toplam lipid.

INTRODUCTION

Tenebrio molitor (Coleoptera: Tenebrionidae) is a stored product pest that causes great damage to products such as flour in warehouses. The optimum temperature for their development is 27-28 ° C and 65-75% relative humidity has been stated. Developmental stages and longevity of insects are directly affected by temperature. Generally, low temperatures extend the growth times, while high temperatures shorten them (Finkel, 1948; Levie et al., 2005; Liu et al., 2020; Mirzaeva et al., 2020). After hatching, *T. molitor* has life stages as larvae, pupae and adult. The types and amounts of lipids, proteins, and carbohydrates they contain in each stage can be different and change with temperature. It has been reported that the most lipid-rich form in all developmental stages is the larvae (Adámková et al., 2017; Halloran et al., 2014; Errico et al., 2021; Ravzanaadii et al., 2012). *T. molitor* larvae are preferred as additive feed or live feed due to the discovery that the protein and lipid contents of the larvae are quite high in recent years. There are many insect farms set up for insect farming, where *T. molitor* is grown massively (Arbab, 2019; Costa et al., 2020; Nevesa et al., 2010; Sørensen et al., 2012). However, one of the most common problems faced by producers in these production facilities is the increase in the rate of cannibalism due to population density. For this reason, especially when the population is very dense, producers separate the insects that are in the pupae or larvae stage from the culture and keep them in coolers such as refrigerators. Insects are ectotherms. Their life cycle and the reserves they contain at different life stages (such as pupae, larvae or adults) are directly dependent on temperature. The cold storage method extends the shelf life of insects. However, long periods of cold storage can damage their life cycle or significantly affect the lipids and protein contents they contain (Adámková et al., 2020).

Sönmez, (2021) determined when the larvae of *T. molitor* are stored at +4 ºC, the amounts of lipid they contain tend to decrease from the 20th day, depending on cold storage time. Dreassi et al., (2017) and Oonincx et al., (2015) stated that *T. molitor* (last larval instar) fed at the same temperature and with different feeds have different nutritional content. Van Broekhoven et al., (2015) determined that *T. molitor* larvae fed with low quality food in terms of protein and starch contents also reduced the lipid reserves required for energy. Studies have found that larvae contain more lipids than pupae and adults (Morales-Ramos et al., 2015; Stanley-Samuelson et al., 1988; Van Broekhoven et al., 2015). Apart from these, conditions such as cannibalism may occur in situations such as hunger and thirst, which may be beneficial for the survival of insect populations and adaptation to changing environmental conditions. It is known that cannibalism is seen in *T. molitor*, especially in stressful situations such as thirst, hunger, or high population density (Morales-Ramos et al., 2015; Zaelor & Kitthawee, 2018). Cannibalism can alleviate the negative effects of the stressful environment and produce individuals with physiological adaptations to the new environment (Ichikawa & Kurauchi, 2009; Via, 1999). Even if the cannibal insect benefits survival, fecundity or developmental time, and this shows little selection advantage for the next generation, cannibalism can develop and spread to the entire population (Via, 1999). This allows the population to survive in times of famine or thirst.

In insects, lipids are used as the primary energy source as they give more energy. Since lipids are very important in insects for energy production, studies on the lipids contained in insects are quite a lot. Especially since insect farming is very popular in recent years, there are many studies on the lipid and protein content of *T. molitor*. However, as mentioned above, there are very few studies reporting that producers extending the shelf life of insects with different methods may have negative effects on the lipid metabolism of these insects. It is not known exactly what the lipid sources would be if *T. molitor* was kept in the refrigerator for too long during mass cultivation. In this study, answers will be sought to the questions of whether *T. molitor* will continue to protect its lipid resources as a result of prolonged cold storage, or whether its physiological adaptations will not be sufficient and will continue to use its energy resources. If they cannot protect the energy sources they store in the larval stages, will they resort to an alternative method such as cannibalism?

MATERIAL AND METHOD

Nutrient Medium: The nutrient medium was modified from Sönmez, (2021). Larvae reared in plastic containers (size 30 × 20 × 5 cm) were given a 1:1 ratio of Flour: Wheat flour (250 g: 250 g) as food. Wood shavings were added to the bottom of the food to facilitate the movement of insects. Egg boxes were added to the containers, where the adults can easily lay their eggs (2 for each container, 4 × 4 × 6 cm). Potatoes wrapped in aluminium foils (3 × 3 × 3 cm) were used for moisture and water needs. Potatoes were changed every 3 days, food was changed every 10 days in all trial groups and control groups.

In this study, larvae reaching a certain weight (Group I) were kept in the cold, while another group (Group II) was kept in the cold after pupation.

Group I: Larval Trials: In the first stage of the study, the larvae were fed as above until they weighed 100-190 mg (larval stages 12-17). The larvae were randomly selected from the cultures and weighed one by one. In order

to avoid population density, larvae were placed in petri dishes in groups of 10. Groups of 10, 20, and 30 days were formed. An unfed cold and control group (UFC: Unfed Cold, UFCont: Unfed Control) and a fed cold and control group (FC: Fed Cold, FCont: Fed Control) were formed for each group. The study was carried out with five repetitions for each group. Thus, a total of 60 groups were created and a total of 600 larvae were used in the first stage of the study. Those with cold groups were kept in the refrigerator (+ 4 ºC), for the specified periods, while the control groups were kept under laboratory conditions at 27 ± 2 ºC and 60 ± 5% relative humidity. Petri dishes, whose cold storage times were over, were taken out of the refrigerator and placed in the deep freezer (Profilo 6600) until lipid analysis. In some groups kept in the cold, it was observed that the larvae turned into pupae. Control groups were also placed in the deep freeze when their times were over. In the control groups, some larvae were observed to be pupae or adults. These adults or pupae were kept in the laboratory conditions and starved until the end of their storage period. When their times were over, they were placed in the deep freeze.

Group II: Pupal Trials: In the second stage of the experiment, the larvae were kept in the aforementioned nutrient medium and laboratory conditions (27 ± 2 and 60 ± 5%) until pupae. Groups of 10, 20, and 30 days were formed. They were placed in petri dishes in groups of 10. Pupae cold storage (FC: Fed Cold) and pupae control (FCont: Fed Control) groups were established. The study was carried out with five repetitions for each group. Thus, a total of 30 groups were created and a total of 300 pupae were used in the second stage of the study. After applying the specified laboratory conditions and cold storage times, it was placed in the deep freezer. It was observed that all pupae matured in pupal control groups. Petri dishes continued to be kept under laboratory conditions until their times were over. Cannibalism was determined in almost every group. These adults were also starved until their periods were over. When their storage period, they were placed in the freezer.

Total Lipid Analyses: The method of Folch et al., (1957) was used to determine the total amount of lipids contained in the larvae and pupae. Each larvae and pupae group were homogenized (Pro 2000) in a 1:2 chloroform-methanol solution. The solution obtained with Whatman 41 paper was filtered. The volatile solution in the solution was evaporated under nitrogen gas. The obtained sample was weighed (initial value) and placed in a desiccator containing silica gel. It was weighed every day until constant weight (final value). The total lipid amount was calculated as "mg" by making the difference between the initial value and the final value when weighing was constant. The total lipid percentages were found by dividing the calculated total lipid mg value by the weight of the larvae and pupae.

***The Cannibalism Rates*:** The cannibalism rates of the adults were obtained by dividing the number of surviving adults in the petri dish by the number of larvae that were first placed in the petri dish.

Data Analyses: SPSS 22.0 package program was used in the analysis of the data. Whether the groups were normally distributed or not was evaluated according to Shapiro Wilk. It was observed that the larval weights and larval total lipid amounts in the groups were normally distributed. One-way Anova was performed on these data and whether there was a difference between which groups was determined by Tukey HSD. It was determined that pupae and adult groups were not normally distributed and the Kruskal-Wallis test was applied to these groups. The difference between which groups was determined by Man-Whitney U. A significance level of 0.05 was taken as the basis.

RESULTS

When the total lipid amounts and percentages of the larvae are examined, the total lipid amount**s** of naturally fed and cold storage larvae (FC) were higher than the unfed larvae for all days. The group with the lowest total lipid content was the 10 days and 30-days unfed control group (UFCont) (respectively 4.8%, 7.7 mg and 5.0%, 6.6 mg). As the exposure to cold increased, the total lipid amount first increased and then decreased in the unfed control group larvae (UFCont). Similarily, the total lipid amounts of the fed group, whose cold storage times are extended, decrease depending on the storage time (FC). As the cold storage time extended (10, 20**,** and 30 days), it was observed that the unfed larval groups (UFC) retained the total amount**s** of lipid (8.95, 10.55, 9.24 mg, Figure 1). What is interesting here is that no statistical difference was observed between the total lipids amounts of the groups kept in the cold from the pupal stage (Figure 3). The reason for this may be that pupae exposed to cold after entering the pupal stage slow down their metabolism and make the necessary preparations to become adults. There was no difference between the total amounts of lipid in adult individuals. According to our findings, it can be thought that especially the lipid metabolism was more active in the larval stage, and that they adjusted the lipid metabolism according to the physiological state and needs of the insect.

When evaluated in terms of cannibalism, cannibalism was not observed in both larvae and pupae groups that were fed and exposed to cold for 10 days (Table 3). The rate of cannibalism increased in direct proportion when the longer the exposure to cold and was higher in the unfed groups. The reason for this may be that the reserves stored by the larvae in the larval stage have been depleted and as a result cannibalism may have been observed to meet

their metabolic demands after they become adults. This may support Sönmez, (2021)'s theory that lipid sources begin to decrease after the 20th day (in larval stage of 13-15th). When we look at the unfed groups, the rates of cannibalism were 44% and 40% in both the Group I and the Group II, especially for 30 days. No cannibalism was observed especially in the groups fed until the pupal stage and exposed to cold at the pupal stage (Group II). This may be because feeding up to the pupal stage may have enabled them to maintain sufficient lipid reserves. In addition, it can be said that the group exposed to cold at the pupal stage was more successful in preserving lipid reserves.

Table 1. Total lipid percentage (%) of *Tenebrio molitor* larvae (Group I) per individual.

	Cold Storage Conditions (Days)											
	10				20				30			
	UFC	UFCont	FC	FCont	UFC	UFCont	FC	FCont	UFC	UFCont	FC	FCont
Total lipid percentage of Larvae (%)	5.8	4.8	9.4	12.2	7.6	8.3	9.9	7.4	6.8	5.0	10.0	8.8
Total lipid percentage of Pupae (%)	3.7	7.2	6.1	6.2	3.2	2.7	---	8.0	---	7.6	---	2.0
Total lipid percentage of Adult (%)	---	5.6	---	---	---	2.1	---	3.1	---	4.0	---	5.2

UFC: Unfed Cold Storage Group, UFCont: Unfed Control Group, FC: Fed Cold Storage Group, FCont: Fed Control Group.

Table 2. Total lipid percentage of *Tenebrio molitor* pupae (Group II) and adults per individual (%).

	Cold Storage Conditions (Days)					
	10		20		30	
	FC	FCont*	FC	FCont*	FC	FCont*
Total Lipid Percentage of Pupae (%)	8.0	---	7.1	---	8.1	---
Total Lipid Percentage of Adult (%)	---	**5.2**	---	**4.8**	---	**3.3**

* Pupae in all control groups became adults. FC: Fed Cold Storage Group, FCont: Fed Control Group.

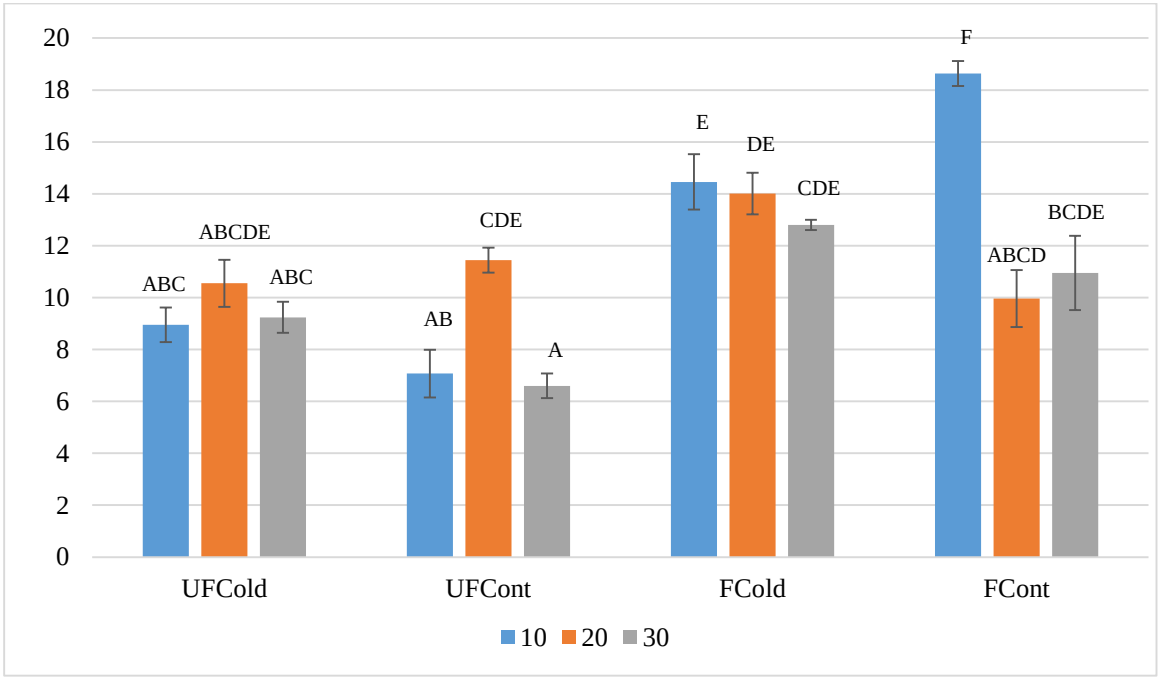

Figure 1. Total lipid amount (mg) of *Tenebrio molitor* larvae per individual (Group I) (There is no statistical difference between the values between the same capital letters. According to Shapiro Wilk, they are normally distributed, OneWay-Anova, Tukey HSD, $F_{11,\ 599}$= 16.45, p<0.001). UFCold: Unfed Cold Storage Group, UFCont: Unfed Control Group, FCold: Fed Cold Storage Group, FCont: Fed Control Group.

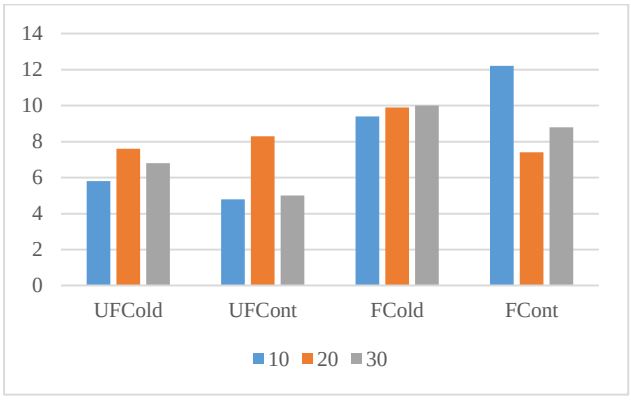

Figure 2. Percentage of total lipids (%) of *Tenebrio molitor* larvae (Group I).

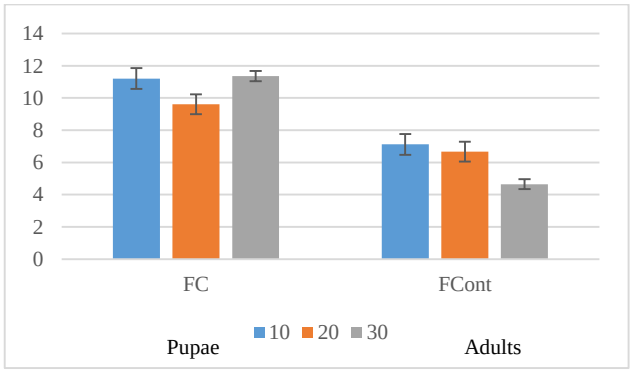

Figure 3. Total lipid amount (mg) of *Tenebrio molitor* pupae (Group II) and adults per individual. All of the FCont Group pupae became adults at the end of their storage times. FC: Fed Cold Storage Group, FCont: Fed

Control Group (It was observed that pupae and adults were not normally distributed, Kruskal Wallis test was performed. No other test was performed because there was no statistical difference between them. For pupae: df=2;15, p= 0.200, Chi-Square: 4.603; For adults: df= 2;15, p= 0.066, Chi-Square: 5.425).

Table 3. Cannibalism rate (%) of Fed and Unfed Control adults stored in the cold storage for 10, 20 and 30 days.

	10 days		20 days		30 days	
	Unfed	Fed	Unfed	Fed	Unfed	Fed
Group I	4	---	24	16	44	20
Group II	16	---	36	---	40	---

Group I: The group exposed to cold from the larval stage.
Group II: The group exposed to cold from the pupal stage.

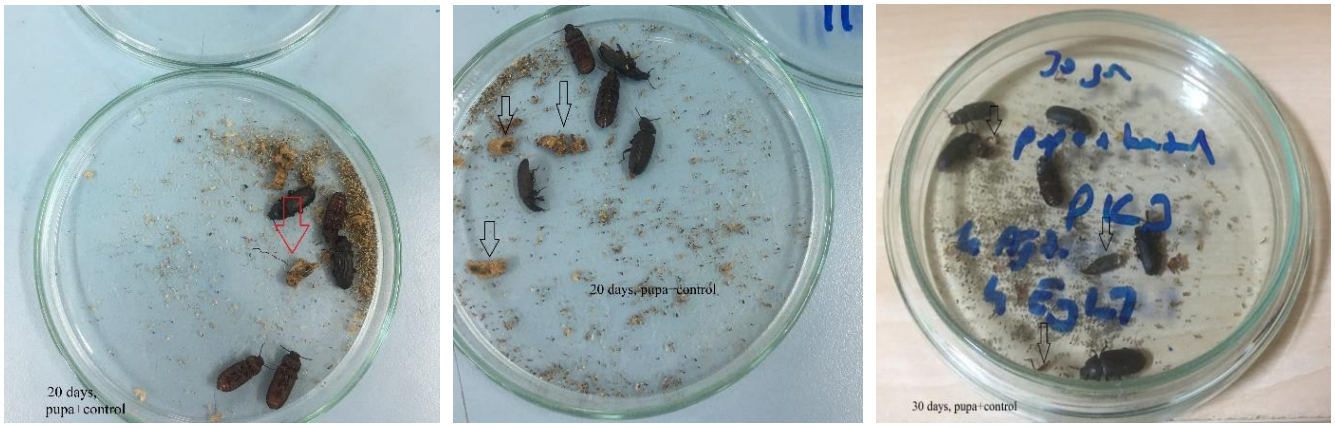

Figure 4. Cannibalistic behavior of unfed *Tenebrio molitor* pupae kept in the cold for 20 and 30 days after they became adults.

DISCUSSION

In this study, the effects of cold storage for different periods on the total lipid amounts and percentages of fed and unfed *T. molitor* larvae and pupae were evaluated. In addition, the effects of starvation on cannibalism rates in these groups were evaluated. Looking at Table 1 and Figure 1, the total lipid amounts and percentages of the larvae that were fed and cold storage for 10, 20, and 30 days were higher than those of the unfed and cold storage groups. This may be because it slowed down the insect metabolism and protected its energy sources. The highest total lipid content and percentage were found in the 10 days fed control group (Table 1 and Figure 1). The reason for this may be due to both the short cold storage period and the maximum use of available resources. It is known that insects slow down their metabolisms in the cold (Arbab, 2019; Morales-Ramos et al., 2015). The slowed metabolism is aimed at protecting energy resources. This shows that the fed control group uses lipids (12.2%, 7.4%, 8.8%, Table 1) for their metabolic needs and prepares for pupation, while the fed-cold group (9.4%, 9.9%, 10.0%, Table 1) tries to protect their existing resources by slowing down their metabolism. Sönmez, (2021), in a study conducted with *T. molitor*, found the total amount of lipid to be 15.8 mg in larvae exposed to cold for 10 days and 15.0 mg in larvae exposed to cold for 20 days. The results of current study (14.46 ± 1.07; 14.01 ± 0.8 for 10 and 20 days, respectively, Figure 1) coincide with the results of the study mentioned above. In the current study, the exposure time to cold was slightly extended and it was found 12.8 ± 0.2 in larvae exposed to cold for 30 days (Figure 1). Based on these results, it can be said that the larvae exposed to cold slow down their metabolisms and protect the existing lipid sources. Ochieng-Odero, (1992) determined that *Cnephasia jactatana* (Lepidoptera: Tortricidae) grown at low temperatures had a slower growth rate.

Lipid contents in living organisms vary according to species (Costa et al., 2020). In insects, the total lipid content is about 30% in Lepidoptera and Coleoptera, and around 20% in Orthoptera and Odonata (Dreassi et al., 2017). *T. molitor* larvae are usually rich in oils. Among the studies carried out, Jones et al., (1972) determined a total lipid percentage of 14.96% in *T. molitor* larvae weighing 98.66 mg. Mlček et al., (2019) found the lipid ratio to be 16.7% g dry weight.

Some researchers suggested that the lipid content increased in *T. molitor* larvae reared at low temperature (Adamkova et al., 2020; Sasmita et al., 2019). The data of the current study also support the theory that the larvae increase their lipid reserves at low temperature and reduce their energy consumption (Adamkova et al., 2020). However, with the prolongation of the storage period, it was determined that the larvae could not maintain the resistance they developed. It is estimated that groups exposed to cold stress try to protect their existing resources, while other groups spend them. This shows that lipids that will facilitate survival in the cold and serve as an energy source are preserved. Therefore, temperature and cold exposure time is a critical parameter to be controlled in commercial insect farming, regardless of whether the producer wants to optimise product quality or not.

The developmental stages such as larvae, pupae, or adult of the insect are also effective in this resistance (Irwin & Lee, 2003). They can store different amounts of lipids, proteins, or carbohydrates according to different developmental stages. Or, larger pupae, larvae, or adult insects may store more lipids, even at the same

developmental stages. Therefore, the insect has more energy reserves and can easily overcome extreme conditions (Irwin & Lee, 2003; Sinclair & Marshall, 2018). The woolly bear caterpillar (Lepidoptera: Noctuoidae), a frost-resistant species, has been found to suppress lipid consumption and metabolic rate at extremely low temperatures (Marshall & Sinclair, 2012). In *Drosophila melanogaster* (Diptera: Drosophilidae), lipids stored in the larval stage have been observed to persist throughout the adult stage (Aguila et al., 2007). These findings suggest that the conservation of energy and lipids is an important component of low-temperature survival success in these insects.

Via, (1999) determined egg cannibalism in a study with *Tribolium castaneum* (Coleoptera: Tenebrionidae). It was found to be quite high in the egg-eating cannibal group, with increased survival rates and fecundity, and saved the surviving *T. castaneum* larvae from adverse demographic effects. These results show that the problems brought by colonization in different environments can be solved by increasing cannibalism. Zaelor & Kitthawee, (2018) found a lower survival rate in *Zophobas atratus* (Coleoptera: Tenebrionidae) with cannibalism in a study conducted with *T. molitor* and *Z. atratus*. They found that cannibalism was observed in *Z. atratus* at the larval stage. They attributed this to the fact that they remained less active for a longer period of time during each moulting.

Egg cannibalism and cannibalism of pupae by larvae have been documented in many insect species (Deruytter et al., 2019; Morales-Ramos et al., 2012). Pupae of tenebrionid insects rotate their abdomens in a circular motion by means of antennae, including legs, maxillary pulp, and wing feeler (Ichikawa & Kurauchi, 2009). Pupae display an effective defense against larval cannibalism with this abdominal rotation movement. Larval-pupal cannibalism was not observed in the present study, since larvae, pupae, and adults were kept in separate containers. However, it was observed that the adults after the pupal stage showed cannibalistic behaviors between adult-pupae and adult-adult due to not feeding and starvation (Figures 1 and 4). Ichikawa & Kurauchi (2009) completely blocked the pupal abdominal rotation responses of *T. molitor* and *Z. atratus* by cutting the ventral nerve cord of the pupae. 20 paralyzed pupae were eaten by 100 larvae within 6 hours. On the other hand, only a few of those whose ventral nerve cords were not blocked were defeated by cannibalism. Weaver & McFarlane (1990) observed cannibalism and incomplete larval-pupae and pupae-adult transformations in *T. molitor* during peak populations. In the current study, it is shown that *T. molitor* adults resort to cannibalism when they are not fed, that is, in cases of hunger and thirst (Figure 4).

ACKNOWLEDGEMENTS

We would like to thank three anonymous reviewers for their contribution to the article.

REFERENCES

Adámková, A., Adámek, M., Mlček, J., Borkovcová, M., Bednářová, M., Kouřimská, L., Skácel, J. & Vítová, E. (2017). Welfare of the mealworm (*Tenebrio molitor*) breeding with regard to nutrition value and food safety. *Potravinarstvo Slovak Journal of Food Sciences*, ***11***(1), 460-465. DOI: 10.5219/779

Adámková, A., Mlček, J., Adámek, M., Borkovcová, M., Bednářová, M., Hlobilová, V., Knížková, I. & Juríková, T. (2020). *Tenebrio molitor* (Coleoptera: Tenebrionidae) optimization of rearing conditions to obtain desired nutritional values. *Journal of Insect Science*, ***20***, 1-10. DOI: 10.1093/jisesa/ieaa100

Arbab, A. (2019). Effect of temperature on pupal development in meal worm *Tenebrio molitor* L. *Indian Journal of Entomology*, ***81***(4), 640-646. DOI: 10.5958/0974-8172.2019.00138.X

Aguila, J.R., Suszko, J., Gibbs, A.G. & Hoshizaki, D.K. (2007). The role of larval fat cells in adult *Drosophila melanogaster*. *Journal of Experimantal Biology*, ***210***(6), 956-963. DOI: 10.1242/jeb.001586

Costa, S., Pedro, S., Lourenço, H., Batista, I., Teixeira, B., Bandarra, N.M., Murta, D., Nunes, R. & Pires, C. (2020). Evaluation of *Tenebrio molitor* larvae as an alternative food source. *NFS Journal*, ***21***, 57-64. DOI: 10.1016/j.nfs.2020.10.001

Deruytter, D., Coudron, C.L. & Teerlinck, S. (2019). Influence of crate size, oviposition time, number of adults and cannibalism on the reproduction of *Tenebrio molitor*. *Journal of Insects as Food and Feed*, ***5***(4), 1-10. DOI: 10.3920/JIFF2019.0018

Dreassi, E., Cito, A., Zanfini, A., Materozzi, L., Botta, M. & Francardi, V. (2017). Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Lipids*, ***52***(3), 285-294. DOI: 10.1007/s11745-016-4220-3

Errico, S., Spagnoletta, A., Verardi, A., Moliterni, S., Dimatteo, S. & Sangiorgio, P. (2021). *Tenebrio molitor as* a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects. *Comprehensive Reviwes in Food Science and Food Safety*, ***21***,148-197. DOI: 10.1111/1541-4337.12863

Finkel, A.J. (1948). The lipid composition of *Tenebrio molitor* larvae. *Physiological Zoology*, ***21***(2), 111-133.

Folch, J., Lees, M. & Stanley, S.G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, ***226***(1), 497-509. DOI: 10.1016/s0021-9258(18)64849-5

Halloran, A., Muenke, C., Vantomme, P. & Van Huis, A. (2014). Insects in the human food chain: global status and opportunities. *Food Chain*, ***4***(2), 103-118. DOI: 10.3362/2046-1887.2014.011

Ichikawa, T. & Kurauchi, T. (2009). Larval cannibalism and pupal defense against cannibalism in two species of Tenebrionid beetles. *Zoological Science*, ***26***(8), 525-529. DOI: 10.2108/zsj.26.525

Irwin, J.T. & Lee, R.E.Jr. (2003). Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis*. *Oikos*, ***100***(1), 71-78. DOI: 10.1034/j.1600-0706.2003.11738.x

Jones, L.D., Cooper, R.W. & Harding, R.S. (1972). Composition of mealworm *Tenebrio molitor* larvae. *The Journal of Zoo Animal Medicine*, ***3***(4), 34-41.

Liu, C., Masri, J., Perez, V., Maya, C. & Zhao, J. (2020). Growth performance and nutrient composition of mealworms (*Tenebrio molitor*) fed on fresh plant materials-supplemented diets. *Foods*, ***9***(2), 151. DOI: 10.3390/foods9020151

Levie, A., Vernon, P. & Hance, T. (2005). Consequences of acclimation on survival and reproductive capacities of coldstored mummies of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiinae*). Journal of Economical Entomology*, ***98***(3), 704-708.

Marshall, K.E. & Sinclair, B.J. (2012). Threshold temperatures mediate the impact of reduced snow cover on overwintering freeze-tolerant caterpillars. *Naturwissenschaften*, ***99***, 33-41.

Mirzaeva, D.A., Khujamshukurov, N.A., Zokirov, B., Soxibov, B.O. & Kuchkarova, DKh. (2020). Influence of temperature and humidity on the development of *Tenebrio molitor* L. *International Journal of Current Microbiology and Applied Sciences*, ***9***(4), 3544-3559. DOI: 10.20546/ijcmas.2020.905.422

Mlček, J., Adámková, A., Adámek, M., Borkovcová, M., Bednářová, M. & Knížková, I. (2019). Fat from Tenebrionidae bugs – sterols content, fatty acid profiles, and cardiovascular risk indexes. *Polish Journan of Food and Nutrition Sciences*, ***69***(3), 247-254. DOI: 10.31883/pjfns/109666

Morales-Ramos, J.A., Rojas, M.G., Kay, S., Shapiro-Ilan, D.I. & Tedders, W.L. (2012). Impact of adult weight, density, and age on reproduction of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Journal of Entomological Science*, ***47***(3), 208-220. DOI: 10.18474/0749-8004-47.3.208

Morales-Ramos, J.A., Rojas, M.G, Shelby, K.S. & Coudron, T.A. (2015). Nutritional value of pupae versus larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) as food for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). *Journal of Economical Entomology*, ***109***(2), 564-571. DOI: 10.1093/jee/tov338

Nevesa, R.C.S., Torresa, J.B. & Zanunciob, J.C. (2010). Production and storage of mealworm beetle as prey for predatory stinkbug. *Biocontrol Science and Technology*, ***20***(10), 1013-1025. DOI: 10.1080/09583157.2010.500718

Ochieng-Odero, J.P.R. (1992). The effect of three constant temperatures on larval critical weight, latent feding period, larval maximal weight and fecundity of *Cnephasia jactatana* (Walker) (Lepidoptera: Tortricidae). *Journal of Insect Physiology*, ***38***(2), 127-130. DOI: 10.1016/0022-1910(92)90041-B

Oonincx, D.G.A.B., Van Broekhoven, S., Van Huis, A. & Van Loon J.J.A. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *Plos One*, ***10***(12), 1-20. DOI: 10.1371/journal.pone.0144601

Ravzanaadii, N., Kim, S., Choi, W.H., Hong, S. & Kim, N.J. (2012). Nutritional value of mealworm, *Tenebrio molitor* as food source. *International Journal of Industrial Entomology*, ***25***(1), 93-98. DOI: 10.7852/ijie.2012.25.1.093

Sasmita, H.I., Tu, W., Bong, L. & Neoh, K. (2019). Effects of larval diets and temperature regimes on life history traits, energy reserves and temperature tolerance of male *Aedes aegypti* (Diptera: Culicidae): optimizing rearing techniques for the sterile insect programmes. *Vectors*, ***12***, 2-16. DOI: 10.1186/s13071-019-3830-z

Sinclair, B.J. & Marshall, K.E. (2018). The many roles of fats in overwintering insects. *Journal of Experimental Biology*, ***7***(221), 1-9. DOI: 10.1242/jeb.161836.

Sørensen, J.G., Addison M.F. & Terblanche J.S. (2012). Mass-rearing of insects for pest management: Challenges, synergies and advancesfrom evolutionary physiology. *Crop Protection*, ***38***, 87-94. DOI: 10.1016/j.cropro.2012.03.023

Sönmez, E. (2021). The effect of different cold storage period on total lipid amount of *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae. *Journal of Anatolian Environmental and Animal Sciences*, ***6***(3), 449-455. DOI: 10.35229/jaes.970307

Stanley-Samuelson, D.W., Jurenka, R.A., Cripps, C., Blomquist, G.J. & Renobales, M. (1988). Fatty acids in insects: composition, metabolism, and biological significance. *Archive of Insect Biochemistry and Physiology*, ***9***(1), 1-33. DOI: 10.1002/arch.940090102

Van Broekhoven, S., Oonincx, D.G., Van Huis, A. & Van Loon, J.J. (2015). Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. *Journal of Insect Physiology*, ***73***, 1-10. DOI: 10.1016/j.jinsphys.2014.12.005

Via, S. (1999). Cannibalism facilitates the use of a novel environment in the flour beetle, *Tribolium castaneum*. *Heredity*, ***82***(3), 267-275. DOI: 10.1038/sj.hdy.6884820

Weaver, D.K. & McFarlane, J.E. (1990). The effect of larval density on growth and development of *Tenebrio molitor*. *Journal of Insect Physiology*, ***36***(7), 531-536. DOI: 10.1016/0022-1910(90)90105-O

Zaelor, J. & Kitthawee, S. (2018). Growth response to population density in larval stage of darkling beetles (Coleoptera; Tenebrionidae) *Tenebrio molitor* and *Zophobas atratus*. *Agriculture and Natural Resources*, ***52***(6), 603-606. DOI: 10.1016/j.anres.2018.11.004