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Dairy Products Added to Rearing Media Negatively Effect *Drosophila melanogaster* (Diptera: Drosophilidae) Egg Production and Larval Development

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Abstract

This study examined the effect of kefir, yogurt, and milk on egg production and development in *Drosophila melanogaster* Meigen. Kefir, yogurt, and milk were added to the *Drosophila* culture medium. First they were fed to mature individuals and then these females laid eggs on medium containing kefir, yogurt, and milk. Later the development of eggs and larvae was examined. The experiments were conducted on two generations, the F_1 generation reared with additives in the media and F_2 without the additives. The effects of these substances on the basic stages of development were also examined. In the experimental groups, the numbers of eggs and larvae decreased considerably in both the F_1 and F_2 generations. The comparison between the experimental groups themselves also showed a difference. In both generations, development of eggs into third instar larvae was reduced and metamorphosis was delayed. In addition, morphological abnormalities were observed in the larvae. Overall the results showed that kefir, yogurt, and milk affected egg and larva development negatively and this negative effect continued in the F_2 generation. The continuation of this negative effect in the F_2 generation, which was not exposed to various milk additives, is an interesting finding. These results indicate that the nutrients from the milk and the milk products used were neither utilized by nor beneficial for this insect.

Key words: kefir, yogurt, milk, development, *Drosophila*

Kefir is a product of symbiotic association of lactic acid and acetic acid bacteria and lactose fermenting yeasts (Dias et al. 2017). Interest in kefir has grown due to a number of studies reporting its health benefits. Consumption of kefir has risen as it is a safe and cheap food that can be produced at home easily by the fermentation of milk (Rosa et al. 2017). An antitumor effect and major delay in tumor development have been observed in mice fed with kefir (Murofushi et al. 1986; de Moreno de LeBlanc et al. 2006, 2007). The application of kefir and β-glucan individually or in combination to the diet of broiler chickens has been reported to improve growth performance and meat quality (Cho 2013). Despite the recent emergence of kefir among probiotic foods, there is little information in the literature about the safe level of consumption of kefir, the amount that should be consumed, the time it takes to show beneficial effects, etc. Based on increasing kefir consumption worldwide, research on these dietary ingredients is urgently required (Rosa et al. 2014). While the majority of the research was conducted in humans and on mice, no studies were found on insects.

Yogurt is a milk product like kefir that is produced by fermentation of milk and is widely consumed around the world. It is easily produced at home, and, due to intensive consumption, industrial production is widespread. Yogurt is produced using *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacterial cultures. It is rich in protein, calcium, vitamin D, riboflavin, and vitamins B6 and B12 (Holden et al. 2008). Probiotics are microorganisms that contribute to host health via their beneficial effects on the intestinal flora. The number of studies showing the contribution of the intestinal flora to health continues to increase (Kabak and Dobson 2011). Experimental studies in animals have shown that probiotic yogurt and fermented milk inhibit tumor formation and proliferation. Mice fed yogurt were reported to have a 28–35% reduction in tumor cells compared to control groups fed milk (Reddy et al. 1983). Probiotic bacteria have been shown to protect the gastrointestinal system and to have a protective effect against cancer (Goldin and Gorbach 1984, Lidbeck et al. 1991). Most of the research on probiotics has been done in humans and on some animals, with almost no research on insects. The effect of lactic acid bacteria on the immune system was investigated in silkworms and it was found that yogurt increased immunity to *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus mundtii* and reduced mortality after infection (Nishida et al. 2016).

Kefir and yogurt are produced by the fermentation of milk. Yogurt was included for comparison with kefir and to have a second fermented product in the study. Moreover, milk, which these products are made from, was also included in the set of experiments to allow a comparison of fermentation. Milk is a quite rich source of nutrition and is known to promote development, improve immunity, and have positive effects against infectious and inflammatory diseases (Heaney 2009, Hill and Newburg 2015). It has been reported that regular consumption of lutein-rich fermented milk enhances the DNA repair capacity of lymphocytes (Herrero-Barbudo et al. 2013). As with fermented milk products, there have been virtually no studies conducted on the effect of milk on insects. Nutritional conditions affecting egg production in *Musca domestica* L. (Diptera: Muscidae) were investigated, and it was found that the number of eggs per day and egg weight decreased when milk was added to the adult diet as a protein source (Pastor et al. 2011). In the present study, the effects of kefir, milk, and yogurt on egg and larval development in *Drosophila melanogaster* Meigen were investigated due to the increased interest in fermented product in recent years and, in spite of this interest, the lack of studies on their in vivo effects on insects. It is known that the early developmental stages of organisms are more sensitive to environmental conditions and nutritional regimes than are the adult stages. Any effect during the early stage of life affects development and growth, and as a result threatens the health of the population (Gonzalez-Doncel et al. 2005). Understanding the impact of nutrition on life history characteristics continues to be a fundamental but difficult problem in biology (May et al. 2015). In the third century BC, Hippocrates stated that 'All disease begins in the gut' and this continues to be upheld by modern science (Barko et al. 2018). Research is needed to optimize both the dose and duration of probiotic, prebiotic, and fermented products that are beneficial to intestinal microbiology and to determine when to use them in the life cycle (Goulet 2015). Furthermore, model systems are needed to understand the relationship between gastrointestinal flora and diseases. In holometabolous insects, alteration in diet quality during development has wide-ranging effects upon many life history characteristics. *Drosophila melanogaster*, due to its short breeding period and cultivable microbiota, provides an ideal model for examining the dynamics of the microbiota throughout its host's lifespan (Blum 2013). In addition, there is 60% homology between *D. melanogaster* and human disease genes (Schneider 2000). *Drosophila* has nearly all of the basic metabolic activation enzymes that mammals have and thus it is a good model for use in genetic and toxicology studies that examine organismal biology (Pandey and Nichols 2011, Ormerod et al. 2017). For all these reasons, *D. melanogaster* was selected to show the effect of food-borne microorganisms in both in vivo conditions and different experimental animals. The present research was planned to reveal the beneficial or deleterious effects of some bacterial strains found in kefir and yogurt on *Drosophila* physiology or early developmental stages. For this purpose, the effect of kefir, yogurt, and milk on egg and larval development in *Drosophila* was examined.

Materials and Methods

The Oregon-R strain of *D. melanogaster* was used. Cultures used in the experiment were kept in 40–60% relative humidity in a refrigerated incubator set to 25 ± 1°C in the dark. Standard medium that does not require cooking or sterilization and is prepared just by adding water was used (Formula 4–24 Instant Drosophila Medium, Carolina Biological Supply Company 2700 York Road, Burlington, NC 27215-3398). This is an easily prepared medium that does not require sterilization or cooking and waiting for a few days after cooking for hardening and solidification. All ingredients are ready to use and prepared in 2–3 min by adding 15 ml of water and a few grains of brewer's yeast to 5 g of instant dry medium containing corn flour, agar, and brewer's yeast. It is used worldwide to save time and ensure standard conditions.

Preliminary studies were conducted to determine the amounts of milk products to be used as dietary additives in the diet media. Accordingly, 15 ml of the nutrients were added to the medium and 100 adults (50 females + 50 males) were placed in each bottle. Glass bottles with volume of 200 ml, 3.5 cm in diameter, and 15 cm in height were used in the experiments. In the experiment groups, 15 ml of kefir, yogurt, or milk was added when preparing the instant medium; in the control group 15 ml of water was added. Since yogurt is not in liquid form, 7.5 ml of yogurt was diluted with 7.5 ml of water and used as a liquid. Natural cow's milk produced in Anatolia was used in the experiments and the kefir and yogurt were made with the same milk by traditional fermentation. The kefir was filtered as it contained kefir grains. The reason for using natural cow's milk and yogurt and kefir fermented naturally in the research was to eliminate the effects of shelf-life-enhancing and preserving additives used during the commercial production of yogurt, kefir, and milk. Thus the aim was to determine the effect of microorganisms found in natural products only.

Milk and fermented milk products were offered as dietary supplements to the F_1 generation by adding them to the medium. In the F_2 generation, however, only water was added. To determine egg production, virgin females and normal males were used. One hundred virgin females were transferred to medium containing milk, yogurt, and kefir and left for 5 d. The same procedures were followed for males regardless of their mating status. However, to prevent mating it was performed in separate bottles. In this medium, female and male individuals that were kept separately for 5 d were transferred to a set of experimental and control groups prepared in Petri dishes. Ten females and 10 males were transferred to each Petri dish. In the experimental group, kefir, yogurt, and milk were added instead of water to the instant medium. Glass Petri dishes with lids 60 mm in diameter were used. After 24 h, the females and males were removed from the Petri dishes and the eggs laid by the females were counted under a dissecting microscope. In order to make the process easier and to prevent incorrect counts, the medium was divided into eight equal parts with the help of a pin. The eggs were counted by examining the Petri dishes under an Olympus SZ61 dissecting microscope. Crossbreeding was done 20 times in both the F_1 and F_2 generations.

For egg development, the Petri dishes with counted eggs were put back in the incubator and left there for 5–6 d for hatching of eggs and larval development. Following emergence of the third instar larvae, the larva to develop. After the third instar larvae emerged, the media for the experimental and control groups were removed with a spoon, diluted with water in a separate glass container, and the larvae counted. The third instar larvae were quite large and mobile and so were easily visible when the medium was diluted. However, in order not to miss any larvae, the medium was removed from each Petri dish with a spoon, diluted with water in a large glass container. The container was then placed over a black cardboard piece for easy detection of larvae, which were counted and collected. In addition, the healthy third instar larvae that had climbed up were also counted in the Petri dish. The number of eggs in the medium at the beginning was compared with the number of larvae that developed at the end of the experiment (Karataş and Bahçeci 2009).

For the F_2 generation data, mature individuals from third instar larvae developed in the F_1 generation were obtained and crossbred separately, according to the dietary supplement used. Ten females

(ensuring they were virgins) and 10 males were transferred to the medium, but fermented milk products and milk were not applied in the media for the F_2 generation. The aim here was to determine whether the effect observed in the F_1 generation continued in the F_2 generation. At this stage, individuals that developed from eggs to which milk, yogurt, and kefir had been applied initially were transferred to the normal medium with relevant labels and laying occurred. That is, the milk label was attached to the medium to which the adults were transferred that had developed from eggs in the medium containing milk, but normal medium to which milk had not been added was used. As in the F_1 generation, eggs were also counted in the F_2 generation and after development was observed and third instar larvae were formed, they were collected from the medium and the numbers of eggs and larvae were compared. Regarding the development of larvae in the F_2 generation, they were counted after the eggs had transformed into larvae (Karataş et al. 2011, Keser and Karataş 2012, McLay et al. 2017, Raj et al. 2017).

Another phase of the study involved investigating the effect of foodstuffs on the duration of metamorphosis. For this purpose, the medium was checked every day, and eggs, larval stages, pupae, and adults were recorded on the first day they were observed. The Petri dishes of both the experimental and control groups were examined every day under the dissecting microscope and the day when the basic stages of metamorphosis were first observed was noted. The eggs were even clearly visible to the naked eye on the medium during a careful examination after an average of 24 h. The first instar larvae were quite small and only their tiny moving heads were seen. Their entire bodies were buried in the medium. The second instar larvae were distinguished from the first instar larvae by their larger, darker heads and larger body structures. Sometimes a part of the body was seen on top of the medium. The third instar larvae were rather large and left the medium and started climbing upwards. Therefore, they were observed on top of the medium and on the glass without medium. Pupae were observed on the side and lid of the Petri dish due to the fact that the third instar larvae secured themselves and created a cocoon around them (Karataş et al. 2011, Keser and Karataş 2012).

The statistical method used to evaluate the results of egg and larval development was the *z*-test for comparison of rates. The rates were converted to *z*-points, and the differences between the counts of two groups were tested. $z = \dfrac{(p_1 - p_2)}{\sqrt{\dfrac{p_1 q_1}{n_1}} + \left(\dfrac{p_2 q_2}{n_2}\right)}; q_2 = 1 - p_1.$

The calculations were performed using Minitab for Windows ver 13.0 (Ratkowsky 1990, Bates and Watts 2007).

Results

In the F_1 and F_2 generations' experimental groups, the numbers of eggs decreased significantly (Table 1). The difference between the control group and the experimental groups was statistically significant in both the F_1 and F_2 generations. Accordingly, kefir, yogurt, and milk reduced egg production in *D. melanogaster*. In addition, the comparison between the experimental groups also showed a difference in both generations. In the F_1 generation, especially the number of eggs in the medium containing milk was lower than in the other experimental groups, and this difference was significant (comparison of G2-G3 and G2-G4). In the comparison of the F_2 generation among the experimental groups, egg production was lower in the medium containing milk and kefir compared to the medium containing yogurt. Another interesting finding is that although the F_2 generation's medium contained only water, egg production was low. Egg production was low in F_2 generation individuals that developed from eggs exposed to substances in the F_1 generation.

There was a statistically significant difference between the larvae of control group and experiment groups of the F_1 and F_2 generations (Table 2). The numbers of larvae in both generations were lower than those of the controls. However, there was no significant difference between the experimental groups of the F_1 generation. A comparison of the experimental groups of the F_2 generation shows that the most negative effect occurred with milk, followed by kefir and then yogurt.

Table 3 shows the larval development rate of the eggs in both generations. The rates in the experimental groups of the F_1 and F_2 generations were considerably lower than those in the control group. In addition, morphological deformations were observed during larval development. Many dead larvae were observed that were black, elongated, and thin. A large number of dead third instar larvae that had not completed their development were found on the glass part of the container (Fig. 1). When the experimental groups of the F_1 generation were compared among themselves, the most negative effect was seen in the group exposed to milk. In the F_2 generation, milk and yogurt had a greater negative effect on development.

Table 4 shows the results of the effects of these dietary supplements on the entire developmental period, showing delayed metamorphosis in all cases except the control. All three experimental

Table 1. Effects of some dairy products on number of eggs in *Drosophila melanogaster*

Generation	Groups	Number of eggs	Z score	P score
F_1	Control (G1)	1,263	(G1-G2) 12.921**	(G1-G2) p1 = 0.33698 p2 = 0.20571
			(G1-G3) 11.101**	(G1-G3) p1 = 0.33698 p2 = 0.222785
			(G1-G4) 9.881**	(G1-G4) p1 = 0.33698 p2 = 0.234525
	Milk (G2)	771	(G2-G3) −1.801*	(G2-G3) p1 = 0.20571 p2 = 0.222785
			(G2-G4) −3.012**	(G2-G4) p1 = 0.20571 p2 = 0.234525
	Kefir (G3)	835	(G3-G4) −1.210	(G3-G4) p1 = 0.222785 p2 = 0.234525
	Yogurt (G4)	879		
F_2	Control (G5)	1,306	(G5-G6) 10.149**	(G5-G6) p1 = 0.323187 p2 = 0.223212
			(G5-G7) 11.206**	(G5-G7) p1 = 0.323187 p2 = 0.213561
			(G5-G8) 8.345**	(G5-G8) p1 = 0.323187 p2 = 0.24004
	Milk (G6)	902	(G6-G7) 1.050	(G6-G7) p1 = 0.223212 p2 = 0.213561
			(G6-G8) −1.793*	(G6-G8) p1 = 0.223212 p2 = 0.24004
	Kefir (G7)	863	(G7-G8) −2.843**	(G7-G8) p1 = 0.213561 p2 = 0.24004
	Yogurt (G8)	970		

*$P < 0.05$; **$P < 0.001$.

Table 2. Effects of some dairy products on number of larvae in *Drosophila melanogaster*

Generation	Groups	Number of larvae	Z score	P score
F$_1$	Control (G1)	948	(G1-G2) 13.811**	p1 = 0.750594 p2 = 0.451362
			(G1-G3) 12.441**	p1 = 0.750594 p2 = 0.487425
			(G1-G4) 12.956**	p1 = 0.750594 p2 = 0.481229
	Milk (G2)	348	(G2-G3) –1.447	p1 = 0.451362 p2 = 0.487425
			(G2-G4) –1.214	p1 = 0.451362 p2 = 0.481229
	Kefir (G3)	407	(G3-G4) 0.256	p1 = 0.487425 p2 = 0.481229
	Yogurt (G4)	423		
F$_2$	Control (G5)	893	(G5-G6) 16.988**	p1 = 0.405356 p2 = 0.179755
			(G5-G7) 13.530**	p1 = 0.405356 p2 = 0.220154
			(G5-G8) 15.672**	p1 = 0.405356 p2 = 0.194734
	Milk (G6)	396	(G6-G7) –3.356**	p1 = 0.179755 p2 = 0.220154
			(G6-G8) –1.274	p1 = 0.179755 p2 = 0.194734
	Kefir (G7)	485	(G7-G8) 2.081**	p1 = 0.220154 p2 = 0.194734
	Yogurt (G8)	429		

**$P < 0.001$.

Table 3. Effects of some dairy products on egg development in *Drosophila melanogaster*

Generation	Groups	Number of eggs	Number of larvae	Developmental rate (%)	Z score (larvae)	P score
F$_1$	Control (G1)	1,263	948	75.059	(G1-G2) 21.000*	p1 = 0.445908 p2 = 0.163688
					(G1-G3) 18.508**	p1 = 0.445908 p2 = 0.191439
					(G1-G4) 17.859**	p1 = 0.445908 p2 = 0.198965
	Milk (G2)	771	348	45.136	(G2-G3) –2.369**	p1 = 0.163688 p2 = 0.191439
					(G2-G4) 2.988**	p1 = 0.163688 p2 = 0.198965
	Kefir (G3)	835	407	48.742	(G3-G4) –0.619	p1 = 0.191439 p2 = 0.198965
	Yogurt (G4)	879	423	48.122		
F$_2$	Control (G5)	1,306	893	68.376	(G5-G6) 11.686**	p1 = 0.683767 p2 = 0.439024
					(G5-G7) 5.735**	p1 = 0.683767 p2 = 0.561993
					(G5-G8) 11.785**	p1 = 0.683767 p2 = 0.442268
	Milk (G6)	902	396	43.902	(G6-G7) –5.204**	p1 = 0.439024 p2 = 0.61993
					(G6-G8) –0.141	p1 = 0.439024 p2 = 0.442268
	Kefir (G7)	863	485	56.199	(G7-G8) 5.154**	p1 = 0.561993 p2 = 0.442268
	Yogurt (G8)	970	429	44.226		

*$P < 0.05$; **$P < 0.001$.

diets inhibited the development of eggs into adults thus delaying metamorphosis. This effect was particularly evident in the transition from egg to first instar larva and from pupa to adult.

Discussion and Conclusion

The results show that when milk and fermented milk products are added to the diet of adult *Drosophila*, the number of eggs and the number of larvae that develop from these eggs are reduced. This reduction is particularly pronounced between the experimental groups and the control group. A comparison between the experimental diets indicates that the most negative effect is caused by milk. The results were similar for both development and metamorphosis.

It can be stated that the adverse effect observed in both F$_1$ and F$_2$ generations are milk, kefir and yogurt, respectively when all the experimental materials are compared among themselves. Many previous studies have reported that the number of eggs is reduced by the application with phenol compound, salicylic acid, insecticides (cypermethrin and diazinon), nanoparticles (copper and silver) to *Drosophila* culture media (Aşkın et al. 2007, Karataş and Bahçeci 2009, Karataş et al. 2011, Keser and Karataş 2012, Han et al. 2014, Raj et al. 2017). However, the very negative effect on *Drosophila* of these ingredients, which are nutritious and beneficial for humans, was an unexpected result in the present study. Milk, yogurt, and kefir, irrespective of fermentation, reduced the numbers of both eggs and larvae in *Drosophila* and negatively affected their development.
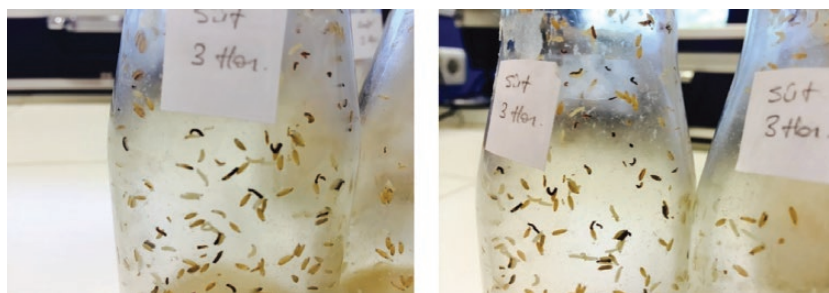
Fig. 1. Malformations seen in the larvae.

Table 4. Effect on development of milk and fermented milk products used as diet additives on the development of *Drosophila melanogaster* generations

	Groups	Eclosion	First instar	Second instar	Third instar	Pupa	Adult
F_1	Milk	Day 1	Day 3	Day 4	Day 5	Day 7	Day 12
	Yogurt	Day 1	Day 3	Day 4	Day 5	Day 7	Day 12
	Kefir	Day 1	Day 3	Day 4	Day 5	Day 7	Day 12
	Control	Day 1	Day 2	Day 3	Day 4	Day 6	Day 10
F_2	Milk	Day 1	Day 3	Day 4	Day 5	Day 7	Day 12
	Yogurt	Day 1	Day 3	Day 4	Day 5	Day 7	Day 11
	Kefir	Day 1	Day 3	Day 4	Day 5	Day 7	Day 11
	Control	Day 1	Day 2	Day 3	Day 4	Day 6	Day 10

In the present study, adverse effects were observed in the F_1 generation as well as in the F_2 generation reared on the normal medium, devoid of experimental ingredients. Thus, it can be concluded that milk and fermented milk products had unexpectedly high negative effects on *Drosophila*. This result in the experimental groups shows that kefir, yogurt, and milk disrupted normal development, contrary to expectations. The findings in the present study related to the F_2 generation are important because it completes its development without being exposed to any of the experimental ingredients. Additionally, the numbers of eggs and larvae and the development rate were low in the F_2 generation. It can also be concluded that despite the development of the F_2 generation on normal medium the adverse effect observed in larval development in the F_1 generation continued. Recessive lethal mutations may be behind the regression observed in this generation. Alternatively, the cause of this negative effect in the F_2 generation may be the genetic changes in the epigenetic mechanisms, possibly occurring due to the change in the nutritional regime, which can be considered an environmental condition. Epigenetic changes are regulatory in the genetic mechanism due to changes in environmental conditions that are not structural but still cause inherited changes (Klug et al. 2018).

An organism is more susceptible to toxicity in the early life stages of development (egg and larval stages) than in the adult stage (El-Toukhy and Girgis 1993). This may be the reason for the adverse effect on the F_1 and F_2 generation but without a negative effect (data not provided) during the concentration determination before the start of the experiment. Dietary protein is a major source of essential amino acids for insects. They also serve as intracellular antioxidants. Several studies have shown that decreases in dietary protein content could potentially increase oxidative stress (Muralidhara 2015). The cause of the retardation has been observed in the eggs and larvae development might be related to the excess amount of experimental ingredients used in the experiment. Increased the excess amount of protein in the medium might have been chronic exposure on the contrary to the normal level. The substance for the living can be harmful in extreme amounts. The useful substance that is necessary for an organism can be harmful effects in the extreme amounts. An increase in lifespan was observed in *Drosophila* with feeding the low protein and high carbohydrate consumption (Bruce et al. 2013). In another study, Van Herrewege (1974) compared survivorship across several casein concentrations in a base medium with sugar, vitamins, nucleic acids, and essential lipids. Mean lifespan was maximized at intermediate concentrations of casein. In the same study, it has been stated that in particular, high levels of a refined nutrient such as casein may be toxic and contribute to mortality that is independent of aging. Furthermore, Min and Tatar (2006) described not only the overall survivorship across varied casein diets but also the trajectories of mortality and the patterns of age-specific fecundity. Male survival is reduced by 38% upon 4% diet compared to one on 1% casein diet. Consistent with our interpretation of the experimental results, flies on reduced casein were not relatively long-lived because flies on high casein suffered a pharmacological artifact which may cause a decrease in fecundity (Min and Tatar 2006).

Yeast is the main protein source for *Drosophila* larvae and is necessary for their development and egg production (Miller et al. 2011). Due to the addition of experimental ingredients, a sufficient amount of yeast might not have been in the medium and so the main protein source of the *Drosophila* larvae might have been reduced. As these three dietary ingredients restrict yeast development, the nutrients available to larvae might have been restricted and their development inhibited. It is also possible that the yeasts' increased metabolic activity depleted the nutrients. Another reason could be that the number of acetic acid bacteria might have been reduced in their surroundings. In its natural medium, acetic acid bacteria, which are a group of bacteria that can pass from the surroundings to the intestine of *Drosophila*, prefer environments rich in fermented sugar and ethanol (Blum et al. 2013, Shingleton et al. 2017). Acetic acid bacteria are the major components of the microbiota of *Drosophila suzukii* and *D. melanogaster* in particular and have been implicated to play a role in the host organism's nutrition physiology as well as its behavior (Vacchini et al. 2017). Moreover, these bacteria support development in *Drosophila* by increasing larval development rate,

body size, intestinal stem cell activity, and energy metabolism and inhibiting the growth of pathogenic bacteria (Blum et al. 2013). Kefir, yogurt, and milk added to the medium may have altered its pH balance, disturbing the optimum conditions for acetic acid bacteria and preventing their growth. Thus, the normal functioning of the symbiotic relationship established in the intestines in *Drosophila* might have been hindered and larval development might have been damaged. In another study supporting our results, oats and milk powder were fed to *M. domestica* females and both the number of eggs and the hatching rate decreased (Pastor et al. 2011). Flies fed on medium enriched with milk and yeast laid three times fewer eggs than those fed on yeast and six times fewer than those fed on sugar. Milk had a negative effect on egg production and development in *M. domestica* (Pastor et al. 2011). It was also reported that food containing protein inhibits egg laying and causes a significant shortening of lifespan in *M. domestica* (Glaser 1923). There are also findings showing the opposite. It has been stated that the addition of protein that are powdered milk, egg solids, whole beef blood, yeast hydrolysate, peptone, and blood albumin to the diet of adult house flies results in an increase in egg production (Turner and Hair 1967). It is possible that the presence of certain feeding deterrent that was introduced in the diet through the milk additives that caused them not to feed properly. For examples, in addition to the rich amino acids, milk contains high levels of D-galactose and kefir contains high levels of D-glucose and D-galactose. Milk contains high levels of D-galactose and kefir contains high levels of D-glucose and D-galactose (Song et al. 1999, Cui et al. 2004, Rosa et al. 2017). It has been stated that D-galactose accelerates aging in different animal models, decreases the average lifespan of *D. melanogaster* and *M. domestica*, and reduces the ability of mice to reproduce (Song et al. 1999, Cui et al. 2004), which is associated with oxidative stress (Qi et al. 2002). Another study found that even low doses of D-galactose caused oxidative stress damage, chronic inflammation, neurodegeneration, and decreased immunity. It has also been stated that changes such as a decrease in lifespan are caused and transcriptional changes are responsible (Song et al. 1999, Cui et al. 2006, Hao et al. 2014). The cause of the adverse effects seen in our research may be D-galactose, which was added to the medium through the three foodstuffs. Another reason may be that the organism is not compatible in terms of metabolic evolution with the metabolism of D-galactose and D-galactose (Rosa et al. 2017) found in the structure of milk, yogurt, and kefir. There appear to be no reported findings showing adverse effects of yogurt, but it has been stated that some strains found in kefir have no beneficial effects on health (Urdaneta et al. 2007).

Furthermore, in addition to all these factors, the functioning and activity of alcohol fermentation, which provides the basic nutrient of *Drosophila*, may change when supplemented with another fermented product, and the development of eggs and larvae may be damaged as they restrict the basic nutrient of the larvae. The functioning of alcohol fermentation (Hernández-Tobías et al. 2011), which creates the basic food of *Drosophila*, may be impaired due to the added nutrients. The ideal environment for larval and adult development in *Drosophila* is one in which microorganisms play a role in fruit decay. The organism prefers the high alcohol content/low pH environment associated with fermentative metabolism (Pohl et al. 2012, Piper 2017). It has also been reported that ethanol induces egg laying in *D. melanogaster* (Sumethasorn and Turner 2016). In this case, the function and activity of alcohol fermentation may have changed and the ethanol concentration in the environment may have decreased. Given the ability of ethanol to stimulate egg laying, reduced ethanol content may have caused the females to lay fewer eggs because of the degraded ethanol fermentation process.

It appears that kefir, yogurt, and milk interfere with the larval development, during transformation from eggs to during the pupa stage with causing delay. The delay have been observed in metamorphosis is between the egg and first instar larvae. This delay is a sign demonstrate that the organisms are more sensitive to environmental changes especially early embryonic periods (El-Toukhy and Girgis 1993). In addition, the darkened dead larvae have been observed in the bottles glass, this can demonstrating the failed larval developmental program. In addition, the delaying has been observed between the last instar larvae and pupae. During the last instar stage, insects generally increase food consumption for reaching to higher larval and/or pupal weight (Salvador et al. 2010). In the same research that has been done with flavonoids in larvae of the velvet-bean caterpillar *Anticarsia gemmatalis* observed the larva attempted to avoid poisoning by eating smaller meals, for a prolonged time, to obtain the ideal weight for pupation. In another research, *A. gemmatalis* fed diets with different rutin concentrations in comparison to rutin-free diets were also shown to have reduced food consumption (Hoffmann-Campo et al. 2006, Piubelli et al. 2006) Although this strategy allows them to survive, the prolonged larval development will certainly make them largely prone to the attack of their natural enemies in field conditions, increasing their mortality risks. In this particular case, experimental ingredients were suggested to alter the activity of enzymes and hormones, blocking biochemical pathways and reducing (Salvador et al. 2010). Experimental ingredients added to the media, especially the milk cause a delay to this developmental program. Experimental ingredients might have hampered the developmental progress by adversely affecting this hormone or enzyme system.

Overall, the results showed that milk and fermented milk products caused a decrease in the number of eggs and larvae in *Drosophila* and adversely affected egg and larval growth, contrary to expectations. These results indicate that milk, kefir, and yogurt inhibited larval development in *Drosophila*. Evaluation of all the findings related to development revealed adverse effects such as decreased numbers of eggs and larvae, lower larval growth rates, and increased larval deaths. It is possible that the fermentation have been relatively reduced the negative effects of the milk because the most negative effect have been observed by milk. Whether the products were fermented or not did not change the effect. When the experimental groups were compared between themselves, no clear difference between milk and fermented milk products was apparent. Considering all these results and the other studies mentioned above, it would be beneficial to investigate the effects of milk and fermented milk products in the diet of other experimental organisms and in vivo.

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