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The effect of streptomycin on survival, development, and some biochemical aspects of *Drosophila melanogaster*

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Abstract: The effects of an aminoglycoside antibiotic, streptomycin, on survival rate, development time, and adult longevity of *Drosophila melanogaster* (Diptera: Drosophilidae) were examined by adding on the artificial diet at different concentrations in laboratory condition. The effect of streptomycin on malondialdehyde (MDA) and protein carbonyl (PCO) contents, superoxide dismutase (SOD), and catalase (CAT) activities in all developmental stages of *D. melanogaster* was also investigated. First-instar larvae were reared into diets with 600 mg/L, 1200 mg/L, and 1800 mg/L of streptomycin concentrations and were fed until the adult emergence. The highest concentration of streptomycin significantly reduced the survival rates of all development stages and the adult longevity of insect when compared to the control diet without streptomycin. Time to the adult stage was 7.73 ± 0.29 days in control, this developmental time in the highest concentration of streptomycin extended to 9.65 ± 0.65 days. Also, MDA content in the highest concentration of streptomycin significantly increased when compared to the control diet in the adult stage. Relative to the control, all concentrations of streptomycin significantly increased the PCO content. SOD activity was reduced in all stages of the insect. Similar results were obtained in CAT activity in the larval and pupal stages. Our results showed that experimented dietary streptomycin concentrations dramatically increased PCO content and caused significant alternations on SOD-CAT activity with the negative effects on the survival and development of *D. melanogaster.*

Key words: *Drosophila melanogaster*, streptomycin, survival rate, development time, antioxidant enzymes

1. Introduction

Antibiotics have been used to treat fungal, helminthic, and bacterial infections in humans and highly organized animals without detriment to host organisms. Several antibiotic agents have been used for mass rearing of model insects in aim to prevent microbial contamination on artificial diets. While insects can able to tolerate applied antibiotic agents in various ways related to dietary concentrations, the right proportion of additional antibiotics on artificial diets should be able to prevent microbial contamination but not exert their negative effects on the insect (Thakur et al., 2016; Nair et al., 2018; Huynh et al., 2019; Li, et al., 2020). Streptomycin, an aminoglycoside antibiotic, have been used to treat bacterial infections such as tuberculosis, *Mycobacterium avium* complex, endocarditis, brucellosis, *Burkholderia* infection by the inhibiting protein synthesis through binding 30S subunit of the ribosome in a broad range of gram-negative bacteria (Kohanski et al., 2010, Holzgrabe et al., 2011; Van and Coenye, 2017). It is also known that streptomycin has been used in the struggle with pest insects as an insecticide because of its bactericidal action on the endosymbiont microorganisms located in the gastrointestinal tract which provides essential metabolites for the development, reprocessing, and immunity of insect (Büyükgüzel and Kalender, 2009; Thakur et al., 2016; Heys et al., 2018). Antibiotics that are applied to the artificial diet of insects exert their bactericidal effects via the production of reactive oxygen species (ROS) regardless of their molecular action (Büyükgüzel and Kalender, 2009; Li et al., 2020). As the maintaining physiological balance between ROS production and antioxidant activity is a key process to the avoiding from destructive impacts of oxidative stress, the enzymatic antioxidant activity of the host provides an essential index for the defining physiological changes of model organisms exposed to antibiotics (Büyükgüzel and Kalender, 2007; Hyršl et al., 2007; Sahayaraj and Balasubramanian, 2016; Huynh et al., 2017; Nair et al., 2018). Insects have also an antioxidant system that protects themselves from the destructive impact of oxidative stress (Güneş and Büyükgüzel, 2017; Aslan et al., 2019; Stanley and Kim, 2020). On the other hand, polyunsaturated fatty acid residues of phospholipids,

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one of the major components of the cell, highly tends to react with ROS and generate toxic aldehydes such as malondialdehyde (MDA) which is used as an indicator for the determining level of oxidative stress (Büyükgüzel and Kalender, 2009; Kobeasy et al., 2011). Also, toxic aldehydes from the lipid peroxidation process can generate protein carbonyl compounds via reaction with functional groups of proteins (Büyükgüzel, 2013). The antioxidant system is composed of enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPX) that work in synergistically to cleanse of ROS. For instance, SOD activity is one of the main responses to dietary pro-oxidants and specifically converts the superoxide anion to hydrogen peroxide, then, CAT and peroxidase (POX) detoxify hydrogen peroxide to molecular oxygen and water. Thus, the SOD and CAT activities are the most important system for determining antioxidant balance which protects living organisms against hydrogen peroxide, superoxide, and hydroxyl radicals (Değer et al., 2008; Büyükgüzel and Kalender, 2009; Büyükgüzel, 2013).

Drosophila melanogaster (Diptera: Drosophilidae) is a widely used model organism due to its high adult yield, short generation time, appropriate for cultivating, and well-known genome. Furthermore, it provides observation of the effect of endogenous and exogenous origin prooxidants on biomolecules (Lee and Brey, 2013; Bonfini et al., 2016). In addition, *D. melanogaster* has been used in novel drug discovery and determining molecular mechanisms of diseases such as cancer, diabetes, and obesity due to its analog tissues and much simpler metabolisms compared to mammals (Men et al., 2016; Krams et al. 2017; Douglas, 2018). Due to its importance at being a model organism for many research areas, the successful rearing of *D. melanogaster* at laboratory conditions is an essential step to understand their physiology (Bronskill 1961; Bernardi et al., 2000). In this study, we investigated the effect of streptomycin on some life parameters of *D. melanogaster* including survival rate, development time, and adult longevity at different dietary concentrations. Also, the oxidative impact of streptomycin on proteins and lipids (PCO and MDA contents), SOD, and CAT activities in the last larval, pupal, and adult stage of the insect were investigated.

2. Materials and methods

2.1. Insect culture

Drosophila melanogaster wild type strain (Diptera: Drosophilidae) was used in all experiments. The stock insect culture was maintained by rearing first instars to adult emergence on the artificial diet at 25 ± 2 °C, 60%–70% relative humidity, and 12 h light-12 h dark photoperiod in laboratory conditions. The artificial diet was composed of 8 g of agar-agar ultrapure (Merck, Darmstadt, Germany), 20 g of sucrose (BioUltra, 99 %, Sigma Chemical Co., St. Louis, MO, USA), 11.78 g of dried powder yeast (Dr. Oetker Food Co., İzmir, Turkey), 0.8 g of ascorbic acid (BioUltra, 99 %, Sigma), 7.72 mL of nipagin (SigmaUltra, p-hydroxybenzoic acid methyl ester, crystal), 36 g of mashed potatoes (Knorr, Unilever Co., İstanbul, Turkey), and 1 L of distilled water (Rogina et al., 2000; Lesch et al., 2007).

2.2. Feeding experiments

To obtain this diet mixture, first, we weighed the agar, sucrose, and dry yeast put into a jar, and added 150 mL of distilled water. Then, we weighed the mashed potato in a separate dish and add 100 mL of distilled water. After the mixture containing agar, sucrose, and dry yeast is thoroughly boiled and homogenized, mashed potato is added to it. When this mixture reached room temperature, ascorbic acid and nipagin were added. To perpetuate the culture, eggs laid by the adults were hatched and the larvae were fed on the same diet until they reached their adult stage. Adults were then transferred to the new culture medium. The culture was carried out in an incubator (Nüve ES 252) of 25 ± 2°C and 60%–70% RH for a photoperiod of 12:12 (L:D) h. Streptomycin sulfate concentrations were determined as 600, 1200, and 1800 mg/L based on the study of Graf and Benz (1970) with several preliminary experiments. Our preliminary experiments showed that tested streptomycin concentrations enable larvae to complete their adult development with gradually increasing mortality. Therefore, we used these actual concentrations for obtaining specific sublethal toxicity at high concentrations of streptomycin. First instar larvae obtained from stock culture were placed into 5 mL bottles as one individual through the fine-tipped brush. Twenty culture bottles of 5 mL and 20 individuals were used for each streptomycin sulfate concentrations.

2.3. Survival rate, development time, and adult longevity experiments of *Drosophila melanogaster*

First instar larvae (n = 20) were placed into the diet without streptomycin, as a control group, and the diets containing 600, 1200, and 1800 mg/L streptomycin using a fine-tipped brush and then covered with a cotton wad. The rates of the larvae that reached the 3rd stage, pupa, and adult stages from the first larval stage were calculated. To determine the development time to reach the 3rd stage, pupa and adult stages were recorded daily. All experiments were conducted in four replicates at 25 ± 2 °C and 60%–70% relative humidity, 12 h light-12 h dark in the laboratory condition. First instars were reared until adult emergence on the artificial diets amended with given concentrations of streptomycin. Newly emerged adults were placed into the diets with and without streptomycin concentrations. The diets were renewed every 2 days to provide a fresh diet

for adult individuals. The adult longevity was determined through recording individuals until the last adult expired. This experiment was conducted under the environmental conditions where the stock culture of insects was maintained.

2.4. Sample preparation

Extracts of 3rd instar larvae, pupae, and adults of the insect were prepared at 4 °C by an ultrasonic homogenizer (Bandelin Sonoplus, HD2070, Berlin, Germany) at 30 W, 15 s in homogenization buffer and subsequent centrifugation (Hettich Zentrifugen Mikro 200 R, Tuttlingen, Germany) at 10,000 g for 15 min at 4 °C. The resulting cell-free extracts were collected for biochemical analysis. Supernatants were centrifuged at 1000 g for 10 min at 4 °C. MDA and PCO contents, and antioxidant enzymes activities (SOD and CAT) were determined by measuring the absorbance of the samples in a dual-beam spectrophotometer (Shimadzu 1700, UV/vis, Kyoto, Japan). Assays were replicated four times each with twenty whole-bodies.

2.5. Malondialdehyde (MDA) and protein content (PCO) assay

Malondialdehyde content was measured based on the method used by Jain and Levine (1995). MDA content was calculated by using constant-coefficient as 1.56×105 M^{-1} cm^{-1} and expressed as nmol/mg protein. Based on the formation of 2,4-dinitrofenilhidrazon, which is formatted by a reaction at strong acid condition between carbonyl groups of proteins and 2,4- DNPH and the measuring of these products at 370 nm, was carried out with the method of Levine et al. (1994) to PCO content assay. PCO content was calculated by using constant-coefficient as 22.000 M^{-1} cm^{-1} and expressed as nmol/mg protein.

2.6. Determination of SOD activity and CAT activity

SOD (EC 1.15.1.1) activity was measured according to Marklund and Marklund, (1974). One unit total SOD activity was calculated as the amount of protein causing 50% inhibition of pyrogallol autooxidation for 3 min at 440 nm with risen absorbance. Units per milligram of protein (U/mg protein) were expressed as the total SOD activity. A blank without homogenate was used as a control for nonenzymatic oxidation of pyrogallol in Tris-EDTA buffer (50 mM Tris, 10 mM EDTA, pH 8.2). CAT (EC 1.11.1.6) enzyme activity was determined by using the method improved by Aebi (1984). The downward absorbance of H_2O_2 degradation was measured at 240 nm for 60 s. CAT activity was expressed as millimoles of H_2O_2 reduced per milligram of protein for a minute, using an extinction coefficient of 0.0394 mM^{-1} cm^{-1}. A blank without homogenate was used as a control for nonenzymatic hydrolysis of peroxide in phosphate buffer (50 mM, pH 7.0) and enzyme-specific activity given as mmol/mg protein/min.

2.7. Statistical analysis

Data obtained from MDA and PCO contents, SOD and CAT activity, and life-history parameters were subjected to one-way analysis of variance. The least significant difference test (SPSS Inc., 1997) was used to determine the significance of differences among the means. Data on survivorship were analyzed using a $\chi 2$ test (Snedecor and Cochran, 1989). When the $\chi 2$ and F estimate exceeded a probability value of 0.05, the differences were considered statistically significant. Regression analysis was also performed to test the correlation between PCO content and the activity of antioxidant enzymes.

3. Results

All dietary streptomycin concentrations (600 mg/L, 1200 mg/L, and 1800 mg/L) reduced the survival rate and extended development time to 3rd instar larvae, pupae, and adults of *D. melanogaster*. In particular, the highest concentration of streptomycin (1800 mg/L) significantly reduced the survival rate of 3rd instar larvae, pupae, and adult stage of the insect. While the development time to 3rd instar larvae and pupal stages of the insect was extended in the highest concentration of streptomycin, elongation of development time to the adult stage of the insect was statistically important relative to control diet without streptomycin. Our results demonstrated that the additional streptomycin concentrations on the artificial diet have an impact on both larval and postlarval development stages of the insect.

3.1. Effect of streptomycin on survival rate, development time and adult longevity of *D. melanogaster*

Streptomycin concentrations (600 mg/L, 1,200 mg/L, and 1,800 mg/L) significantly decreased survival rate and prolonged development time of 3rd instar larva, pupal, and adult stages of insect compared to the diet without streptomycin. Experimented streptomycin concentrations including 600 mg/L, 1,200 mg/L, and 1,800 mg/L significantly reduced survival rates of 3rd instar larva to 67.25 ± 4.14%, 61.25 ± 5.41%, and 43.75 ± 2.72%, respectively when compared to control diet without streptomycin (95 ± 3.26%). Similar results were obtained from the survival rates of pupal and adult stages of the insect (Table 1).

Development time to 3rd instar larva, pupal, and adult stages of the insect was significantly extended with increasing streptomycin concentrations (600 mg/L, 1200 mg/L, and 1800 mg/L). When compared to control, the highest concentration of streptomycin significantly extended the development time of the 3rd instar larva, pupal, and adult stages to 3.56 ± 0.28 days, 5.61 ± 0.38 days, and 9.65 ± 0.65 days, respectively. Also, experimented streptomycin concentrations reduced adult longevity of the insect. The highest concentration of streptomycin

Table 1. Effect of streptomycin on survival rates of *D. melanogaster*.

Streptomycin (mg/L)	Survival to 3rd instar larva (%) (mean* ± S.D)†	Survival to pupal stage (%) (mean* ± S.D)†	Survival to adult stage (%) (mean* ± S.D)†
0.0§	95 ± 3.26a	91.25 ± 3.69a	87.5 ± 2.79a
600	67.5 ± 4.14b	66.25 ± 4.8b	65 ± 5.3b
1200	61.25 ± 5.41a	52.5 ± 6.25b	56.6 ± 6.2ba
1800	43.75 ± 2.72c	38.75 ± 2.07c	31.25 ± 2.07c

* Four replicates with 20 larvae per replicate.
† Values followed by the same letter are not significantly different from each other, $p > 0.05$ (chi-square test).
§Control diet (noncontaining streptomycin).

(1800 mg/L) shortened adult longevity by 3-fold (8.16 ± 0.77) when compared to control (21.89 ± 2.46 days) (Table 2).

3.2. Effect of streptomycin on MDA and PCO content in 3rd instar larvae, pupae, and adults of *D. melanogaster*

While the MDA content was significantly reduced in the diet with streptomycin concentrations in 3rd instar larvae and pupae of *D. melanogaster,* the highest concentration of streptomycin significantly increased MDA content in the adults of insect when compared to the control diet without streptomycin (2.12 ± 0.51 nmol/mg protein to 8.31 ± 2.11 nmol/mg protein) (Figure 1).

Diet with streptomycin concentrations significantly increased the PCO content in the 3rd instar larvae, pupae, and adults of insect (Figure 2). Particularly, the highest concentration of streptomycin dramatically increased the PCO content in 3rd instar larvae (9.75 ± 1.85 nmol/mg to 46.94 ± 8.36 nmol/mg protein), pupae (6.53 ± 1.07 nmol/mg protein to 86.4 ± 20.32 nmol/mg protein) and adults of insect (12.88 ± 2.64 nmol/mg protein to 163 ± 50.52 nmol/mg protein) when compared to all development stages in control diet by 5-fold, 14-fold, and 13-fold, respectively.

3.3. Effect of streptomycin on SOD and CAT enzyme activities in 3rd instar larvae, pupae, and adults of *D. melanogaster*

Except for CAT activity in the adults of insect, streptomycin concentrations significantly decreased SOD and CAT activities in 3rd instar larvae, pupae, and adults compared to control. Experimented streptomycin concentrations significantly reduced SOD activity in all development stages of insect (Figure 3). Particularly, the highest concentration of streptomycin dramatically reduced SOD activity in 3rd instar larvae (0.2 ± 0.73 U/mg protein) by 30-fold when compared to the control diet without streptomycin (6.24 ± 0.53 U/mg protein).

CAT activity in 3rd instar larvae, pupae and adults of the insect determined as 8.78 ± 0.49 mmol/mg protein/min, 29.88 ± 1.93 mmol/mg protein/min, and 0.91 ± 0.08 mmol/mg protein/min, respectively. While CAT activity significantly decreased in 3rd instar larvae and pupae treated with streptomycin concentrations, experimented streptomycin concentrations increased CAT activity in the adults of insect when compared to control diet without streptomycin (Figure 4).

4. Discussion

Streptomycin has been commonly used for improving the nutritional quality of artificial diets to mass rearing of model insects including *Corcyra cephalonica* (Pyrallidae: Lepidoptera), *Diabrotica virgifera virgifera LeConte* (Coleoptera: Chrysomelidae), *Spodoptera litura* (Lepidoptera: Noctuidae), *Spodoptera exigua* (Lepidoptera: Noctuidae), *Rhynocoris marginatus* (Hemiptera: Reduviidae) for various purposes at laboratory condition (Sahayaraj and Balasubramanian, 2016; Nair et al., 2018; Arun Kumar et al., 2018; Huynh et al., 2019). In the case of struggle with microbial contamination in mass rearing of insects, the right proportion of antibiotic agents in artificial diets should be able to prevent microbial contamination but not exert or have less detrimental effects on host insect (Thakur et al., 2016; Nair et al., 2018; Huynh et al., 2019; Li et al., 2020). Recent studies pointed out that the high concentration of antibiotics increased the mortality rate and prolonged development time of insect through the changing physical and chemical properties of artificial diets (Büyükgüzel and Kalender, 2009; Lin et al., 2015; Aslan et al., 2019). For instance, Lin et al. (2015) demonstrated that dietary streptomycin reduced the diversity of intestinal flora and caused a dramatic increase in larval and pupal mortality of *Plutella xylostella* (Lepidoptera: Plutellidae) with the increasing streptomycin concentrations amended with leaf discs. Similarly, we determined that experimented streptomycin concentrations (600 mg/L, 1200 mg/L, and 1800 mg/L) negatively affected the survival rates of larvae and pupae of *D. melanogaster.* Diet with 600 mg/L, 1200 mg/L and 1800 mg/L concentrations of streptomycin significantly reduced survival rates of 3rd instar larvae ($R = -0.97$; $p < 0.05$)

Table 2. Effects of streptomycin on development time and adult longevity of *D. melanogaster*.

Streptomycin (mg/L)	Time to 3rd instar larva (day) (mean* ± S.D†)	Time to pupal stage (day) (mean* ± S.D†)	Time to adult stage (day) (mean* ± S.D†)	Adult longevity (day) (mean* ± S.D†)
0.0§	2.79 ± 0.26a	4.45 ± 0.33ab	7.73 ± 0.29a	21.89 ± 2.46b
600	3.15 ± 0.46a	4.33 ± 0.26a	7.82 ± 0.29a	14.27 ± 0.5a
1200	3.26 ± 0.37a	4.89 ± 0.39ab	8.25 ± 0.14a	10.61 ± 0.43ac
1800	3.56 ± 0.28a	5.61 ± 0.38b	9.65 ± 0.65b	8.16 ± 0.77c

* Four replicates with 20 larvae per replicate.
† Values followed by the same letter are not significantly different from each other, p > 0,05 (chi-square test).
§ Control diet (noncontaining streptomycin).

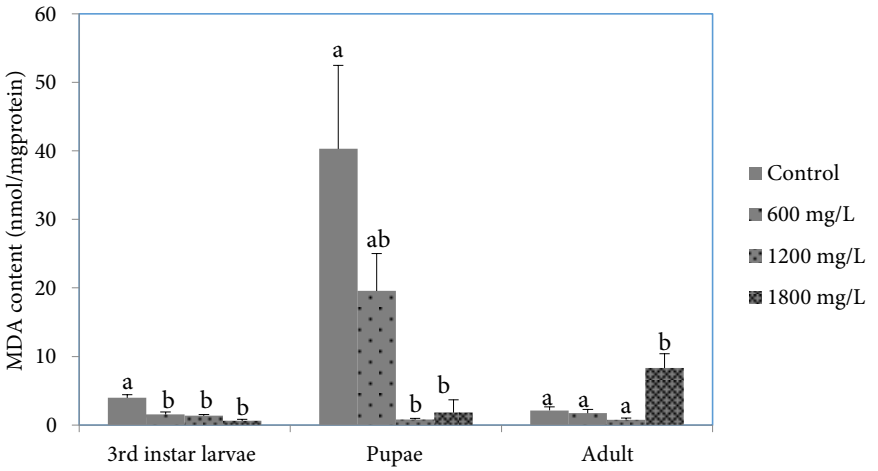

Figure 1. Effects of dietary streptomycin on MDA content in 3rd instar larvae, pupae, and adults of *D. melanogaster*. Bars represent the means of four replicates. Means followed by different letters are significantly different (p < 0,05, LSD test).

by 28%, 36%, and 46%, respectively when compared to control without streptomycin (95 ± 3.26%). In our study, the negative effect of dietary streptomycin concentrations on survival rate and development time of insect may arise from the impairment of the nutritional value of diet and the imbalanced gastrointestinal homeostasis.

Streptomycin concentrations negatively affected adult longevity when compared to the control diet without streptomycin (R = –0.96; p < 0.05). Antibiotics significantly altered life parameters of model insects including adult longevity (Dimou et al., 2010; Raina et al., 2015; Çelik et al., 2019). Similar to our results, Aslan et al. (2019) reported that dietary gemifloxacin reduced adult longevity and extended development time to pupa and adult emergence of *D. melanogaster*. In addition, Raymann et al. (2017) demonstrated that exposure to tetracycline, a broad-spectrum antibiotic, significantly reduced adult longevity of honeybees by disturbing intestinal microbiota and increasing susceptibility to infection by pathogens. To maintain the balance between beneficial commensals and diversity of the intestinal microbiome, the host's immunity must be tightly regulated by epithelial cells of the intestine. Poor diversity of these microorganisms or the reduction in the abundance of commensal communities of intestinal flora, dominated by Lactobacillus and Acetobacter genera in *D. melanogaster,* makes insect more susceptible to fungal and viral infections and paralyzes the essential physiological process of the host (Mistry et al., 2016; Krams et al., 2017; Douglas, 2018). In our study, the negative effect of dietary streptomycin on adult longevity of *D. melanogaster* may arise from the hypersusceptibility of the insect as a result of the distribution of intestinal flora. In this view, ROS production is one of the key immune responses for sustainable intestinal homeostasis due to its microbicidal activity and being secondary modulators for intestinal cell renewal (Wu et al., 2012; Lee and Brey, 2013; Lambeth and Neish, 2013; Bonfini et al., 2016). Furthermore, the regulative actions of host on beneficial

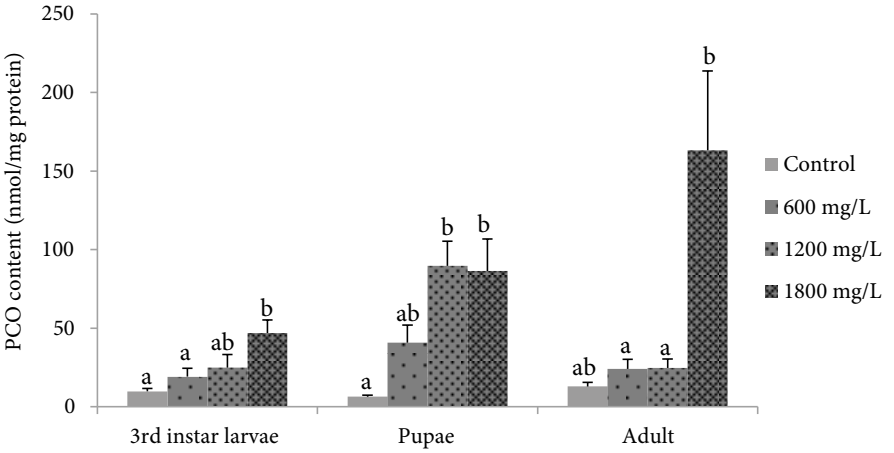

Figure 2. Effects of dietary streptomycin on PCO content in 3rd instar larvae, pupae, and adults of *D. melanogaster*. Bars represent the means of four replicates. Means followed by different letters are significantly different ($p < 0.05$, LSD test).

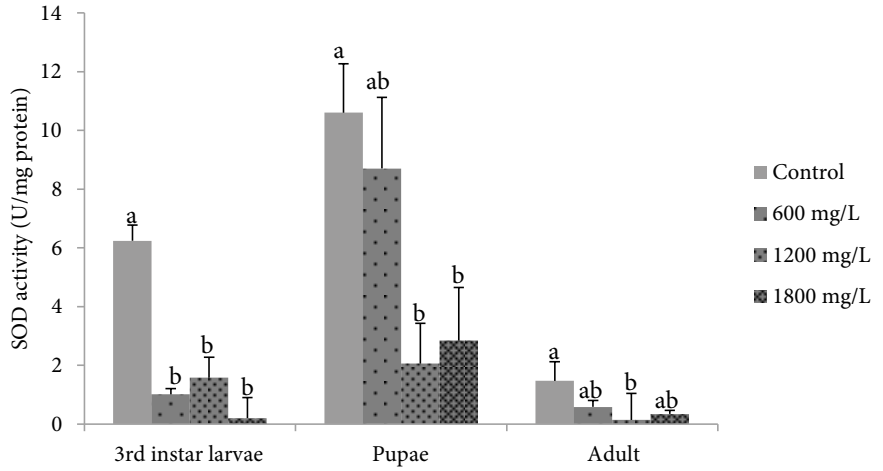

Figure 3. Effects of dietary streptomycin on SOD enzyme activities in 3rd instar larvae, pupae, and adults of *D. melanogaster*. Bars represent the means of four replicates. Means followed by different letters are significantly different ($p < 0.05$, LSD test).

communities of gut is driven primarily by signaling pathways including immune deficiency and Toll pathways, and also antimicrobial peptide production which are moderated with ROS generation (Lee et al., 2015; Marra et al., 2021). In a broader comparative context, our results can suggested that the high concentrations of dietary streptomycin changed the intestinal flora or disturb the biotransformation process of xenobiotic (streptomycin) by accessing into the gastrointestinal tract of insect and caused a physiological imbalance on gut-fat body feedback communications because of the excessive production of ROS.

We obtained significant alternation on MDA and PCO content with the negative effects of streptomycin on survival and development parameters. It is known that the exposure to high concentrations of antibiotics is strongly inducible for the production of MDA and PCO content by leading to excess accumulation of reactive oxygen species ROS, regardless of their molecular targets (Büyükgüzel and Kayaoğlu, 2014; Sahoo et al., 2015; Van and Coenye, 2017). Although insects can tolerate applied antibiotic agents related to dietary concentrations in primarily by their antioxidant enzymes such as SOD and CAT, these substances can change the structure of antioxidant enzymes and reduce enzymatic antioxidant activity by increasing to the level of oxidative stress in tissues that can be determined by evaluating the amount of MDA and PCO contents in living organisms (Kohanski et al., 2007; Suganya et al., 2016; Li et al., 2020). On the other hand, insects, like the other animals, have adaptive metabolic

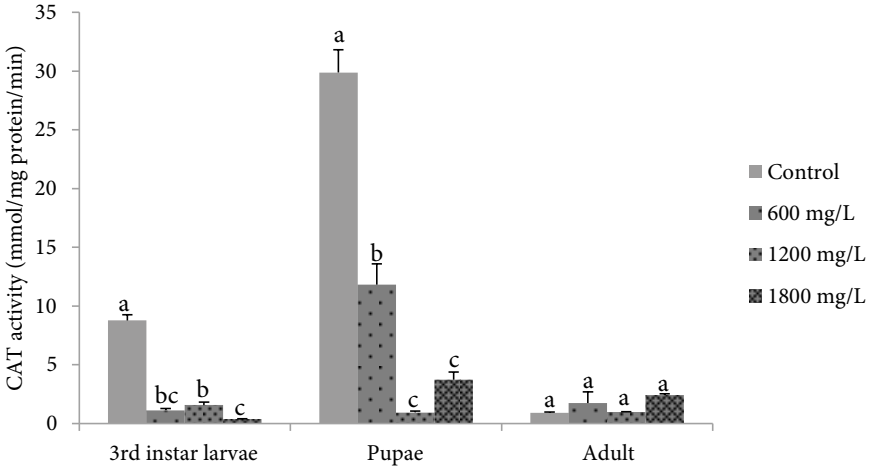

Figure 4. Effects of dietary streptomycin on CAT enzyme activities in 3rd instar larvae, pupae, and adults of *D. melanogaster*. Bars represent the means of four replicates. Means followed by different letters are significantly different ($p < 0.05$, LSD test).

and physiological mechanisms to cope with destructive effect of oxidative stress. To maintain tissue homeostasis under oxidative stress environments, insect can inhibit peroxidation of membrane phospholipids by use of lipid-binding proteins such as Materazzi which purges lipids from the hemolymph (Bailey et al., 2015; Li et al., 2020). In this study, the significant increase in the PCO content of all stages of *D. melanogaster* indicates intense protein damage with increasing streptomycin concentrations (especially 3rd instar larvae, $R = 0.96$; $p < 0.05$). Also, streptomycin concentrations significantly reduced MDA contents in all stages of insect except for the adult stage. Although it is known that proteins are less sensitive to the direct effect of ROS's than unsaturated fatty acids of lipids, toxic aldehydes such as MDA, glyoxal, 2-hydroxy heptanal, and 4-hydroxy-2-nonenal, end products of lipid peroxidation, can change protein structures by cross-linking with amino and carboxyl groups and increase the production of protein carbonyl compounds (Büyükgüzel and İçen, 2004; Büyükgüzel and Kalender, 2008; Li et al., 2020). In our study, the known end product of lipid peroxidation, MDA, might have interacted with functional groups of protein structures and caused the excess generation of protein carbonyls. This situation may clarify the considerable results of PCO and MDA contents relative to increasing dietary streptomycin concentrations in larval and pupal stages of insect. Also, in the study by Esther et al. (2015), active detoxification of nicotine in honey bees (*Apis mellifera*) was associated with increased energetic investment and also antioxidant and heat shock responses by the end of metabolomics and proteomic analyses. Similarly, the increased MDA and PCO contents on adults of *D. melanogaster* in the highest concentration of streptomycin may be originated from the adult metabolism which requiring higher oxidation processes for providing more energy than other development stages of insect due to its behavioral differences. We determined a significant reduction in SOD and CAT activities with a negative correlation in response to increased PCO content ($R^2 = 0.76$; $p < 0.01$). Similarly, Li et al. (2020) demonstrated that additional dietary antibiotics (vancomycin and chloramphenicol) significantly reduced the intestinal SOD and CAT activity of *Bombyx mori* L. Although the significant induction of antioxidant activities is a common situation in model insects exposed to the high concentration of antibiotics (Aslantürk et al., 2011; Büyükgüzel and Kayaoğlu, 2014; Aslan et al., 2019), however, several studies reported the decreasing in enzymatic antioxidant activity due to applied antibiotics on artificial diet of the insects (Hyršl et al., 2007; Büyükgüzel and Kalender, 2009; Thakur et al., 2016). The reduction in SOD and CAT activities in our study may have reasoned from the impairment of enzyme structures due to excessive accumulation of protein carbonyls with relative to increasing dietary streptomycin concentrations. In addition, the use of antibiotics on artificial diets of insects can lead to significant alternations on the intestinal flora that has an important role in a variety of biochemical processes including enzymatic antioxidant activity (Rosengaus et al., 2011; Thakur et al., 2016). Considering that streptomycin has a broad-range antibacterial activity, reduction in SOD and CAT activities in our results could be originated from the impact of dietary streptomycin on gut microflora which provides essential micronutrients such as Mn, Fe, Zn, and Cu that are required as a cofactor for the SOD activity (Rosengaus et al., 2011). These situations may explain the possible mechanisms of streptomycin to alter the enzyme activities in our study.

5. Conclusion

Several studies evaluated the effect of streptomycin on various model insects by the observing alternations on some life parameters of insect (Singh and House, 1970a, 1970b; Graf and Benz, 1970). Also, recent studies on this aim mentioned above demonstrated the physiological effects of dietary streptomycin on model insects with additional assays such as the content of oxidative stress indicators, antioxidant and digestive enzyme activities, and quantitative analysis of possible regulatory factors for intestinal homeostasis (Büyükgüzel and Kalender, 2009; Lin et al., 2015; Thakur et al., 2016). However, there is no information regarding the effect of dietary streptomycin on biochemical aspects of *D. melanogaster*. So, we showed that 600 mg/L, 1200 mg/L, and 1800 mg/L of dietary streptomycin concentrations negatively effect of survival and development parameters of *D. melanogaster*. Furthermore, with the biochemichal approaches, we demonstrated that experimented dietary streptomycin concentrations increased oxidative stress and lead to significant changes on important detoxifying antioxidant enzymes. Therefore, obtained results from the present study may yield significant considerations for the physiological effect of dietary streptomycin on *D. melanogaster* and contribute to adjusting the right proportion of streptomycin that is commonly used in artificial diets to prevent microbial contamination for successful insect rearing at laboratory conditions.

Conflict of interest

The authors declare that there are no conflicts of interest.

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