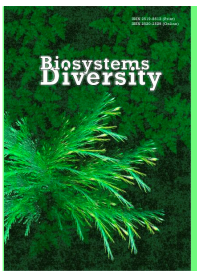

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The influence of synthetic food additives and surfactants on the body weight of larvae of *Tenebrio molitor* (Coleoptera, Tenebrionidae)

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The broad spectrum of negative effects of food additives and surfactants on living organisms and the environment in general indicate a necessity of a detailed study on this issue. The aim of this article is to evaluate the impact of food additives and surfactants in a concentration of 350 mg/kg of fodder on the body weight of third age *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae) larvae. A significant change in the body weight of *T. molitor* larvae was observed when they consumed a diet containing 350 mg/kg of sodium glutamate, sodium cyclamate and sodium benzoate. We observed a tendency towards increase in body weight after addition of the food colouring Allura Red, saccharin, benzoic acid, betaine, emulsifying wax, AOS and SLES, and also we observed a decrease in body weight after addition of Tartrazine and Indigo Carmine in the same concentration. Out of the 18 tested food additives, 3 significantly stimulated an increase in the body weight of third age *T. molitor* larvae, and 3 manifested the same effect at the level of tendency (stimulated an increase in mass on average by 43–58% over the 14-day experiment), and 2 caused decrease in the body weight of larvae. Also, the 4 studied surfactants manifested a tendency towards increase in the body weight of *T. molitor*. This study on the impact of food additives and surfactants on organisms of insects is of great significance for protecting rare species of insects.

Keywords: source materials; taste modifiers; food colourings; sweeteners; body weight; healthy diet

Introduction

The use of food additives in developed countries continues to increase (Bobyliov et al., 2014). Organic molecules used as synthetic food additives are heterogenous to living organisms. In humans or animal organisms they may cause various diseases (Ashida et al., 2000; Boyko and Brygadyrenko, 2017). Food additives enter the environment in different ways and become the food of litter and soil saprophages. A considerable amount of these substances which were unused in production are deposited on dumping grounds for solid municipal waste; the substances used in human food, after passing through the intestines, enter the municipal sewage treatment facilities. Therefore, the substances one way or another become included in the food of terrestrial and aquatic saprophage animals (Kroes and Kozianowski, 2002). On the whole, the impact of food additives on the organisms of insects remains virtually unstudied (Bobyliov et al., 2014; Boyko and Brygadyrenko, 2017).

Food additives are classified into colourings (E_{100}–E_{199}), preservatives (E_{200}–E_{299}), antioxidants (E_{300}–E_{399}), stabilizers (E_{400}–E_{499}), emulsifiers (E_{500}–E_{599}), modifiers of taste and flavor (E_{600}–E_{699}), antiflamings (E_{900}–E_{999}).

Preservatives are used for preventing decomposition and bacterial infestation of products. In the intestine of insects these substances are assumed to selectively eliminate certain species of microorganisms, thus causing intense breeding of other species of microorganisms (including those pathogenic for insects). Many preservatives have a broad negative impact on organisms of animals and humans (migraines, depression, irritable bowel syndrome, etc) (Moutinho et al., 2007). For example, consumption of monosodium glutamate causes increase in the body weight of animals as a result of increase in the amount of the consumed product. Also, decrease in the mass of kidneys, pancreas, spleen, and a hepatoxic effect caused by this substance have been determined (Alirezaei et al., 2011). The food industry uses colourings for improving the appearance of products, (Hansen et al., 1963; Maekawa et al., 1987; Gao et al., 2011). Unlike natural colourings, synthetic colourings are widely used due to their intense colouring properties, homogeneity, stability and low cost. Many synthetic colourings have a toxic impact following their long term consumption, causing digestive problems, allergic reactions, causing damage to the brain, kidneys, liver, and resulting in anomalies in development of progeny (Mannell et al., 1962; Ashida et al., 2000; Kroes and Kozianowski, 2002; Moutinho et al., 2007).

The most common environmental pollutants also include surfactants (Fitzhugh and Nelson, 1948). In the environment, they can be quickly destroyed by bacteria and fungi, or, on the contrary, can accumulate in the organisms of animals and in high concentrations (Nair, 1998). Most surfactants have a broad range of negative impact on the organisms of humans and animals. They take part in the rearrangement of other groups of pollutants (heavy metals, petrochemicals, pesticides, etc.), slow down the decomposition of carcinogens, inhibit the consumption of oxygen, etc. The sources of surfactants include detergents, the textile and chemical industries, oil production, agriculture, and public utilities.

Darkling beetles are usually saprophages or phytophages (Medvedev, 1968; Brygadyrenko and Nazimov, 2014, 2015; Nazimov and Brygadyrenko, 2013), and are able to digest many types of food, including food contaminated with additives. Most species of this family have adapted to life in conditions of insufficient moisture and feed on dry food with minimum content of moisture (Chen et al., 2004). The sheer abundance of many Tenebrionidae species, the slow development of their larvae, their ability to accumulate toxins from food in their body, make them convenient objects for ecotoxicological studies.

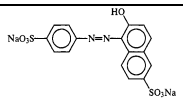
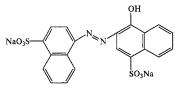
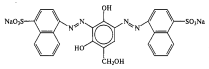
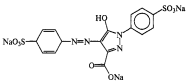
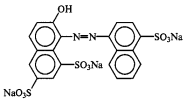
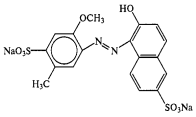
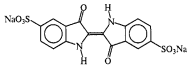
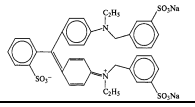
The broad spectrum of negative impacts of food additives and surfactants on living organisms and the environment in general,

indicates the necessity of scientific studies on the question. The objective of this research was to evaluate the impact of food additives and surfactants on the body weight of *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae) larvae.

Materials and methods

The experiment used third age *T. molitor* larvae. Before the experiment, larvae were kept in a common vessel with standard diet for their maintenance. The experiment was conducted in plastic cups of 0.2 l capacity with 37.5 ± 0.21 g of dry rolled oats in each cup (the accuracy of weighing the food and larvae equals 0.1 mg). Using a pipette, the substrate was moistened with aqueous solutions of the studied substances (the accuracy of measuring the volume of the solution equals 0.05 ml). As a result, the concentration of the substance in the food substrate equaled 350 mg/kg of fodder (Table 1–3). For the control group of beetles, the rolled oats were moistened with 3.75 ml of distilled water per 37.5 g of fodder.

Table 1
Food colourings used in the experiment

The name of the substance	Chemical formula	Structural formula	Properties	Usage in food industry
Sunset Yellow, E_{110}	$C_{16}H_{10}N_2O_7S_2Na_2$		orange powder, fully soluble in water	colouring of beverages, confectionary, etc.
Carmoisine, E_{122}	$C_{20}H_{12}N_2O_7S_2Na_2$		dark red powder or granules	colouring of beverages, confectionary, etc.
Chocolate Brown HT, E_{155}	$C_{27}H_{18}N_4O_9S_2Na_2$		brown powder or granules	colouring of confectionary
Tartrazine, E_{102}	$C_{16}H_9N_4OS_2Na_3$		yellow powder or granules	colouring of confectionary, ice cream, etc.
Ponceau 4R, E_{124}	$C_{20}H_{11}N_2O_{10}S_3Na_3$		red powder or granules	colouring of beverages, confectionary, etc.
Allura Red, E_{129}	$C_{18}H_{14}N_2O_8S_2Na_2$		dark red powder or granules	stamping of meat, colouring beverages, confectionary, etc.
Indigo Carmine, E_{132}	$C_{16}H_8N_2O_8S_2Na_2$		dark blue powder or granules	colouring of liquor, confectionary, etc.
Brilliant Blue, E_{133}	$C_{37}H_{34}N_2O_9S_3Na_2$		violate powder or granules	colouring of beverages, confectionary, etc.

The objective of the experiment was to compare the actions of the different food additives in equivalent concentrations and determining the variability of the body weight of *T. molitor* larvae in different variants of the experiment. There is no data on average lethal doses of the studied substances for insects. An experiment on the toxicity of food colourings for rats El-Wahab and Moram (2013) used Carmoisine in the concentration of 70 mg/kg of fodder. In our experiment the concentration of substances (350 mg/kg) corresponded to a five-fold dose of Carmoisine for rats, which was observed to have a toxic effect. Apart from the food additives, the experiment analyzed addition of surfactants to the diet; some of them are considered relatively safe chemical compounds.

After adding the solutions, the substrate was dried and uniformly mixed for uniform distribution of the active substance through the whole mass of fodder and prevention of mould. In each variant, the experiment was conducted in 8 cups on 8 individuals of third age *T. molitor* larvae (one in each cup). Before and after the 14-day experiment, all individuals were weighed. In the laboratory, we did our best to achieve constant temperature (+19…+20 ºC), illumination and air moisture. The cups with larvae were randomly placed on laboratory tables out of direct reach of sunrays.

Table 2
Food additives of different classes, used in the experiment

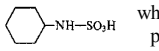
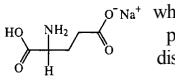
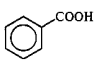
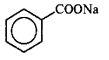
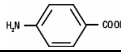
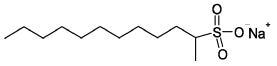
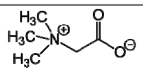
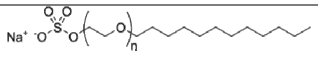
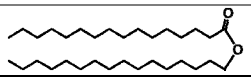
The name of the substance	Chemical formula	Structural formula	Properties	Usage in food industry
Saccharin, E_{954}	$C_7H_5NO_3S$		white crystalline powder with sweet and metallic taste	sweetener
Cyclamic acid, E_{952}	$C_6H_{13}NO_3S$		white crystalline powder with sweet taste	sweetener
Monosodium glutamate, E_{621}	$C_5H_8NaNO_4$		white crystalline powder with distinctive taste	taste and flavour modifier, salt substitute
Benzoic acid, E_{210}	$C_7H_6O_2$		white crystalline powder with distinctive flavour	preservative
Sodium benzoate, E_{211}	$C_7H_5O_2Na$		white crystalline powder with no flavour	preservative
4-Aminobenzoic acid	$C_7H_7NO_2$		white crystalline powder	vitamin additive

Table 3
Surfactants used in the experiment

The name of the substance	Structural formula	Properties	Usage
Sodium Alpha-Olefin Sulfonate (AOS)		white or light yellow powder with no flavour	production of cleaning substances, shampoos, cosmetics, etc.
Betaine		zwitterionic compound, trimethyl derivative of glycine	humidifiers and osmoprotectants
Sodium Lauryl Sulfate (SLES)		amphiphile substance in the form of partly-transparant gel	is basis for cleaning substances, shampoos, etc.
Cyclonette Wax		shaft or solid homogenous mass	cosmetology and production of medicines

Average initial body weight of *T. molitor* larvae equaled 41.4 ± 3.7 mg (x ± SD, n = 134), 14 days after the beginning of the experiment the mass of the larvae increased to 59.4 ± 7.6 mg.

The statistical analysis of the results was conducted in Statistica 8.0 (StatSoft Inc., USA). The differences between the selections were found using one-way ANOVA and considered significant at $P < 0.05$.

Results

During the experiment, we did not observe death or moulting of the larvae. The changes in the body weight of the *T. molitor* larvae in the 14-day laboratory experiment on adding different substances to the food substrates are presented in Figures 1, 2 and 3. Following the addition of food additives to the fodder, the most clearly manifested differences were observed in the groups fed with substrate containing monosodium glutamate ($P < 0.01$). A significant increase in body weight was also observed after addition of sodium cyclamate ($P < 0.05$) and sodium benzoate ($P < 0.05$).

An insignificant impact on the body weight of *T. molitor* larvae was caused by Allura Red, Tartrazine, Indigo Carmine, saccharin, benzoic acid, AOS, betaine, emulsifying wax and SLES ($P > 0.05$).

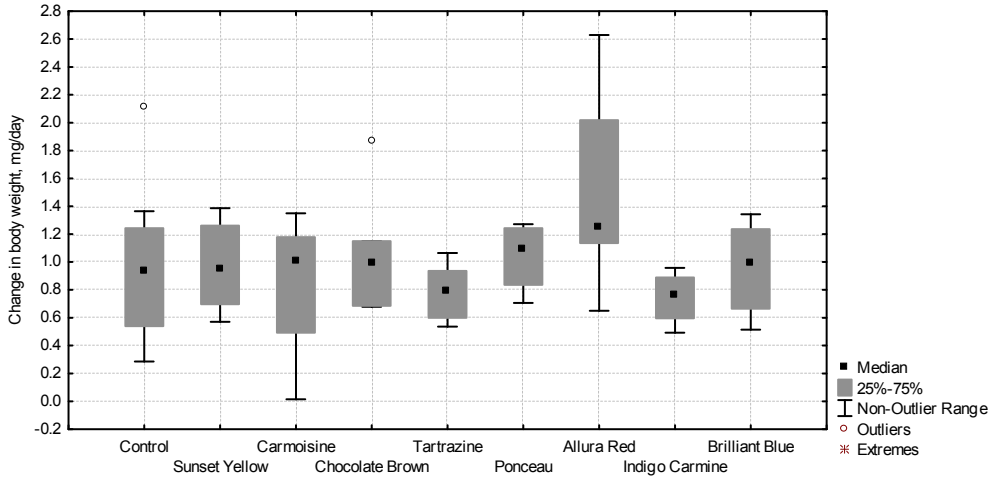


Fig. 1. Change in the body weight of *T. molitor* larvae over the 14-day experiment involving feeding with the substrate saturated with food colourings (n = 8)

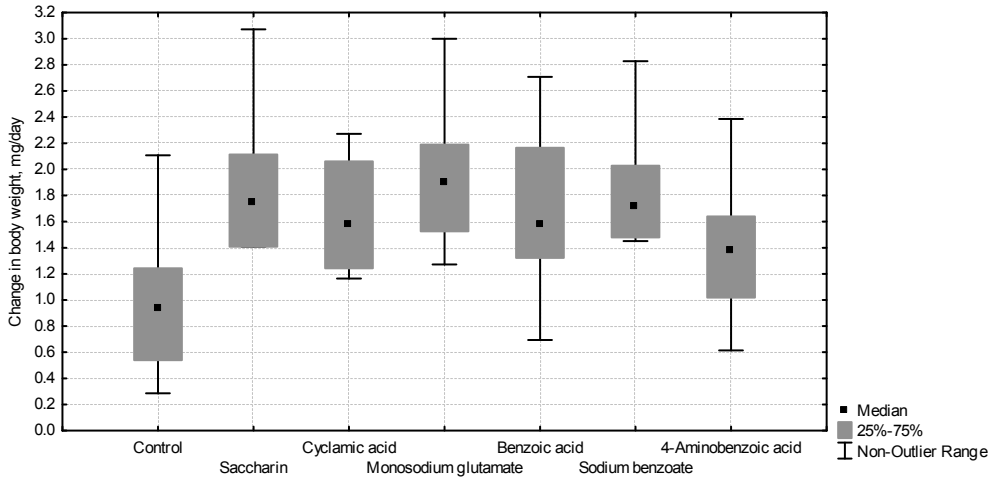


Fig. 2. Change in the body weight of *T. molitor* larvae over the 14-day experiment involving feeding with the substrate saturated with food additives (n = 8)

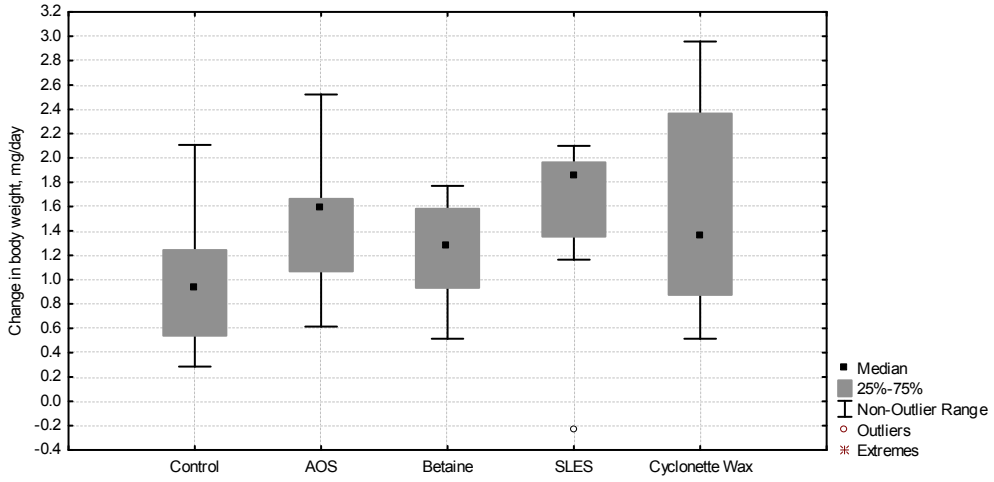


Fig. 3. Change in the body weight of *T. molitor* larvae over the 14-day experiment involving feeding with the substrate saturated with surfactants (n = 8)

Discussion

The results indicate that the concentrations included in the diet of *T. molitor* larvae caused increase in the body weight rather than decrease. Such effect can be explained by disorders in the insects' metabolism and increased consumption of food. Decrease in the body weight can be caused by binding of the food additives to the surface of bacterial cells in the insects' intestine, which leads to reduction in the population of bacteria and inhibition of the consumption capacity of the intestine.

There are few studies on the impact of food additives on the variability of the mass and other characteristics of insects. Nevertheless, many studies have been devoted to the toxic effect of the substances which we have studied on rats and some other vertebrates.

The toxicity of food colourings for rats (Spargue Dawley) has been studied by El-Wahab and Moram (2013). The experiments used the following colourings Brilliant Blue (124 mg/kg diet), Carmoisine (70 mg/kg), Tartrazine (75 mg/kg), and also all these colourings in combination with vanilin, propylene glycol and trans-Anethole. All colourings were observed to cause significant decrease in the body weight, concentration of hemoglobin and the number of erythrocytes. Also, a significant decrease was observed in the content of glutathiline, glutathione-S-transferase and superoxide dismutase in the blood and liver compared to the control. On the other hand, all groups were observed to have a significant increase in activity of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activity, concentration of bilirubin, urea, creatinine, total protein and albumin compared to the control. A similar effect on the main biochemical markers was observed by Amin et al. (2010) in their study of the impact of Tartrazine and Carmoisine. Reyes et al. (1996) studied the effect of colourings including Ponceau, Allura Red, Sunset Yellow, Tartrazine, Brilliant Blue and others on breathing in mitochondria. All tested colourings inhibited mitochondrial breathing. This inhibition significantly varied for mitochondrial protein: from 100% to 16% for Tartazine at the concentration of 0.1 mg.

The toxicity of the colouring Sunset Yellow was studied by Gaunt et al. (1974). The colouring in the dose of 1–6% over 80 weeks caused no negative effect on the rats' survival, growth rate and weight of organs. Doses up to 10 g/kg for rats and 6 g/kg for mice were received with no side effects (Gaunt et al., 1967). The colouring is not a carcinogen and does not cause a long term toxic effect. Hashem et al. (2010) studied the impact of peroral administration over 4 weeks of Sunset Yellow in doses of 47.3 and 157.5 mg/kg of body weight on rats. The colouring did not cause increase in the body weight and weight of spleen, nevertheless the mass of thymus and content of monocytes significantly decreased. Synthetic colourings used in doses 10 times higher than the daily intake inhibit the cell response, but not the humoral immune response. According to Gaunt (1969), feeding pigs with food containing Sunset Yellow in concentrations of 250, 500 and 1000 mg/kg over 98 days caused no change in increase in the body weight, hematological and other indicators in comparison with the control.

Saxena and Sharma (2014) studied the toxicity of Sunset Yellow and Tartrazine on Swiss Albino Rats *Rattus norvegicus* (Berkenhout, 1769). The concentration of this substance equaled 25, 50 and 75 mg/kg of body weight; the experiment lasted 30 days. No death among the rats was observed. The colourings caused increase in the body weight in all doses. Decrease in total protein and albumin, increase in the level of alkaline phosphatase and bilirubin were observed in all groups compared to the control.

Tartrazine is a yellow colouring, widely used in food products, medications and cosmetics. Acceptable daily intake (ADI) for humans is 0–7.5 mg/kg of body weight. Himri et al. (2013) studied the toxicity of Tartazine and its main metabolite, sulfanilic acid, for *Caenorhabditis elegans* (Maupas, 1900) nematodes and *Artemia salina* (Linnaeus, 1758) larvae. Among *C. elegans* nematodes, concentrations of Tartrazine 3 mM and sulfanilic acid 1 mM caused disorders in the cell cycle, without causing death. *A. salina* were affected by Tartazine and sulfanilic acid in concentrations 1, 2.5, 5, 7.5, 10, 25, 50, 75, 100 μg/ml for 48 hours. Tartrazine showed no toxicity, and sulfanilic acid had low toxicity (LC_{50} = 82.3 μg/ml). Paterson and Butler (1982) demonstrated that the effect of Tartazine in concentrations of 5–20 μg/ml induces chromosomal aberrations in the cells of *Muntiacus muntjak* (Zimmermann, 1780) fibroblasts. Carcinogenicity of Tartazine was studied by Maekawa et al. (1987) on F_{344} rats. Tartazine concentrations of 0.1% and 2.0% in potable water over 2 years caused no toxic damages, the colouring was not found to be a carcinogen. Davis et al. (1964) studied the chronic toxicity of Tartazine on rats and dogs. The experiments used Osborne-Mendel rats, which received diet containing Tartazine in 0, 0.5, 1.0, 2.0 and 5% for 2 years. Insignificant effects on growth were observed, but no effects on survival, hematology and body weight. The dogs received a diet containing Tartazine in 0.1% and 2% for 2 years. There were no symptoms of toxicity or hematologic anomalies observed. Gao et al. (2011) mentions the toxic effect of Tartazine on the memory and learning functions of rats and mice. A study of the neurotoxic impact of Tartazine was conducted by Mohamed et al. (2015). The rats were given Tartazine perorally in concentration of 500 mg/kg of body weight for 30 days. The analysis found significant decrease in concentrations of brain neurotransmitters, serious deficiency in the level of antioxidant biomarkers (superoxide dismutase, catalase and recovered glutathione), notable increase in the levels of malondialdehyde and numerous apoptotic cells in the cerebral cortex in comparison to other groups.

Mason et al. (1974) studied the toxicity of Carmoisine administered to mice in 0.01, 0.05, 0.25 and 1.25% concentrations during 80 weeks. They observed no changes in death rate, no increase in the body weight and mass of the organs. At 1.25% concentration, a light anemia was observed. Carcinogenic potential of Carmoisine is absent at 1.25% concentration. Studying metabolism of Carmoisine in rats, mice and guinea pigs, Phillips et al. (1987) found that after administration of one peroral dose of 0.5 or 50 mg/kg of body weight, practically the entire dose is removed through the feces during 72 hours.

Drake et al. (1978) studied the toxicity of the colouring Chocolate Brown HT in 0, 0.01, 0.1 and 0.5% concentrations on mice for 80 weeks. Insignificant decrease in the body mass and heart of the males was observed at the 0.5% concentration. Carcinogenic effect at doses of up to 700 mg/kg·day was not observed. In a similar study, Chambers et al. (1966) gave rats Chocolate Brown HT in 0, 0.02, 0.06, 0.2, 0.6, 1.0 and 2.0% concentrations for 90 days. Almost no side effects were observed, and the gain in body weight did not change, although, an insignificantly reduced growth rate among males was observed at 1% and 2% concentrations. The analysis found decrease in the content of hemoglobin, erythrocytes and hematocrit.

The toxicity of the colouring Ponceau was studied by Hansen et al. (1963) and Davis et al. (1966). They used 0.5, 1.0, 2.0 and 5.0% concentrations. Over 2 weeks, these concentrations caused a high death rate of rats, reduced their growth rate, increased the mass of the liver and kidneys, and caused the formation of malignant and benign tumours. Loss of weight and high death rate was observed among dogs at 1% and 2% concentrations. A study of the acute toxicity of Ponceau was conducted on mice and rats by Gaunt et al. (1967). Similar studies were conducted by Brantom et al. (1987): LD_{50} was higher than 8 g/kg orally in both species. Feeding rats with 0.0, 0.5, 1.0 and 2.0% concentrations of the colouring for 90 days caused no negative effects. At 2% concentration of Ponceau, the females were observed to have an insignificant decrease in the number of erythrocytes, and also increased activity of transaminases in the blood serum. The level of absence of the effect for rats is 500 mg/kg a day. Meyer and Hansen (1975) conducted a study of embryotoxicity of Ponceau for Wistar rats. The colouring was given using a stomach pump from 1 to 20th period of pregnancy in doses of 0, 1000, 2000 and 4000 mg/kg a day. The analysis found no negative effects on the fetus.

Borzelleca et al. (1989) studied the toxicity of the colouring Allura Red for Sprague-Dawley rats. The colouring was administered in 0.0, 0.37, 1.39 and 5.19% concentrations during the period of mating, feeding and lactation. No side effects related to the colouring were observed, except decrease in the body weight among females at high doses. The absence of side effects was observed at 2829 mg/kg·day among males and 901 mg/kg·day among females. A similar study was conducted by Vorhees et al. (1983). Physical and behavioural toxicity of the colouring was observed at concentrations of up to 10% of diet. A study of genotoxicity of Allura Red was conducted on mice by Honma (2015). At maximum accepted doses, genotoxic or mutagenic effects were not observed.

Hooson et al. (1975) conducted an 80 week study on the toxicity of Indigo Carmine in 0.2, 0.4, 0.8 and 1.6% concentrations on mice. No effect on the death rate, gain in body weight, mass of organs and results of histopathological examination was found. An insignificant

anemia was observed at 0.8% and 1.6% concentrations. This content of Indigo Carmine does not cause carcinogenic effect. Studying active transport of insects, Maddrell et al. (1974) determined that Indigo Carmine concentration is subject to passive penetrability of malpighian tubule walls. The speed of transfer of the colouring in insects is related to speed of liquid withdrawal.

The toxicity of the colouring Brilliant Blue was studied by Mannell et al. (1962). The rats were fed with the colouring in concentrations of 0.03, 0.30 and 3.00% of the diet for 75 weeks. The negative effect of the substance on growth was not observed.

The toxicity of saccharin was studied on rats by Munro et al. (1975). The diet contained sodium saccharin in concentrations of 0, 90, 270, 810 and 2430 mg/kg·day of saccharin. Bladder tumours were found at doses of 90 and 810 mg/kg. Decrease in the body weight and in life expectancy was observed among male rats. Taylor et al. (1980) studied chronic toxicity of sodium saccharin for rats. They used a diet with concentrations of 0, 0.01, 0.1, 1.0, 5.0 and 7.5%. Effects on hematological indicators, organ mass and survival were not observed. Increase in the frequency of the bladder hyperplasia was observed among male rats, which received 7.5% of sodium saccharin. Fitzhugh et al. (1951) provide data on insignificant effects of toxicity of saccharin for rats at a dose of 5%.

Studying the toxicity of sweeteners for *Mesocricetus auratus* (Waterhouse, 1839) hamsters, Althoff et al. (1975) found that chronic administration of saccharin and sodium and calcium cyclomates caused no tumours even at maximum doses. Luini et al. (1981) report that rats, which received saccharin in 1.0, 2.5 and 5.0% concentrations for 54 days, were observed to have inhibition of formation of primary humoral antibodies. Zhang et al. (2017) report high indicators of death rate of *Solenopsis invicta* (Buren, 1972) ants under the impact of saccharin and state that it could be used as a low cost insecticide.

The toxicity of sodium cyclamate was studied on mice by Brantom et al. (1973). The substance was used in concentrations of 0.7, 1.75, 3.5 and 7.0%. A decrease in gain of body weight and mild anemia were observed. Effects related to the death rate, mass of organs and histological characteristics were absent. At a sodium cyclamate content of 7%, no carcinogenic effect was observed. Ershoff (1972) studied the toxicity of natrium cyclamate for rats using concentrations of 2.5, 5.0 and 10.0% of the diet. He observed decrease in the mass, reduced growth rate, random alopecia and diarrhea. The concentration of 10% was lethal. The effects were manifested three days after the feeding. Friedman et al. (1972) studied the toxic effect of sodium cyclamates in 0.4–10.0% concentrations on rats during 101 weeks. They observed decrease in the survival rate, disorders of kidneys (nephrocalcinosis and polyposis) and bladder (hyperplasia).

The toxicity of the mixture "cyclamate + saccharin" for rats was studied by Oser et al. (1975). The study used the mixture in concentrations of 500, 1120 and 2500 mg/kg of body weight. The only result of the study was carcinomas in the bladders of the rats which received the maximum dose. Roe et al. (1970) report absence of observed toxic effects among mice which were fed with sodium cyclamate in 5% concentration during 18 months. Taylor et al. (1968) studied the toxicity of sodium cyclamate and saccharin for mice, rats and dogs. Administration of 2% of cyclamate or mixture of cyclamate and saccharin (or intragastrically 1 g/kg•day) caused no effects on the rats. Intragastic administration of cyclamate (4 g/kg·day) had no effects on the dogs. Decrease in food consumption at high levels was observed among some of the rats. Adding up to 3% sodium cyclamate to the diet of rats or 0.5 g/kg·day to the diet of dogs during 11 months was observed to have no negative effects.

The toxicity of sodium glutamate was studied by Mushahwar and Koeppe (1971). Intragastic injection of 4 mg/g to baby rats caused increase in glutamine in the brain. The toxicity manifested in spasms. Farombi and Onyema (2006) reported an increase in the formation of malonic aldehyde in the liver, kidneys and brain of rats. Eweka and Om'Iniabohs (2008) studied the impact of sodium glutamate on rats' kidneys. The animals received 3 and 6 g of sodium glutamate for 14 days. A negative effect was found, disorders in the cell structure and cell necrosis of the kidneys.

According to Olney (1969), hypodermic injection of sodium glutamate to new born mice caused brain necrosis. Mature individuals were observed to have retarded skeletal development, obesity, infertility, pathologies of the organs of the endocrine system. Malik and Ahluwalia (1994) studied the toxicity of sodium glutamate for concentrations 2, 4 and 8 mg/kg of body weight, which was injected subdermally to mice during 6 days: carbohydrate metabolism tends to lipogenesis and results in hyperlipidemia. Injection of 4 and 8 mg/g of body weight significantly increased the activity of glutathione reductase, glutathione-S-transferase and glutathione peroxydase. Concentration of sodium glutamate above 4 mg/g of body weight causes oxidative stress and maintains the level of glutathione. This was achieved by increase in the activity of its metabolizing enzymes.

Pszczolkowski and Brown (2002, 2004) studied the impact of sodium glutamate on *Cydia pomonella* (Linnaeus, 1758) larvae which fed on the leaves of apple-trees. Addition of 25 ppm of sodium glutamate increased the food consumption up to 20–30%. Leaves with sodium glutamate (0.05 and 0.10 mg/ml) increased the consumption of leaves up to 60% among the first age larvae. The effect of MSG also speeded up the moulting to the second age (Pszczolkowski et al., 2002). MSG is a feeding stimulator and intensifies the toxic properties of spinosad, which is a good regulator of pest insect populations.

The toxicity of benzoic acid was studied by Nair (2001). Benzoic acid can cause pathologies in the development of hamsters. Nevertheless, the studies on rats and mice found no negative effects on reproductive function, development, and no genotoxicity and carcinogenicity. Studying the acaricidal activity of benzoic acid in the cortex of roots of *Paeonia suffruticosa* (Andrews, 1804) against *Tyrophagus putrescentiae* (Schrank, 1781) acari, Tak et al. (2006) determined that it equals 4,80 μg/sm^2.

A study on the toxicity of sodium benzoate for *Danio rerio* (Hamilton, 1822) was conducted by Tsay et al. (2007): LD_{50} equaled 1450 ppm. These fishes were observed to have manifestations of neurotoxicity and nephrotoxicity. O'Connor et al. (1987) reported that sodium benzoate inhibits the synthesis of urea, which increases the death rate.

The toxicity of α-olefin sulphonate for rats was studied by Hunter and Benson (1976). The animals were given the substance in 1000, 2500 and 5000 ppm concentrations for 2 years. No negative effects or decrease in survival rate were observed. Decrease in weight gain and in food consumption was observed in the group, which was given 5000 ppm. According to Nair (1998) peroral value of LD_{50} for Sodium Alpha-Olefin Sulfonates equaled 1.3–2.4 g/kg for rats and 2.5–4.3 g/kg for mice. No effects were observed among mice at concentrations below 0.5–1.0 g/kg. Fetal anomalies were observed only at the doses toxic to the mother. Fitzhug and Nelson (1948) reported on the toxicity of sodium dodecyl sulfate. Peroral administration of Sodium dodecyl sulfate for two years caused no toxic effect at 1% dose. Sodium dodecyl sulfate in 4% concentration caused reduced growth rate.

Betaine is not toxic and is a typical component of intermediary metabolism. It is used in production of cosmetics and surfactants. Ramakrishna et al. (1998) showed that betaine is efficient in memory recovery (in case of memory loss caused by Al^{3+}) through increase in the level of choline. Betaine can prevent formation of plaques in early stages of Alzheimer's disease. Betaine is a natural product of beet (*Beta vulgaris* Linnaeus, 1758), and in the process of production of sugar from beet this product can therefore be efficient in treating diseases related to dysfunctions of the cholinergic system, which cause memory loss. Alirezaei et al. (2011) report that betaine can prevent hyperhomocysteinemia and oxidative stress. The protective effect of betaine is related to its ability to strengthen the cells of the cerebellum membrane by intensification of antioxidative enzymes.

Despite numerous studies devoted to the toxic impact of food additives and surfactants on mammals, similar data on insects and other invertebrate organisms is absent. Our research demonstrates that for insects the effects of food additives can be different from those for animals and require further study. Also, *T. molitor* is a good model for studying the impact of compounds introduced into food.

Conclusion

A significant change in the body weight of *T. molitor* larvae was caused by adding 350 mg/kg of sodium glutamate, cyclamate and sodium benzoate to their food. A tendency towards increase in body weight was observed with addition of Allura Red, saccharin, benzoic acid, betaine, emulsifying wax, AOS and SLES, and decrease in the body weight was observed with addition of the same concentrations of Tartrazine and Indigo Carmine.

Out of the 18 studied additives, 3 significantly stimulated increase in the body weight of third age *T. molitor* larvae, 3 manifested these capacities at the level of tendency (stimulated increase in the body weight on average by 43–58% over the 14-day experiment), and 2 caused decrease in the body weight of the larvae. Also, the 4 studied surfactants showed a tendency towards increasing the body weight of *T. molitor*. The study on the impact of food additives and surfactants on the organisms of insects is of great significance for protecting rare species of insects.

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