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BROCCOLI SPROUTS MODULATE THE EFFECT OF CARBOHYDRATES ON DROSOPHILA PUPATION: ACCELERATION ON HIGH-SUGAR DIETS, DELAY ON LOW-SUGAR DIETS

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Abstract: Diet-induced obesity has become a global health concern, driving the search for effective preventive measures and functional food interventions. This study evaluates the potential of broccoli sprouts as a functional supplement to counteract the effects of an obesogenic diet in *Drosophila melanogaster*, a widely accepted model organism for studying metabolic diseases. The research examined the influence of broccoli sprout supplementation on larval pupation rates, adult fly body mass, and the content of proteins and triacylglycerols (TAGs) in adult flies. Larvae were fed diets with varying sucrose concentrations, with or without broccoli sprouts. Results showed that broccoli sprouts independently slowed larval development, irrespective of sucrose-induced delays. While no significant effects on body mass were observed in adult males, female flies exhibited reduced body mass in some broccoli-supplemented groups. Metabolically, TAG levels and protein content varied across diets, with high sucrose diets generally increasing TAG levels. However, broccoli sprouts mitigated some adverse metabolic changes, particularly in male flies on high-sucrose diets. These findings suggest that broccoli sprouts impact developmental timing in *Drosophila* and highlight their potential modulatory effects on metabolic markers associated with diet-induced obesity. The study underscores the need for further investigation into optimal doses and the underlying mechanisms of broccoli's bioactive compounds in mitigating obesity-related metabolic alterations. Future research should also address parameters such as food intake, survival rates, and oxidative stress markers to understand the broader implications of broccoli supplementation better.

Keywords: broccoli sprouts, fruit fly, functional food, obesity, triacylglycerols.

1. INTRODUCTION

Hypercaloric diets are a common feature of the modern world (Bayliak et al. 2022). Fast food has been endorsed due to its accessibility, good advertising, quick service, long-term storage, ease of preparation, and taste qualities. Unfortunately, such meals were not designed to keep consumers healthier. A combination of junk food, overeating behaviour, heredity, and a sedentary lifestyle is threatened by the development of metabolic disorders like obesity and its comorbidities (Lushchak et al. 2023).

Obesity is not only recognized as excessive accumulation of storage lipids. This pathological condition is recognized as a non-communicative disease that can indirectly lead to mortality. Due to the aggravation of oxidative stress and inflammation, obesity can lead to hyperglycaemia, hypertension, atherosclerosis, hepatic diseases, etc. (Bayliak et al. 2019). Patients and physicians mostly deal with consequences of the obesity while scientists study various approaches to prevent

the development of obesity. Nowadays, functional food supplements with bioactive compounds are considered as a remedy for the prevention and treatment of obesity.

Broccoli (*Brassica oleracea var. italica*) is a well-known verdure that is used for cooking and investigations due to its healthy properties. Broccoli is rich in polyphenols and vitamin compounds with antioxidant action in addition to bioactive compounds that stimulate defensive mechanisms (Le et al. 2019). The main interest is related to 3-5 days-old sprouts (sprouted seeds) and isothiocyanate sulforaphane (SFN), one of the most known component of broccoli sprouts (Houghton 2019). Broccoli sprouts are proposed as an available, cheap, simple functional food to treat obesity although the effectiveness of therapeutic usage, as well as optimal dose consumption, remains unclear (Ivanochko et al., 2024).

Fruit fly (*Drosophila melanogaster*) is a popular model organism to study obesity (Semaniuk et al. 2018, Rovenko et al. 2015a). Despite the visual difference between flies and humans, they both had similar metabolic pathways, and cell signalling pathways, and about 75% of disease-relating genes in humans have homologs in *Drosophila* (Semaniuk et al. 2021). They both are at the risk of development of obesity by sucrose increase in the diet (Rovenko et al. 2015b). Nevertheless, the obtained results should be properly interpreted in humans, because of the difference in nutritional needs, appropriate food sources, development, and physiology between insects and mammals (Rovenko et al. 2015b).

Broccoli preparations are well-studied for treating obesity (Martins et al. 2022). Although there is an excess of publications about broccoli supplementation in rodents and humans, the studies on the benefits of broccoli consumption for fruit flies are limited (Li et al. 2008)(Lyles et al. 2021). This work aimed to determine the effect of supplementation with broccoli sprouts on the pupation of larvae, body mass of imago flies, their body protein and triacylglycerols content in the body of flies fed foods with different sucrose content.

2. MATERIALS AND METHODS

2.1. Cultivation of broccoli sprouts and preparation of sprouts as a food supplement

Broccoli (*Brassica oleracea var. italica*, cultivar calabrese) was chosen as a plant material for the experiment. Seeds were purchased from SemyaSvet Company (Odesa, Ukraine). They were planted on a moist cotton substrate in sealed plastic transparent containers. Cultivation was kept under conditions as follows: 12h:12h (light: dark) photoperiod, 25°C, and 50-60% humidity. The three-day-old broccoli sprouts were harvested and quickly frozen in liquid nitrogen and stored at -20°C until food preparation procedures. Plant material was ground using mortar and pestle in a ratio of 1:5 (g of broccoli to ml of water) right before addition to fly food.

2.2. Flies and rearing conditions

The experiment was conducted with *Drosophila melanogaster* line w^{1118}. The stock flies were obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537, Indiana University, USA). The parental generation of flies was reared on food containing 5% sucrose, 5% dry yeast (PJSC Enzym, Lviv), 1% agar and 0.18% methyl parahydroxybenzoic acid to inhibit mold growth. All flies were maintained at 25 °C, 55-60% humidity and a 12:12-hour light cycle.

The offspring eggs were placed on six experimental media. The first medium contained a basal diet consisting of 2.5% sucrose and 2.5% dry yeast (denoted as BD2.5). The second medium contained the previously listed components with the addition of 5% broccoli sprouts extract (denoted as BS2.5). The third medium contained 12.5% sucrose, named the high sucrose diet (HS12.5). The fourth medium was a combination of a high sucrose diet with the addition of 5% broccoli (BS12.5). The fifth medium contained the highest content of sucrose (32.5%) and yeast (7.5%) (HS32.5). And sixth medium was a combination of the previous one with 5% broccoli sprouts (BS32.5).

2.3. Pupation rate and imago collection

The dynamics of fly development was assessed by counting the number of pupae twice a day (9 am and 6 pm), starting from the 6th day after egg laying. The time of 9 am on the 6th day was presented as zero hour of larval development. The total number of pupae formed for each group was expressed as 100%. After hatching, five-day flies were used for further experiments (Bayliak et al., 2018). Flies were anaesthetized using light CO_2 anesthesia, separated by sex and frozen at -20°C for further biochemical determination.

2.4. Determination of wet body mass

Measuring of fly wet body mass was performed before the procedure of homogenization for the parameters described below. Ten flies were weighted on aluminium foil using RADWAG AS220.R2 analytical scales (Radom, Poland) and the mass value was divided by the number of flies. The results are presented as the mean mass of one fly in milligrams (mg).

2.5. Determination of triacylglycerol and protein levels

An assay of triacylglycerols (TAG) was performed by the modified protocol described earlier (Yurkevych et al. 2020, Demianchuk et al. 2023). For the procedure, 8-12 frozen flies (to keep approximately 6.5 mg of weight) were transferred into microcentrifuge tubes with sterile pestles and homogenized in a ratio 1:50 (mg flies: µl buffer) using cooled buffered phosphate saline (PBST) buffer (pH 7.4) containing (final concentrations) 10 мM $Na_2HPO_4{\times}2\ H_2O$, 2 мM KH_2PO_4, 137 мM NaCl, 2,7 mM KCl, and 0.05% Triton X-100. After homogenization, total protein content was determined in homogenates using Bradford assay (Bradford 1976).

Next, homogenates were incubated at 70°C for 10 min for heat inactivation of endogenous enzymes. After that, samples were centrifuged (15 min, 16000 g, and 21°C). TAG levels were measured using a commercial diagnostic kit (“Reagent”, Dnipro, Ukraine) and following the manufacturer’s recommendations. Samples were incubated for 20 min at 37 °C. Absorption was determined at 540 nm using a plastic cuvette and Ulab 102UV spectrophotometer (Ulab Scientific Instruments, Nanjing, China). Standard TAG solutions provided by the manufacturer were used for the calibration curve building. TAG levels were determined as milligrams per gram of wet mass (mg/gwm).

2.6 Statistical analysis and visualization

For statistical processing of the results, the program "Microsoft Excel" and "GraphPad Prism 8" were used. Results were presented as mean and standard error of the mean (SEM) for each set of data. Pupation curves were compared using the Log-rank (Mantel-Cox) test. P values < 0.05 were considered as a criterion for significant differences between the experimental groups.

3. RESULTS AND DISCUSSION

The rate of pupation of larvae on experimental diets is presented in Fig. 1. Analysis showed slower pupation of HS32.5 larvae compared to the control BD2.5 group. Similarly, the pupation of HS12.5 larvae was slower than BD2.5, making both HS12.5 and HS32.5 the slowest pupation groups. Interestingly, BS2.5 larvae showed an intermediate rate of pupation between BD2.5 and HS32.5 groups, suggesting that broccoli compounds are potentially unfavorable for fruit fly development. Next, we did not determine any significant difference between HS12.5 and BS12.5, suggesting that the addition of broccoli sprouts extract to a high sucrose diet did not prevent the slowed rate of pupation. It seems that broccoli and sucrose diet at this combination of concentrations did not influence each other's effect. In addition, a significant difference was determined between the HS32.5 and BS32.5 groups. In particular, the impact of high sucrose alone was more harmful for pupation than in combination with broccoli.

It is considered that glucose is toxic at high doses for flies. In addition, fructose is more tolerated at higher doses by flies, but it promotes obesity as well as glucose (Rovenko et al. 2015b). During development larvae consume high amounts of low-sucrose food. An experimental medium is important to provide the appropriate level of carbohydrate intake to the body. It was determined, that a high-sucrose diet promotes juvenile hormone synthesis and release, thus suppressing pupation (Rovenko et al. 2015a). Although there is a lack of studies about the consumption of broccoli preparation by flies, we suggested that bioactive compounds of broccoli sprouts affected the development mechanisms of larvae. It is important to mention that isothiocyanates were shown to be beneficial for mammals, rodents and humans, while toxic for herbivores, potentially insects and flies. Those compounds are part of defense mechanisms against plant-eating pests (Hopkins et al. 2009, Mortazavi et al. 2008). This state partially explained why larvae on broccoli food had slower pupation. However, it was not clear why the harmful effect of broccoli and sucrose was not amplified together. In addition, the same content of broccoli extract affected pupation at the same level despite a combination with three different sucrose concentrations, while two high-sucrose diets showed the slowest pupation in a dose-dependent manner. Finally, pupation on low-sucrose food and such medium with broccoli demonstrated different results.

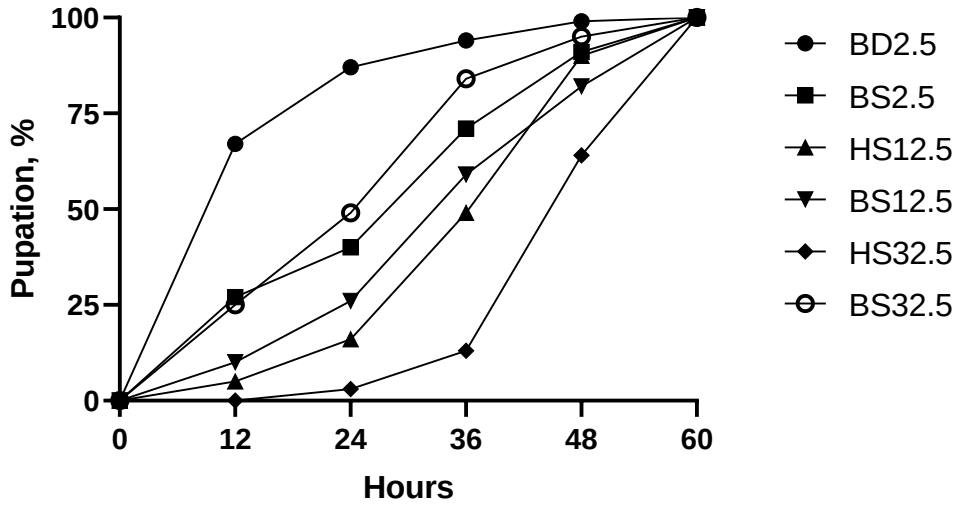

Figure 1. Pupation of larvae on diets containing different concentrations of sucrose - 2.5%, 12.5% and 32.5% without (BD, HS) or with the addition of 5% broccoli sprouts (BS), n=3

To determine metabolic changes and the development of obesity-like symptoms in *Drosophila* several parameters were determined and calculated per single fly. At first, the wet body mass of flies had a trend to be higher in high-sucrose groups males (HS12.5 and HS32.5) compared to BD2.5 ones (Tab. 1). Next, the presence of broccoli extract did not critically modulate body mass as a response to high-sucrose food or to low-sucrose basic food. Moreover, HS32.5 and BS32.5 had the highest body mass.

The total protein parameter was taken into analysis to characterize the possibility of general metabolic changes (Table 1). The HS12.5 male group had the lowest total protein content (7.91 mg/gwm), while in the BS12.5 group this parameter was higher compared to BD2.5 or BS2.5 groups. Interestingly, the total protein content of HS32.5 was 11.37 mg/gwm and this value was the highest among experimental groups. We modified the HS32.5 medium to contain more yeasts as a nutritional protein source and an additional source of energy as well. This partially explains why experimental flies on this food had higher protein values. However, the BS32.5 value was 9.49 mg/gwm which was lower than the BS12.5 value (9.93 mg/gwm) despite higher yeast content. Literature data claims that the protein content

of fly bodies is not affected by the concentration of carbohydrates in the diet (Rovenko et al. 2015b). In our experiment we received opposite results – from one side the protein content was lower in HS12.5 flies and from another, it was higher in BS12.5, HS32.5 and BS32.5 groups.

Triacylglycerols are one of the main metabolites that react to the caloric change in diet (Table 1). This parameter was 3.51 mg/gwm in the BD2.5 (control) group. In BS2.5 flies, this value was lower (3.17 mg/gwm). In the HS12.5 group, Tag levels were determined as 3.18 mg/gwm and in BS12.5 TAG levels were 3.64 mg/gwm. Like with protein levels, the TAG value of HS12.5 was lower compared to the BD2.5 group. In HS32.5, we determined a higher level of TAG than in the BS32.5 group. The ratio of TAG: protein was the highest in the HS12.5, HS32.5 and BS32.5 groups (Table 1). This parameter was similar to BD2.5 in the BS2.5 and BS12.5 groups.

Gain of body mass, higher lipid content and lower protein content were described as features of obesity development in flies (Rovenko et al. 2015a). In our study with male flies, we observed only a trend of enlargement of wet body mass.

Table 1. Body mass and some metabolite levels in male flies reared on different diets

Parameter/Group	BD2.5	BS2.5	HS12.5	BS12.5	HS32.5	BS32.5
Wet body mass, mg	0.45	0.50	0.53	0.50	0.58	0.62
Total protein, mg/gwm	8.92	8.83	7.91	9.93	11.37	9.49
TAG, mg/gwm	3.51	3.17	3.18	3.64	5.07	4.72
TAG: Protein ratio	0.39	0.35	0.44	0.36	0.44	0.49

Note. Data are from one measurement (6-8 flies).

Analysis of female groups showed that flies had higher mass than males as a part of normal *Drosophila* sex physiology (Table 2) (Mathews et al. 2017, Rovenko et al. 2015a). However, we observed the stability of wet body mass among experimental groups. Lower body mass values were observed in the BS2.5 (0.72 mg) and BS12.5 (0.67 mg) groups compared to the BD2.5 and HS12.5 groups, respectively. This fact testified about the small modulation effect of broccoli sprouts extract on the body mass of female flies during consumption of low and high sucrose food. Such modulation was not observed between the HS32.5 and BS32.5 groups.

The total protein content in the female BD2.5 group was 10.83 mg/gwm. In the BS2.5 and BS32.5 groups, this value was similar to BD2.5. In HS12.5, BS12.5 and HS32.5 groups the protein values were slightly lower.

TAG content in females of the BD2.5 group was the lowest among experimental female groups – 3.71 mg/gwm (Table 2). In BS32.5 this value was the highest (5.47 mg/gwm). Broccoli supplementation demonstrated a light higher TAG content in BS2.5 compared to BD2.5 and in BS32.5 compared to HS32.5 groups. However, HS12.5 and BS12.5 groups demonstrated opposite results. TAG: protein ratio values showed a similar pattern, as was determined for TAG.

Table 2. Body mass and some metabolite levels in male flies reared on different diets

Parameter/Group	BD2.5	BS2.5	HS12.5	BS12.5	HS32.5	BS32.5
Wet body mass, mg	0.82	0.72	0.85	0.67	0.80	0.85
Total protein, mg/gwm	10.83	10.63	9.60	9.83	9.46	10.86
TAG, mg/gwm	3.71	4.01	4.63	4.21	4.51	5.47
TAG: Protein ratio	0.34	0.37	0.48	0.42	0.47	0.50

Note. Data are from one measurement (6-8 flies).

Previously, sucrose concentration in larval food was excluded as the factor that affects wet body mass (Rovenko et al. 2015a). However, in our study wet body mass of fly males was slightly higher in groups with high sucrose medium compared to low sucrose medium. The wet body mass of females remained unchanged in response to high sucrose diet, while broccoli supplementation had a trend to lower body mass on low sugar or high sugar medium. The total protein content was not associated with sucrose or yeast content in females. In males, higher protein content was determined on three high sucrose diets with one exception. In males, TAG content was higher on both, without or with broccoli sprouts, 32.5% sucrose mediums, while in females all TAG values were higher than those on the basal diet one. TAG: protein ratio between female groups was in line with TAG content.

4. CONCLUSIONS AND PERSPECTIVES

This study demonstrated that broccoli sprouts added to *D. melanogaster* food affected the rate of larval pupation but had no critical effects on the studied metabolic parameters in imago. We established slower pupation due to a sucrose content in food in a dose-dependent manner. Slower pupation was determined with the addition of broccoli sprouts but it was not clear if different amounts of broccoli sprouts could cause changes in the rate of pupation, as we chose only 5% content of the sprouts. This suggestion is an avenue for further investigation. A high sucrose diet (HS32.5) caused light metabolic changes in protein and TAG contents compared to a low sucrose diet (BD2.5) in male experimental groups. Those changes were attenuated in combination with broccoli sprouts (BS32.5). Such effects were absent in females.

Further research is needed to determine whether broccoli as part of a high sucrose medium affects larval food intake. It is also interesting to establish some parameters such as pupal survival or to prove the harmful effects of broccoli compounds against larvae as plant pests. From a biochemical view, it would be necessary to determine fruit fly response to the consumption of broccoli such as markers of oxidative stress and parameters of glutathione-dependent antioxidant enzymes, which can be affected by diet-induced obesity or bioactive compounds of broccoli sprouts.

Conflict of interest

The authors declare that they have no conflict of interest.

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Іваночко Мар'ян, Харук Святослав, Лилик Марія, Господарьов Дмитро. Проростки броколі сповільнювали заляльковування личинок *Drosophila* замість усунення ожиріння, спричиненого їжею. *Журнал Прикарпатського університету імені Василя Стефаника. Біологія*, Том **11** (2024), С.115–С.122.

Ожиріння, спричинене дієтою, є частим явищем у сучасному світі. Для запобігання та усунення ожиріння та його супутніх захворювань вчені досліджують використання функціональних продуктів харчування, таких як проростки броколі. Плодова мушка є популярною моделлю ожиріння, але ця лабораторна тварина ще не використовувалася для дослідження властивостей броколі проти ожиріння. Метою цього дослідження було визначити вплив проростків броколі на заляльковування, масу тіла дорослої особини мух, вміст білка і триацилгліцеролів в тілі мух, які перебували на ліпогенній дієті. Результати продемонстрували здатність препарату броколі уповільнювати розвиток личинок. Цей ефект був незалежним від уповільнення розвитку до лялечки, спричиненого високим вмістом сахарози. Також були встановлені деякі відмінності рівнів білку та ТАГ в мух, які споживали броколі чи їжу з високим вмістом сахарози.

Ключові слова: проростки броколі, плодова мушка, функціональна їжа, ожиріння, триацидгліцероли.