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INFLUENCE OF AMYLOSE STARCH ON DEVELOPMENT AND LIFESPAN OF FRUIT FLY DROSOPHILA MELANOGASTER

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Abstract. Last years, the concept of resistant starch (RS) has evoked a new interest in researchers in the context of bioavailability of starch and its use as a source of dietary fiber. Based on clinical and animal research, RS has been proposed to be the most potentially beneficial starch fraction for human health. In this study, the effects of amylose starch as a fraction of RS on development and lifespan of fruit fly *Drosophila melanogaster* were investigated. In both Canton S and w^{1118} strains, the diet with 20% amylose RS delayed fly development, increased triacylglyceride level in the body of adult insects and reduced their lifespan compared to the diet with 4% amylose starch. Thus, our data clearly demonstrate that amylose starch at high concentrations may negatively affect fruit fly.

Keywords: *Drosophila melanogaster*, resistant starch, pupation, triacylglycerides, lifespan.

Abbreviations: RS, resistant starch; TAG, triacylglycerides.

1. INTRODUCTION

In recent years, there has been an increasing interest in metabolic syndrome, a phenomenon describing clinical profiles of some of the world's major health problems today: obesity, heart diseases and diabetes [7, 19]. It is known that an excessive intake of all macronutrients, particularly carbohydrates, contributes to the development of obesity [2, 4, 6]. The rising prevalence of obesity in both, adults and children, is one of the most important public health concerns in developed and developing countries [1]. Therefore, slowly digestible nutrients has been proposed as a possible intervention to decrease the risk and complications related to metabolic disorders such as obesity and metabolic syndrome [1, 5, 15, 19]. Regarding starch, it is known that this component contains different fractions which are digested and absorbed at different rates in the human small intestine, resulting in varied glycemic responses [15]. Based on speed of digestion, starch is divided into such categories as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [1, 3, 15]. Rapidly digestible starch is rather quickly digested and hydrolysed products are absorbed in the duodenum and proximal regions of the small intestine leading to a rapid elevation of blood glucose and usually a subsequent episode of hypoglycemia, hungry feeling and overconsumption. Fast and substantial increase in blood glucose level can further lead to cellular, tissue and organ damages [1]. Resistant starch is digested partly and very slowly in the upper part of gastrointestinal tract, but it is also fermented by the colonic microflora producing short chain fatty acids that provide additional energy to the body. Slowly digestible starch is hydrolysed within the small intestine providing

sustained glucose release with low initial glycemia, leading to prolonged energy availability, compared to more rapidly digestible starch [1]. It is considered, that RS in the diet may assist to prevent and manage conditions associated with the metabolic syndrome via its potential effects on delaying of glucose delivery with subsequent fat utilisation and appetite control benefits [17, 19]. However, studies related to effects of resistant or slowly digestible starches on parameters of the metabolic syndrome have been relatively scanty.

The fruit fly *Drosophila melanogaster* is one of models that has become important for use in the investigation of metabolism. It is well established that central metabolic and regulatory pathways, including metabolism of carbohydrates, fat, proteins, and insulin signaling are conserved throughout evolution [2]. This creates a solid basis for the use of fly model to reveal certain metabolic disturbances and transfer gained knowledge to human. The current study aimed to examine the beneficial and deleterious effects of the diet with amylose starch as one of fractions of resistant starch [15, 19] on fat metabolism, development and lifespan of D. melanogaster.

2. Materials and Methods

2.1. Drosophila Melanogaster Stock and Media

The *D. melanogaster* strains w^{1118} and Canton S were obtained from Bloomington Stock Center (Bloomington, Indiana, USA). Stock flies and larvae were reared on yeast-corn-molasses (regular) food with 12 hour illumination at 25 ± 1°C and relative humindity of 55-60%. Nipagin (methyl-p-hydroxybenzoate) at concentration of 0.18% was added to the medium to inhibit mold growth [9]. Experimental media contained 4% yeast extract, 0.18% nipagin, 1% agar and starch in a range of concentrations from 0.25 to 20%.

2.2. Pupation

After egg laying for 3-4 h, the eggs were transferred into bottle containing food with different concentrations of starch (about 150 eggs per bottle containing 15 mL of food). In these vials eggs hatched and larvae developed until pupation. The number of formed pupae was recorded every day for 5 days. Mean larval development time was calculated accordingly to Olcott [12] as time at which 50% of larvae had pupated.

2.3. Triacylglyceride Assay

Pre-weighed flies were homogenized in chilled 10 mM phosphate buffered saline with tween (PBST buffer) (pH 7.4) in a ratio 1:50 (milligram flies per buffer microliter) at 4 °C. Homogenates were heated at 70 °C for 10 min to denature proteins followed by cooling to 4 °C. To precipitate denatured proteins, supernatants were centrifuged (16,000 g, 15 min, 21 °C). Final supernatants were used for assay of body triacylglycerides (TAG) which were measured using a diagnostic kit Liquic Cor-TG (PZ Cormay S.A., Poland) following kit guidelines. Standard TAG solutions in concentration range from 3 to 30 μg/mL were used for determination of TAG content in flies. Ttriacylglyceride levels in fly bodies were expressed as micrograms per fly (μg/fly).

2.4. Lifespan Assay

Experimental flies were raised at standard densities of 150 eggs per bottle with 15 mL of yeast–molasses medium. Newborn flies were transferred without anesthesia to fresh medium and maintained for 2 days. After separation by sexes, 100 two-day-old flies were placed into containers with 5 mL of the experimental food. Food was changed every second day, and died flies were counted. To minimize any density effects on mortality, two vials within cohorts were merged when the density of flies reached 50% of initial one [10]. Two or three independent trials with about 300 flies were performed.

2.5. Statistics

The values are presented as means ± S.E.M. Statistical analysis of all data was performed using one-way ANOVA followed by the Dunnett's test to compare multiple experimental treatments to the single

control value with the use of the Mynova program. The difference in survival between cohorts was calculated with log-rank test with a use of JMP 9.0 statistical software (SAS Institute) [14].

3. Results and Discussion

The effect of carbohydrate diets on physiological parameters is generally studied in relatively simple model organisms like nematodes, mice and rats. Recently, fruit fly *Drosophila melanogaster* has been started to be intensively used in nutritional studies [2, 4, 10, 16, 18].

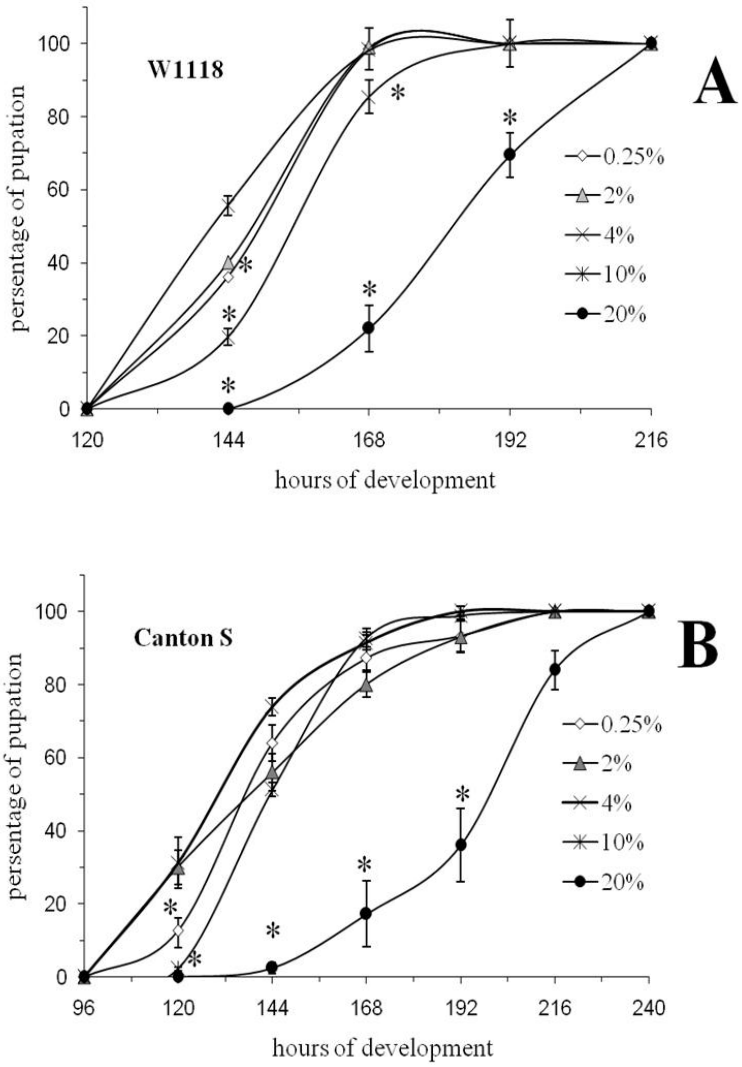

*Fig. 1. Developmental curves of D. melanogaster w^{1118} (A) and Canton S (B) grown on media containing amylose starch in different concentrations. Data are presented as means ± S.E.M.; cohorts with 1500 flies were used (n = 4). Diet with 4% starch was taken as a control group. *Significantly different from the control group with P < 0.05.*

Many *Drosophila* organs that regulate food intake and energy metabolism have analogs in humans, in part those that are potential targets of diabetic complications: heart, brain, kidney (nephrocytes, Malpighian tubules), liver and adipose (fat body), gastrointestinal tract, and blood (hemolymph) [2, 11]. In the current study, we used *D. melanogaster* as a model to investigate biological effects of starch with high amylose content. This type of starch belongs to so-called resistant starch and up to present time there is a too little information about mechanisms of action of this fraction.

First we evaluated the developmental patterns of fly larvae after *per os* uptake of amylose starch in different concentrations (Fig 1). Since previous studies demonstrated that effects of diets could be strain-specific [8], all experiments on the effects of dietary amylose were carried out with two *D.*

melanogaster strains, Canton S (wide type) and its derivative w^{1118}. Experimental medium supplemented with 4% starch was selected as the control and all parameters further were compared with flies fed under these conditions.

Strain	Starch concentration, %				
	0.25	2	4	10	20
w^{1118}	146±4	146±7	144±3	154±6*	175±6*
Canton S	137±3♣	139±2♣	130±6♣	144±5*,♣	202±7*,♣

Data are presented as the means ± S.E.M. (n = 3).
**Significantly different from the control group (4% carbohydrate) and ♣from w^{1118}.*

Tab. 1. Fly half-pupation time (time at which 50% of larvae pupated) on different starch-containing diets.

Consumption of food with 0.25-2% starch showed the tendency to retard normal development of insects relatively to the control (Fig. 1A-1B). This might be due to deficiency of energy nutrients required for normal fly development. High-caloric starch diet (10-20%) significantly prolonged development time in both strains.

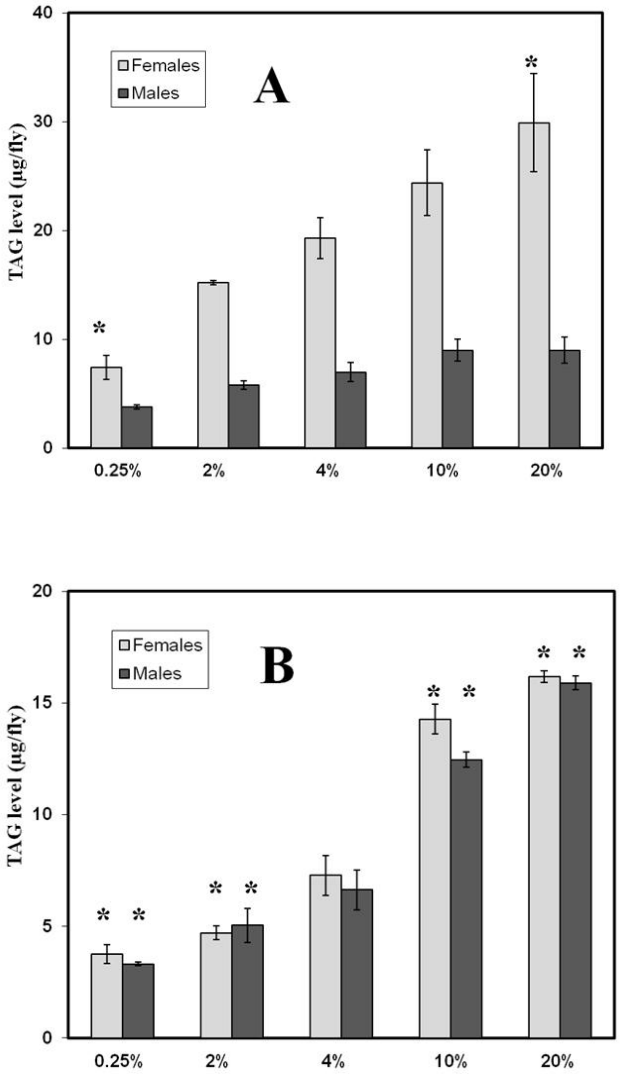

Fig. 2. The levels of triacylglicerides in the bodies of eight-day old w^{1118} (A) and Canton S (B) flies, kept on media containing amylose starch in different concentrations. Data are presented as means ± S.E.M. (n = 4). Significantly different from the control group (4% starch diet) with P < 0.05.

To determine the length of developmental delay, we calculated the time at which 50% of larvae had pupated (Tab. 1). For 20% starch groups in w^{1118} and Canton S strains, this parameter increased by 1.2- and 1.6-fold, respectively, compared the control diet with 4% starch. At the same time, pupae survival and pupation height on low (0.25%) and high (20%) starch diets were significantly lower in comparison with 2-10% starch diet (data not shown). Our results demonstrate that adverse effects of resistant starch at high concentrations on fly development were not related to the strains. We suppose that toxic effects of high concentrations of the starch could be connected either with excessive solidification of the culture medium making the food very hardly accessible for larvae, or with metabolic rearrangements in larvae bodies delaying their development. It can not be excluded that both mechanisms could be involved.

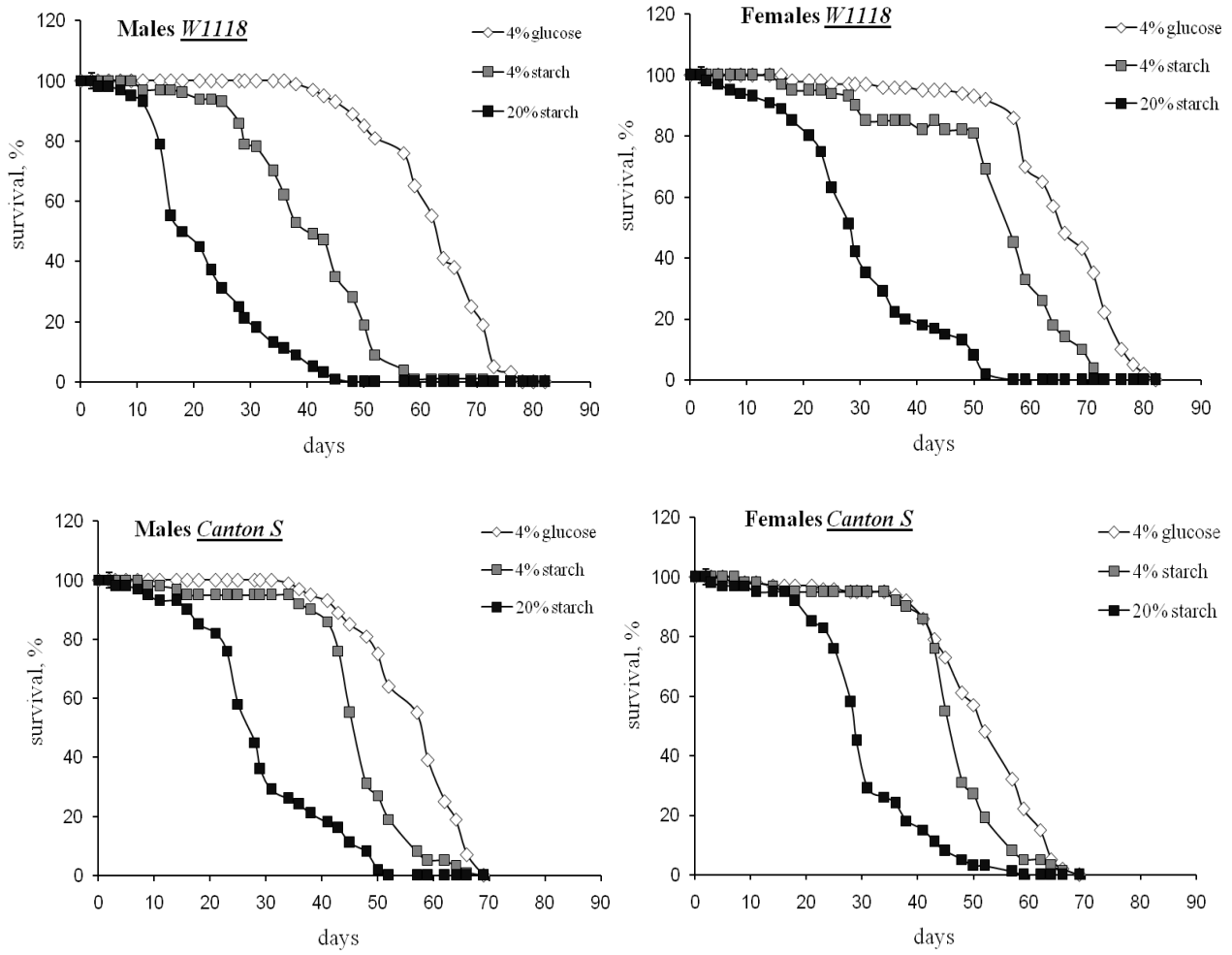

Fig. 3. Survival curves of D. melanogaster w^{1118} and Canton S flies, maintained on glucose or starch diet. Each curve represents survivorship about 300 flies.

Previously it has been shown, that high-sugar feeding enhances triglyceride (TAG) level in insect bodies [11, 16]. In this study, we examined the level of TAG in adult fruit flies consumed different starch diets (Fig. 2). As it could be expected, with increase in starch concentration the content of TAG proportionally increased in w^{1118} females (Fig. 2A) and both sexes of Canton S flies (Fig. 2B). Thus, our results regarding effects of starch diet are in a very good agreement with previous studies in *Drosophila* on other carbohydrates [11, 16]. Interestingly, under consumption of the high-starch diet the wet mass of fly bodies did not differ from other groups (data not shown). This is consistent with previous study which revealed that a diet high in RS reduced rat adipose tissue with no changes in body mass [13]. This work indicated that despite similar mean body mass, rats given high-starch food had more body fat and less lean body mass than those given low-starch food [13].

Since diet with different starch concentrations modified fly development and level of TAG in adult flies, next we investigated the long-term effects of amylose starch consumption on *D. melanogaster* lifespan. Again, the medium with 4% glucose was used as the control. The presence of starch in the food reduced the survival of male and female flies of both strains in dose-dependent manner compared to one on glucose medium (Fig. 3). For w^{1118} strain, median survival time on 4% glucose, 4 and 20% starch, was 65, 45 and 23 days for males, and 68, 58 and 30 days for females, respectively. For Canton S flies, this parameter was 63, 48 and 28 days for females, and 56, 47 and 28 days for males, respectively. Thus, dietary amylose starch does not appear to offer any benefits for lifespan of fruit fly and is actually toxic at higher concentrations. It seems that amylose starch induced such metabolic perturbations in fly bodies which lead to shortening of lifespan, but this statement needs the further investigations.

In summary, our results demonstrate that dietary amylose starch at moderate and high concentrations demonstrates deleterious effects on fly development and lifespan, and induces metabolic reorganization accompanied by increased triacylglycerol levels. The results point out the need for further research to review the concept of resistant starch as beneficial food component.

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Абрат О.Б. Вплив крохмалю з високим вмістом амілози на розвиток та тривалість життя плодової мушки. *Журнал Прикарпатського університету імені Василя Стефаника*, **2** (1) (2015), 100–106.

В останні роки використання харчових продуктів на основі резистентного крохмалю (РК) викликало новий інтерес у дослідників з позиції біодоступності його волокон. На підставі клінічних та експериментальних досліджень РК було запропоновано як потенційно найбільш корисну для здоров'я людини фракцію крохмалистих продуктів. Метою даної роботи було вивчення впливу крохмалю з високим вмістом амілози, що належить до фракції резистентного крохмалю, на розвиток та тривалість життя плодової мушки *Drosophila melanogaster*. Показано, що споживання резистентного крохмалю у високих дозах (20%) призводило до затримки розвитку двох ліній мух (Canton S and w^{1118}), збільшення рівня триацилгліцеридів у їх тілі та зменшення тривалості життя комах, порівняно з дієтою на 4% крохмалі. Таким чином, наші результати наштовхують на думку, що крохмаль на основі амілози у високих концентраціях може негативно впливати на плодову мушку.

Ключові слова: *Drosophila melanogaster*, резистентний крохмаль, лялькування, триацилгліцериди, тривалість життя.