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Dietary wheat germ oil and age influences fatty acid compositions in adult oriental fruit flies

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ABSTRACT

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a serious pest of fruit crops in Asia, several Pacific Islands and sometimes the western United States, particularly California. Sterile insect technique programs have been developed for management of several tephritid fruit fly pests. These programs are based on continuous production of adult fruit flies. The high expense of mass-rearing oriental fruit flies drives research to improve the cost effectiveness of rearing programs. One recent improvement for mass rearing oriental fruit flies involves adding wheat germ oil (WGO) to the larval culture medium, which improved several parameters of biological performance. The performance enhancing influence of WGO is due to the presence of polyunsaturated fatty acids (PUFAs), some of which are nutritionally essential for many insect species. We considered the issue of whether WGO supplementation of the larval culture medium influences the fatty acid make up of adult tissues. We report that WGO supplementation led to substantial increases in adult tissue C18 PUFAs. Unlike the outcomes of unrelated nutritional studies on moths, the PUFA components of WGO did not improve adult fruit fly performance. Taken with recent publications reporting that WGO in larval diets influences gene expression, we conclude that dietary WGO improves biological performance of adults through changes in tissue C18 PUFAs and gene expression.
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Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a serious pest of fruit crops in Asia, several Pacific Islands and sometimes the western United States, particularly California. Area wide integrated pest management programs, including the sterile insect technique (SIT), strive to achieve very low pest prevalence or pest-free status while reducing applications of classical insecticides. SIT programs have been developed for management of several tephritid fruit fly pests, including the Mediterranean fruit fly, the Mexican fruit fly and the congeneric melon fruit fly, *B. cucurbitae*. SIT programs are based on continuous mass-rearing, sterilization and release of adult fruit flies. The high expense of mass-rearing oriental fruit flies drives research to improve the cost effectiveness of rearing programs. Chang et al. (2006) contributed a major improvement by developing a low-waste liquid culture medium for larval oriental fruit flies. Chang and Vargas (2007) added another improvement to rearing oriental fruit flies by amending their liquid culture medium with small amounts of WGO. Supplementing their basal liquid culture medium with WGO (at 0.15%) improved several performance parameters, including pupal recovery, proportions of adult fliers, egg production and egg hatch. Wheat germ was first used to improve the culture medium for the pink bollworm (Adkisson et al., 1960) and has since been included in artificial media for many insect species (Vanderzant, 1974). Wheat germ oil (WGO) consists of several saturated and unsaturated fatty acids and vitamin E and it satisfies the nutritional requirements for essential fatty acids (EFAs) for many insect species.

With respect to insect nutrition, the performance enhancing influence of WGO is due to the presence of polyunsaturated fatty acids (PUFAs), some of which are nutritionally essential for many insect species (Dadd, 1985), and to biologically active tocopherols, or vitamin E. The PUFAs and tocopherols in WGO probably serve entirely different roles in insects. The essentiality of dietary PUFAs for most animals, including most insect species, is now universally accepted. PUFAs make up important components of biomembrane phospholipids (PLs) and some of them are oxygenated into various eicosanoids (Stanley, 2000). Long recognized as an important antioxidant

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in human and pet nutrition, the potential roles of vitamin E in insects were controversial for some time, before discovery that it is necessary for spermatogenesis in the house cricket (Meikle and McFarlane, 1965) and likely for many other insect species. WGO is a widely used dietary supplement that fulfills several essential nutritional requirements.

Dietary PUFAs influence the fatty acid compositions of mammals and insects, although insects are understudied in this regard. Stanley-Samuelson and Dadd (1981) demonstrated that increasing levels of dietary arachidonic acid (AA; 20:4n−6) led to accumulation of higher proportions of tissue AA in mosquitoes, *Culex pipiens*. Similarly, increased amounts of dietary C18 PUFAs led to higher proportions of C18 PUFAs and some C20 PUFAs in tissues PLs of waxmoths, *Galleria mellonella* (Stanley-Samuelson and Dadd, 1984). The authors interpreted the increased proportions of C20 PUFAs in terms of the desaturation/elongation pathways of PUFA metabolism (Stanley-Samuelson et al., 1988). It might be concluded that dietary PUFAs generally influence insect fatty acid patterns, however understanding the situation with some dipterans remains clouded.

A key question is whether higher Diptera, such as *Drosophila melanogaster*, requires essential dietary PUFAs as seen in the majority of other insects that have been studied. Rapport et al. (1984) reared a wild-type *D. melanogaster* stock through 10 consecutive axenic generations on a fatty acid-free chemically defined culture medium. Detailed gas chromatographic analysis of the tissue fatty acids in males and females consistently produced a very small peak (<1.0% of total PL fatty acids) with retention time exactly corresponding to the C18 PUFA, linoleic acid. By the time of this work, Bloomquist et al. (1982) had demonstrated de novo biosynthesis of 18:2n−6 in several insects and the authors concluded that *D. melanogaster* is one of the insect species that may be capable of producing the small amounts of 18:2n−6 required for growth and reproduction. On the understanding that insects are able to convert 18:2n−6 into AA (Stanley-Samuelson et al., 1988), it was thought that *D. melanogaster* was independent of any dietary PUFA need because it was capable of producing C18 and C20 PUFAs.

Other fruit flies, such as the oriental fruit fly, may also be independent of dietary PUFA requirements, however this does not appear to be likely because supplementing the basal liquid culture medium for larval rearing with WGO improved the biological performance of adults (Chang and Vargas (2007). This raises the possibility that WGO supplementation of the larval culture medium influences the fatty acid make up of adult tissues. Here we report on the outcomes of experiments designed to investigate the point.

Materials and methods

Insects

The newly collected eggs (<24 h) of oriental fruit flies, *Bactrocera dorsalis* (Hendel), were provided by the Tropical Crop and Commodity Protection Research Unit of the USDA's Agricultural Research Service (ARS) in Hilo, Hawaii. The fruit fly colony has been maintained at 25 °C, 65% relative humidity, and 12D:12L for more than 360 generations.

Culture medium preparation

We carried out all experiments in small plastic containers (17W × 22.5L × 3.7D cm), using a slight modification of the culture medium formula and preparation methods described by Chang et al. (2004, 2006). The medium includes brewer's yeast in one of three formulations. One included 32.64 g of FNI200 and GSH (8.16 g) (gift from Lallemand, Montreal, Canada), one was composed of LS65 and GSH, and another composed of LBI2240 and LS65. We completed the medium with sugar (24.36 g), the anti-fungal, nipagen (0.4 g), sodium benzoate (0.4 g), streptomycin (0.3 g), citric acid (7.5 g), oil (2 ml, 1% of water volume), and water (200 ml).

We weighed the dry diet ingredients into a lidded 200-ml plastic container, then added water (150 ml) to the container with all other ingredients except citric acid and wheat germ oil. After shaking to mix the dry ingredients with water, we gently poured the mix into a blender (Magic bullet), and used 50 ml of water to rinse the ingredients into the container for further homogenization (total 200 ml). After blending the medium for 1–2 min, we added citric acid and wheat germ oil or fatty acids and adjusted the pH to 3.5. We poured the culture medium into a plastic container (12.7W × 17.78L × 2.5 cm) bedded with one piece of dry sponge cloth (10.16 × 15.24 cm) and a garden net. Note: The culture medium mixture should be leveled to the thickness of the sponge cloth (4 mm) to avoid over-flooding the cloth. We seeded another piece of wet sponge cloth 2 × 4 cm with 0.5 ml of eggs (<24 h old) and placed it onto the center of the larger sponge cloth. We covered the box with a plastic lid and placed it inside a Rubbermaid plastic container (30.38 × 25.4 × 7.62 cm) located in a room set to 25 °C, 65% RH and 12D:12L for larval development and collection.

Chemicals

All fatty acids including palmitic acid, stearic acid, linoleic acid, oleic acid, linolenic acid, and α-tocopherol (vitamin E) were purchased from MP Biomedicals, Inc. (Dolon, Ohio, USA). Cold pressed wheat germ oil was purchased from KIC Chemicals, Inc. (New York, USA).

Fatty acid analysis

Lipid extraction and preparative chromatography

Adult oriental fruit flies, ages 1 through 12 days old, were reared from larvae on stock culture media with and without WGO were sexed and collected into cryogenic vials. Each sample contained 50 flies (approximately 0.3–0.5 g). Lipids were extracted following the method of Bligh and Dyer (1959). Specifically, two ml of Folch's solution (2:1 methanol:chloroform containing 0.005% w/v butylated hydroxytoluene as an antioxidant to protect double bonds from autoxidation were added to each sample. Each sample was homogenized for approximately 30 s using a Tissue Master 125 (Omni International). One ml of water was added to each tube before vortexing for about 30 s. Samples were then centrifuged for 5 min at 4400 ×*g* in a clinical centrifuge, which generally separated the preparations into three layers. The bottom methanol:chloroform layer contains lipids of interest and was separated from the upper aqueous layer by a semi-solid infranatant containing proteinaceous and other non-lipid components. In some cases it was advantageous to increase the proportion of the aqueous layer to aid separation. The lower methanol:chloroform layer was removed by pipetting. A second extraction with methanol:chloroform was performed to ensure high recovery of lipids. The two organic fractions were combined and reduced in volume under a nitrogen gas stream prior to thin-layer chromatography.

Phospholipid (PL) and triacylglycerol (TG) fractions of the total lipids were isolated for further analysis via thin-layer chromatography (TLC). Whatman 20 × 20 cm plates (250 μm silica gel coating) were developed in a 80:20:1 petroleum ether:diethyl ether:acetic acid mobile phase. PLs remain at the starting line in this system and canola oil was used as a standard to identify TG fractions. Lipid fractions were visualized by brief immersion in an iodine chamber. The PL and TG fractions were individually scraped from the TLC plates for further processing.

Saponification and transmethylation

The fatty acid compositions of the PL and TG components were determined by gas chromatography. PLs and TGs were saponified and transmethylated to individual fatty acid methyl esters (FAMEs) using a modified version of the protocol described by Rubinson and Neyer-Hilvert (1997). TLC fractions were soaked in approximately 1 ml of 0.5 M potassium hydroxide in methanol and heated to just