Dietary wheat germ oil influences gene expression in larvae and eggs of the Oriental fruit fly

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Abstract: Proteomics holds the promise of providing valuable information about the impact of nutrition on a wide range of biochemical parameters by investigating how nutrition alters global gene expression patterns. Our goal is to use proteomics to identify insect molecular markers that could be used as early indicators of insect response to different nutritional sources. The objective of our current study was to illustrate the potential discovery of molecular markers using the oriental fruit fly, *Bactrocera dorsalis,* when reared separately on a diet devoid of, or supplemented with, wheat germ oil (WGO), and analyze the presence of differentially expressed genes in larvae and F_1 eggs compared to that previously reported for adults. The analysis of spots from 2D-electrophoresis gels revealed significant changes in expression levels of 8 proteins in larvae samples and 23 proteins in egg samples which were identified by mass spectrometry on MALDI TOF/TOF. These findings support the premise that three proteins expressed in the egg and two proteins previously reported to be expressed in the adult that are differentially regulated as a result of the level of WGO in the diet would make good biomarker candidates for additional study of the optimal concentration of dietary lipids for the fruit fly.

Results

Table 1. Bioassay results for larvae fed diet containing +/– WGO.

Parameters	-WGO	+WGO
Larval Duration (Days)	10.1	9.5*
Pupal Recovery (%)	61.2	74.3
Pupal Weight (mg)	9.2	8.3
Adult Emergence (%)	89.1	91.6
Adult Fliers (%)	83.3	96.7*
Mating (%)	43.2	81.2*
Eggs/Female/Day (No.)	0.3	2.5*
Egg Hatch (%)	32.2	75.9*

*Indicates parameter is significantly different between treatments.

Fig. 1. Gel images of proteins from larvae fed +/- WGO diet.

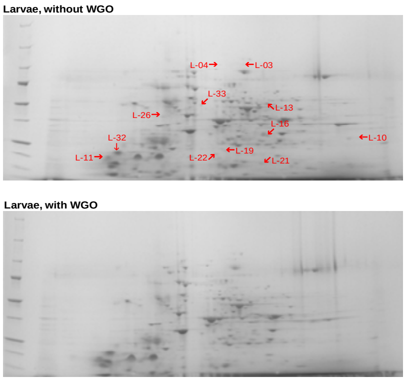
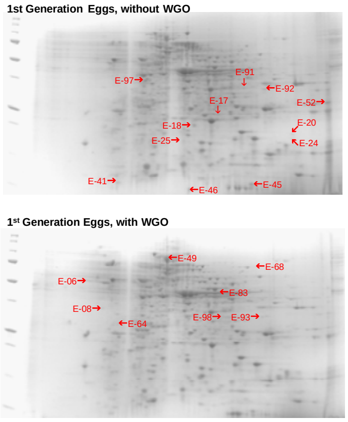


Fig. 2. Gel images of egg proteins from +/- WGO diet.



Materials and Methods

Fruit fly liquid diet

Liquid diet formulation

Sugar	121.8 g (8.99%)
LBI2240: FNI LS65 (3:1)	204.0 g (15.06%)
Nipagen	2.0 g (0.15%)
Sodium benzoate	2.0 g (0.15%)
Citric acid	23.1 g (1.70%)
Water	1000 ml (73.84%)
Streptomycin	1.5 g (0.11%)
WGO	10.00 ml (1% of water)

Larval diet

Protein analysis protocol

Protein resolution
↓
Protein separation- 2-Dimensional gel electrophoresis
↓
Protein quantification – intensity of dye
↓
Protein cleavage into peptides with trypsin
↓
Peptide ID by Mass spectrometry (MALDI-TOF/TOF)
↓ match → Protein/peptide identity sequence tag ↓ Data search using Mascot program (E-value) ↓ Identification of up-regulated proteins
↓ no match → *de novo* or partial ↓ NCBI-BLAST

References
Chang CL, Vargas RI (2007). J Econ Entomol 100:322
Chang CL, Coudron TC, Goodman CL, Stanley D, An S, Song Q (2010). J Insect Physiol 56:356.

Conclusions

1. There were significant differences in egg production and egg hatch between adults from larvae reared on +/– WGO diet (Table 1).
2. More than 380 larval and 800 egg adult proteins were detected through 2D gel electrophoresis (Figs. 1 & 2). Densitometry indicated that 8 larval proteins and 43 egg proteins changed significantly in their expression levels between treatments. Of these, 8 larval and 23 egg proteins were identified using mass spectrometry (Tables 1 & 2).
3. Larval protein changes mainly involved down-regulation and included heat shock proteins & proteins involved in cell protection.
4. Egg proteins were both up- and down-regulated and included glyceraldehyde-3-phosphate dehydrogenase, cyclophilin, heat shock proteins, and signaling proteins.
5. Protein changes was confirmed by qPCR of selected genes.
6. Expression of genes encoding sensitive biomarker proteins, such as E-20 and E-93, may be applied to assist in the formulation of high quality culture media for selected beneficial insect species.

Table 2. Up-regulated proteins from larvae fed +/- WGO diets.

Spot No.	Percent +WGO/-WGO	Protein Identification and Function
		Protein Structure, Function and Degradation
L-10	47	HSP70-like protein (involved in protein folding and signal transduction).
L-16	56	20S proteasome subunit 1 (Involved in nonlysosomal protein degradation).
L-26	55	HSP70-like protein (involved in protein folding and signal transduction).
		Cell Protection
L-13	57	Glutathione reductase (catalyzes the reduction of oxidized glutathione).
L-19	78	Peroxiredoxin (thiol-specific antioxidant reducies peroxides).
L-22	55	Glyoxalase/Bleomycin resistance protein/Dioxygenase (detoxifies endogenous toxins).
L-30	69	Peroxiredoxin (thiol-specific antioxidant reduces peroxides).
		Signal Transduction and Cytoskeleton
L-21	54	Actin-binding domain (in cytoskeletal and signal transduction proteins).

Table 3. Up-regulated proteins from F1 eggs of larvae fed +/- WGO diets.

Spot No.	Percent (+WG)/-WGO)	Protein Identification and Function
		Energy and Metabolism
E-17	40	Glyceraldehyde-3-phosphate dehydrogenase (glycolysis/gluconeogenesis).
E-45	23	Mitochondrial ATPase inhibitor (involved in nucleotide processing).
E-49	157	AAA+ ATPase domain (functions as molecular chaperones, e.g., proteases).
E-52	43	Malate dehydrogenases (important in the TCA cycle).
E-68	243	Aconitate hydratase (plays important role in TCA cycle).
E-83	148	Vitellogenin 1 precursor (major yolk protein; nutrient source for embryos).
E-91	44	Glyceraldehyde-3-phosphate dehydrogenase (glycolysis/gluconeogenesis).
E-92	37	Glyceraldehyde-3-phosphate dehydrogenase (glycolysis/gluconeogenesis).
E-98	321	Glyceraldehyde-3-phosphate dehydrogenase (glycolysis/gluconeogenesis).
		Protein Metabolism, Structure and Function
E-06	543	26S proteasome subunit (plays a major role in protein breakdown).
E-08	248	2 Protein domains (reversibly binds ribosomes; ubiquitin associated domain).
E-20	1	Cyclophilin (involved in protein folding, can modulate protein function).
E-24	9	Cyclophilin (involved in protein folding, can modulate protein function).
E-46	12	Polyubiquition (involved in protein turnover during cell cycle progression).
		Protein Structure and Signal Transduction
E-53	36	HSP70-like protein (nvolved in protein folding and signal transduction).
E-69	185	HSP70 (involved in protein folding and signal transduction).
E-97	49	HSP-like protein (involved in protein folding and signal transduction).
		Intracellular Signaling
E-64	188	14-3-3 epsilon isoform C (numerous roles, e.g., subcellular localisation).
E-93	680	RACK1 (anchors activated protein kinase C's to subcellular compartments).
		Lipid Transport
E-102	36	Lipocalin family (transports small hydrophobic molecules; PGD synthase).
		Cell Protection
E-18	11	Thioredoxin peroxidase (reduces hydrogen peroxides).
		DNA Replication and Repair
E-25	41	dUTPase (hydrolyses dUTP to dUMP and pyrophosphate).
E-41	26	Exact function unknown (binds tubulin in cytoskeleton; Ca2+ high affinity).