Changes in gene expression associated with altering protein and fat deposition in yellow mealworms treated with exogenous pyriproxifen

Victoria Hill, Tim Parr, John Brameld, Andrew Salter
School of Biosciences, University of Nottingham

Insects, such as the larvae of *Tenebrio molitor* (TM, yellow mealworms), are a potential alternative protein source for animal feed and human food. However, like many larval stage insects, they contain comparatively high fat contents (25-36%). Reducing this fat content could diminish the need for processing to generate a high protein feed or food ingredient. Our previous work has demonstrated that application of exogenous pyriproxifen (a juvenile hormone analogue) decreases fat and increases protein content of TM. This study aimed to identify changes in gene expression associated with this phenotype.

Introduction

Insects have been garnering attention as a potential alternative protein source and feed supplement to help reduce dependence on soya, which is associated with deforestation in certain regions. Insect larvae can contain high quantities of fat which is not conducive to effective feed formulation. Reducing fat content of insects via application of exogenous hormones could highlight significant changes in gene expression which may provide insight into targets for selective breeding.

Methods

<u>TM feeding trial</u>

Treatment groups were fed wheat bran (WB) containing

- Vcont: 12 ml/ kg acetone in WB.
- Juvenile hormone (JH) analogue = pyriproxifen
- JH-PL: 2 mg/kg pyriproxifen in WB.
- JH-PH: 15 mg/kg pyriproxifen in WB.

TM fed *ad libitum* over 28 days, de-ionised water provided through soaked cotton wool. Kept in darkness in incubator at 25^{o}C, 60% humidity. TM culled with liquid nitrogen and stored at -80^{o}C.

<u>Proximate nutrient analysis</u>

- TM freeze dried for 72 hours at <0.4 mbar, -55^{o}C.
- 1 g material used for Gerhardt SOXTHERM fat extraction for fat content determination.
- 50 mg material used for crude protein content determination through nitrogen content (EA 1112 Elemental Analyser).

<u>Transcriptomic analysis</u>

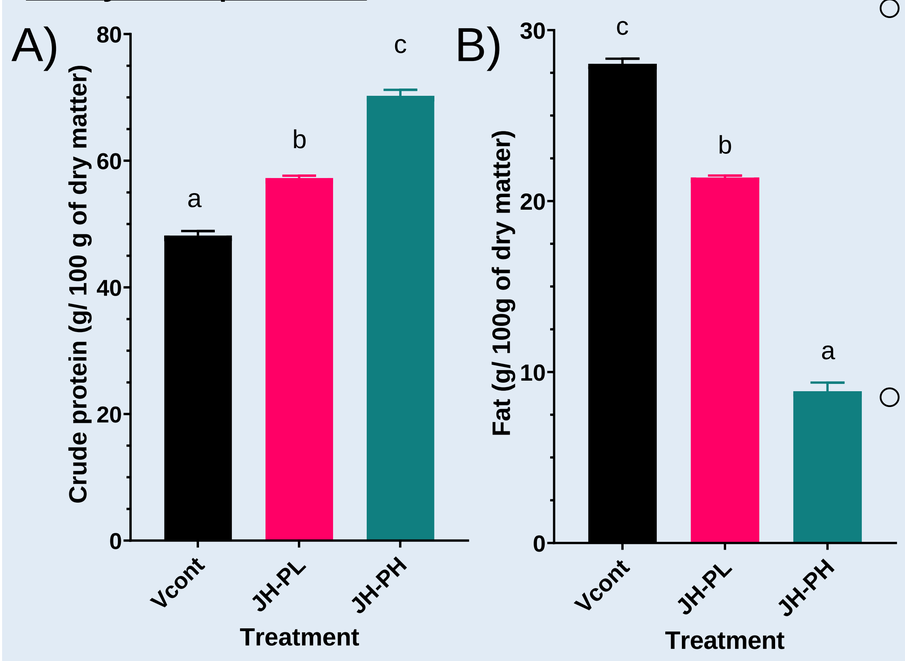
- RNA extracted - RNeasy Mini kit (Qiagen).
- Transcriptomic analysis via Next Generation Sequencing (Eurofins Genomics, Germany).
- SALMON and STAR used to map transcriptome to genome, derived from TM egg gDNA (Genomics Platform: Deep Seq).
- Bioinformatics to identify differentially expressed (DE) genes.

<u>Statistical analysis</u>

- One-Way and Two-Way ANOVA used to analyse data in Genstat (20th Edition).

Results

<u>Body composition</u>



- 46% increase in crude protein in JH-PH TM compared to Vcont TM.
- 63% decrease in fat content in JH-PH TM compared to Vcont TM.

Figure 1: The effect of the Juvenile hormone analogue pyriproxifen on whole TM composition after 28 days treatment. (A) Crude protein content. (B) Fat content. Significant effect results at P<0.001 following One-Way ANOVA, different letters denote a significant difference between treatment groups (P<0.05). Data are presented as average ± standard error of the mean.

<u>Gene expression</u>

- RNAseq analysis of the JH-PH treated TM identified 508 genes that were differentially expressed (P<0.05) (see Figure 2).
- 236 of these genes were upregulated, whilst 273 genes were downregulated.

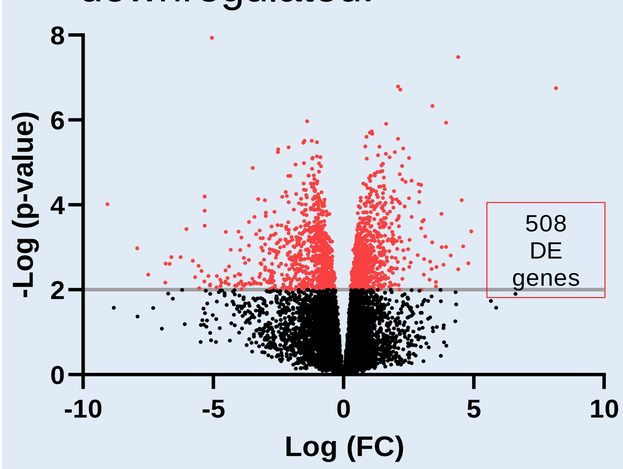


Figure 2. Volcano plot of the effect of pyriproxifen treatment for 28 days on whole MW transcriptome, illustrated through Log Fold Change (FC) vs –Log (p-value) of 508 DE genes of JH-PH treated TM relative to Vcont TM. Significant results correspond to –Log (p-value) > 2.

Conclusions

Changes in lipid and protein content of TM were associated with changes in expression of large numbers of genes. Work is ongoing to identify which of the genes are most likely to be responsible for the changes in protein and fat metabolism. This information will provide an insight into potential gene targets that could be used for selective breeding programmes to improve the efficiency of insect protein production to serve the animal feed industry.