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RESEARCH ARTICLE

Changes in nutrient composition and gene expression in growing mealworms (*Tenebrio molitor*)

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Abstract

Insects are of high interest as a sustainable source of nutrients to be included in the food production system. The larvae of *Tenebrio molitor*, commonly known as yellow mealworms (MW), have a high protein content, which means potential applications in the animal feed and human food sectors. However, previous reports have shown considerable variability in the nutrient composition of mealworms, which may in part, be due to harvesting at different developmental stages. A better understanding of the regulation of composition during development would potentially facilitate future attempts to manipulate nutrient content, perhaps through gene editing, to maximize commercial value. In the present study, mealworms were harvested at various time points within a 24 day period leading up to the start of pupation. At the earliest time points (between days –24 and –17), a 44% increase in fat content was seen, which was maintained throughout the rest of development. By day –12, protein content fell by 12%, a change that was also maintained. Throughout development there was a change in fatty acid composition, with a shift from oleic acid being the major fatty acid at day –24, to linoleic acid being predominant at later time points. In an attempt to better understand the genetic basis of these changes, an analysis of the transcriptome was undertaken. In the absence of a specific annotated genome for the mealworm, an Affymetrix GeneChip microarray for Drosophila was utilized. The hybridisation of RNA extracted from five developmental stages (larvae and pupae) showed differential gene expression; and some potential orthologs were identified which may be involved in regulating nutrient composition during development. However, we were unable to identify a significant proportion of the most highly regulated genes, highlighting the need for a fully annotated mealworm genome.

Keywords

fat – fatty acids – protein – transcriptome

1 Introduction

There is increasing concern about the sustainability of protein production for both direct human consumption and use in animal feed (Salter and Lopez-Viso, 2021). With an expanding global population, the use of human-edible protein-rich crops for animal feed is seen as an inefficient and unsustainable use of land and water resources. As a result, there has been considerable interest in alternative protein sources, of which insects

have received particular attention (Hawkey *et al.*, 2021; van Huis *et al.*, 2013). In the last decade, one of the species of insect that has received a lot of interest from industry is *Tenebrio molitor*, whose larvae (yellow mealworms, MW) have high nutritional value (Ghaly and Alkoaik, 2009; Hawkey *et al.*, 2021), as well as the versatility to grow on different low quality substrates (Alves *et al.*, 2016; Yang *et al.*, 2019).

Improving the efficiency and affordability of mealworm larvae is essential in establishing them as a viable alternative for livestock feed. In general, this is likely to be seen as maximizing the protein yield. However, for human consumption the amount and composition of lipid may also be important. The reported ranges of protein and fat content per unit dry weight are 46-54% and 25-36% respectively (Hawkey *et al.*, 2021). Most of the data available on MW nutrient composition is from a single timepoint (Gowda *et al.*, 2022), often examining relative changes in composition in response to different diets (Dreassi *et al.*, 2017) or a variety of environmental conditions (Adámková *et al.*, 2020). Mealworms have a relatively long larval stage (8-10 weeks) and in insects that undergo metamorphosis it appears the critical body weight (also known as threshold size), associated with the accumulation of protein and fat, is a key factor in determining the onset of metamorphosis (Malita and Rewitz, 2021; He *et al.*, 2020). During metamorphisis, feeding ceases and body tissues are degraded and remodelled requiring nutrient use and mobilization. Therefore the point at which mealworms are harvested may impact on their nutrient composition. One of the primary aims of the current study was to investigate mealworm protein and lipid composition at different time points as they progress towards pupation. With this novel information it may be possible to identify key timepoints most appropriate to harvest the larvae for specific uses.

In addition, gaining a better understanding of the changes in gene expression that accompany any compositional changes, might also help to develop strategies to manipulate growth and composition. Thus, in addition to analysing the composition of the mealworms we also analysed changes in the transcriptome. It is only very recently that a partly annotated genome of *Tenebrio molitor* has started to emerge (Eriksson *et al.*, 2020; Eleftheriou *et al.*, 2022, Kaur *et al.*, 2023). Although genetic resources are avaliable, the basic understanding of MW regulatory mechanisms is lacking, which is also true for most other species of insect. GeneChip microarrays (Thermo Fisher Scientific, Loughborough, UK) are a useful tool to study differential gene expression and pathway enrichment analysis associated with phenotypes of interest, but commercial arrays are only available for a limited number of species. However species like human, mouse, zebra fish and *Drosophilia* have extensively studied genomes and transcripts. Therefore microarrays for such species usually have a high degree of annotation. We previously (Graham *et al.*, 2011) showed that a cross-species microarray approach can successfully provide useful information on gene expression and indications of associated transcript annotation in the target species, for instance in sheep when using a human microarray. This cross-species approach has now been applied to a range of species (Graham *et al.*, 2010; Nilsson *et al.*, 2014).

The aims of this study were to investigate (i) changes in body composition and nutritional value of *Tenebrio molitor* at five different stages of larval growth and at pupation and (ii) the potential use of a cross-species microarray (GeneChip) to identify differentially expressed genes related to the phenotype. Thus, we have used an Affymetrix GeneChip microarray for Drosophila in an attempt to identify changes in gene expression which may underlie changes in composition.

2 Material and methods

Insect growth study

A single batch of mealworms (MW) were obtained from Monkfield Ltd UK (Mepal, UK). They were initially sieved to exclude any larvae <0.7 mm or >2 mm. The included MW were acclimatised for 3 days in an incubator (Weiss Technik) at 25 °C, 60% humidity and 100% darkness, with *ad libitum* access to organic wheat bran (BuyWholeFoodsOnline, Ramsgate, UK) and deionised water. The latter was provided via soaked organic cottonwool balls which were replaced twice a week. The same feed, water and conditions were used throughout the study. After acclimatisation, MW were manually transferred from the original single batch to 3 replicate containers, with dimensions of 36 × 55 × 12 cm, each containing 1000 MW. They were provided with 100 g of wheat bran which was topped up every time the MW were weighed. At the start of the trial, the mealworms were approximately 6 weeks old, however the exact hatch date was not provided by the suppliers. As the mealworms were obtained from the same batch, day 0, the end point of the experiment, was when 30% of the mealworms across all 3 replicates had pupated, which was 24 days after the start of the trial. By day 40-

41 after the start of the trial virtually all (97%) of the mealworms across all 3 replicates had pupated. Comparisons were done between time points. Growth was monitored throughout the trial and samples of 25 larvae were collected at different time points (days –24, –17, –12, –11, –7, –3 and 0) for proximate nutrient analyses and were culled by freezing and storage at –20 °C. A further 25 MW were collected at days –24, –17, –11, –3 and 0 for total RNA extractions and were culled using dry ice and stored at –80 °C.

Proximate nutrient analysis

Frozen MW samples (–20 °C) were freeze dried (Christ Freeze Dryer) for 72 hours at –55 °C and 0.4 mbar until they reached a constant weight. Samples were ground to a fine powder in a liquid nitrogen cooled pestle and mortar before being stored in a desiccator until analyses.

Crude protein content was determined via the nitrogen content using an EA 1112 elemental analyser (Thermo Fisher Scientific, Loughborough, UK) and 50-mg freeze-dried MW powder and compared to aspartic acid standards (10.52 (w/w) N%; Elemental Lab, Okehampton, UK). A standard conversion factor of 6.25 was used to convert nitrogen into total protein.

Total fat content was determined via Soxtherm extraction (Gerhardt Analytical Systems, Germany) using 1-g freeze-dried MW as described previously (Plamquist and Jenkins, 2003). In brief, the sample was boiled in petroleum ether at 150 °C for 30 minutes followed by refluxing for 90 minutes to ensure all fat was collected. The solvent was evaporated to dryness and the weight of fat determined. Fat samples were then resuspended in hexane and kept at –20 °C for subsequent fatty acid analysis.

To determine fatty acid contents, the triacylglycerol (TAG) was hydrolysed followed by fatty acid methyl esterification (FAME), as described by Lock *et al.* (2005), with samples stored in hexane at –20 °C before analysis using gas chromatography mass spectrometry (GCMS) (Thermo Fisher Scientific, Loughborough, UK) as previously described (Gedi *et al.*, 2017). The system consists of the Thermo Scientific Trace 1300 gas chromatography system (Thermo Fisher Scientific, Loughborough, UK) which utilised helium as a carrier gas and column operating parameters of a 5-min retention time at 140 °C then temperature gradient of 4 °C /min up to 240 °C, then held at this temperature for 10 minutes to complete the run. A Thermo Scientific ISQ 7000 (Thermo Fisher Scientific, Loughborough, UK) was used for mass spectrometry. The standards used were Supelco 37 Component FAME mix (Merck, Gillingham, UK). Data outputs were analysed using Chromeleon 7 (Thermo Fisher Scientific, Loughborough, UK). Proportions of individual fatty acids were calculated relative to total amount of all fatty acids detected by GCMS.

Fibre and ash were determined on samples previously defatted via Gerhardt's Soxtherm. Fibre analysis was carried out using the Fibretherm methodology (Gerhardt Analytical Systems, Königswinter, Germany) and their standard method (AN-04-203). The defatted dry matter was subjected to acid (0.128M sulphuric acid) and then alkaline digestion (0.313M sodium hydroxide) to remove starch/ sugars and then protein, with the remaining material being crude fibre and ash. This was combusted in an oven at 600 °C for 4 hours to remove the fibre and thereby allow the determination of ash contents.

RNA extractions

Frozen (–80 °C) groups of 25 MW collected for RNA extractions were crushed using a liquid nitrogen cooled mortar and pestle to produce an homogeneous powder. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Manchester, UK), following the manufacturer's instructions including the use of a DNase treatment step to remove any DNA present. The purity and concentration of the extracted RNA was determined by Nanodrop 2000 (Thermo Fisher Scientific, Loughborough, UK). Total RNA samples were run on agarose electrophoresis gels (1% w/v) at 100 V for 30 minute to validate their integrity. Any poor quality samples were re-extracted.

Microarray (GeneChip) analyses

The use of the microarray was as previously described (Graham *et al.*, 2011). First strand cDNA was produced by reverse transcription followed by second strand synthesis. Double stranded cDNA were then used to synthesise biotinylated complementary RNA *in vitro*, which was purified and fragmented in different sizes (200-2000 bp). These fragments were hybridised onto a GeneChip™ Drosophila gene 1.1 st array (Thermo Fisher Scientific, Loughborough, UK) using the GeneAtlas™ System (Thermo Fisher Scientific, Loughborough, UK).

Statistical analysis

Data were analysed using Genstat statistical software (21st Edition). The growth and nutrient composition data obtained from groups across the time course (n = 3) was analysed using one-way (time) ANOVA. When

$P < 0.05$ was observed for ANOVA, post hoc Bonferroni tests were used to identify differences between time points.

Gene expression profile data was generated as CEL files and initially subjected to analysis by the Partek Genomics Suite (PGS) 6.6 software (Partek, Chesterfield, MO, USA). Quality Control (QC) metrics were checked by examining surface defects, hybridization, labeling, and a ratio of the 3′ probe set to the 5′ probe set (3′/5′ ratio) to provide the quality of the microarray data. The values were log2 transformed and quantile normalization using the Robust Multi-array Average (RMA). The list of genes of interest comprised genes upregulated or downregulated by at least one fold with an un-adjusted *P*-value <0.05. Gene Ontology (GO) analysis (PGS 6.6) was performed selecting the Fisher's Exact test to compare the proportion of gene lists in a functional group to the proportion of genes in the background for that group. An enrichment score >1 corresponds to overrepresentation of a particular functional group.

3 Results and discussion

Changes in nutrient composition during growth

Preliminary work showed that following hatching, mealworms show minimal growth in the first 6 weeks after hatching. Therefore, from a starting point of between 6-7 weeks old, a time course study was conducted from day –24 to day 0 (when pupation started), over which time mealworms progressively increased in weight (Figure 1, $P < 0.001$) with those at day 0 being 89% heavier than those at day –24. There was a significant ($P < 0.001$) increase in the moisture content of the MW between days –24 and –12, after which it plateaued until pupation when there was a decrease (Figure 2). There was a significant ($P < 0.001$) increase in fat content between days –24 and –17 (44% increase – Figure 2), with no further changes at subsequent time points, nor in the pupae, which agrees with a previous report (Dreassi *et al.*, 2017). By contrast, protein content significantly decreased ($P < 0.001$), with the greatest drop (–12%) being between days –17 and –12. At the end of the time course, MW larvae contained 50% protein, which was maintained in the pupae (Figure 2). Although crude fibre content showed a significant difference ($P < 0.001$), this was entirely due to a lower fibre content of the pupae compared to MW. Overall the nutritional composition of the MW was similar to that previously reported from a range of studies (Hawkey *et al.*, 2021), with protein content being within the range 46-54%, and fat within the range 25-36%. These reported ranges probably reflect the lack of standardisation in the rearing conditions and use of MW at different stages of development. Our results show a change in the total fat and protein contents in the mid-late stages of larval growth, suggesting changes in metabolism, which likely relate to the preparation for pupation (Chapman, 2013; Li *et al.*, 2019). This has also been seen in *H. illucens* (black soldier fly) larvae, where body fat accumulates in the larval stage in order to support reproductive processes in the adult fly (Georgescu *et al.*, 2021).

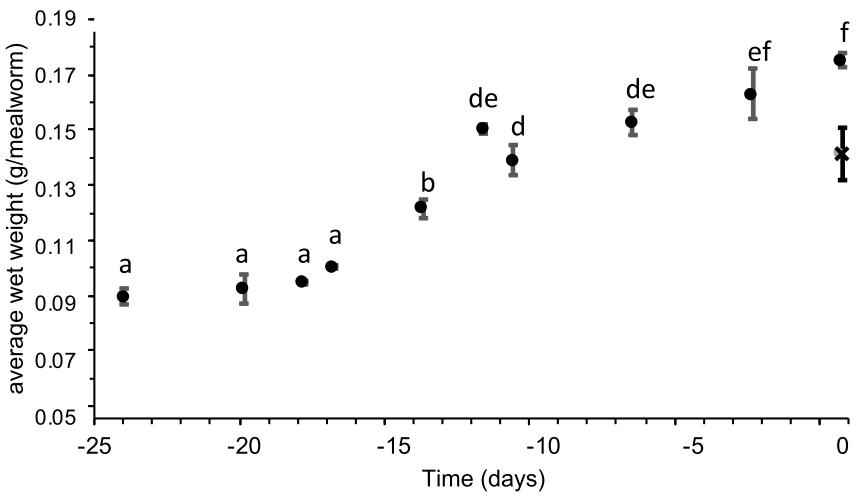

FIGURE 1 Average weight of Mealworms (MW) across the growth period prior to pupation. Average weight of individual MW was calculated from replicates (n = 3) of 1000 MW. Day 0 is the start of pupation, with negative values indicating number of days before pupation started. Data are presented as means ± standard deviation. The single data point shown by a cross is the average weight of pupae. There was a significant effect of time ($P < 0.001$, one-way ANOVA). [a,b,d,e,f] Different letters indicate significant differences ($P < 0.05$, Bonferroni host-hoc test).

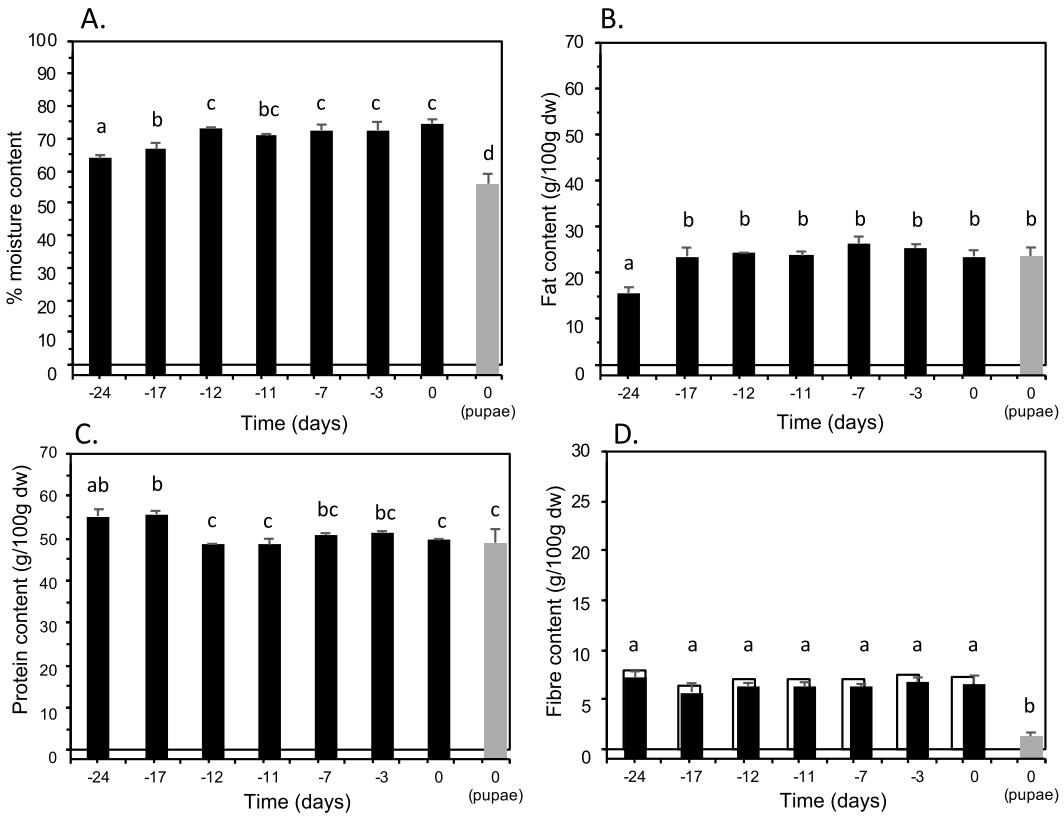

FIGURE 2 Changes in composition of mealworms (MW) across the growth period and pupation. Changes in (A) Moisture (%), (B) fat (g/100 g DM), (C) protein (g/100 g DM) and (D) fibre (g/100 g DM) contents across the time course. Day 0 is the start of pupation, with negative values indicating the number of days before pupation started. Data are presented as means ± standard deviation for n = 3 replicates. The grey bar at Day 0 indicates the data for pupae obtained at day 0. There was a significant effect of time for all 4 constituents ($P < 0.001$, one-way ANOVA). [a,b,c] Different letters indicate significant differences ($P < 0.05$, Bonferroni host-hoc test).

In addition to a reduction in total fat content of the MW, changes in fatty acid composition were also seen across the time course (Table 1). As shown previously (Dreassi *et al.*, 2017; Kroncke *et al.*, 2023), palmitic, oleic and linoleic acids were the predominant fatty acids found in mealworms, and this was the case at each time point, while small amounts of myristic, stearic, palmitoleic and α-linolenic acids were also found. While all fatty acids showed some variability over time, the biggest changes were seen in oleic and linoleic acids. At the earliest time point, oleic acid was the predominant fatty acid, though this fell with increasing time. By contrast, the proportion of linoleic acid increased with time, such that by the later time points this was the predominant fatty acid. The reasons for the change in relative proportion of these unsaturated fatty acids remains to be established, particularly as to whether this represents a change in deposition of dietary fatty acids, or changes in *de novo* synthesis and/or desaturation. Linoleic acid is the predominate fatty acid in the wheat bran (which was used as feed) representing over 50% of the total fatty acids present, compared to 15% oleic acid (Feed Tables, 2021). It is however of note that *Tenebrio molitor* may have the capacity to synthesize linoleic acid from oleic acid, as the closely related *Tribolium castaneum* species has been shown to express the delta-twelve-desaturase enzyme required for such a transformation (Zhou *et al.*, 2008).

Differential gene expression

Total RNA was successful extracted from MW from days –24, –17, –11, –3 and 0. Total RNA concentrations ranged between 100 and 700 ng/μl and had A_{260}/A_{280} and A_{260}/A_{230} absorbance ratios greater than 1.96 and 1.70, respectively. Our previous work (Graham *et al.*, 2011) showed that it was possible to use a human GeneChip array (Affymetrix; Thermo Fisher Scientific, Loughborough, UK) to detect changes in the ovine transcriptome using a cross species approach. In the current study, we used the *Drosophila* GeneChip (Affymetrix) to identify differentially expressed genes in MW across the growth time course. This approach appeared to produce promising results. Principal component analysis (PCA) clearly showed the similarities between biological repli-

TABLE 1 Fatty acid composition of mealworms (MW) across the growth period and in pupae

	Saturated fatty aAcids[1]			Unsaturated fatty acids[1]			
Days	MA[2] (C14:0)	PA (C16:0)	SA (C18:0)	POA(C16:1)	OA(C18:1n9c)	LA(C18:2n6c)	ALA(C18:3n3)
–24	1.77 ± 0.12^{ab}	19.33 ± 1.00^{b}	4.20 ± 0.11^{b}	1.73 ± 0.07^{c}	41.73 ± 1.17^{b}	29.60 ± 0.64^{a}	1.60 ± 0.10
–17	1.97 ± 0.06^{ab}	17.57 ± 0.36^{ab}	3.30 ± 0.22^{a}	1.67 ± 0.18^{bc}	38.30 ± 1.68^{ab}	34.73 ± 2.11^{ab}	1.77 ± 0.15
–12	1.73 ± 0.18^{ab}	17.13 ± 0.13^{a}	2.93 ± 0.08^{a}	1.53 ± 0.06^{bc}	36.17 ± 0.85^{a}	37.93 ± 1.18^{b}	1.97 ± 0.09
–11	1.70 ± 0.10^{a}	17.47 ± 0.23^{ab}	2.93 ± 0.06^{a}	1.40 ± 0.10^{ab}	35.53 ± 0.75^{a}	38.37 ± 0.10^{b}	2.00 ± 0.06
–7	2.17 ± 0.24^{ab}	18.47 ± 1.23^{ab}	3.30 ± 0.36^{a}	1.53 ± 0.05^{bc}	37.93 ± 3.83^{ab}	33.70 ± 6.0^{ab}	1.47 ± 0.56
–3	2.23 ± 0.14^{b}	17.70 ± 0.43^{ab}	3.07 ± 0.03^{a}	1.37 ± 0.13^{ab}	35.47 ± 1.41^{a}	37.57 ± 2.00^{b}	1.73 ± 0.15
0	1.93 ± 0.19^{ab}	16.97 ± 0.38^{ab}	3.13 ± 0.17^{a}	1.20 ± 0.01^{a}	34.03 ± 0.83^{a}	40.10 ± 1.27^{b}	1.90 ± 0.07
0 (Pupae)	2.77 ± 0.16^{c}	18.40 ± 0.46^{a}	3.50 ± 0.29^{a}	1.37 ± 0.10^{ab}	35.03 ± 0.28^{a}	36.10 ± 0.45^{ab}	1.40 ± 0.01
P-value[3]	<0.001	<0.01	<0.001	<0.001	0.001	<0.01	<0.05

1 Individual FAs are expressed as mean % of the total of all fatty acids detected by GCMS ± SD. Fatty acids with <1% abundance are not shown.

2 MA = myristic acid, PA = palmitic acid, SA = stearic acid, POA = palmitoleic acid, OA = oleic acid, LA = linoleic acid, ALA = α-linolenic acid.

3 *P*-value for one-way ANOVA.

a,b,c For each fatty acid, means with different superscript letters are significantly different ($P < 0.05$, Bonferroni host-hoc test).

cates for time points but clear differences between the four larval groups (days –24, –17, –11 and –3) and the pupae (day 0). There were large differences between larvae and pupae were likely due to the large changes relating to the metamorphosis occurring with pupation.

Since the highest proportional change in nutritional composition was observed between days –24 and –17, where total fat increased by 44%, these two time points were compared by cross-species microarray. Hybridisation of MW RNAs to the *Drosophila* GeneChip identified 685 differentially expressed genes ($P < 0.05$) out of 16,322 probes on the array (Figure 3A and Supplementary Table S1). Of these differentially expressed genes, 624 were associated with an annotated *Drosophila* gene and 61 were classified as unknown transcripts. Relative to day –24, there were 361 transcripts that were up-regulated at day –17 and 324 transcripts that were down-regulated (Supplementary Table S1). The GO enrichment based on the different biological processes highlighted effects on a range of processes including a number relating to growth and metabolism (Figure 3B).

The top 20 genes that were down- or up-regulated are shown in Tables 2 and 3. Functions of some of the down-regulated genes (Table 2) included chitin metabolism (Muc68D and Cpr47Ef), proteolysis (CG9586 and Nepl8) and vitellogenesis (yl), whose principal hormone (vitellogenin) is synthesized in the fat body tissue that contains lipid storage droplets and associated proteins and lipases (Corona *et al.*, 2007). For the up-regulated genes there were a number of transcripts of unknown function, but there were a number associated with proliferation, differentiation and morphogenesis (Idgf2, Sox21b and lin-28).

In the cross-species array approach, labelled transcripts from the species under investigation hybridise to known and often annotated probe sequences on the array. This approach has often been applied to examine transcript expression across biological orders, for example, human (Order – *Primates*) microarrays have been used for sheep (Order – *Antiodactyla*) (Graham *et al.*, 2011), horses (Order – *Perissodactyla*) (Graham *et al.*, 2010) and elephants (Order – *Proboscidea*) (Nilsson *et al.*, 2014). Positive transcripts are identified by high sequence similarity hybridisation of multiple probes spanning a probe set for that annotated gene, meaning that it is very likely that the transcripts detected are homologues, potentially orthologues with similar biological function. This then allows the application of GO analysis to identify major processes being affected by treatments. There may be a degree of misidentifi-

TABLE 2 Top 20 genes down-regulated in mealworms (MW) when comparing day –17 to –24 of the growth time course

	Probe set ID[1]	Fold change[2]	*P*-value no.[3]	Gene symbol[3]	Accession	Description[3]
1	18140727	–1.54	0.0032	NimB4	NM_176032	Involved in apoptosis, phagosome maturation
2	18198571	–1.44	0.0108	HHEX	NM_142681	Homeobox transcription factor activity response to sucrose
3	18149496	–1.39	0.0018	CG14759	NM_001299262	Transcript of unknown function
4	18142739	–1.38	0.0489	CG9586	NM_001273350	Orthologue of human CCDC43, associated with cell proliferation
5	18216902	–1.38	0.0039	Vha36-3	NM_001297899	ATP hydrolysis coupled proton transport
6	18180422	–1.35	0.0433	CG33160	NM_176278	Proteolysis – serine-type endopeptidase
7	18158259	–1.33	0.0167	CG6362	NM_137424	Transcript of unknown function
8	18152426	–1.33	0.0101	Ice1	NM_137942	regulates small nuclear RNA (snRNA) gene transcription
9	18147545	–1.31	0.0383	retn	NM_001299844	Transcription factor -involved in glial cell development
10	18157707	–1.31	0.0047	fus	NM_001169690	Regulation of mRNA splicing
11	18185998	–1.31	0.0079	CG7091	NM_141986	Transmembrane transport
12	18177590	–1.30	0.0055	Muc68D	NM_140247	Chitin metabolism
13	18168483	–1.30	0.0217	Tsp66E	NM_079261	Cell surface receptor signaling pathway
14	18206130	–1.30	0.0021	yl	NM_078596	Vitellogenesis
15	18132555	–1.30	0.0188	robl37BC	NM_136087	Microtubule-based cell movement
16	18155485	–1.29	0.0178	CG44243	NM_001202029	Involved in protein lipoylation
17	18135289	–1.28	0.0088	Nepl8	NM_135951	Proteolysis – metalloendopeptidase activity
18	18167827	–1.28	0.0282	CG11350	NM_139653	Transcription co-activator
19	18159586	–1.28	0.0146	Cpr47Ef	NM_001201976	Chitin metabolism
20	18159139	–1.27	0.0490	PPO2	NM_136599	Formation of pigments

1 Probe set ID for GeneChip™ *Drosophila* gene 1.1 st array (Thermo Fisher Scientific).

2 Fold change of transcripts with $P < 0.05$ for day –17 compared to –24 of the MW growth time course.

3 Gene symbol, accession number and description are assigned from the *Drosophila melanogaster* genome.

cation of paralogues due to hybridisation across conserved regions of transcripts, but due to the need for multiple probes to produce a strong signal, cross-hybridisation will not mis-identify entirely unrelated genes. The biggest concern is that inefficient hybridisation to highly divergent homologues may result in missed candidates, however, measures of differential expression will be quantitative between samples for all identified homologue candidates, even if the size of probe sets is reduced. Our previous work using a human array for cross-species detection of ovine transcripts indicated this to be the case, with transcripts from liver or muscle being identified by the appropriate probes known to have tissue specific expression (Graham *et al.*, 2011). The advantage of using the *Drosophila* array in a cross-species approach is that it has a large number of probes (16,322) with a wide coverage of the genome and that the array has extensive gene annotation. The availability of gene annotation allows the potential mining of data through pathway enrichment analysis to identify gene clusters which due to annotation allows the identification of potential interactions associated biological processes. This can then help the understanding of the development of phenotypes. More information *Tenebrio molitor* genome is becoming available with the partly annotated genome starting to emerge (Eriksson *et al.*, 2020; Eleftheriou *et al.*, 2022; Kaur *et al.*, 2023), which will hopefully in the future for allow a complete species transcriptome to be identified. The partly annotated genome could be utilised in combination with the cross-species microarray approach to clarify some of the gaps in either resource. For insect species where the genome has not been fully sequenced, or there is little or no annotation of that genome, the approach described in this study potentially allows the study of gene expression profiles.

TABLE 3 Top 20 genes up-regulated in mealworms (MW) when comparing day –17 to –24 of the growth time course

	Probe set ID[1]	Fold change[2]	*P*-value	Gene symbol[3]	Accession no.[3]	Description[3]
1	18146297	6.61	0.0046	CG42680	NM_001201881	Transcript of unknown function
2	18171376	2.39	0.0187	CG32214	NM_001275116	Transcript of unknown function
3	18178258	1.71	0.0075	CG13050	NM_140602	Transcript of unknown function
4	18198737	1.64	0.0006	CG5386	NM_142766	Transcript of unknown function
5	18178862	1.61	0.0051	CG18294	NM_140858	Transcript of unknown function
6	18140900	1.61	0.0444	osp	NM_001169504	Cytoskeletal protein
7	18170030	1.55	0.0016	CG12519	NM_001275117	Transcript of unknown function
8	18218384	1.50	0.0170	inaF-D	NM_001103488	Membrane channel regulator
9	18202244	1.45	0.0019	CG45105	NM_001300415	Microtubule organisation
10	18208408	1.39	0.0077	CG2157	NM_001298164	Transcript of unknown function
11	18182932	1.38	0.0108	sim	NM_001275598	Transcription factor associated with Ectoderm development
12	18141683	1.37	0.0491	mRpS2	NM_078749	Part of mitochondrial small ribosomal subunit
13	18131850	1.36	0.0008	Idgf2	NM_001259113	Imaginal disc growth factor 2 – stimulates proliferation
14	18187565	1.36	0.0140	CG13856	NM_142779	Transcript of unknown function
15	18168910	1.36	0.0208	CG7377	NM_206330	Transcript of unknown function
16	18179686	1.35	0.0342	Sox21b	NM_001274899	Transcription factor associated with morphogenesis
17	18167974	1.34	0.0086	lin-28	NM_139726	Regulation of stem cell maintenance and differentiation
18	18206391	1.34	0.0181	Cp7Fa	NM_001038748	Chorion-containing eggshell formation
19	18164146	1.33	0.0304	CG42382	NM_078941	Predicted ion binding activity
20	18179673	1.33	0.0058	Cpr65Ax1	NM_001104053	Chitin-based cuticle development

1 Probe set ID for GeneChip™ *Drosophila* gene 1.1 st array (Thermo Fisher Scientific).
2 Fold change of transcripts with $P < 0.05$ for day –17 compared to –24 of the MW growth time course.
3 Gene symbol, accession number and description are assigned from the *Drosophila melanogaster* genome.

4 Conclusions

We have shown changes in fat and protein composition across the growth period of MWs and that these changes are associated with significant changes in gene expression, as determined by the cross-species microarray approach. Hence, the use of cross-species microarrays has the potential to enhance our understanding of the fundamental biology and molecular mechanisms that regulate the nutritional value of edible insects.

Supplementary material

Supplementary material is available online at:
https://doi.org/10.6084/m9.figshare.24160281

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Conflict of interest

All authors declare that they have no conflicts of interest.

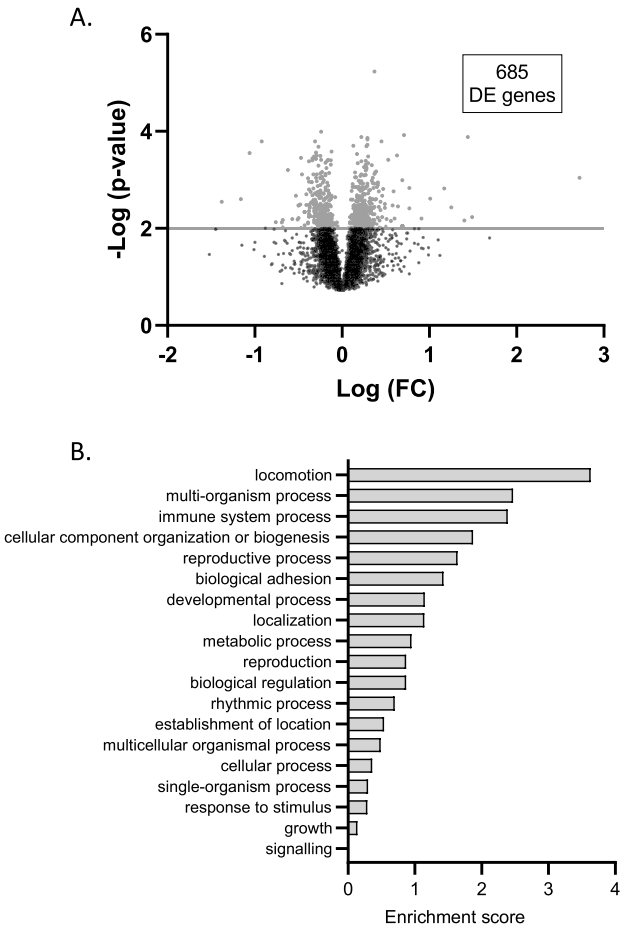

FIGURE 3 Differentially expressed gene transcripts detected using the GeneChip™ *Drosophila* Genome 2.0 Array and comparing mealworms (MW) at days –17 and –24 of the growth period. Days –24 and –17 (before pupation) were compared as that is when the biggest change in fat contents was observed. (A) Volcano plot showing the –Log (*P*-value) against log fold change (FC) for array probes detecting MW transcripts. A total of 685 genes/ transcripts were differentially expressed using the cut off of –Log(*P*-value) > 2. (B) Enrichment scores for biological processes identified for array probes detecting differentially expressed MW transcripts.

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