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Octopamine alters yellow mealworm body composition

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ABSTRACT

There is the potential to increase the production yield within the emerging insect industry in order to produce high-quality, sustainable protein. In invertebrates, the monoamine, octopamine (**OA**), has a similar role to that of noradrenaline in mammals. Beta-2 adrenergic agonists increase protein and decrease fat deposition in mammals, thereby inducing favourable changes in body composition. We hypothesised that OA would have similar effects in insects. *Tenebrio molitor* larvae, commonly called yellow mealworms, were fed for 35 days on either control wheat bran or wheat bran containing OA at 5 μg OA/g. There were trends for treatment × time interactions for mealworm group weight ($P = 0.075$) and individual mealworm weight ($P = 0.069$), with the OA group becoming heavier/bigger after 18 days. In addition, there was a trend for a treatment × time interaction on cumulative pupation ($P = 0.099$), with OA–treated mealworms having delayed pupation. After 35 days of OA treatment, there were significant effects on mealworm final body proximate nutrient composition on a DM basis, with fat content being significantly decreased (by 8%, $P = 0.006$), whilst CP was significantly increased (by 6%, $P = 0.019$) in OA–treated mealworms compared to control. There was little effect of OA on the fatty acid composition of the mealworms, with small reductions in palmitoleic acid ($P < 0.001$) and oleic acid ($P = 0.082$). Despite a significant increase in protein content with OA treatment, SDS-PAGE did not reveal any changes in the proteins being expressed. Hence, OA treatment of mealworms resulted in an increase in the proportion of protein and a decrease in fat, demonstrating that mealworm nutrient composition can potentially be manipulated to provide a higher–value feed ingredient.

Implications

Comparatively, insects are at an earlier stage of use as food production animals and therefore, the extent body composition can be altered to improve their nutritional quality has not been explored. In insects, monoamines have similar roles as adrenergic agonists in mammals, in which beta-adrenergic agonists have been shown to impact body composition. In this study, treatment of mealworms (*Tenebrio molitor* larvae) with the monoamine, octopamine, resulted in decreased fat and increased protein content, with a tendency for octopamine to improve growth characteristics. This indicates that mealworms can potentially be manipulated to improve their body composition and nutritional value.

Introduction

Insects have been identified as a novel alternative protein source (Hawkey et al., 2021; van Huis et al., 2013). The ability of *Tenebrio molitor* larvae (yellow mealworms) to grow on low-quality substrates (Alves et al., 2016; Yang et al., 2019) and have a high nutritional value (Ghaly and Alkoaik, 2009; Hawkey et al., 2021) makes them attractive as a novel alternative protein source. Their protein and fat per unit dry weight has been reported as 46–54% and 25–36%, respectively (Hawkey et al., 2021). Therefore, this variation in values indicates mealworm composition potentially could be manipulated to maximise the protein and minimise the fat content. Typically, manipulations involve the alteration of mealworm feed composition. For example, Kröncke and Benning (2023) reported that adding increasing quantities of protein (such as pea or rice protein isolates) to wheat bran (their normal feed) decreased growth rate, but the mealworm protein DM increased and fat DM decreased. Mealworms, like many insects that undergo metamorphosis, appear to have a critical BW (known as threshold size) in determining the onset of metamorphosis (He et al., 2020; Malita and Rewitz, 2021). To reach threshold size, both protein and fat are accumulated, which, along with growth and metamorphosis, are regulated by a range of endocrine factors. The predominant endocrine factors associated with larval growth are the ecdysteroids, which are involved in inducing moulting (Lafont et al., 2005) as well as metamorphosis activation (Cherbas et al.,

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2003). The sesquiterpene, juvenile hormone (**JH**), also has a significant role in regulating growth during life stage progression and metamorphosis (Jindra et al., 2013). The monoamines, octopamine (**OA**) and tyramine (**TA**), have similar roles in insects to those of the adrenergic agonists, noradrenaline and adrenaline in mammals. Like the mammalian adrenergic system, where adrenaline is produced from noradrenaline, OA is produced from TA (Roeder, 2020), and TA and OA are involved in regulating behaviour, physiological processes and metabolic pathways (Li et al., 2016).

In mammals, adrenergic agonists, especially adrenaline, are known to have effects on nutrient mobilisation, inducing glycogenolysis (i.e. mobilisation of the glucose stored as glycogen), as well as lipolysis (i.e. mobilisation of lipids stored as triacylglycerol to release glycerol and fatty acids (Lanham-New et al., 2010)). In addition, stimulation of beta-2 adrenergic receptors via synthetic agonists such as clenbuterol and cimaterol have long been known to increase protein deposition (Bohorov et al., 1987). Derivatives of the beta-adrenergic agonists are licenced for use as growth promoters for production animals in several countries in the world, but not in the European Union (Brameld and Parr, 2016). As these agents regulate nutrient deposition in production animals, they have been investigated to gain an understanding of this process (Brown et al., 2018) and the consequences of growth manipulation and nutrient deposition on product quality (Kemp et al., 2010). From this research, genes and polymorphisms have been identified for molecular marker-assisted breeding selection (Zalewska et al., 2021). Comparatively, insects are at a much earlier stage of use as food production animals, however, the identification of exogenous agents that affect insect body composition would help lead to a better understanding of the mechanisms involved in altering insect nutrient composition. Work in our labs has already shown that inclusion of JH in the diet can alter mealworm body composition by repartitioning nutrients, significantly increasing protein and decreasing fat (Hill et al., 2024).

However, increasing animal size is a fundamental objective for animal production systems, ideally through enhancing production efficiency (i.e. growth per unit feed), along with nutrient deposition as protein rather than fat. This is also one of the main objectives of the insect production industry. Mealworms accumulate more lipid as they mature during the larval stages of their life cycle, which is associated with a decreased protein content (Lopez-Viso et al., 2024, Kröncke et al., 2023), changes which appear to be associated with the preparation for pupation (Li et al., 2019). OA acts as an endocrine factor in a range of invertebrate species. For example, in *Caenorhabditis elegans,* it has been shown to stimulate lipolysis (Tao et al., 2016), while *Drosophila melanogaster* lacking OA have increased fat deposition, and increased release from internal OA stores results in a decrease in body fat (Li et al., 2016). In terms of nutrient metabolism and deposition in invertebrates, it appears that OA may have similar effects on the adrenergic system in mammals, at least at the level of fat deposition. Our hypothesis therefore was that OA would decrease fat deposition and this would be associated with an increase in the proportion of protein in mealworms. Hence, the objectives of this study were to determine the impact of administering dietary OA to mealworms for 35 days on larval growth (before pupation) and the protein and fat composition of mealworms.

Material and methods

Study design

The study was an experimental randomised block design assessing the change in mealworm BW, pupation and death over a 35–day treatment period. Our previous study (Hill et al., 2024) observed significant effects of a 28–day exposure to the exogenous endocrine factor JH; therefore, we used a similar time period, but as OA is not expected to be as efficacious as JH, we extended the time period to 35 days. At the end of the intervention, mealworm proximate nutrient composition (fat and protein) was determined. The experimental replication unit was the container, each holding 300 mealworms, with n = 4 replicates per treatment. Replication number was based on our previous study where, on the same level of experimental replication, administration of the exogenous endocrine factor JH significantly affect mealworm growth and proximate nutrient composition relative to the control (Hill et al., 2024).

Mealworm feeding trial

Mealworms at a length of 18–20 mm were supplied by Ynsect Ltd (France) at approximately 6 weeks old, although the supplier did not provide a hatch date. This age was used because the start of pupation was expected over the period of the trial, thereby allowing us to assess the effect of OA on this life cycle event, as well as growth. Following their arrival, mealworms were fed *ad libitum* on organic wheat bran (Buy Whole Foods Online) at a particle size between 2 and 0.5 mm (standardised by sieving), in a dark incubator (Fitotron®, Weisstechnik) at 27 °C and 60% (range 59.4–60.4%) humidity. Water was provided using a 1 cm diameter cotton wool ball soaked in deionised water; this was replaced twice a week. Following 3 days of acclimatisation, small mealworms which were able to pass through a 2 mm sieve (Retsch) were removed. Mealworms larger than 2 mm were randomly allocated to 6x9x16cm containers (300 mealworms counted per container, average individual mealworm weight 74.1 ± 0.9 mg) and allocated to one of two treatment groups (four containers per treatment). Vehicle control (control) was 1.2 ml deionised water added per 100 g wheat bran. The OA (Merck Life Science) was made using the same volume of deionised water but at a concentration that resulted in 5 μg OA/g wheat bran. This dose was derived from a study previously conducted examining the role of OA in endurance exercise adaptations in Drosophila (Sujkowski et al., 2017). The proximate nutrient composition of wheat bran (determined in our labs as described below) was 14.8/100 g DM CP and 2.3/100 g DM crude fat. The OA and vehicle control solutions were first added to 10 g of wheat bran and mixed by shaking in a sealed plastic bag, to this was added 90 g of wheat bran and then mixed again. The wheat bran was then left to air dry before use. To each container was added 10 g wheat bran per container (day 0), this gave a height of substrate of approximately 5 mm. This was equivalent to a minimum of 8.3 mg/mealworm per day, but this value increased during the trial as the number of pupated or dead mealworms increased. This amount of wheat bran was identical to that used previously (Hill et al., 2024) to investigate the impact of another exogenous endocrine treatment, the JH analogue pyriproxyfen, which was also included in the diet of mealworms. In addition, other as yet unpublished studies, as well as the JH study, have clearly demonstrated that this quantity of wheat bran is sufficient to allow *ad libitum* feeding, but reduces the amount of excess feed left in the containers, which could potentially become spoiled by water leakage and thereby compromise mealworm health. Hence, the amount used reduces feed wastage and maintains health. The containers were all placed in the same incubator (Weiss Technik), on the same shelf, in randomised control and OA –treated pairs, under the same incubation conditions as described above. On days 4, 7, 11, 14, 18, 21, 25, 28, 32 and 35, mealworms were measured, and then feed, as well as water were replaced. This involved the contents of each container being sieved through a 2 mm sieve to catch the mealworms. Uneaten feed was retained

by catching it in a 0.5 mm sieve, below the 2 mm sieve, while the frass passed through both sieves, all this material was then discarded. All dead or pupated mealworms were removed from the live, and their numbers were recorded, but were not used in any further analysis. The average individual mealworm weight was calculated by dividing the group weight of live mealworms by the number present. The live mealworms were returned to their respective container and 10 g wheat bran containing vehicle or OA was added, along with fresh water, and the containers were returned to the incubator. All remaining live mealworms at the end of the trial (day 35) were culled by being submersed in liquid nitrogen and stored at −80 °C for subsequent proximate nutrient analysis.

Proximate nutrient analysis

The groups of mealworms (n = 4 per treatment) culled at the end of the trial (dead or pupated mealworms being excluded) were freeze–dried (Christ Freeze Dryer) to a constant weight, to determine moisture content. They were then ground in liquid nitrogen and mixed to a fine powder, then stored in a desiccator until analysed. Using 50 mg freeze-dried samples the nitrogen content was determined using an EA 1112 elemental analyser (Thermo Fisher Scientific) along with aspartic acid standards (10.52 (w/w) N%, Elemental). To convert nitrogen into total CP, the conversion factor of 4.76 was applied, as suggested by Janssen et al. (2017) for the estimation of CP content in whole larvae.

Soxtherm extraction (Gerhardt Analytical Systems) method was used to determine total fat content as described previously (Plamquist and Jenkins, 2003). A 1 g aliquot of freeze-dried mealworms was boiled at 150 °C in petroleum ether for 30 min followed by refluxing for 90 min. The collected solvent was evaporated to dryness and the weight of fat was determined. For subsequent fatty acid analysis, the extracted fat was resuspended in hexane and kept at −20 °C. Fatty acid contents were determined by first hydrolysing the triacylglycerol followed by fatty acid methyl esterification, as described by Lock et al. (2005). These samples were stored in hexane at −20 °C before analysis by gas chromatography–MS as previously described (Gedi et al., 2017). The Thermo Scientific Trace 1 300 gas chromatography system (Thermo Fisher Scientific™) was used for the analysis of samples. Helium was the carrier gas and column operating parameters were a 5 min retention time at 140 °C, followed by a temperature gradient of 4 °C/min up to 240 °C, this temperature was held for 10 min to complete the run. The Thermo Scientific ISQ 7 000 (Thermo Fisher Scientific™) was used for MS. Supelco 37 Component FAME mix (Merck) were used as standards. Chromeleon 7 (Thermo Fisher Scientific™) was used for data analysis with the proportions of individual fatty acids calculated relative to the total amount of all fatty acids detected by gas chromatography–MS.

SDS-PAGE

SDS-PAGE was carried out to determine whether the types of proteins in whole mealworms were altered by OA treatment. SDS-PAGE was carried out to determine whether the types of proteins in whole mealworms were altered by OA treatment. Samples (200 mg) of freeze-dried mealworm powder (the same as used for nutrient analysis) were prepared for SDS-PAGE as previously described by Hill et al. (2024). Constant protein was then loaded onto a 4–15% polyacrylamide gel (Criterion™ TGX Stain-Free™, Bio-Rad) and separated by electrophoresis for 30 min. Stain-free gels were exposed to UV, with an activation time of 5 min and images were captured using the ChemiDoc™ Imaging System (Bio-Rad), according to the manufacturer's instructions.

Statistical analyses

Outliers of data were identified as being greater than 2x SD (there were none), and data were not tested for normality, based on the advice of van Emden (2008). Data were analysed using Genstat statistical software (21st Edition, 2021) by two–sample *t*-tests (to examine effects of treatment) or two-way repeated measures ANOVA (to examine treatment × time, with time being the repeated factor) and, when appropriate, ($P < 0.05$), subsequently analysed by *post hoc* Bonferroni tests. Data were presented as the means ± SE of the mean, with significant differences accepted at $P < 0.05$, with a trend being considered at $P < 0.1$.

Results

Effects of octopamine on mealworm growth, pupation and survival

There was a trend for a treatment × time interaction ($F_{10,60} = 4.71$, $P = 0.069$) for average individual mealworm weight, with the OA–treated mealworms being bigger/heavier than the controls from day 21, until day 35, the OA mealworms were 173.0 ± 5.9 mg per larvae whilst control were 135.0 ± 17.6 mg per larvae (Fig. 1A). As expected, there was a significant effect of time ($F_{10,60} = 83.71$, $P < 0.001$), with the mealworms growing over the 35 days, but there was also a trend ($F_{1,3} = 8.35$, $P = 0.063$) for a treatment effect. There was also a trend for a time × treatment interaction ($F_{10,60} = 3.38$, $P = 0.099$) for the cumulative number of pupated mealworms as a proportion of the remaining viable larvae, with more pupation seen in the control group, particularly after day 14 (Fig. 1B). Pupation appeared to be delayed in the OA–treated group, with pupae only seen after day 28, and by day 35, there were fewer percent of total mealworm pupating relative to the starting number of mealworms, OA being 27.3 ± 7.4% and control 48.7 ± 12.1%. As expected, there was a significant effect of time ($F_{10,60} = 23.94$, $P < 0.001$), with the number of pupae increasing over the 35 days, with a trend for an effect of treatment ($F_{1,3} = 5.91$, $P = 0.093$), with OA–treated mealworms tending to have fewer pupae than controls. There was no treatment × time interaction ($F_{10,60} = 0.55$, $P = 0.621$) for mealworm survival (Fig. 1C). The numbers of surviving mealworms declined over time ($F_{10,60} = 60.23$, $P < 0.001$), to 92.8 ± 0.6% surviving, with no effect of treatment ($F_{1,3} = 1.13$, $P = 0.366$). Commercially, mealworms are harvested before pupation. Therefore, extending the time before pupation may further increase the yield, by having more live mealworms with higher weights. Therefore, we have reported the group mealworm weight, which is the live mealworm larvae per container, excluding dead and pupated. For the total group weight, there was a trend for a treatment × time interaction ($F_{10,60} = 4.06$, $P = 0.075$). As there was a trend for the weight per larvae to increase in the OA–treated group, and there were fewer pupating larvae in this group, it was not surprising that the OA –treated group weight was heavier than the control from day 21, with weights peaking at day 28 when the OA group were 46.1 ± 1.1 g and control 32.0 ± 9.2 g. Group weights then declined in both groups until day 35, but the OA was still heavier, with the OA group weight being 34.0 ± 10.3 g and control group weight being 19.8 ± 14.7 g (Fig. 1D). This decrease in group weight corresponded to increased pupation in both groups after day 28. As might be expected, there was a significant effect of time ($F_{10,60} = 13.96$, $P = 0.005$) and a trend for a treatment effect ($F_{1,3} = 6.37$, $P = 0.081$).

Effects of octopamine on mealworm body composition

At the end of the study (day 35), the body composition of the remaining live mealworms was determined. The moisture content

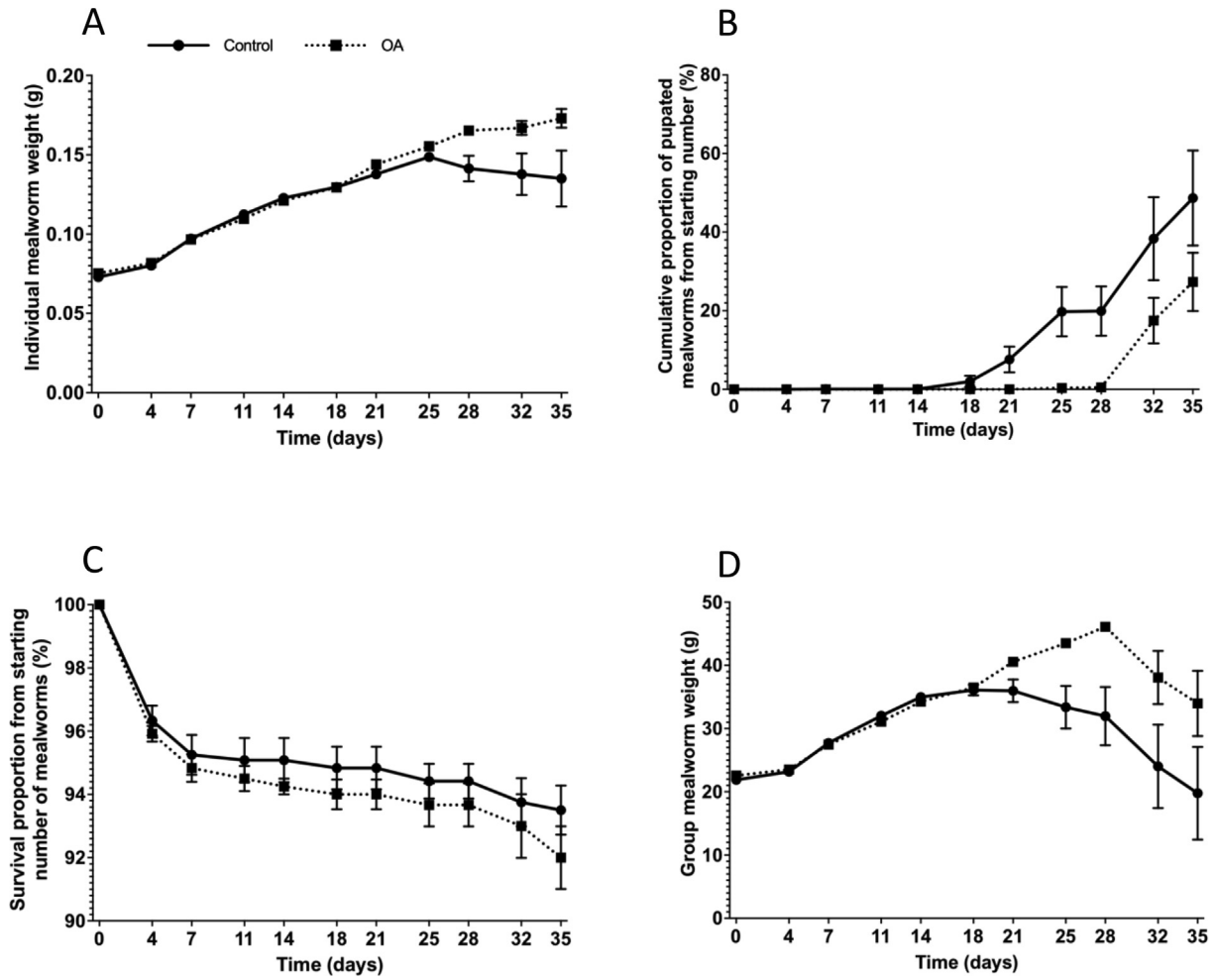

Fig. 1. Effect of 35 days treatment with octopamine (OA) on mealworm growth characteristics. Control = wheat bran feed, OA = 5 μg OA/g wheat bran feed, n = 4 per treatment group. Data are presented as average ± SEM and were analysed by 2-way repeated measures (time being the repeated factor) ANOVA. (A) The average individual mealworm weight. There was a trend for a treatment × time interaction ($P = 0.069$). (B) The cumulative proportion of mealworms pupating across the trial. There was a trend for a treatment × time interaction ($P = 0.099$). (C) Mealworm survival, indicated by the proportion of viable larvae remaining (excluding pupated and dead mealworms). There was a significant effect of time ($P < 0.001$) but no effect of treatment nor a treatment × time interaction (both $P > 0.3$). D) The average group mealworm weight (average weight of the total number of live mealworms in container treatment replicates). There was a trend for a treatment × time interaction ($P = 0.075$).

was not affected by OA treatment (t = –1.25, df = 6, $P = 0.872$, Table 1), but there were significant effects on both CP and fat contents on a DM basis. The CP content of OA–treated mealworms was significantly greater than the controls (t = –3.19, df = 6, $P = 0.019$, Table 1). Concurrently, there was a significant reduction in fat content in OA–treated mealworms (t = 4.11, df = 6, $P = 0.006$, Table 1). As the fat content was significantly decreased in OA–treated mealworms, the fatty acid composition (Table 2) was determined at the end of the study (day 35). As has been previously described for mealworms fed wheat bran diets, oleic and linoleic acids were the predominant fatty acids present (Kröncke et al., 2023; Lopez-Viso et al., 2024). The only significant effect of OA treatment was a small decrease in palmitoleic acid (t = 6.62, df = 6, $P < 0.001$), whilst there was trend for a decrease in oleic acid (t = 2.09, df = 6, $P = 0.082$).

SDS-PAGE

The SDS-PAGE indicated potential differences in major bands located at approximately 56, 35 and 16 kDa (Fig. 2). However, following quantitation via densitometry analysis, there were no significant differences in the average densities of those bands (t = –0.031, df = 6, $P = 0.976$ (56 kDa); t = –0.259, df = 6, $P = 0.805$ (35 kDa); and t = 0.339, df = 6, $P = 0.746$ (16 kDa)).

Table 1
The effect of octopamine (OA) treatment for 35 days on whole mealworm nutrient composition.

Component	Control	OA	t-value[1]	*P*-value[2]
Moisture (g/100 g mealworms)	62.5 ± 0.84	63.7 ± 0.46	–1.25	0.872
CP (g/100 g DM)	38.0 ± 0.50.	40.1 ± 0.46	–3.19	0.019
Crude Fat (g/100 g DM)	29.2 ± 0.34	26.9 ± 0.47	4.11	0.006

Abbreviations: Control = wheat bran feed, OA = 5 μg OA/g wheat bran feed.
[1] The t-value for two–sample *t*-test for the effect of treatment, with 6 df.
[2] *P*-value for two–sample *t*-test for the effect of treatment.

Table 2
The effect of octopamine (OA) treatment for 35 days on whole mealworm fatty acid composition.

Fatty acids	Proportion of fatty acid profile (g/100 g total fatty acids)[1]		t- value[2]	*P*-value[3]
	Control	OA		
Myristic (C14:0)	2.0 ± 0.10	2.1 ± 0.07	−0.73	0.491
Palmitic (C16:0)	17.0 ± 0.28	16.6 ± 0.55	0.66	0.531
Stearic (C18:0)	2.0 ± 0.08	2.1 ± 0.08	1.13	0.302
Palmitoleic (C16:1)	1.4 ± 0.03	1.1 ± 0.01	6.62	<0.001
Oleic (C18:1n9c)	35.3 ± 0.32	34.2 ± 0.42	2.09	0.082
Linoleic (C18:2n6c)	40.4 ± 0.51	41.9 ± 0.87	−1.55	0.171
α-Linolenic (C18:3n3)	1.1 ± 0.07	1.1 ± 0.13	0.14	0.894

Abbreviations: Control = wheat bran feed, OA = 5 μg OA/g wheat bran feed.
[1] Individual fatty acids are expressed as mean of the total of all fatty acids detected by gas chromatography–MS ± SEM (n = 4). Fatty acids with <1/100 g total fatty acid abundance are not shown.
[2] The t-value for two–sample *t*-test for the effect of treatment, with 6 df.
[3] *P*-value for two–sample *t*-test for the effect of treatment.

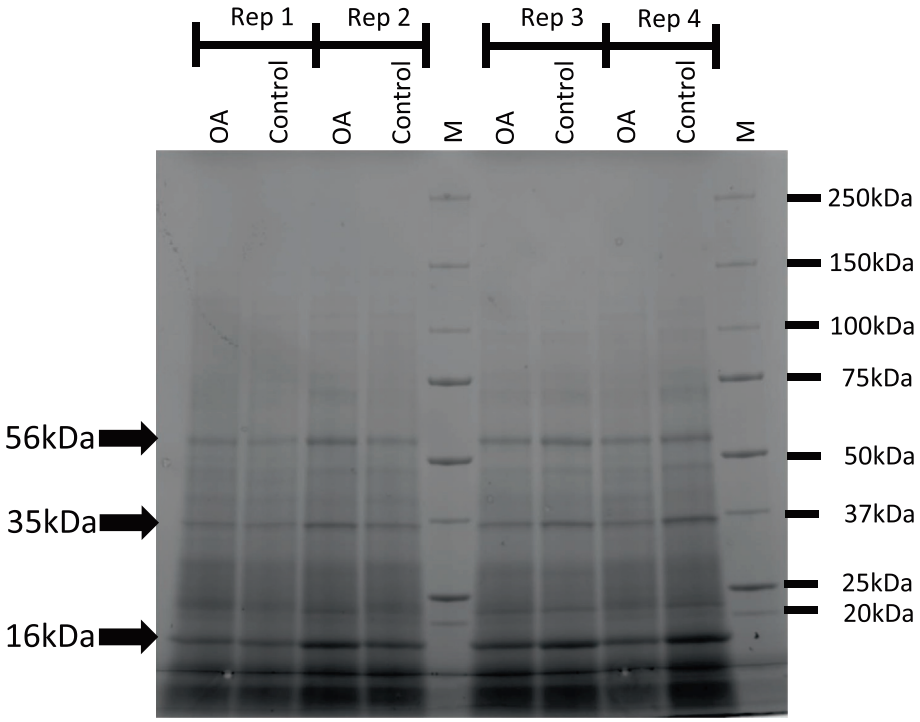

Fig. 2. Effect of octopamine (OA) treatment for 35 days on whole mealworm protein composition. A 4–15% polyacrylamide gel (Criterion™ TGX Stain-Free™, Bio-Rad) was used for SDS-PAGE of soluble mealworm protein (20 ug protein per lane). Molecular weight markers (kDa) are indicated. Arrows indicate predominant bands subjected to densitometry analysis (no significant differences). Control = wheat bran feed, OA = 5 μg OA/ g wheat bran feed, M = molecular weight markers, Rep = independent experimental replicate.

Discussion

During normal mealworm growth, the DM proportion of protein decreases and fat content increases as mealworms move towards pupation (Kröncke et al., 2023; Lopez-Viso et al., 2024; Yu et al., 2021). In this current study, OA was shown to have an impact on the body composition of mealworms, with a shift towards a leaner phenotype. The growth (at a container-group and individual larvae level) and pupation were only slightly affected by treatment with OA, with trends for increased weights observed with OA, but there were no effects on survival. There was a trend for the proportion of pupated mealworms being lower in the OA–treated mealworms, with 27% of total mealworms pupating, compared with 49% in the control group. This reduction in pupation may be linked to the reduced fat phenotype. Typically, as holometabolous insects grow older in their larval stage, they accumulate fat (Lopez-Viso et al., 2024) which appears to be associated with their preparation for pupation (Li et al., 2019). Given that there was a significant reduction in fat content in OA–treated mealworms at day 35, it could be postulated that this means they did not reach the critical level of fat stores required for pupation (Enriquez et al., 2022). However, the reduction in fat was insufficient to completely inhibit metamorphosis, suggesting that some were able to reach the threshold weight needed for pupation (Mirth and Riddiford, 2007). As might be expected, CP content was increased in the OA –treated mealworms, likely because a decrease in the proportion of fat in DM content (i.e. fat) means that the proportion of another component increases (i.e. protein). Hence, the impact of metabolic

changes could be on either synthesis and/or degradation of lipid and/or protein or potentially these combined. Further research is needed to clarify this, but the lack of any differences in the proteins observed in the SDS-PAGE analyses suggests that there are no gross changes in the types of proteins being expressed, and therefore the protein composition, in response to the OA treatments.

The fatty acid composition observed in this study agrees with previous studies (Kröncke et al., 2023; Lopez-Viso et al., 2024), whereby the majority of the fatty acids present are unsaturated, with the highest proportions being linoleic and oleic acids (in that order). This is thought to reflect uptake from the diet (wheat bran), which contains > 50% linoleic acid as a proportion of total fatty acid content (Feed Tables, 2021). Whilst there was a significant reduction in palmitoleic acid, a monounsaturated fat, the magnitude of this change was minimal. Overall, the effects of OA are potentially beneficial in terms of mealworm production, whereby there are no significant effects on growth (and therefore, presumably indicating a lack of detrimental effects), whilst enhancing the body composition (i.e. more protein, less fat) is more favourable.

OA is generally considered an important regulator of metabolic traits, similar to that of the mammalian adrenergic system. When *Drosophila* were exposed to ectopically increased OA they were lean and had a higher activity level (Li et al., 2016), this movement increases energy expenditure, which is typically seen as part of an increased attempt to find food, particularly in starvation conditions (Roeder, 2020). In addition, OA is involved in mobilising fat stores during times of nutrient deprivation (Tao et al., 2016), stimulating the release of the energy substrate mobilising metabolic neuropeptide, adipokinetic hormone (Pannabecker and Orchard, 1986). Adipokinetic hormone is the invertebrate equivalent of glucagon and similarly stimulates lipolysis, activating proteins involved in lipolysis such as lipid droplet-associated protein (Miura et al., 2002) and cytosolic enzyme, triglyceride lipase (Arrese et al., 2006). Hyperlipidaemic or hyperglycaemic responses can be stimulated by OA, but not both concurrently (Woodring et al., 1989) and OA is also involved in the release of trehalose, an energy-containing compound within the haemolymph (Park and Keeley, 1998). In addition, Li et al. (2016) showed that *D. melanogaster* unable to produce OA had increased fat deposition. Therefore, some of OA's effects are similar to the role of glucagon/adrenergic axis in mammals, which stimulates glycogenolysis and lipolysis. In mammals, noradrenaline inhibits insulin release from pancreatic islets (Porte and Williams, 1966), thereby reducing lipogenesis. In insects, insulin-like peptides are the homologs of mammalian insulin (Rulifson et al., 2002). OA binds to specific receptors, Octα-1R, on neurosecretory cells which synthesise insulin-like peptides. Therefore, OA may perhaps reduce the secretion of insulin/insulin-like peptide in insects (Straub and Sharp, 2012), thereby resulting in increased mobilisation of fat and glycogen stores, and reduced lipid deposition. However, the role of OA modulating insulin–like activity and its impact on nutrient deposition has not yet been fully characterised.

OA also stimulates the release of JH in some insect species (Rachinsky, 1994). We (Hill et al., 2024) have recently shown that treatment of mealworms with the JH analogue, pyriproxyfen has a potent effect of reducing fat and increasing the protein content of mealworms, as well as inhibiting pupation. However, the effect of OA on JH release is not consistent across insects, since the application of exogenous OA has also been shown to inhibit the biosynthesis and release of JH (Woodring and Hoffmann, 1994), as well as increase the activity of JH esterase (Hirashima et al., 1999), which would potentially increase the degradation of JH. Therefore, although there is a potential interaction between OA and JH, the effect is not clear and not characterised in mealworms. However, given that the effect of OA on fat (8% decrease relative to control) and protein (6% increase relative to control) proportions is relatively small compared with the effects of JH (68% decrease in fat, 46% increase in protein) (Hill et al., 2024), the likely effect of OA effect on JH is small.

In insects, OA has similar roles to those of the adrenergic agonists in mammals. In mammals, the effects of derivatives of the beta-adrenergic agonists on decreasing fat and increasing protein deposition have been well characterised along with the mechanisms which cause these effects (Mersmann, 1998). The magnitude of their effects on fat and protein deposition in production animals (8% decrease in fat deposition in pigs) are similar to the effects of OA in insects in this study. As for beta-adrenergic agonists and other experimental anabolic interventions that alter growth and body composition, investigating the metabolic and transcriptomic effects of OA on nutrient deposition in insects may yield insights into the regulation of these processes and thereby identify gene targets which may have a positive or negative effect on the efficiency of growth and product quality. An example of the success of this kind of approach is the identification of the inhibition of calpain–mediated proteolysis by the endogenous calpain-specific inhibitor, calpastatin, in beta agonist-related growth (Bohorov et al., 1987, Parr et al., 1992) and its negative impact on meat proteolysis (Kemp et al., 2010). This understanding has led to the identification of calpastatin genomic markers to select for high meat quality in production animals (Ciobanu et al., 2004, Shackelford et al., 1994).

In this study, only one dose of OA was used. This was derived from the work described by Sujkowski et al. (2017) who demonstrated four OA receptors found on different tissues in *Drosophila* which conveyed differing tissue–specific effects, with the main target tissues being skeletal and cardiac muscle. The speculation might be that increasing the dose of OA could increase the effect on muscle and fat. However, as described above, given the range of mechanisms for how OA mediates its effects and the range of target tissues, this would require a more detailed investigation to establish dose responses. The effects might be similar to those seen in other species in response to adrenergic agonists (analogues of OA), where high doses often cause negative and unfavourable cardiac (tachycardia) and skeletal muscle (tremor) effects (Sears, 2002). Treatment of mealworms with OA at the dose used in this trial resulted in mealworms exhibiting a decrease in fat and an increase in protein contents, but no impacts on the proteins or fatty acids, other than a minor reduction in palmitoleic acid. All of these effects are likely to be beneficial for commercial production for use as food or feed, However, the prohibition of use of pharmaceutical growth promoters in food production livestock (in the EU) as well as the potential impacts of contamination of food or feed would potentially prevent OA use or its analogues in insect food production systems. However, determining the mechanisms for how OA alters insect body composition would help establish the capacity and potential limits of manipulation and may allow the identification of biological processes or key genes that could be selected to improve the production of mealworms for food and feed.

Conclusion

Treating mealworms with OA resulted in them exhibiting a decrease in fat and an increase in protein contents, indicating that mealworm body composition can be manipulated. However, associated changes in growth characteristics were not substantive, with a tendency for the average individual mealworm weight to be greater than controls after 18 days of OA treatment, and OA –treated mealworms tending to have delayed pupation. Overall, these changes could benefit commercial mealworm production.

Ethics approval

Insects, as invertebrates, do not come under the Animals (Scientific Procedures) Act of 1986. However, the work was completed in line with the principles of the 3Rs (Refinement, Reduction, Replacement). The dose of OA used in the trial was derived from a study previously conducted examining the role of OA in endurance exercise adaptations in Drosophila (Sujkowski et al., 2017). The number of replicates used in the study was informed by our previous published work investigating the effects of orally administering (via feed) another endocrine factor, Juvenile Hormone, on mealworm growth (Hill et al., 2024).

Data and model availability statement

The data/models were not deposited in an official repository. The data that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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