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Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products

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ABSTRACT

Insects receive increasing attention as an alternative protein-rich food source for humans. Producing edible insects on diets composed of organic by-products could increase sustainability. In addition, insect growth rate and body composition, and hence nutritional quality, can be altered by diet.

Three edible mealworm species *Tenebrio molitor* L., *Zophobas atratus* Fab. and *Alphitobius diaperinus* Panzer were grown on diets composed of organic by-products originating from beer brewing, bread/cookie baking, potato processing and bioethanol production. Experimental diets differed with respect to protein and starch content. Larval growth and survival was monitored. Moreover, effects of dietary composition on feed conversion efficiency and mealworm crude protein and fatty acid profile were assessed. Diet affected mealworm development and feed conversion efficiency such that diets high in yeast-derived protein appear favourable, compared to diets used by commercial breeders, with respect to shortening larval development time, reducing mortality and increasing weight gain. Diet also affected the chemical composition of mealworms. Larval protein content was stable on diets that differed 2–3-fold in protein content, whereas dietary fat did have an effect on larval fat content and fatty acid profile. However, larval fatty acid profile did not necessarily follow the same trend as dietary fatty acid composition. Diets that allowed for fast larval growth and low mortality in this study led to a comparable or less favourable n6/n3 fatty acid ratio compared to control diets used by commercial breeders. In conclusion, the mealworm species used in this study can be grown successfully on diets composed of organic by-products. Diet composition did not influence larval protein content, but did alter larval fat composition to a certain extent.

1. Introduction

Insects are consumed in most tropical countries, whereas in the Western world they currently do not form a significant part of the human diet. Due to a growing world population and increasing welfare, there is a rising demand for animal-derived protein, and the consumption of insects (entomophagy) receives increasing attention as an alternative protein-rich food source (Van Huis, 2013; Van Huis et al., 2013).

Production of conventional livestock is associated with detrimental environmental effects such as global warming, land degradation, air and water pollution, and loss of biodiversity (Mekonnen and Hoekstra, 2010; Steinfeld et al., 2006). Insects, being poikilotherms, do not use metabolic energy to maintain a constant body temperature as homeotherms do and can therefore invest more energy in growth, resulting in a higher feed conversion efficiency (Nakagaki and DeFoliart, 1991). Furthermore, compared to conventional livestock, insects require less land (Oonincx and De Boer, 2012), are expected to use less water (Van Huis, 2013) and emit less greenhouse gases (Oonincx et al., 2010), making them a more sustainable source of animal protein.

In the Western world, insects are produced in closed farming systems rather than harvested from nature. For example, three species of edible larvae of the beetle family Tenebrionidae, better known as mealworms, are currently commercially produced: the Yellow mealworm (*Tenebrio molitor* L.), the Giant mealworm (*Zophobas atratus* Fab.) and the Lesser mealworm (*Alphitobius diaperinus* Panzer). These insects are commonly produced on mixed grain diets. Recently, separate production lines have been set up in The Netherlands to facilitate the production of *T. molitor* and *A. diaperinus* for human consumption. *Zophobas atratus* is currently not yet produced for human consumption; however, larvae of this

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species are suitable for human consumption. Mealworm mass production is well-documented (Ghaly and Alkoaik, 2009; Van Huis, 2013). Mealworm species are considered suitable for introducing unaccustomed consumers to entomophagy since they feed on cereals directly used in food production.

When introducing edible insects as a more sustainable alternative to conventional meat, it is advantageous to use diets from a local and more sustainable source than is currently the case. This can be achieved by producing the insects on diets composed of industrial by-products, for example from the food industry.

Insect growth rate and body composition, and hence nutritional quality, can be altered by diet (Anderson, 2000; Davis and Sosulski, 1974). This offers opportunities to increase production and alter the nutritional composition of mealworms to better suit consumer needs.

Literature is available on dietary effects on the growth and chemical composition of *T. molitor* (Davis and Sosulski, 1974; Gao et al., 2010; Morales-Ramos et al., 2010; Ramos-Elorduy et al., 2002), but is very scarce for *A. diaperinus* (Hosen et al. 2004) and seems unavailable for *Z. atratus*. Furthermore, it is thus far unknown how diet composition influences feed conversion efficiency of these insects. In this study, growth performance, feed conversion efficiency and nutritional composition of the three mealworm species on diets composed of organic by-products were determined.

2. Materials and methods

2.1. Insects

Newly hatched larvae of *T. molitor*, *Z. atratus* and *A. diaperinus* were obtained from the insect rearing company Kreca (Ermelo, The Netherlands). During the experiment, insects were maintained in a climate chamber (28 °C, 65% RH, 12 h photoperiod).

2.2. Diet preparation

Side streams were selected as ingredients for the experimental diets based on local availability and deemed suitability as feed for insects and included: spent grains and beer yeast (*Saccharomyces cerevisiae* Meyen *ex* Hansen; Anheuser-Busch, Dommelen, The Netherlands), bread remains (Bakkersland BV, Hedel, The Netherlands), cookie remains (Banketbakkerij Van Strien, Oud-Beijerland, The Netherlands), potato steam peelings (Hedimix BV, Boxmeer, The Netherlands) and maize distillers' dried grains with solubles (DDGS; Groan BV, Giessen, The Netherlands). The ingredients were lyophilised, ground and then mixed to compose four diets either high in both protein and starch (HPHS), high in protein and low in starch (HPLS), low in protein and high in starch (LPHS) and low in both protein and starch (LPLS) (Table 1). Because high starch diets based on cookie remains caused high larval mortality, they were replaced with high starch diets based on potato steam peelings (see Sections 3.2 and 4). Diets obtained from commercial insect rearing companies (referred to as A and B) were used as control diets. Company A uses the same diet for *T. molitor* and *Z. atratus*, but does not produce *A. diaperinus*. Hence, that same diet (control diet A) was used for this species in this experiment. Company B also uses the same diet for *T. molitor* and *Z. atratus* (control diet B-Tm/Za), but a different diet for *A. diaperinus* (control diet B-Ad). Diets were stored at −20 °C until use.

2.3. Larval growth and development experiment

Fifty newly hatched larvae were transferred to a plastic container (17.5 × 9.3 × 6.3 cm) with aeration slits in the sides. Each container contained 4 g of diet and 1 g of carrot. Per diet and species, five replicate containers were used. Larvae were allowed to feed *ad libitum* and diet was refreshed when needed, based on visual observation of remaining diet and accumulated faeces. To provide moisture, 2 g of fresh carrot was added twice a week. Old carrot pieces were removed.

Larvae were allowed to feed undisturbed for four weeks. After 4 weeks, larval weight and survival were monitored weekly as a group until 50% of the surviving larvae had pupated. Because *Z. atratus* larvae failed to pupate under crowded condition, individual larvae were moved to containers containing 1 g of diet and 0.25 g of carrot once 50% of the larvae reached or exceeded a body length of 5 cm. Pupae were collected, weighed and kept separate until adult eclosion after which adult weight was determined.

2.4. Feed conversion efficiency experiment

Diet LPLS was excluded from further experiments because larvae failed to consume large portions of it (personal observation). Control diet A was also excluded from further experiments, because of relatively low survival and because this diet was not used by commercial companies to produce *A. diaperinus*.

For each diet, batches of newly hatched larvae were allowed to feed *ad libitum* prior to the experiment. The experimental period was chosen because mortality among newly hatched larvae was higher than in later larval stages and growth rates can vary considerably between individual larvae. The larval age during which the experiment was conducted was based on the results from the growth and development experiment and differed per species, but was equal in duration for each diet: from day 45 to day 60 for *T. molitor*; from day 70 to day 112 for *Z. atratus*; and from day 25 to day 40 for *A. diaperinus*. Per replicate, 50 larvae for *T. molitor*, 30 larvae for *Z. atratus* and 70 larvae for *A. diaperinus* were weighed as a group at the start of the experimental period. They were subsequently placed in a plastic container (17.5 × 9.3 × 6.3 cm) on 5 g diet for *T. molitor*, 7 g diet for *Z. atratus* and 3 g diet for *A. diaperinus*. Per diet and species, five replicate containers were set up. Throughout the experiment, carrot (2 g for *T. molitor*, 3 g for *Z. atratus* and 1 g for *A. diaperinus*) was replaced twice a week. Non-consumed carrot was removed and dried at 100 °C until constant weight, which was then compared to the dry weight of a carrot piece of the same original fresh weight cut from the same carrot as the pieces used in the experiment. Before the diet was completely consumed (determined based on visual observation of diet and faeces), larvae were transferred to a container with fresh diet and carrot. The residue, consisting of a mixture of leftover diet and faeces, was removed and stored at −20 °C. After termination of the experiment, larvae were starved for 24 h and were then killed by freezing at −20 °C and stored at this temperature until further analysis.

In order to determine diet consumption, for each diet-species combination, a separate batch of larvae was allowed to consume the diet and carrot entirely. Larvae were then removed and pure faeces were stored at −20 °C. Uric acid analysis (see Section 2.5) was performed on pure faeces and on residues to quantify diet consumption in the feed conversion experiment. Thereafter, diets, pure faeces and residues were dried at 100 °C to a constant weight. Uric acid concentration in pure faeces and diets was corrected for dry weight percentage.

Feed conversion efficiency was expressed on a dry matter base as the Efficiency of Conversion of Ingested food (ECI; Waldbauer, 1968), calculated as:

$$\text{ECI} = (\text{weight gained}/\text{weight of ingested food}) \times 100\%$$

and expressed on a fresh matter base as the feed conversion ratio (FCR), calculated as:

$$\text{FCR} = \text{weight of ingested food} \;/\; \text{weight gained}$$

Table 1
Composition of experimental diets made from organic by-products, and approximate composition of experimental diets and control diets.

	HPHS	HPHS[b]	HPLS	LPHS	LPHS[b]	LPLS	Control A	Control B-Tm/Za	Control B-Ad
Ingredient (%)									
Maize DDGS	10	10	20	–	–	–			
Beer yeast	40	40	40	5	5	10			
Bread remains	10	10	10	10	10	50			
Spent grains	–	–	30	–	–	40			
Potato steam peelings	40	–	–	85	–	–			
Cookie remains	–	40	–	–	85	–			
Approximate composition (%)[a]									
Crude protein	24.1	26.4	32.5	10.7	10.7	20.0	18.8	15.5	16.0
Crude fat	4.0	7.1	7.0	1.8	8.4	6.2	6.0	4.0	4.4
Starch	28.4	26.9	7.4	49.8	46.7	19.4	43.6	23.0	~

~: No information available.
Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch); LPLS (low protein, low starch).
[a] Values calculated based on available values for organic by-products (www.duyniebeuker.nl, www.groan.nl).
[b] Discontinued.

2.5. Uric acid analysis

Ten milligram of either residue or pure faeces sample was extracted in 50 mL of 0.5% borax solution for 2 h, after which the uric acid concentration was determined by spectrophotometry at OD 450 nm according to Van Handel (1975). Uric acid content of the residues was compared to uric acid content of pure faeces to determine the amount of faeces in the residues, calculated as:

weight of pure faeces in the residue = amount of uric acid in the residue / amount of uric acid in the pure faeces

2.6. Analysis of nutrient composition

After termination of the feed conversion experiment, insects were harvested and pooled for each species and diet. Insect samples were then lyophilised at −50 °C and 1.5 mbar. Total lipid content was determined as described by Folch et al. (1957) and fatty acid composition was determined according to Metcalfe et al. (1966). Nitrogen content was determined according to Novozamsky et al. (1984). Crude protein content was calculated by multiplying nitrogen content by 6.25.

2.7. Statistical analysis

Data of pupal and adult weight were distributed normally and analysed by One way Analysis of Variance (ANOVA) at a significance level of 0.05, followed by a Šidák correction for multiple comparisons. Data of larval survival and development time, percentage of emerged adults, as well as uric acid concentration of faeces, ECI, FCR and consumed carrot/food ratio did not conform to the normal distribution and were analysed by a Kruskal–Wallis test at a significance level of 0.05, followed by Mann–Whitney U tests with applying Šidák correction. For larval survival and development time and percentage of eclosed adults, the level of significance was corrected to $1-(1-0.05)^{1/5}=0.010$ for post hoc analysis. For uric acid concentration of faeces, ECI and FCR, the level of significance was corrected to $1-(1-0.05)^{1/3}=0.017$ for post hoc analysis. Correlation between larval survival and development time was analysed by Spearman's rank correlation coefficient. All statistical analyses were performed using IBM SPSS statistics v. 20.

3. Results

3.1. Diet nutrient composition

Diets were prepared to differ in protein and starch content (Table 1). Calculated approximate protein content of high protein diets ranged from 24.1% to 32.5% and was 10.7% for both LPHS diets. Approximate starch content was 26.9% and 28.4% for the HPHS diets, 7.4% for diet HPLS and 46.7% and 49.8% for the LPHS diets. Diet LPLS was only slightly lower in both protein and starch (20% and 19.4% respectively) than the HPHS diets. Approximate fat content of the experimental diets was between 6.2% and 8.4% for all experimental diets except for the potato-based high protein diets (4.0% for HPHS and 1.8% for LPHS).

Protein and fat content was determined for diets used for the feed conversion experiment. Protein content of high protein diets was between 33% and 39% and was 17–18% in control diets (Table 2). The control diets and HPHS had similar DM contents, whereas diets HPLS and LPHS had a DM content of *ca.* 95%. High protein diets contained 5–6% fat whereas diet LPHS and control diets contained 5% or less. Analysis of fatty acid composition showed that linoleic acid was prevalent in all diets, in particular in control diet B-Tm/Za (Table 3). Other predominant fatty acids were palmitic and oleic acid. Oleic acid content in control diet B-Ad and the experimental diets exceeded 20% of total fatty acids but was *ca.* 13% for control diet B-Tm/Za. The ratio between ω-6 and ω-3 polyunsaturated fatty acids (n6/n3 ratio) ranged from 10:1 to 18:1 for the control diets and high protein diets, and was 5:1 for diet LPHS.

3.2. Larval survival

The original, and discontinued high starch diets contained cookie remains as starch source. For all species reared on diets containing cookie remains, survival was 0% on diet LPHS and <40% on diet HPHS (results not shown). Therefore, the experiment was

Table 2
Dry matter (DM) percentage, crude protein and crude fat content of different diets.

Diet	DM[a] (% of whole)	Crude protein[b] (% DM)	Crude fat[a] (% DM)
Control B-Tm/Za	89.0	17.1	3.0
Control B-Ad	87.4	17.8	5.0
HPHS	86.5	32.7	5.5
HPLS	95.1	39.1	5.8
LPHS	95.7	11.9	2.3
Carrot[c]	11.7	7.9	2.1

Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch).
[a] Values based on single analysis.
[b] Values based on analysis in duplo.
[c] Values for carrot are based on USDA SR-12 nutrient data for carrot.

Table 3
Fatty acid profile of control diets and experimental diets. Values are in g/100 g of total fatty acids. Fatty acids not detected in any of the diets were excluded. Values based on single analysis.

Fatty acid			Diet					
Common name	Lipid number	ω-*n*	Control B-Tm/Za	Control B-Ad	HPHS	HPLS	LPHS	Carrot[a]
Saturated								
Lauric acid	C 12:0		–	–	0.09	–	0.41	–
Myristic acid	C 14:0		–	–	0.41	0.28	1.21	–
Pentadecanoic acid	C 15:0		–	–	0.16	–	–	–
Palmitic acid	C 16:0		17.04	13.50	13.16	14.90	14.04	14.93
Margaric acid	C 17:0		–	–	0.32	0.20	1.33	–
Stearic acid	C 18:0		0.76	3.13	2.35	2.09	2.90	0.87
Arachidic acid	C 20:0		–	–	0.27	0.33	0.57	–
Behenic acid	C 22:0		0.17	–	0.18	–	0.60	–
Lignoceric acid	C 24:0		–	–	0.24	0.24	0.54	–
Monounsaturated								
Myristoleic acid	C 14:1	ω-5	–	–	0.08	–	–	–
Palmitoleic acid	C 16:1	ω-7	0.17	–	3.28	1.79	3.31	0.87
Oleic acid	C 18:1	ω-9	12.58	23.68	26.20	24.48	22.88	5.13
Vaccenic acid	C 18:1	ω-7	1.06	1.19	0.67	0.65	1.45	–
Gadoleic acid	C 20:1	ω-12	0.83	–	–	–	–	–
Gondoic acid	C 20:1	ω-9	–	–	0.26	0.39	0.42	–
Nervonic acid	C 24:1	ω-9	0.22	–	0.36	0.24	–	–
Polyunsaturated								
Hexadecadienoic acid	C 16:2	ω-4	–	–	0.27	–	1.31	–
Hexadecatrienoic acid	C 16:3	ω-4	–	–	0.48	0.26	0.72	–
Linoleic acid	C 18:2	ω-6	60.66	53.87	47.28	51.03	32.02	49.00
α-Linolenic acid	C 18:3	ω-3	5.90	4.63	2.67	2.86	4.32	0.87
Stearidonic acid	C 18:4	ω-3	–	–	0.23	–	2.34	–
Eicosadienoic acid	C 20:2	ω-6	–	–	0.08	–	0.68	–
Eicosatetraenoic acid	C 20:4	ω-3	–	–	–	–	0.42	–
n6/n3 ratio			10:1	12:1	16:1	18:1	5:1	56:1

–: Not detected.
Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch).
[a] Values for carrot are based on USDA SR-12 nutrient data for carrot.

repeated using new high starch diets with potato steam peelings as starch source.

For all three mealworm species, diets affected survival (Table 4, Figs. 1–3; $p = 0.034$ for *T. molitor*, $p < 0.001$ for *Z. atratus* and *A. diaperinus*) and strongly affected development time ($p < 0.001$). For *T. molitor*, survival on diets HPLS and LPHS was higher (>80%) than on control diet A (71%; $p = 0.008$). For *Z. atratus*, survival was higher on experimental high protein diets ($\geqslant$84%) than on control diets ($\leqslant$78%; $p = 0.008$). Survival on diet LPHS was very low compared to other diets (27%; $p = 0.008$). For *A. diaperinus*, survival was lower on control diet A (*ca.* 80%) than on experimental diets (>90%, $p = 0.008$).

3.3. Development time

For *T. molitor* and *Z. atratus*, development time until 50% pupation was similar on both control diets but lower on high protein experimental diets ($p = 0.008$; Table 4). For *A. diaperinus*, development time on control diet B-Ad was longer than on diet HPLS and shorter than on diet LPLS ($p = 0.008$). Development time on control diet A was longer than on control diet B and experimental diets HPHS, HPLS and LPLS ($p = 0.008$). For all three species, development time on diet LPHS was much longer than on the other diets ($p = 0.008$). Furthermore, larvae grown on diet LPHS were lighter in colour than larvae grown on the other diets, while faeces were darker. Both diets LPHS and HPHS containing potato steam peelings were light in colour as were control diets A and B-Ad, and diet LPLS. Development time and survival were only correlated for *Z. atratus* ($\rho = -0.759$, $p < 0.001$).

3.4. Pupal and adult weight

Number of days from pupation until adult eclosion was not influenced by diet and was 7 days for *T. molitor*, 12 days for *Z. atratus* and 5 days for *A. diaperinus*.

For *T. molitor*, pupal weight was higher on diet HPLS (Table 4), and lower on diet LPHS compared to the control diets ($p \leqslant 0.001$). Adult weight was lower on diet LPHS than on control diet A ($p < 0.01$) and higher on diets HPLS and LPLS than on control diet B-Tm/Za ($p < 0.001$). For *Z. atratus*, both pupal weight and adult weight differed between the control diets and the experimental diets, where weight was higher on both high protein diets and diet LPLS, and lower on diet LPHS ($p < 0.001$). For *A. diaperinus*, pupal weight was lower on diet LPHS than on control diets ($p < 0.001$), as was adult weight ($p = 0.004$ for control diet A and $p < 0.001$ for control diet B-Ad). Rather than turning black in colour, *A. diaperinus* adults remained dark brown when larvae had consumed diet LPHS. Percentage of adults eclosing intact (viz. normal elytra and wings) was over 90% for *T. molitor* and over 80% for *Z. atratus*. No difference was observed between diets. For *A. diaperinus*, successful eclosion was lower for diet HPLS (77%) than for diet LPHS (91%, $p = 0.008$). No differences were observed when comparing other diets.

3.5. Feed conversion efficiency

Uric acid concentration in pure mealworm faeces differed depending on species and diet consumed (Table 5). For all three species, more uric acid was present in faeces when larvae fed on diet HPLS than on LPHS or control diet. For *A. diaperinus*, faeces pro-

Table 4
Average development time, survival at 50% pupation, pupal weight, adult weight and percentage of successfully eclosed adults of three mealworm species grown on different diets. Values are given as mean ± SD. Superscripts denote significant differences; $n = 5$.

Diet	Development time (days)		Survival (%)		Pupal weight (g)		Adult weight (g)		Adults (% of pupae)	
Tenebrio molitor										
Control A	117[a]	±1.5	71[a]	±12.7	0.149[ab]	±0.022	0.133[ab]	±0.020	94[a]	±0.071
Control B-Tm/Za	123[a]	±2.4	86[ab]	±9.6	0.144[ab]	±0.023	0.127[a]	±0.019	95[a]	±0.048
HPHS	79[b]	±3.2	88[ab]	±5.2	0.146[ab]	±0.021	0.126[a]	±0.018	90[a]	±0.114
HPLS	95[c]	±3.6	92[b]	±2.6	0.161[b]	±0.023	0.140[b]	±0.020	97[a]	±0.054
LPHS	168[d]	±11.5	88[b]	±0.9	0.117[c]	±0.017	0.100[c]	±0.015	93[a]	±0.068
LPLS	95[c]	±7.1	84[b]	±10.5	0.145[b]	±0.021	0.127[a]	±0.020	98[a]	±0.028
Zophobas atratus										
Control A	139[a]	±8.5	68[a]	±3.8	0.603[a]	±0.063	0.499[a]	±0.049	86[a]	±0.074
Control B-Tm/Za	140[a]	±11.4	78[ab]	±11.4	0.584[a]	±0.065	0.485[a]	±0.056	85[a]	±0.147
HPHS	117[b]	±2.9	96[c]	±2.6	0.664[b]	±0.059	0.551[b]	±0.047	94[a]	±0.092
HPLS	103[c]	±1.7	91[cd]	±2.3	0.722[c]	±0.062	0.604[c]	±0.054	98[a]	±0.032
LPHS	225[d]	±6.1	27[e]	±16.9	0.482[d]	±0.114	0.391[d]	±0.074	80[a]	±0.326
LPLS	152[a]	±7.9	84[bd]	±6.2	0.651[b]	±0.038	0.538[b]	±0.037	95[a]	±0.075
Alphitobius diaperinus										
Control A	66[a]	±0.9	79[a]	±6.6	0.021[a]	±0.003	0.018[a]	±0.003	89[ab]	±0.078
Control B-Ad	42[b]	±1.7	82[ab]	±15.4	0.023[a]	±0.004	0.019[a]	±0.003	74[ab]	±0.112
HPHS	44[bc]	±2.2	95[b]	±4.1	0.022[a]	±0.003	0.019[a]	±0.003	90[ab]	±0.076
HPLS	38[d]	±1.5	94[b]	±2.6	0.021[a]	±0.003	0.020[a]	±0.013	77[a]	±0.078
LPHS	106[e]	±8.1	91[b]	±5.2	0.018[b]	±0.003	0.015[b]	±0.003	91[b]	±0.031
LPLS	48[c]	±2.6	97[b]	±1.1	0.023[a]	±0.003	0.019[a]	±0.003	86[ab]	±0.092

Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch); LPLS (low protein, low starch).

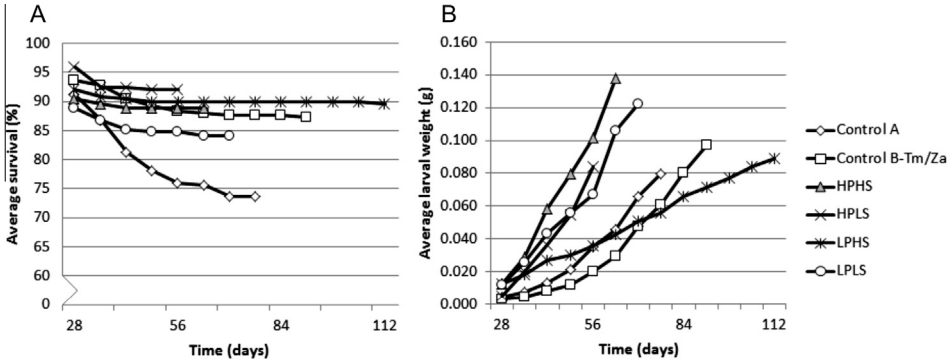

Fig. 1. Average larval survival (A) as percentage of the total number of larvae at week 0 ($n = 50$) and average larval weight (B) of *Tenebrio molitor*, determined weekly until the first pupa was observed. HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch); LPLS (low protein, low starch).

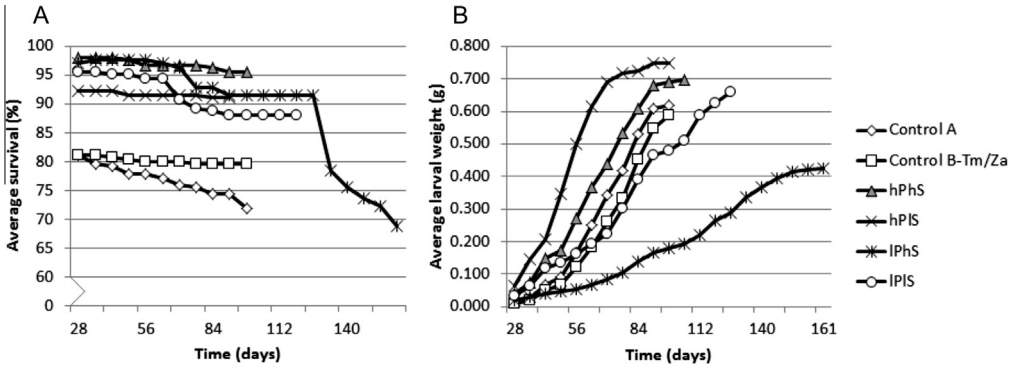

Fig. 2. Average larval survival (A) as percentage of the total number of larvae at week 0 ($n = 50$) and average larval weight (B) of *Zophobas atratus*, determined weekly until the first pupa was observed. HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch); LPLS (low protein, low starch).

duced on diet LPHS contained less uric acid compared to both high protein diets.

For all three mealworm species, diet had an effect on the ECI (Table 6). For *T. molitor*, ECI was lowest on diet LPHS ($p = 0.008$) but did not differ between the other diets. For *Z. atratus*, ECI was highest on both high protein diets ($p = 0.008$). ECI on diet LPHS was approximately half of that for the other experimental diets (*ca.* 15 vs. 30%, $p = 0.016$). For *A. diaperinus*, ECI was highest on diet

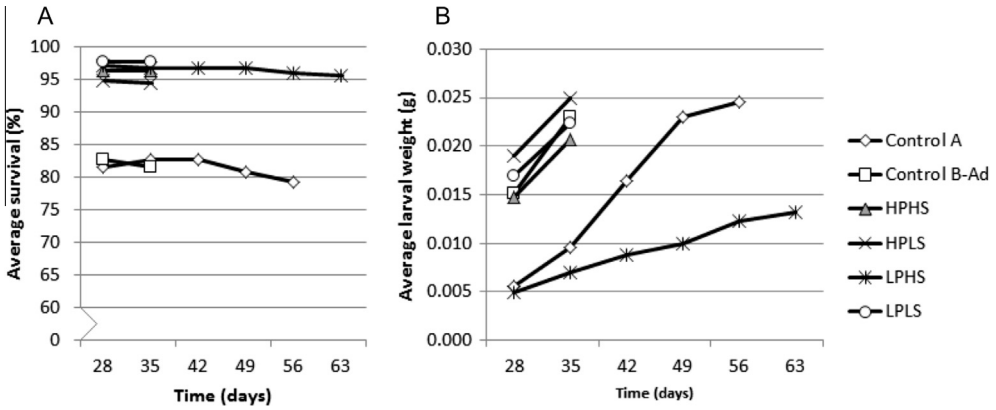

Fig. 3. Average larval survival (A) as percentage of the total number of larvae at week 0 ($n = 50$) and average larval weight (B) of *Alphitobius diaperinus*, determined weekly until the first pupa was observed. HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch); LPLS (low protein, low starch).

Table 5
Uric acid concentration in pure faeces (mg/mg DM) produced by three mealworm species grown on different diets. Values are given as mean ± SD. Superscripts denote significant differences; $n = 4$.

Diet	*Tenebrio molitor*		*Zophobas atratus*		*Alphitobius diaperinus*	
Control B-Tm/Zm	0.042^{ac}	±0.002	0.043^{ac}	±0.003	–	
Control B-Ad	–		–		0.108^{ab}	±0.004
HPHS	0.151^{abc}	±0.008	0.073^{abc}	±0.001	0.186^{b}	±0.012
HPLS	0.188^{b}	±0.012	0.182^{b}	±0.002	0.202^{b}	±0.017
LPHS	0.041^{c}	±0.002	0.049^{c}	±0.004	0.050^{a}	±0.001

Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch); LPLS (low protein, low starch).

Table 6
Feed conversion efficiency (ECI) and carrot consumed per gram diet on dry matter (DM) basis and feed conversion ratio (FCR) on fresh weight (FW) basis. Values are given as mean ± SD. Superscripts denote significant differences; $n = 5$.

Diet	ECI (DM) (%)		FCR (FW)		Carrot consumed per g diet (g DM)	
Tenebrio molitor						
Control B-Tm/Za	18.96^{a}	±0.70	3.44^{a}	±0.24	0.159^{ab}	±0.011
HPHS	28.93^{a}	±3.56	3.04^{a}	±0.21	0.211^{ac}	±0.036
HPLS	28.47^{a}	±0.75	2.62^{b}	±0.10	0.155^{b}	±0.007
LPHS	16.76^{b}	±0.77	6.05^{c}	±0.44	0.248^{c}	±0.013
Zophobas atratus						
Control B-Tm/Za	23.78^{a}	±0.90	3.64^{a}	±0.17	0.186^{a}	±0.008
HPHS	28.93^{b}	±1.41	3.11^{b}	±0.14	0.162^{b}	±0.002
HPLS	33.33^{c}	±2.37	2.73^{c}	±0.12	0.177^{a}	±0.011
LPHS	15.76^{d}	±1.45	5.63^{d}	±0.64	0.226^{c}	±0.017
Alphitobius diaperinus						
Control B-Ad	23.03^{a}	±6.93	8.11^{a}	±2.69	0.508^{a}	±0.149
HPHS	34.37^{b}	±6.09	3.01^{b}	±0.34	0.257^{b}	±0.047
HPLS	25.41^{a}	±2.21	3.24^{ab}	±0.48	0.208^{b}	±0.037
LPHS	6.36^{c}	±1.73	24.60^{c}	±6.86	0.424^{a}	±0.082

Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch).

HPHS ($p = 0.016$) and very low on diet LPHS (6.36%, $p = 0.008$). A high ECI corresponded to a low FCR. However, for *T. molitor*, FCR on diet HPLS was lower than on the control diet and diet HPHS ($p = 0.008$ and $p = 0.016$ respectively), whereas ECI values were similar. For *A. diaperinus*, FCR on diet HPLS did not differ from the control diet and diet HPHS.

Differences were observed in the amount of carrot consumed per gram of diet (DM base, Table 6). Larvae of *T. molitor* consumed more carrot per gram of diet on diet LPHS than on control diet ($p = 0.008$). For *Z. atratus*, carrot consumption was lowest on diet HPHS and highest on diet LPHS compared to the other diets ($p = 0.008$ and $p = 0.016$, respectively). Large differences were observed for *A. diaperinus*, where carrot consumption per gram of diet on control diet B-Ad and diet LPHS was approximately twice as high as on the high protein diets.

3.6. Nutritional composition

Dry matter content of the three mealworm species was *ca.* 30% (Table 7). Crude protein content was *ca.* 47% for *T. molitor*, *ca.* 40% for *Z. atratus* and *ca.* 64% for *A. diaperinus*. Fat content was *ca.* 25% for *T. molitor*, *ca.* 38% for *Z. atratus* and *ca.* 19% for *A. diaperinus*.

The predominant fatty acids in all three species were palmitic acid, oleic acid and linoleic acid, together comprising 72–91% of total fatty acids (Table 8). Fatty acid data of *A. diaperinus* larvae on diet HPHS were excluded because of insufficient quality of the sample, creating a high background in gas chromatography. Palmitic acid content was *ca.* 16% for *T. molitor*, whereas for *Z. atratus* this was *ca.* 25% on diet HPLS to 33% on diet LPHS. For *A. diaperinus*, palmitic acid concentrations showed a wider range (*ca.* 16% on diet HPLS to 25% on control diet B-Ad). Oleic acid content was *ca.* 40%

Table 7
Dry matter (DM) percentage, crude protein and crude fat content of three mealworm species grown on different diets.

Diet	DM[a] (% of fresh weight)	Crude protein[b] (% DM)	Crude fat[a] (% DM)
Tenebrio molitor			
Control B-Tm/Za	27.3	45.1	25.0
HPHS	33.4	48.6	26.3
HPLS	29.4	47.5	27.6
LPHS	33.3	46.9	18.9
Zophobas atratus			
Control B-Tm/Za	33.3	41.5	36.2
HPHS	36.7	41.1	43.5
HPLS	35.7	42.5	40.0
LPHS	30.8	34.2	32.8
Alphitobius diaperinus			
Control B-Ad	33.3	61.7	24.3
HPHS	31.8	64.3	21.8
HPLS	30.0	65.0	18.1
LPHS	33.3	–	13.4

–: Values not available due to insufficient sample.
Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch).
[a] Values based on single analysis.
[b] Values based on analysis in duplo.

on diet HPLS to 58% on diet LPHS for *T. molitor*, *ca.* 31% on diet HPLS to 45% on diet LPHS for *Z. atratus* and *ca.* 20% on diet HPLS to 44% on diet LPHS for *A. diaperinus*. Linoleic acid content showed a wide range for all three species and was *ca.* 15% on diet LPHS to 31% on diet HPLS for *T. molitor*, *ca.* 10% on diet LPHS to 29% on diet HPLS for *Z. atratus* and *ca.* 17% on diet LPHS to 36% on diet HPLS for *A. diaperinus*. Other fatty acids comprised ⩽3.19% of total fatty acids, with the exception of stearic acid in *Z. atratus* and *A. diaperinus* (*ca.* 7–8% and 9–11%, respectively).

The ratio between ω-6 and ω-3 polyunsaturated fatty acids (n6/n3 ratio) was *ca.* 19:1 for larvae on control diets, 19–25:1 on diet HPLS and *ca.* 30:1 for larvae on high starch diets. For *T. molitor* on diet LPHS, no ω-3 fatty acids were present in values above the detection limit and an n6/n3 ratio could therefore not be calculated.

4. Discussion

This study shows that when the three edible mealworm species were produced on diets composed of organic by-products, diet affected larval performance and feed conversion efficiency. Furthermore, larval chemical composition differed between species and dietary treatments.

Larval mortality was very high on the original high starch diets containing cookie remains. These diets smelled strongly of spices such as cinnamon and clove, of which the vapours can be toxic to insects (George et al., 2010; Işıkber et al., 2009). On the alternative high starch diets, containing potato steam peelings as starch source, larval mortality was similar to that observed on the other diets.

Larvae showed higher survival and shorter development time on diets higher in protein and lower survival and longer development time on diet LPHS, compared to control diet used by commercial mealworm producers. Several studies reported increased growth rate of mealworms on high protein diets, in particular when derived from yeast (Davis and Sosulski, 1974; Morales-Ramos et al., 2010). High protein diets in the present study contained 40% beer yeast. In addition to protein (Aghdamshariar et al., 2006; Rumsey et al., 1991), yeast supplies B vitamins (Copping and Honora Roscoe, 1937; Fraenkel et al., 1950) and works as a feeding stimulant for *T. molitor*, as does wheat flour (Murray, 1960). Similar literature data are lacking for *Z. atratus* and *A. diaperinus*. Suboptimal performance of larvae can be due to low feeding stimulation, rather than a low nutritional value or the presence of deterring components. For this reason, each experimental diet in the present study contained at least 10% bread remains and 5% yeast. Similar to protein source, starch source could influence larval performance, rather than the absolute amount of starch. Hosen et al. (2004) observed differences in performance of *A. diaperinus* when grown on different types of cereal flour, despite minor differences in carbohydrate levels. Potato starch is more resistant to digestion by Tenebrionidae than starch from wheat or maize (Applebaum, 1966; Mereiles et al., 2009). Furthermore, potato glycoalkaloids, which persist after processing (Po and Sinha, 2010) can have a toxic effect on insects that do not consume potato in nature (Nenaah, 2011; Ventrella et al., 2014). Long-time exposure to a high content of potato steam peelings could in part explain the high mortality of *Z. atratus* on diet LPHS, which predominantly occurred after 20 weeks. Induced detoxification of secondary plant metabolites commonly begins in earlier larval stadia in herbivorous insects (Glendinning, 2002; Yu and Hsu, 1993) and lower mortality is therefore expected in older larvae. However, chronic toxicity resulting in among others reduced growth and lower feed conversion efficiency has been described (Chaubey, 2008; Wheeler and Isman, 2001). In addition to retarded growth on diet LPHS, larvae of all three species and beetles of *A. diaperinus* were lighter in colour than those that consumed other diets. Lee et al. (2008) observed that larvae of *Spodoptera littoralis* (Boisduval) had less strongly melanised cuticles when feeding on diets lower in protein quality. Stronger melanisation is associated with increased direct immune function (Barnes and Siva-Jothy, 2000; Lee et al., 2008). Possibly, protein quality of diet LPHS was lower for mealworms, hence causing decreased melanisation of the cuticle. This would cause the larvae to be more vulnerable to infection by pathogens, reducing their survival.

Pupal weight can be used as a measure for insect dietary quality (Chapman, 1998). For all three mealworm species, pupal weight, and concomitantly adult weight, was lowest on diet LPHS. This is a further indication that this diet was of lower quality for development of the three mealworm species. However, diet LPHS did not have a detrimental effect on successful eclosion of adults.

Carrot consumption was significantly higher on diet LPHS than on the other diets for all three species. Possibly, larvae consumed more carrot to compensate for nutrients lacking in the diet.

Feed conversion efficiency was higher on high protein diets and lower on diet LPHS. This confirms the explanation that diet LPHS might not only lack nutrients, but might also contain compounds which are harder to digest or toxic to mealworms. Feed conversion ratio (FCR) on the high protein diets was *ca.* 3 and was higher than the value reported by Oonincx and De Boer for *T. molitor* (2.2; Oonincx and De Boer, 2012), although carrot consumption was not taken into account in this study, and thus values found in the present study are expected to be higher. No FCR values have been published for *Z. atratus* and *A. diaperinus*. The extremely high FCRs found for *A. diaperinus* on diet LPHS and control diet B are in part due to the large amounts of carrot consumed. Other ways of providing water could dramatically decrease the FCR, provided that carrot was mostly consumed as a source of moisture and not as a source of other nutrients. However, results from the present study suggest that carrot is likely consumed as a source of nutrients to compensate for poor diet quality. When comparing the FCRs of mealworm species on high protein diets (*ca.* 3) with FCRs for conventional livestock, mealworms are comparable to poultry (2.0) and pigs (3.6), and compare favourably to beef (7.8; Wilkinson, 2011). However, the edible portion of mealworms (100%) is greater

Table 8
Fatty acid profile of three mealworm species grown on different diets. Values are in g/100 g of total fatty acids. Fatty acids not detected in any of the diets were excluded. Values based on single analysis.

Fatty acid			*Tenebrio molitor*				*Zophobas atratus*				*Alphitobius diaperinus*[a]		
Common name	Lipid number	ω-*n*	Control B-Tm/Za	HPHS	HPLS	LPHS	Control B-Tm/Za	HPHS	HPLS	LPHS	Control B-Ad	HPLS	LPHS
Saturated													
Caprylic acid	C 8:0		–	–	–	–	0.15	0.13	0.12	0.15	–	0.28	–
Capric acid	C 10:0		–	–	–	–	–	–	–	–	–	0.24	–
Lauric acid	C 12:0		–	0.38	–	–	–	–	–	–	–	–	–
Myristic acid	C 14:0		2.32	3.19	2.20	2.79	0.78	0.76	0.70	0.75	0.57	0.57	0.82
Pentadecanoic acid	C 15:0		–	0.19	–	–	0.16	0.24	0.31	0.13	–	0.22	0.21
Palmitic acid	C 16:0		16.19	16.96	16.13	16.67	31.09	28.90	25.04	33.00	24.89	15.73	22.30
Margaric acid	C 17:0		–	0.34	0.49	–	0.29	0.84	1.10	0.22	–	1.14	0.53
Stearic acid	C 18:0		2.97	2.72	2.64	–	7.73	7.06	6.77	7.14	9.50	8.95	10.44
Arachidic acid	C 20:0		–	0.16	–	–	0.18	0.18	0.17	0.13	0.49	0.34	0.55
Behenic acid	C 22:0		–	–	–	–	–	–	–	–	–	0.16	–
Monounsaturated													
Palmitoleic acid	C 16:1	ω-7	1.56	2.88	2.67	1.56	0.77	1.88	1.85	1.85	–	0.51	0.67
Oleic acid	C 18:1	ω-9	46.41	48.68	39.78	57.63	33.17	39.56	30.72	44.72	37.25	20.55	44.25
Vaccenic acid	C 18:1	ω-7	0.39	0.26	0.40	0.20	0.36	0.25	0.36	0.34	–	0.24	–
Gondoic acid	C 20:1	ω-9	–	–	–	–	0.13	–	0.12	–	–	–	–
Polyunsaturated													
Hexadecatrienoic acid	C 16:3	ω-4	–	0.37	–	–	–	–	–	–	–	0.44	0.28
Linoleic acid	C 18:2	ω-6	27.83	20.99	31.25	15.45	22.54	17.84	29.31	9.86	22.65	36.41	16.84
γ-Linolenic acid	C 18:3	ω-6	–	–	–	–	–	–	–	–	–	–	0.41
α-Linolenic acid	C 18:3	ω-3	1.48	0.67	1.29	–	1.25	0.62	1.16	0.29	0.70	0.65	0.38
Eicosadienoic acid	C 20:2	ω-6	–	0.10	0.34	–	–	–	–	–	–	0.18	–
Dihomo-γ-linolenic acid	C 20:3	ω-6	–	–	–	–	–	–	–	–	–	0.23	0.18
Eicosatetraenoic acid	C 20:4	ω-3	–	–	–	–	–	–	–	–	–	0.31	–
Eicosapentaenoic acid	C 20:5	ω-3	–	–	0.21	–	–	–	–	–	–	–	–
Docosadienoic acid	C 22:2	ω-6	–	–	0.24	–	–	–	–	–	–	1.55	0.18
Docosatrienoic acid	C 22:3	ω-3	–	–	–	–	–	–	–	–	0.50	1.03	0.19
n6/n3 ratio			19:1	32:1	21:1	–	18:1	29:1	25:1	34:1	19:1	19:1	31:1

–: Not detected.
Diet abbreviations: HPHS (high protein, high starch); HPLS (high protein, low starch); LPHS (low protein, high starch).
[a] Due to insufficient quality of *Alphitobius diaperinus* material grown on diet HPHS, data were excluded.

than that of poultry and pigs (*ca.* 50%; De Vries and De Boer, 2010), making them more efficient production animals.

Larval crude protein content (*ca.* 47% for *T. molitor*, 40% for *Z. atratus* and 64% for *A. diaperinus*) fell within the ranges reported in literature (*ca.* 45–69% for *T. molitor*, 40–52% for *Z. atratus* and 58–67% for *A. diaperinus*; Barker et al., 1998; Despins and Axtell, 1995; Finke, 2002; Ghaly and Alkoaik, 2009; Pennino et al., 1991; Ramos-Elorduy et al., 2002; Yi et al., 2013). Despite the 2–3-fold differences in dietary crude protein contents, larval protein content was similar among the dietary treatment groups, except for the 18% lower value for *Z. atratus* on diet LPHS. Similarly, Ramos-Elorduy et al. (2002) and Gao et al. (2010) observed small differences in *T. molitor* protein content when grown on different diets. This suggests that the mealworms are well able to regulate body protein content. Insects regulate dietary intake of nutrients in order to obtain their nutrient target (Behmer, 2009; Raubenheimer and Simpson, 1999). Being restricted to one diet complicates intake regulation, although Behmer (2009) lists several modes of post-ingestive regulation. For example, locusts (*Locusta migratoria* L.) excrete excess ingested protein in the form of uric acid and ammonium. In the present study, larvae feeding on diet HPLS excreted more uric acid through faeces than larvae feeding on control diet or diet LPHS, indicating mealworms species might use a similar strategy to cope with excess dietary protein.

Larval fat content as well as fatty acid profile were more strongly influenced by diet. Fat content in this study (*ca.* 19–28% for *T. molitor*, 33–44% for *Z. atratus* and 13–24% for *A. diaperinus*) fell within the ranges reported in literature (*ca.* 23–40% for *T. molitor*, 40–45% for *Z. atratus* and 21–24% for *A. diaperinus*; Barker et al., 1998; Despins and Axtell, 1995; Finke, 2002; Ghaly and Alkoaik, 2009; Pennino et al., 1991; Ramos-Elorduy et al., 2002; Tzompa-Sosa et al., 2014; Yi et al., 2013). However, larvae grown on diet LPHS had a lower fat content, as did the diet itself. In addition, larvae use fat reserves for energy when diet nutritional quality is low (Arrese and Soulages, 2010). A low nutritional quality of diet LPHS could have contributed to the lower fat content of larvae on this diet. Raubenheimer and Simpson (2003) observed a rather stable lipid content in the desert locust *Schistocerca gregaria* (Forsskål) on increasingly protein deficient diets. A similar result was not observed in the present study. Insects can synthesise lipids out of different dietary components such as carbohydrates. Possibly, the more difficult to digest potato starch present in diet LPHS interfered with biosynthesis of lipids out of carbohydrates.

With respect to fatty acid composition, larvae of all three species were predominantly high in palmitic acid, oleic acid and linoleic acid. This corresponds to results from previous studies (Finke, 2002; Howard and Stanley-Samuelson, 1996; Tzompa-Sosa et al., 2014), although values for the three fatty acids reported by Finke (2002) were relatively low compared to those reported in the present study. Diet composition was, however, not specified by Finke (2002). The present results show a rather wide range of individual fatty acid contents depending on the diet, in particular for oleic acid and linoleic acid. Though larval fatty acid composition was altered by diet, it did not necessarily follow the same trend as dietary fatty acid composition in this study, indicating physiological regulation of larval fatty acid composition. Control diet B-Tm/Za contained the highest amount of palmitic acid and linoleic acid, but larvae fed on these diets did not contain the highest amount of these fatty acids. Larval oleic acid content was highest on diet LPHS, whereas this diet contained the lowest amount of oleic acid. In contrast, linoleic acid content was lowest in diet LPHS, and also in the larvae fed on this diet. These results show that larval fatty acid composition can be altered by diet, but to what extent differs between individual fatty acids and mealworm species. Insects are known to synthesise certain fatty acids *de novo*, such as palmitic, oleic and stearic acid (Beenakkers et al., 1985; Stanley-Samuelson et al., 1988; Canavoso et al., 2001). This could be an explanation for larval levels of palmitic and oleic acid not following a similar pattern to dietary levels. In contrast, linoleic acid is more likely to be an essential fatty acids which needs to be obtained from a dietary source. According to Stanley-Samuelson et al. (1988), increasing levels of dietary polyunsaturated fatty acids generally leads to a higher proportion of insect tissue polyunsaturated fatty acids and a lower proportion of monounsaturated fatty acids. This also appeared to be the case in the present study, where higher proportions of dietary polyunsaturated C18 fatty acids led to relatively lower proportion of larval C18 monounsaturated fatty acids (results not shown).

On all diets in this study, the three mealworm species had a high n6/n3 ratio, ranging from *ca.* 18:1 on control diet to >30:1 on diet LPHS. This was unexpected as diet LPHS had a lower n6/n3 ratio (5:1) than the other diets (*ca.* 10–12:1). However, the n6/n3 ratio of carrot exceeded 50:1. Possibly, high carrot consumption contributed to the high n6/n3 ratio of mealworms fed on diet LPHS. The n3/n6 ratios found in this study were comparable to the ratios found by Tzompa-Sosa et al. (*ca.* 25:1 for *T. molitor* and *ca.* 20:1 for *A. diaperinus*; 2014). An n6/n3 ratio of $\leqslant$5:1 is considered optimal for the human diet (Kouba and Mourot, 2011). When producing mealworm species for human consumption, it would improve dietary quality to lower the n6/n3 ratio through dietary fatty acid composition. Controlling fatty acid composition is, however, difficult to achieve when using organic by-products. In conventional meat, the ratio ranges from 10:1 to 15:1 (Kouba and Mourot, 2011), but can be lowered through diet. The extent to which the n6/n3 ratio can be lowered for the mealworm species used in this study remains a topic of investigation.

In conclusion, the mealworm species used in this study can be grown successfully on diets composed of organic by-products. Diet affects mealworm growth, development and feed conversion efficiency, where diets high in yeast-derived protein appear favourable with respect to reduced larval development time, reduced mortality and increased weight gain. However, studies spanning several insect generations should be performed to determine the effect of diet composition on adult fecundity. Dietary protein content had a minor effect on mealworm protein content, whereas larval fat content and fatty acid composition varied over a wider range. Diets that allowed for fast larval growth and low mortality in this study led to a less favourable n6/n3 ratio than control diets. Further studies are needed in order to compose diets which support optimal growth and development rate, while simultaneously facilitating a more optimal nutritional composition for human consumption.

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