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ORIGINAL ARTICLE

The effects of the particle size of four different feeds on the larval growth of *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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Abstract. Diet is one of the most important factors affecting the growth and lifecycle of *Tenebrio molitor* L. The chemical and nutritional properties of the diet of mealworms are well studied whereas its physical properties are almost neglected. This work aims to study the effects of four different particle sizes (0–0.8, 0.8–2, 2–3 and 3–4 mm) of four different feeds (wheat bran, chicken feed pellets, grounded corn kernels and alfalfa dried pellets). Four-week-old larvae were reared on the experimental substrates for four to six weeks depending on the feed. Our results indicate that particle size can significantly influence larval growth and that particles smaller than 2 mm improve larval growth on all feeds except alfalfa pellets. The maximum larval weight was slightly different for wheat bran (12%) when comparing particle sizes smaller than 2 mm with greater than 2 mm but increased up to 70% when corn kernels were used as feed. Significant differences were found between the different feeds for growth rate, larval weight and variability in larval weight. The larvae that reached an average weight of 100 mg the fastest were those reared on wheat bran followed by chicken feed and corn kernels. Larvae reared on alfalfa pellets did not reach an average weight of 60 mg. The variability in larval growth was lowest when fed wheat bran followed by chicken feed and alfalfa pellets, and the highest variability was recorded when fed corn kernels. In conclusion, both the type (wheat bran, chicken feed and corn kernels) and particle size (< 2 mm) of the feed were important determinants of larval growth.

INTRODUCTION

The yellow mealworm, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae), is an edible insect that is indigenous to Europe (Ramos-Elorduy et al., 2002). It is a good candidate for industrial-scale production as it is easy to breed and has a good amino acid profile with a low environmental footprint (van Huis et al., 2013; Mariod, 2020). Food has a central role in the life cycle of *T. molitor* and affects many parameters, such as development time, fertility, number of larval instars and percentage survival (Ribeiro, 2017). It also markedly affects the lifespan and the nutritional composition of mealworms (Rho & Lee, 2016). Several studies report the optimal composition of the diet (nutrients and components) for *T. molitor* larvae and its effect on the growth and nutritional composition of the different stages (Morales-Ramos et al., 2011, 2013; Oonincx et al., 2015; van Broekhoven et al., 2015).

Morales-Ramos et al. (2011) formulate an optimal composition of the diet for larvae of *T. molitor* based on choice tests. This diet consists of 80% wheat bran and 20% potato flakes (60% carbohydrates, 32% protein and 8% fat) and results in optimal larval growth. However, when larvae are not given a choice, different diets result in optimal growth. The optimum no-choice diet consists of 90% wheat bran and 10% potato flakes (64% carbohydrates, 29% protein and 7% fat) (Morales-Ramos et al., 2011). Further optimization resulted in an increase in adult fecundity, but no improvement in food utilization efficiency, growth, development time or survival during the larval stage. The requirement for protein is confirmed by both Oonincx et al. (2015) and van Broekhoven et al. (2015). They show that larvae have higher survival rates and shorter development times when fed high protein diets (> 20%). Several studies provide a more in-depth assessment of the nutritional needs. Davis & Sosulski (1977) and John et al. (1979) assess the amino acid requirements of mealworm larvae. In addition, there are detailed reports on the effect of different carbohydrate sources (Leclercq, 1948; Fraenkel, 1955; Meireles et al., 2009). Moreover, Dreassi et al. (2017) evaluate the fatty acids requirements of mealworms by rearing them on diets with different fat contents. Francardi et al. (2017) also studied the fat requirements and the composition of mealworm larvae reared on enriched diets.

That is, there are many studies on the nutritional aspects of feed and in particular on the protein to carbohydrate ratio (P : C), but few on the influence of physical characteristics. The physical aspects of insect diets include texture, viscosity, homogeneity, specific heat capacity and many other qualities. However, these properties have remained a much-neglected aspect of insect diet science (Cohen, 2015).

The particle size of feed is one physical characteristic that may influence the preference and ease the consumption of diets. The particle size preference of ants depends on the head width of the species (Hooper-Bui et al., 2002). Migratory locust, *Locusta migratoria* (L.) (Orthoptera: Acrididae), consumes more powdered cellulose than fibrous cellulose (Vanderzant, 1969). *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) does not oviposit in material with a particle size smaller than that needed for larval development (Mason & McDonough, 2012). Manley et al. (2018) also report the effects of substrate particle size on the oviposition sites of the coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae), which show that both wild and laboratory-reared beetles lay eggs preferentially in mulches of small rather than large particle sizes.

Murray (1960) assesses whether mealworm larvae can discriminate between the different particle sizes of growth-stimulating and non-stimulating constituents. The results indicate they can reject powdered cellulose (non-stimulating) when mixed with wheat bran flakes or wheat endosperm granules, but not with fine particles (flour). In addition, Rumbos et al. (2020) state that the form of a specific feed substrate may also affect the larval growth and reproductive performance of *T. molitor*. Fecundity is highest when adults are fed millet flakes and growth of larvae is the best when fed millet grains, although both the flakes and grains have the same nutritional composition. Although none of the above examples explicitly assess the effect of particle size on the growth of insect larvae, they provide a strong indication that it may be important. For completeness, the influence of particle size should also be evaluated for very different substrates.

Wheat bran and chicken feed are already frequently used for rearing mealworms (Ludwig & Fiore, 1960; Bumroongsook & Nahuanong, 2018; Melis et al., 2019), whereas alfalfa pellets and corn kernels are nutritionally different and are of interest. Alfalfa is rich in protein, but rarely used as a mealworm diet. So far, it has been included in self-selection tests (Morales-Ramos et al., 2020) and is reported as an ingredient in mealworm diet on a farm in Austria (Dreyer et al., 2021). Corn kernels are rarely used in diets and need to be studied further. The products of corn kernels (maize gluten, corn flour, cornmeal) are among the commodities assessed by Rumbos et al. (2018) and Rumbos et al. (2020) as a potential diet for mealworms. The corn dry distillers' kernels, a by-product of ethanol production, are among the ingredients of diets in self-selection tests (Morales-Ramos et al., 2020).

Therefore, the main objective of this study is to obtain basic information on the effects of diets of different particle sizes on the larval growth and development of *T. molitor*. This assessment was based on four different "single" ingredient diets to ensure the broader applicability of the results.

MATERIAL AND METHODS

Feed preparation

In this experiment, four different feeds were used: wheat bran which is the by-product of organic durum wheat (*Triticum durum*) without prior sieving or mixing (Molens Joye nv, Belgium), whole corn kernels (Molens Joye nv, Belgium), chicken feed (commercial product: Farm 1 Crumble, Natural Granen Gebr De Scheemaecker nv, Belgium) and alfalfa pellets usually used as dry fodder (Desaele-De Loof, Belgium). Each feed was first sieved using a 2 mm mechanical sieve (finex separator, Russellfinex) to homogenize them and to remove any small particles that may not have the same nutritional composition as the larger particles (e.g., remaining flour in wheat bran). Thereafter, the feeds were shredded by means of short bursts of a Blixer (Robot coupe) to ensure that there were no alterations to the feed due to the heat of friction. Finally, each feed was sieved again using four different mesh sizes (0.8 mm, 2.0 mm, 3.0 mm and 4.0 mm) to create four different particle sized feeds of 0–0.8 mm, 0.8–2 mm, 2–3 mm and 3–4 mm.

Insect culture

The mealworms used in this study were reared at Inagro Insect Research Centre (Rumbeke-Beitem, Belgium). Four-week-old adults of *T. molitor* were allowed to lay eggs for 24 h in a commercial substrate (Insectus®). Eight plastic crates (60 × 40 cm) with 250 g of adults/crate were used for this purpose. The eight crates of ovipositing adults were set on 4 consecutive days (2 crates/day). The eggs were left undisturbed for two weeks after which they received ad libitum their first water source (agar 2.5%). At the age of four weeks, the mealworm larvae were separated from the initial feed on 4 consecutive days and were gently mixed by hand to ensure a unique homogenous starting population. Throughout the experiment, the insects were kept in a controlled climate room at a temperature of 27 ± 1°C, 60 ± 5% humidity and always dark except during checks and when renewing the water source.

Experiment

At the beginning of the experiment the initial weight of the four-week-old larvae was measured and the crates with the larvae were stacked on 4 consecutive days for the four feeding substrates (wheat bran, chicken feed, grounded corn kernels and alfalfa pellets). The initial larval weight was based on a single but large random subsample and therefore only a single initial weight was recorded for each of the feeds as follows: 3.9 mg for wheat bran, 3.9 mg for chicken feed pellets, 3.1 mg for corn kernels and 5.3 mg for alfalfa pellets. The larvae were divided between 48 crates (60 × 40 cm) with 12 crates per feed (4 particle sizes and 3 replicates per particle size) with an estimated 2500 larvae/crate. Each crate was provided with 1200 g of feed of a particular particle size. Agar (2.5%) was used as a source of water and was added ad libitum and well distributed. A sample of the 0–0.8 mm and 3–4 mm particle sizes of each feed was kept at –20°C for further analysis. The experimental crates were stacked randomly in columns of six crates. Each column had an empty crate on the floor and the top (total of 8 crates/column) and was placed at least 30 cm from any wall to minimize any possible border effects. Each day the crates were checked visually and agar was added if needed. Dry or mouldy agar was removed. Every week, a representative subsample was taken from each crate to determine the average larval weight. The minimum subsample size was 100 individuals per crate and all the larvae in the subsample were counted and weighed to avoid any (sub)conscious bias towards smaller or bigger larvae. For each feed, all the larvae were harvested when the average larval weight reached 100 mg or when the first pupae were observed (case of alfalfa). The total weight of the larvae and final average weight were recorded. The individual weight of at least 100 larvae was measured at harvesting to determine the variability in larval growth between different particle sizes of the four different feeds.

Chemical analysis

The largest (3–4 mm) and smallest (0–0.8 mm) particle sizes of the four feeds were chemically analysed to check the macronutrient composition of the dry matter. The analysis was replicated three times for each feed of each particle size (largest and smallest). The materials were dried at 105°C for 24 h in an oven to determine the dry matter content of the samples (ISO 6496:1999). Electric Muffle Furnace set at 550°C was used for determining the total ash content (inorganic matter) (ISO 5984:2002). Kjeldahl method was used to determine the total nitrogen content of the samples (ISO 1871:2009). After total nitrogen content determination, crude protein content was calculated by multiplying nitrogen content by 6.25. The Soxhlet method was used to measure the fat content (petroleum ether extract) (ISO 6492:1999). The crude fibre was determined by boiling consecutively in sulfuric acid and potassium hydroxide, each step followed by filtration. The residue was dried, weighed and then transformed into ash. The resulting loss in mass corresponded to the crude fibre content (ISO 6865:2000). The total amount of the non-fibre carbohydrates in the dry matter was calculated by subtracting the amounts of protein, ash, fat and fibre from 100.

Statistical analysis

The statistical analysis was performed using R statistical software. A linear mixed-effect model was used (Lme4 package) to assess the influence of feed and particle size on the growth of mealworm larvae. The mixed effect modelling was necessary due to the longitudinal nature of the data. The different particle sizes were treated as factorial variables and the analysis was done for each feed separately. The analysis started using the following full model (equation 1):

$$\text{Log}_{10}(\text{Average weight}) = T \times PS + T^2 \times PS \qquad \text{(equation 1)}$$

(T = time in weeks and PS = particle size)

To determine the optimal model the full model (equation 1) was reduced via backward selection until all parameters in the model were significant (p-value < 0.05).

A similar approach was used to assess the differences in growth between different feeds. Only data from 0.8–2 mm was used for this analysis as this particle size resulted in the best growth in all feeds. The analysis started using the following full model (equation 2):

$$\text{Log}_{10}(\text{Average weight}) = T \times S + T^2 \times S \qquad \text{(equation 2)}$$

(T = time in weeks and S = substrate)

Finally, the final average larval weight and the variability in growth of the mealworms were analysed using one-way analysis of variance (ANOVA) and their means compared among substrates and repetitions using the Tukey-Kramer honestly significant difference (HSD) post-hoc test ($P < 0.05$). As a proxy of the variability in growth, the Coefficient of Variation (standard deviation of the larvae weight / mean weight) was used. This was done for each feed separately to compare different particle sizes and for particle size 0.8–2 mm to compare different feeds. Before the analyses, the data were checked for normality and homoscedasticity using Shapiro-Wilk test and Levene's test, respectively, with a significance level of 0.05. All data conformed to the assumptions of parametric tests.

RESULTS

Diet nutrient composition

The macronutrient composition of the smallest (0–0.8 mm) and the largest (3–4 mm) particle sizes of each diet are presented in Table 1. The corn kernel feed contained the lowest amount of protein (7.5% and 7.9% for the smallest and largest particle size, respectively) and the highest amount of non-fibre carbohydrates (83.3% and 83.9%, respectively). The highest amount of protein was recorded in the wheat bran (16.0% and 15.7% for the smallest and largest particle sizes, respectively). Chicken feed and alfalfa pellets had a slightly lower amount of protein compared to wheat bran. The protein content of chicken feed was 15.0% for the smallest and 15.6% for the largest particle size; in

Table 1. Moisture (%), crude protein, non fiber carbohydrates, crude fat and ash contents (g/100g) of the dry matter (mean ± standard deviation) of the four experimental feeds of the smallest (0–0.8 mm) and biggest (3–4 mm) particle sizes.

Feed	Particle size (mm)	Moisture	Crude protein (g/100 g)	Non fiber carbohydrates[1] g/100 g)	Crude fat (g/100 g)	Crude fiber (g/100 g)	Ash (g/100 g)	P : C[2]
Wheat bran	0–0.8	10.9 ± 0.44	16.04 ± 0.31	67.44	2.65 ± 0.04	9.56 ± 1.67	4.31 ± 0.16	1 : 4.2
	3–4	11.3 ± 0.11	15.74 ± 0.51	64.56	2.24 ± 0.03	11.51 ± 0.15	5.95 ± 0.07	1 : 4.1
Chicken Feed	0–0.8	8.9 ± 0.43	15.00 ± 0.35	61.96	4.07 ± 0.03	5.26 ± 0.10	13.71 ± 0.23	1 : 4.1
	3–4	8.3 ± 0.16	15.58 ± 0.29	66.20	3.67 ± 0.04	4.07 ± 0.19	10.48 ± 0.63	1 : 4.2
Corn	0–0.8	11.1 ± 0.13	7.54 ± 0.13	83.31	4.96 ± 0.04	2.61 ± 0.18	1.58 ± 0.11	1 : 11.1
	3–4	9.6 ± 0.16	7.85 ± 0.59	83.94	3.99 ± 0.05	2.98 ± 0.17	1.24 ± 0.02	1 : 10.7
Alfalfa	0–0.8	8.6 ± 0.19	15.15 ± 0.45	47.37	2.50 ± 0.07	23.54 ± 0.52	11.44 ± 0.17	1 : 3.1
	3–4	7.9 ± 0.22	14.96 ± 0.24	44.05	2.22 ± 0.06	27.58 ± 0.13	11.19 ± 0.19	1 : 2.9

[1] The total amount of the non-fiber carbohydrates was calculated by subtracting the amounts of protein, ash, fat and fiber from 100.
[2] P : C is the protein to carbohydrate (including hemicellulose) ratio.

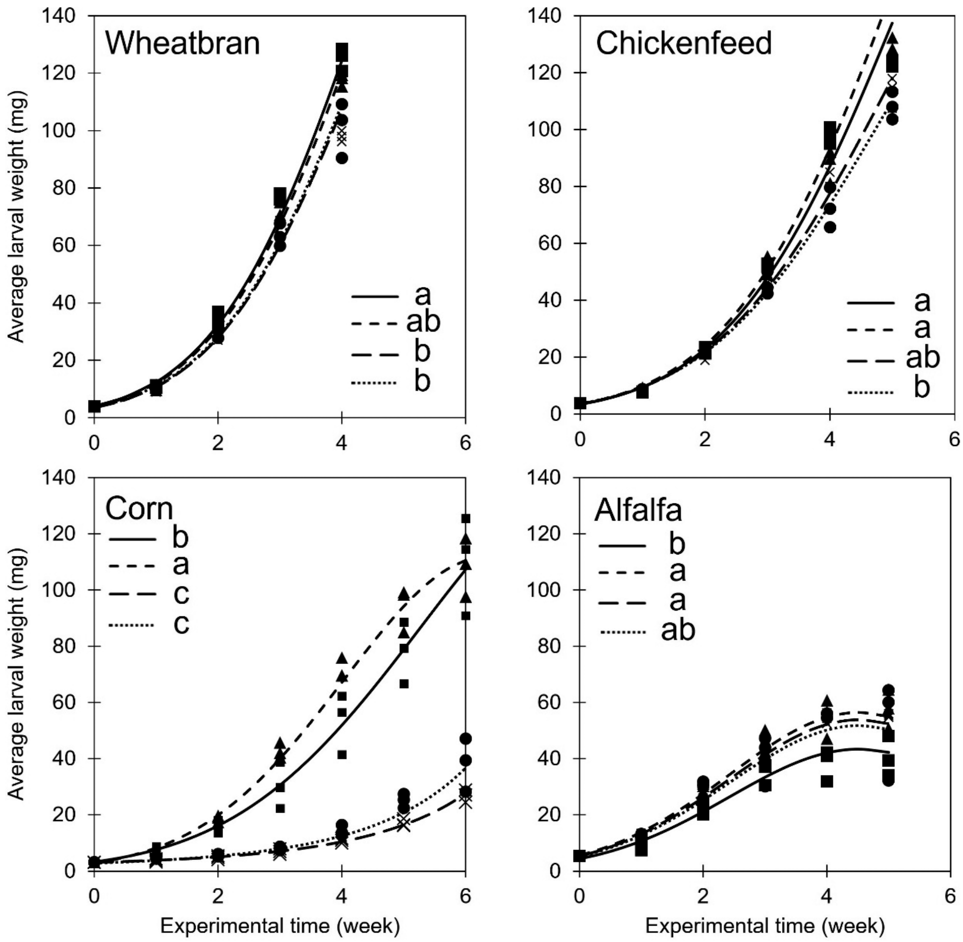

Fig. 1. The trends in the average weights of mealworm larvae recorded over six weeks when reared on four feeds of different particle sizes. The symbols indicate different particle sizes: ■ – 0–0.8 mm, ▲ – 0.8–2 mm, X – 2–3 mm and ● – 3–4 mm. The model predictions for the different particle sizes are visualized via the solid line (0–0.8 mm), dashed line (0.8–2 mm), long dashed line (2–3 mm) and dotted line (3–4 mm). The letters (a, b and c) indicate significant differences between different growth curves for the different particle sizes.

alfalfa pellets, the protein content was about 15.0%. The alfalfa pellets had the lowest amount of digestible carbohydrate (small particle size: 47.4% and large particle size: 44.1%) and intermediate amounts were detected in wheat bran (67.4% and 64.6% for small and large particle sizes, respectively) and chicken feed (62.0% and 66.2% for small and large particle sizes, respectively). The feeds used in this experiment are either grain-based, legume-based or mixtures of different raw materials. They are characterized by a wide range of protein to carbohydrate ratios (P : C). However, the P:C ratios of the two particle sizes of each feed analysed were very similar. The lowest P:C ratios were recorded for corn kernels (from 1 : 11.1 to 1 : 10.7) and the highest P : C ratios for alfalfa pellets (1 : 3.1 and 1 : 2.9 respectively). The intermediate values of the P : C ratios were recorded for wheat bran and chicken feed (1 : 4.1 and 1 : 4.2 for each substrate and particle size, respectively).

Larval performance

There were significant differences in the average final weights of larvae fed on almost all of the feeds of different particle sizes (Table 2). For the wheat bran feed (one-way ANOVA: $F = 14.97$; $df = 3, 8$; $P = 0.001$), the two small particle sizes (0–0.8 mm and 0.8–2 mm) resulted in a higher larval weight (126 mg and 117 mg, respectively) than the large particle sizes (2–3 mm and 3–4 mm; 98 mg and 101 mg, respectively) (Tukey-Kramer HSD post-hoc test). For the chicken feed (one-way ANOVA: $F = 11.54$; $df = 3, 8$; $P = 0.003$), the maximum larval weight of larvae reared on the 0.8–2 mm particle size (129 mg) was significantly higher than that of those reared on the two feeds with large particle sizes (112 mg and 107 mg for 2–3 mm and 3–4 mm particle sizes, respectively). However, the weight of larvae reared on the smallest particle sizes (0–0.8 mm: 123 mg) chicken feed was not significantly different from those

Table 2. The maximum larval weight ± standard deviation (mg) recorded for each particle size (in mm) and feed. The letters indicate significant differences between the maximum larval weight when fed the same feed but of different particle sizes.

Particle size (mm)	Wheat bran	Chicken feed	Corn	Alfalfa
0–0.8	126 ± 4 a	123 ± 1 ab	110 ± 18 a	41 ± 7 NS
0.8–2	117 ± 2 a	129 ± 5 a	107 ± 10 a	58 ± 7 NS
2–3	98 ± 2 b	112 ± 5 bc	27 ± 2 b	55 ± 1 NS
3–4	101 ± 10 b	107 ± 5 c	38 ± 10 b	52 ± 17 NS

NS – not significant.

reared on the 0.8–2 mm (123 mg) and 2–3 mm (112 mg) particle sizes, but statistically different from the maximum weight of larvae reared on the large particle size (3–4 mm; 107 mg) (Tukey-Kramer HSD post-hoc test). More markedly, for the corn kernels (one-way ANOVA: $F = 56.65$; df = 3, 8; $P < 0.001$), the two small particle sizes (0–0.8 mm and 0.8–2 mm) had a greater significant effect on the maximum weight (110 mg and 107 mg respectively) compared to the two large particle sizes (27 mg and 38 mg for 2–3 mm and 3–4 mm particle sizes, respectively) as the former were 3 to 4 times bigger than those reared on the large particle sized feed. Finally, for alfalfa pellets, different particle sizes did not affect the larval weight (one-way ANOVA: $F = 1.73$; df = 3, 8; $P = 0.240$) as the maximum larval weights were, statistically, the same on the different particle sized feeds (Table 2). However, the reduction in maximum average weights recorded for the 0–2 mm and 2–4 mm feeds was approximately 12% for chicken feed and 18% for wheat bran, but 70% for corn kernels.

The larval growth rate was also significantly affected by particle size (Fig. 1), the statistical growth models of which are listed in Table 3. The larvae reared on the small particle size (0–0.8 mm) of wheat bran grew significantly faster than those reared on the feed with large particle sizes (2–3 mm and 3–4 mm). The growth curve of larvae reared on the

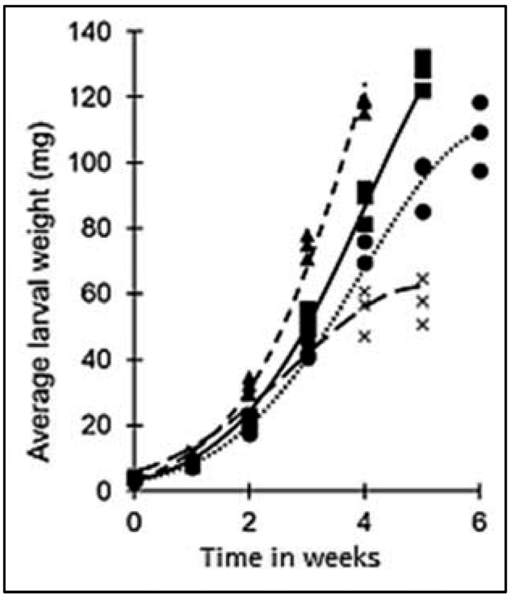

Fig. 2. The trends in the average weights of mealworm larvae recorded over six weeks when reared on 0.8–2 mm particle size of the four feeds. Triangles, squares, circles and crosses are, respectively, the larval weights recorded for wheat bran (▲), chickenfeed (■), corn (●) and alfalfa (**x**). The dashed line, solid line, dotted line and long dashed line are the trends predicted for the weights of larvae fed on wheat bran, chickenfeed, corn and alfalfa.

Table 3. The growth models for the different feeds and particle sizes (0–0.8 mm as reference). T – time (weeks), PS – particle size, df = 3, 8. The letters in column (Sig.) indicate a significant difference between particle size curves of the same feed ($p < 0.05$ in one or more parameters).

Feed	PS	intercept	T	PS	T × PS	T²	T² × PS	Sig.
Wheat bran	0-0.8	0.603 (0.016)	0.534 (0.014)	0	NS	–0.0403 (0.0034)	NS	a
	0.8-2	0.603 (0.016)	0.534 (0.014)	–0.0149 (0.016)	NS	–0.0403 (0.0034)	NS	ab
	2–3	0.603 (0.016)	0.534 (0.014)	–0.064 (0.016)	NS	–0.0403 (0.0034)	NS	b
	3–4	0.603 (0.016)	0.534 (0.014)	–0.0567 (0.016)	NS	–0.0403 (0.0034)	NS	b
Chicken feed	0–0.8	0.543 (0.0200)	0.470 (0.012)	0	0	–0.0302 (0.0021)	NS	a
	0.8–2	0.543 (0.0200)	0.470 (0.012)	0.0108 (0.027)	–0.00496 (0.00876)	–0.0302 (0.0021)	NS	a
	2–3	0.543 (0.0200)	0.470 (0.012)	0.0135 (0.027)	–0.0160 (0.00876)	–0.0302 (0.0021)	NS	ab
	3–4	0.543 (0.0200)	0.470 (0.012)	0.0203 (0.027)	–0.0233 (0.00876)	–0.0302 (0.0021)	NS	b
Corn	0–0.8	0.504 (0.0296)	0.399 (0.016)	0	0	–0.0241 (0.0026)		b
	0.8–2	0.504 (0.0296)	0.399 (0.016)	–0.0425 (0.0419)	0.0975 (0.0232)	–0.0241 (0.0026)	–0.0147 (0.0037)	a
	2–3	0.504 (0.0296)	0.399 (0.016)	–0.0173 (0.0419)	–0.319 (0.0232)	–0.0241 (0.0026)	0.0372 (0.0037)	c
	3–4	0.504 (0.0296)	0.399 (0.016)	–0.0425 (0.0419)	–0.288 (0.0232)	–0.0241 (0.0026)	0.0362 (0.0037)	c
Alfalfa	0–0.8	0.634 (0.0326)	0.444 (0.015)	0	NS	–0.0491 (0.0028)	NS	b
	0.8–2	0.634 (0.0326)	0.444 (0.015)	0.1133 (0.0415)	NS	–0.0491 (0.0028)	NS	a
	2–3	0.634 (0.0326)	0.444 (0.015)	0.0934 (0.0415)	NS	–0.0491 (0.0028)	NS	a
	3–4	0.634 (0.0326)	0.444 (0.015)	0.0762 (0.0415)	NS	–0.0491 (0.0028)	NS	ab

NS – not significant.

0.8–2 mm particle size of wheat bran was not statistically different from the others. The growth curves of the larvae reared on small particle sized chicken feed (0–0.8 mm and 0.8–2 mm) were significantly different from those reared on the 3–4 mm particle sized feed. Whereas the growth curve when reared on the 0.8–2 mm particle sized corn kernel feed differed significantly from that of larvae reared on the smallest particle size (0–0.8 mm) there was no significant difference in the final maximum weights on the two large particle sized (2–3 mm and 3–4 mm) feeds (Table 2). Although the alfalfa pellets were not the optimum substrate for mealworms, the larvae succeeded in pupating even at low weight. The growth curves of larvae reared on 0.8–2 mm and 2–3 mm feeds differed significantly from that of those reared on the 0–0.8 mm particle sized feed. The curve for those reared on the 3–4 mm particle sized alfalfa feed was statistically similar to the others.

Significant differences in mealworm growth were recorded for the different feeds (0.8–2 mm particle size) as reported in Fig. 2 and Table 4. The fastest larval growth rate was recorded for wheat bran followed by chicken feed and corn kernels. The growth rate on alfalfa pellets initially was similar to those recorded on the other experimental diets but then decreased rapidly.

The analysis of the variability in larval growth (based on individual larval weights) depicted in Fig. 3 indicates that there were significant differences in the variability in growth associated with particle sizes for 3 of the 4 feeds (wheat bran: $F = 6.17$; $df = 3, 8$; $P = 0.018$; chicken feed: $F = 3.65$; $df = 3, 8$; $P = 0.064$, corn kernels: $F = 30.21$; $df = 3, 8$; $P < 0.001$; alfalfa pellets: $F = 4.62$; $df = 3, 8$; $P = 0.037$). The variability in larval growth when reared on wheat bran, corn kernels and alfalfa pellets were significantly different for the different particle sizes. The highest variability (94 ± 9%) was recorded for corn kernels of 2–3 mm particle size and the lowest for wheat bran of less than 0.8 mm particle size (25 ± 1%). The variability in larval growth when reared on different feeds of particle size 0.8–2 mm was significantly different ($F = 8.12$; $df = 3, 8$; $P = 0.008$) with the lowest recorded for wheat bran.

DISCUSSION

Some insects are not discriminatory (generalists) in their feeding habits whereas others are highly selective in their choice (specialists) in which their nutritional needs may be important. The nutritional needs of insects in general and mealworms in particular are already well studied (Morales-Ramos et al., 2011, 2013; Oonincx et al., 2015; van Broekhoven et al., 2015). However, very little information is available on the physical properties of the feed and how these affect larval growth. One of the physical properties is the particle size of the feed and a few studies report that this may be important (Vanderzant, 1969; Cornelisse & Hafernik, 2009; Mason & McDonough, 2012; Manley et al., 2018).

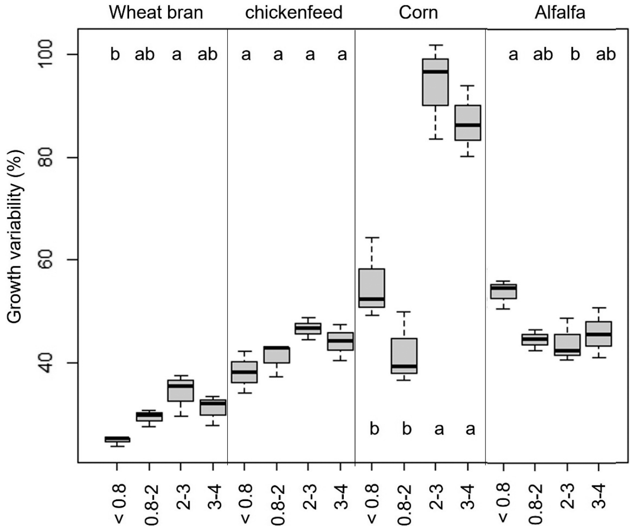

Fig. 3. The variability (%) in larval growth when reared on 4 different feeds of different particle sizes (in mm).

This study provides firm evidence that the particle size of feed is important for mealworm larval growth and using the correct particle size can significantly improve production. The nutritional compositions of the small and large particles are similar and this provides strong support for particle size being the main factor and not nutritional composition. However, the magnitude of the influence of particle size was variable: low for wheat bran and chicken feed, both of which are good for rearing mealworms (Klasing et al., 2000; Bumroongsook & Nahuanong, 2018) and high for corn kernels. This influence is also reported by Rumbos et al. (2020) in which different particle sizes of millet led to different results. Millet flakes improved the beetles' reproduction while millet grains favoured the growth of mealworm larvae.

Mealworms grew significantly better and achieved higher larval weights when reared on the two smaller particle sizes (< 2 mm) of wheat bran, chicken feed and corn kernels. As particle size increased (> 2 mm up to 4 mm), larval

Table 4. The growth models for the different feeds of particle size 0.8–2 mm (bran as reference). T – time (weeks), F – feed. The letters indicate a significant difference between feeds (p < 0.05 in one or more parameters).

Feed	Intercept	T	F	T × F	T²	T² × F	Sig.
Wheat bran	0.572 (0.023)	0.547 (0.027)	0	0	–0.0418 (0.0065)	0	a
Chicken feed	0.572 (0.023)	0.547 (0.027)	–0.0165 (0.032)	–0.0838 (0.0346)	–0.0418 (0.0065)	0.0120 (0.0077)	b
Corn	0.572 (0.023)	0.547 (0.027)	–0.111 (0.032)	–0.050 (0.032)	–0.0418 (0.0065)	0.0306 (0.0071)	c
Alfalfa	0.572 (0.023)	0.547 (0.027)	0.139 (0.032)	–0.0744 (0.0345)	–0.0418 (0.0065)	–0.0110 (0.0077)	d

performance declined on all feeds except alfalfa pellets. The reason may be that the larger particles are harder for the larvae to chew, especially the small larvae. However, different results were recorded when alfalfa pellets were used, as the smallest sized particles were less suitable. Alfalfa pellets were not a suitable feed (at least as a single component) as the larvae did not reach the same maximum weight achieved on other diets. Although alfalfa pellets are a good quality feed for ruminants due to their high content of protein and fibre, it is currently unclear why it is a poor quality feed for mealworms, especially the smallest particle sizes. Probably, the reason for the poor performance was that this feed contains a low amount of non-fibrous carbohydrate and as a consequence was not easily assimilated by *T. molitor* (Renaud, 2002). In fact, alfalfa pellets are low in easily digestible carbohydrates like starch and soluble sugars (Heuzé et al., 2016). The high fibre content could also be problematic. Although mealworms can partially degrade hemicellulose (Wang et al., 2017; Yang et al., 2019), their digestion is similar to that of monogastric omnivores that are not able to completely decompose and digest the fibrous components (cellulose and hemicellulose) of alfalfa. This assumption needs to be tested and it is likely that the availability of the nutrients might affect the performance of a feed (Cohen, 2015). This is particularly important for alfalfa pellets, on which the larvae did not die but grew slowly and pupated at a lower weight.

The larvae that reached the maximum weight the quickest were those reared on wheat bran and the slowest were those reared on corn kernels. The reason for this difference in larval growth rate may be the lower protein content of corn kernels (less than 8%) and their lower P : C ratio (about 1 : 11). The development time of larvae reared on low protein diets is longer than those reared on high protein diets (Oonincx et al., 2015). However, the protein content of corn kernels is lower than that of the low protein diets (12.9% and 14.4%) used by Oonincx et al. (2015). Another reason may be particle hardness as corn kernels are harder and tougher than wheat bran, but this needs to be tested.

This study was based on the growth of larvae in their latter stages of development and did not consider the relation between the larval stage and particle size. Individuals of different sizes might react differently to feed particle sizes; in ants it was shown that small food particles are easier and more available to small adults and vice versa (Hooper-Bui et al., 2002). Future studies should be based on different combinations of larval weight and particle size and include observations on the early larval stages when adults lay eggs in the diet used by the larvae.

In conclusion, the results of this study indicate that along with the nutritional quality of feed, particle size can affect the growth of mealworms. In general, small particles were more suitable than large ones for all feeds except alfalfa pellets. This was most evident for the corn kernels of 2–3 mm and 3–4 mm particle sizes in which the larvae did not reach the weight for pupating before they were harvested. The case of corn kernels clearly indicates that reducing particle size of a diet can make it more acceptable and easier to consume for mealworms.

Therefore, the production and rearing processes can be improved by using agricultural by-products shredded to the appropriate size. Finally, the physical characteristics of the diets of mealworms is important and should be studied further as poor growth should not automatically be attributed to their nutritional quality.

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