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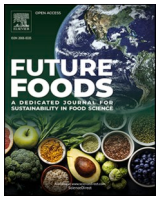

Replacing conventional substrate with linseed cake improves the omega-3 profile of *Zophobas atratus* fabricius (Coleoptera: Tenebrionidae) larvae

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ABSTRACT

Edible insects emerge as sustainable sources of protein, lipids, and minerals for human consumption, with the potential for transformation and bioaccumulation of nutritional compounds from alternative feeds. This study examined the nutritional enrichment of *Zophobas atratus* larvae using linseed cake, an agro-industrial by-product, as a feed substitute. Larvae were fed diets containing varying proportions of linseed cake (0 %, 25 %, 50 %, 75 %, and 100 %), and their nutritional profile and feed efficiency were evaluated. Feed conversion efficiency remained comparable to the control group with up to 75 % linseed cake incorporation. Larvae fed 100 % linseed cake showed higher protein content (46.68 % vs. 41.38 % in control) and significantly improved fatty acid profiles, with alpha-linolenic acid increasing from 1.03 % to 28.66 %. This resulted in a favorable shift in the omega-6/omega-3 ratio from 23.61 to 0.58. Mineral analysis revealed high levels of phosphorus, manganese, zinc, and magnesium for larvae fed 100 % linseed cake. The results demonstrate that *Zophobas atratus* larvae can be nutritionally enhanced through linseed cake feeding, offering improved protein content and beneficial fatty acid profiles for sustainable food systems.

1. Introduction

Concerns about environmental sustainability occupy a central place in global discussions. A current example is the efficiency of livestock production as the main source of meat and protein for the food market. Animal rearing, especially cattle, has high water and land consumption, with considerable greenhouse gas emissions, when aimed at large-scale production (Xu et al., 2021). This adds pressure on the environment and raises questions about the sustainability of these production systems. As an alternative, there is a need to explore more sustainable and efficient production methods. One such potential method is the rearing of edible insects for human consumption (Siddiqui et al., 2022), which are already part of the diet of at least 2 billion people worldwide, with >1900 edible species identified (Tanga and Ekesi, 2024).

Among edible insects, mealworms stand out, as they are already widely used as feed for animals such as reptiles, birds, and monkeys and food for humans (Bordiean et al., 2020). Representative species are *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) and *Zophobas atratus* Fabricius (Coleoptera: Tenebrionidae) (Peng et al., 2020). Insects of this order are distributed worldwide due to their adaptability to different environments and substrates, as well as their rich nutritional composition and resistance to harmful compounds such as mycotoxins and pesticides (Bordiean et al., 2020).

Mealworms have a nutritional content considered suitable for human and animal consumption, offering proteins, fats, minerals, and fibers (Nascimento et al., 2022). In addition, these larvae have demonstrated

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the ability to biodegrade different agro-industrial by-products, including rye bran, rapeseed meal, brewery spent grain, bread scraps, rice straw, and grape pomace (Bordiean et al., 2022; Montalbán et al., 2022; Nascimento et al., 2022), transforming them into insect biomass and suggesting highly functional and adaptable digestive systems. The fatty acid profile for these insects shows high levels of monounsaturated fatty acids (34.80 % for *Zophobas atratus* and 50.01 % for *Tenebrio molitor*) and saturated fatty acids (43.28 % for *Zophobas atratus* and 29.60 % for *Tenebrio molitor* (Nascimento et al., 2022; Dreassi et al., 2017). However, a lower level of polyunsaturated fatty acids has been identified (20.80 % for *Zophobas atratus* and 19.89 % for *Tenebrio molitor*), with a low value of omega-3 (PUFA n-3) in *Zophobas atratus* (1.40 %) (Nascimento et al., 2022) and 0.36 % for *Tenebrio molitor* (Lawal et al., 2021).

Omega-3 fatty acids, such as alpha-linolenic acid (ALA), are precursors to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), compounds integral to cardiovascular, cognitive, and inflammatory regulation (DiNicolantonio and O'Keefe, 2021). Conversely, omega-6 fatty acids, while necessary, are pro-inflammatory when consumed in excess, underscoring the importance of maintaining an optimal dietary omega-6 to omega-3 ratio. Current Western diets are often skewed towards higher omega-6 intake, which is linked to an increased risk of chronic inflammatory diseases (DiNicolantonio and O'Keefe, 2021). Enhancing the omega-3 content in edible insects through targeted dietary modifications could improve their nutritional value, positioning them not only as a sustainable protein source but also as a functional food with potential health benefits.

Currently, the commercial rearing of these larvae mainly uses wheat bran or oats as their substrate. However, it should be considered that these ingredients are also used for human food, which leads to competition for inputs and increases the cost of rearing insects. Due to the ability of mealworms to metabolize various components, it is important to assess the feasibility of replacing traditional feeds with more sustainable alternatives (Brandon et al., 2018).

Linseed, the seed of *Linum usitatissimum*, is known to be an important source of alpha-linolenic acid (C18:3n3), with a high lipid content (30.07–37.37 %) and a significant concentration of protein (19.45 - 21.71 %) and fiber (10.04 - 12.04 %) (Qiu et al., 2020; Khare et al., 2021). Global linseed production in 2020 reached approximately 3.5 million tons (Yadav et al., 2022), and lipid is a component of great commercial interest due to its oil extraction. On the other hand, the by-product of the oil industry, known as linseed cake, is often used as animal feed (Schasteen, 2024), as it is still a rich source of protein (27.80 to 39.40 %) and fiber (7.60 to 12.80 %), as well as containing moderate levels of lipids (up to 16.90 %), maintaining the seed's fatty acid profile, rich in alpha-linolenic acid (C18:3n3; 42.90 to 68.60 % of total fatty acids) (Kokić et al., 2024).

Some studies have investigated the use of linseed in feeding Tenebrionidae larvae. Examples include: the use of its oil as a way of improving the lipid profile of *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) and *Tenebrio molitor*, obtaining a significant reduction in the omega-6/omega-3 ratio (Oonincx et al., 2019; Rossi et al., 2022); use of its by-product (cake), replacing the conventional feed of *Tenebrio molitor* larvae by 50 %, resulting in larvae with 17.00 % alpha-linolenic acid (C18:3n3) compared to 1.73 % in the Control, and also with a better omega-6/omega-3 ratio, from 1.71 to 20.55 in the Control (Bordiean et al., 2022). These studies indicate the potential to use the linseed and its by-products to feed mealworms, intending to enhance their nutritional fatty acid profile.

Most research focuses on *Tenebrio molitor* (Tavares et al., 2022). However, *Zophobas atratus* has some distinct and interesting characteristics compared to *Tenebrio molitor*, such as its dependence on isolation during metamorphosis and its ability to reach larger sizes (Kim et al., 2015). *Zophobas atratus* has a higher biomass yield, reaching more than twice the weight of *Tenebrio molitor* (Harsányi et al., 2020). Therefore, studies evaluating the use of linseed by-products to feed *Zophobas atratus* larvae may be of interest both from an economic point of view and to elucidate scientific hypotheses regarding the possibility of nutritional enrichment and the development of more sustainable rearing methods.

Considering the nutritional richness of linseed cake, the ability of larvae of the Tenebrionidae family to metabolize various nutritional substrates, and the need to evaluate alternative methods of sustainable food production, this study aimed to assess the feasibility of nutritional enrichment of *Zophobas atratus* larvae, with emphasis on the bioaccumulation of omega-3 fatty acids through its feeding with linseed cake.

2. Material and methods

2.1. Biological material

2.1.1. Larvae

The company SuperBugs - Alimentos Funcionais (Salvador-BA, Brazil) donated 2000 *Zophobas atratus larvae* of up to 15 days from ecloding (average larval unit weight of 39.58 mg ± 4.50 and length between 0.5 and 1.0 cm). Prior to the experiment, the larvae were exclusively fed wheat bran (Relva Verde, Ibiporã-SC, Brazil).

2.1.2. Material for formulating substrates

The linseed cake from the cold pressing process for oil extraction was donated by the company Vital Âtman Ltda (São Paulo-SP, Brazil).

The Control substrate was developed with a mixture of 70 % commercial poultry growth feed (Imbramil, São Paulo-SP, Brazil) purchased from a local business in the city of Salvador-BA, Brazil, consisting of ground whole corn, soybean meal, wheat bran, meat and bone meal and a mineral/vitamin mixture; and 30 % wheat bran (Relva Verde, Ibiporã-SC, Brazil), also purchased from a local business in the city of Salvador-BA, Brazil.

2.2. Methods

2.2.1. Substrate formulation

The Control substrate and the linseed cake were ground in a grain grinder (80,393 Hamilton Beach, United States of America) until they reached a particle size of <0.71 mm (25 mesh). After this, 5 substrates were prepared, which made up the distinct treatments in this study: 0 (Control) (70 % commercial poultry growth feed + 30 % wheat bran); 25 (75 % Control + 25 % linseed cake); 50 (50 % Control + 50 % linseed cake); 75 (25 % Control + 75 % linseed cake); and 100 (100 % linseed cake). Table 1 presents the nutritional composition of each substrate used to feed the *Zophobas atratus* larvae.

2.2.2. Larvae rearing conditions

One hundred larvae were placed in a plastic box measuring 25.5 cm x 11.5 cm x 15 cm (length x height x width), with an opening at the top covered by a tulle mesh. Each box contained the respective substrate (Table 1) at a ratio of 2 g of substrate to 1 g of larval weight (Boukid et al., 2021). In addition, 0.3 g of fresh potato (*Solanum tuberosum*) per 1 g of larval weight was offered in cotton layers and replaced twice each week as a water source (Ruschioni et al., 2020). The experiment was conducted for 90 days with 4 replicates per treatment. The boxes were arranged randomly on a vertical shelf and kept in an air-conditioned room with temperature control (25 °C ± 1) and relative humidity (50 % ± 5), using a digital thermo-hygrometer (KR42 Instrubras, Brazil) and a 12 h day/night photoperiod (Latney et al., 2017).

Every two weeks, fresh substrates were renewed (considering the larval weight per box) and the uneaten substrate and feces were weighed. The larvae were weighed weekly on an analytical balance (AY220 Shimadzu, Japan). At the end of the experiment, the larvae were separated from the substrates to empty the digestive tract for 24 h (Kulma et al., 2020), followed by freezing at −80 °C and freeze-drying (Lyophilizer L101 Liobras, Brazil) for 48 h. The slaughtered and freeze-dried larvae were ground in a grain grinder (80,393 Hamilton

Table 1
Nutritional composition of substrates containing different percentages of linseed cake used to feed *Zophobas atratus* larvae.

Nutrient	Treatments				
	0 (Control)	25	50	75	100
Proximate composition (% wet matter)					
Carbohydrates	46.63^{a} ± 0.36	39.13^{b} ± 0.25	31.63^{c} ± 0.14	24.13^{d} ± 0.04	16.64^{e} ± 0.08
Crude fiber	16.09^{e} ± 0.22	19.48^{d} ± 0.17	22.87^{c} ± 0.13	26.25^{b} ± 0.08	29.64^{a} ± 0.03
Protein	14.55^{e} ± 0.23	18.28^{d} ± 0.17	22.01^{c} ± 0.11	25.74^{b} ± 0.05	29.47^{a} ± 0.01
Moisture	12.38^{a} ± 0.38	11.75^{ab} ± 0.29	11.13^{bc} ± 0.21	10.51 ^{cd} ± 0.12	9.89^{d} ± 0.04
Lipid	4.01^{e} ± 0.26	5.27^{d} ± 0.19	6.54^{c} ± 0.11	7.80^{b} ± 0.04	9.06^{a} ± 0.03
Ash	6.35^{a} ± 0.11	6.09^{b} ± 0.08	5.83^{c} ± 0.06	5.57^{d} ± 0.04	5.31^{e} ± 0.03
Fatty acid (%)					
Saturated					
C8:0	0.03^{a} ± 0.00	0.02^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	–
C12:0	0.07^{a} ± 0.00	0.05^{b} ± 0.00	0.04^{c} ± 0.00	0.02^{d} ± 0.00	–
C14:0	0.88^{a} ± 0.02	0.71^{b} ± 0.02	0.53^{c} ± 0.01	0.35^{d} ± 0.01	0.17^{e} ± 0.03
C15:0	0.08^{a} ± 0.00	0.06^{b} ± 0.00	0.04^{c} ± 0.00	0.02^{d} ± 0.00	–
C16:0	21.11^{a} ± 1.19	18.35^{b} ± 0.89	15.50^{c} ± 0.50	12.57^{d} ± 0.00	9.53^{e} ± 0.61
C18:0	8.18^{a} ± 0.26	7.35^{b} ± 0.21	6.51^{c} ± 0.13	5.63^{d} ± 0.03	4.73^{e} ± 0.11
Monounsaturated					
C16:1	0.11^{a} ± 0.00	0.08^{b} ± 0.00	0.06^{c} ± 0.00	0.03^{d} ± 0.00	–
C18:1n9 *cis*	26.11^{a} ± 0.37	25.24^{b} ± 0.24	24.34^{c} ± 0.13	23.42^{d} ± 0.06	22.48^{e} ± 0.00
Polyunsaturated					
C18:2n6 *cis*	39.19^{a} ± 0.28	33.39^{b} ± 0.09	27.45^{c} ± 0.30	21.33^{d} ± 0.32	15.04^{e} ± 0.13
C18:3n3	2.27^{e} ± 0.05	12.94^{d} ± 0.40	23.89^{c} ± 0.43	35.16^{b} ± 0.10	46.76^{a} ± 0.61
Other fatty acids	1.98^{a} ± 0.76	1.81^{a} ± 0.57	1.64^{a} ± 0.38	1.46^{a} ± 0.18	1.29^{a} ± 0.01
∑ SFA	30.34^{a} ± 1.47	26.54^{b} ± 1.12	22.63^{c} ± 0.64	18.60^{d} ± 0.03	14.43^{e} ± 0.75
∑ MUFA	26.22^{a} ± 0.37	25.32^{a} ± 0.24	24.40^{ab} ± 0.14	23.45^{ab} ± 0.06	22.48^{b} ± 0.00
∑ PUFA	41.46^{e} ± 0.34	46.33^{d} ± 0.31	51.34^{c} ± 0.13	56.49^{b} ± 0.21	61.80^{a} ± 0.74
omega-6/omega-3	17.26^{a} ± 0.29	2.58^{b} ± 0.09	1.15^{c} ± 0.03	0.61^{d} ± 0.01	0.32^{e} ± 0.00
Metal (mg/100 g wet matter)					
Potassium	780.81^{e} ± 5.74	840.42^{d} ± 12.38	900.04^{c} ± 1.92	959.66^{b} ± 8.54	1019.28^{a} ± 5.74
Phosphorus	840.77^{c} ± 1.17	876.06^{bc} ± 14.75	911.34^{abc} ± 28.33	946.63^{ab} ± 41.91	981.91^{a} ± 55.50
Calcium	872.33^{a} ± 109.64	727.53^{ab} ± 81.84	582.73^{bc} ± 54.05	437.92 ^{cd} ± 26.25	293.12^{d} ± 1.54
Magnesium	322.33^{a*} ± 16.20	308.70^{a*} ± 10.88	318.93^{a*} ± 5.68	315.52^{a*} ± 1.93	312.11^{a*} ± 5.74
Sodium	103.97^{a} ± 6.63	85.21^{b} ± 4.94	66.46^{c} ± 3.26	47.70^{d} ± 1.58	28.94^{e} ± 0.10
Iron	13.32^{a} ± 0.44	11.70^{b} ± 0.27	10.09^{c} ± 0.10	8.48^{d} ± 0.08	6.86^{e} ± 0.25
Zinc	9.25^{a} ± 0.04	8.81^{b} ± 0.05	8.38^{c} ± 0.07	7.94^{d} ± 0.08	7.50^{e} ± 0.10
Manganese	9.21^{a} ± 0.04	8.43^{b} ± 0.03	7.65^{c} ± 0.02	6.87^{d} ± 0.01	6.10^{e} ± 0.00
Copper	0.78^{e} ± 0.04	1.06^{d} ± 0.02	1.33^{c} ± 0.01	1.61^{b} ± 0.03	1.89^{a} ± 0.05
Nickel	0.05^{e} ± 0.00	0.10^{d} ± 0.00	0.14^{c} ± 0.01	0.19^{b} ± 0.01	0.24^{a} ± 0.01
Arsenic	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**
Cadmium	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**

Data presented as mean ± standard deviation. Different letters on the same line indicate a statistical difference between substrates ($p \leq 0.05$) according to Tukey's test, except when the line shows.
*, which indicates the Mann-Whitney U test.
** indicates results below the detection limit. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake. ∑ PUFA: sum of polyunsaturated fatty acids; ∑ MUFA: sum of monounsaturated fatty acids; ∑ SFA: sum of saturated fatty acids.

Beach, United States of America) until they reached homogeneous granulometry and stored in plastic containers at −80 °C for later analysis.

2.2.3. Larval growth and feed conversion efficiency

The unit larval mass growth curve throughout the experiment period was generated and adjusted in the JMP pro12 program by applying the three-parameter Gompertz growth model (Eq. (1)), where a = asymptote, b = growth rate, c = inflection point, e = exponent, time = rearing days.

$$\text{Larval mass (g)} = a * e^{e^{-b*(\text{Time}-c)}} \quad \text{(Eq. 1)}$$

To assess the adaptation of the larvae to the substrates and the efficiency of converting food into larval biomass, the following parameters were calculated: Conversion Efficiency of Ingested Feed (ECI (%), Eq. (2) - dry matter), Feed Conversion Ratio (FCR, Eq. (3) - wet matter) and Mortality Rate (MR (%) Eq. (4)) (Oonincx et al., 2015; Zhang et al., 2019) according to the following equations:

$$\text{ECI (\%)} = (\text{weight gained})/(\text{weight of ingested feed}) \times 100 \quad \text{(Eq. 2)}$$

$$\text{FCR} = (\text{weight of ingested feed})/(\text{weight gained}) \quad \text{(Eq. 3)}$$

$$\text{MR (\%)} = (\text{number of dead insects})/(\text{number of initial insects}) \times 100 \quad \text{(Eq. 4)}$$

2.2.4. Proximate composition

The proximate composition was determined according to AOAC standards (2019). Moisture was determined by oven drying (Tecnal, TE-394/I, Brazil) at 105 °C until constant weight was obtained, ash was determined in a muffle furnace (Lavoisier 402-D, Brazil) by incineration at 550 °C, crude fiber was determined by acid and alkaline extraction. The cold extraction method (Bligh and Dyer, 1959) was used to determine total lipids in order to preserve the fatty acids so that they could be properly profiled afterward. Carbohydrates were calculated using the difference between the other macronutrients (Bhattacharjee et al., 2013). The energy value was calculated considering 4 kcal/g for carbohydrates and proteins and 9 kcal/g for lipids (Bhattacharjee et al., 2013). Crude protein was determined using the Kjeldahl method (AOAC, 2019), where the nitrogen conversion factor used was 4.76 for the larvae (Janssen et al., 2017) and 6.25 for the substrates (Nascimento et al., 2022).

2.2.5. Fatty acid profile and lipid quality

The identification and quantification of fatty acids was carried out according to the methodology proposed by Souza et al. (2017). To this end, an aliquot of the total lipids was subjected to the saponification reaction with NaOH in methanol (0.5 N), followed by methylation with BF_3 (12 % in methanol) and extraction with isooctane. The extracted fatty acid methyl esters were stored in an amber vial in an inert atmosphere (N_2). A gas chromatograph (Perkin Elmer Clarus 680) with flame ionization detector and DB - Fast FAME column (30 m × 0, 25 mm × 0, 25 μm) was used to separate the fatty acid methyl esters. Helium was used as the carrier gas at a flow rate of 1.0 mL/min, and injections of 1 μL were made in split mode (1:50). The fatty acids were identified by comparing the retention times of the peaks in the samples with the retention times of a standard mixture (189–19, Sigma Aldrich, USA). The fatty acids in the samples were quantified by normalizing the peak areas (% area). The sums of total saturated fatty acids (∑SFA), total monounsaturated fatty acids (∑MUFA), and total polyunsaturated fatty acids (∑PUFA) were calculated, as well as the omega-6/omega-3 ratio. The following lipid quality indices were calculated for *Zophobas atratus* larvae: Atherogenicity Index (AI, Eq. (5)), Thrombogenicity Index (TI, Eq. (6)), Hypocholesterolemic/Hypercholesterolemic Ratio (H/H, Eq. (7)), Health Promotion Index (HPI, Eq. (8)), Unsaturation Index (UI, Eq. (9)), Oxidizability Index (COX, Eq. (10)) and Peroxidizability Index (PI, Eq. (11)). Their respective equations are presented below (Chen and Liu, 2020; Duarte et al., 2022):

$$AI = (C12:0 + 4 \times C14:0 + C16:0)/(\Sigma MUFA + omega-6 + omega-3) \quad \text{(Eq. 5)}$$

$$TI = (C14:0 + C16:0 + C18:0)/((0.5 \times \Sigma MUFA) + (0.5 \times omega-6) + (3 \times omega-3) + (omega-3/omega-6)) \quad \text{(Eq. 6)}$$

$$H/H = (cis-C18:1 + \Sigma PUFA)/(C12:0 + C14:0 + C16:0) \quad \text{(Eq. 7)}$$

$$HPI = \Sigma UFA/(C12:0 + (4 \times C14:0) + C16:0) \quad \text{(Eq. 8)}$$

$$UI = (1 \times \%\ monoenoic) + (2 \times \%\ dienoic) + (3 \times \%\ trienoic) + (4 \times \%\ tetraenoic) + (5 \times \%\ pentaenoic) + (6 \times \%\ hexaenoic) \quad \text{(Eq. 9)}$$

$$COX = ((C18:1) + (10.3 \times C18:2) + (21.6 \times C18:3))/100 \quad \text{(Eq. 10)}$$

$$PI = (0,025 \times C18:1) + (C18:2) + (2 \times C18:3) \quad \text{(Eq. 11)}$$

2.2.6. Analysis of metals

Metals were determined using inductively coupled plasma optical emission spectrometry (ICP OES; Agilent Technologies, series 720, United States of America). The analyzed metals were copper, sodium, manganese, magnesium, selenium, iron, calcium, zinc, potassium, phosphorus, cobalt, arsenic, cadmium, and nickel. The accuracy of the method was established by analyzing reference material and certified apple leaves (NIST 1515) under the same analysis conditions as the treatments.

2.2.7. Statistical analysis

To assess statistical differences, One-way ANOVA and Tukey's test were applied to all the results with a normal distribution. The Kruskall-Wallis and Mann-Whitney U tests were conducted for results with a non-normal distribution. Spearman's correlation was carried out between the data from the larvae and the substrates. For all tests, a significance level of 5 % was considered.

3. Results and discussion

3.1. Larval efficiency in mass gain and feed conversion

Fig. 1 shows the larval mass curve over the 90-day experiment. At the end of the period, there was no statistical difference between the average mass of the Control larvae (0.57 g), with treatments 25 (0.59 g) and 50 (0.60 g) ($p > 0.05$), while treatments 75 and 100 resulted in curves with lower final mass (0.43 g and 0.27 g respectively) ($p \leq 0.05$). Furthermore, according to Table 2, the asymptote, or maximum larval mass, was statistically higher for treatments 50 (0.733 g), 25 (0.698 g), and Control (0.692 g). Treatment 75 showed an intermediate value (0.554 g), followed by treatment 100 with the lowest value for this parameter (0.347 g).

Table 3 shows the parameters for the efficiency of converting feed into larval mass (ECI and FCR) and the mortality rate. The results indicate a good Conversion Efficiency of Ingested Feed (ECI), and Feed Conversion Ratio (FCR). In terms of ECI, the Control larvae (26.59 %) and treatments 25 (24.03 %), 50 (24.26 %), and 75 (22.22 %) showed statistically equal results, suggesting that partially replacing the Control substrate with up to 75 % linseed cake does not compromise the efficiency of the larvae in converting such by-products. Treatment 100 showed the lowest efficiency, with a statistically lower result (16.58 %).

A study conducted by Nascimento et al. (2022) found an ECI for *Zophobas atratus* larvae fed a control substrate (70 % poultry growth feed + 30 % ground corn) of approximately 25 %, a result close to that of the present study.

As for FCR, Control (3.78), 25 (4.08), 50 (4.07), and 75 (4.54) treatments resulted in better efficiency in the consumption and transformation of the substrate into larval mass. Treatment 100 (6.96) resulted in the statistically highest feed conversion ratio. A substrate obtained from a commercial *Zophobas atratus* rearing company was used in a study by van Broekhoven et al. (2015) and the FCR found was 3.64, close to the 25, 50, and 75 treatments, which corroborates the commercial applicability of the substrates developed in this study.

The exclusive use of linseed cake as a substrate proved to be insufficient to provide adequate rearing for *Zophobas atratus* larvae. Substrates with high protein content and low carbohydrate content showed lower feed conversion efficiencies (Table 1), in line with the results described by Zhang et al. (2019), as the linseed cake had a significantly higher protein content (102.54 % higher) and a 64.31 % lower carbohydrate content than the control feed. This may indicate that the larvae tend to grow more adequately on substrates with a balance of nutrients, which may justify the better results on substrates with intermediate proportions of linseed cake.

Kröncke and Benning (2022) reported that *Tenebrio molitor* larvae tended to develop better when consuming substrates high in carbohydrates and proteins and low in lipids (around 67.3 to 71.5 % for carbohydrates, 19.9 to 22.8 % for proteins and 8.6 to 10.0 % for lipids). This corresponds to a carbohydrate:protein ratio of between 3.13:1 and 3.38:1. For the present study, the minimum ratio found was 0.56:1

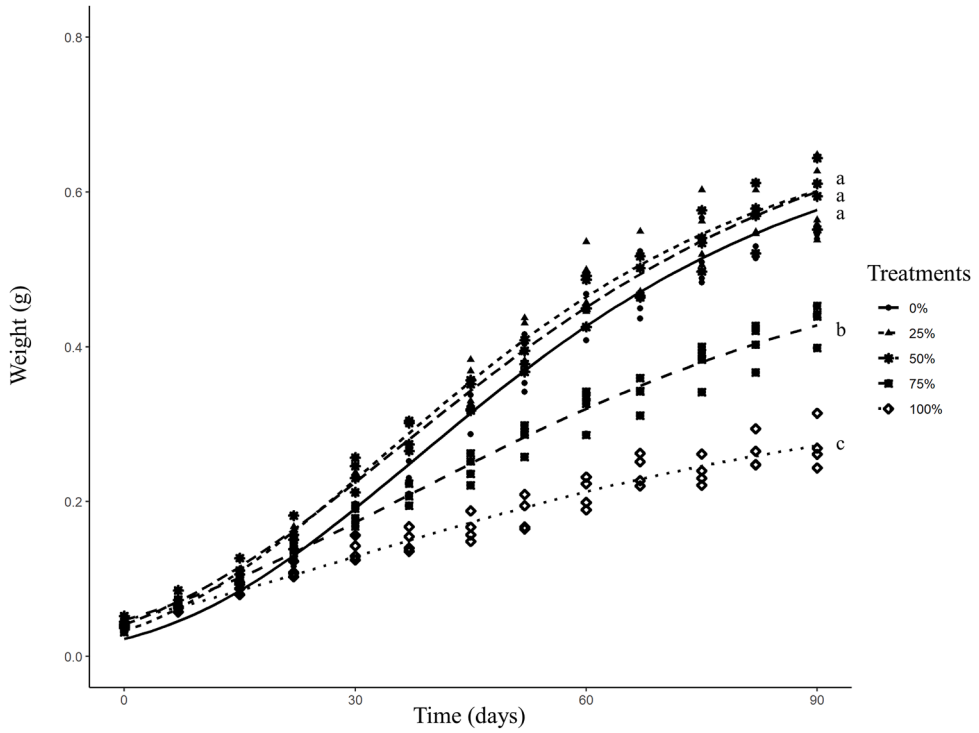

Fig. 1. Growth curve of *Zophobas atratus* larvae over 90 days on diets with varying linseed cake content. The fitted curves represent the average mass (g) of larvae fed on substrates containing different percentages of linseed cake, modeled using the Gompertz growth model with three parameters. Different letters (a, b, c) at the end of the experiment indicate statistically significant differences among treatments ($p \leq 0.05$), as determined by Tukey's test. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake.

Table 2
Parameters estimated according to the Gompertz (3 Parameters) model for the growth rate of *Zophobas atratus* larvae fed substrates containing different percentages of linseed cake.

Treatments	a	b	c	SEM.a	SEM.b	SEM.c
100	0.347^{d}	0.0234^{c}	29.50	0.041053	0.004379	5.902931
75	0.554^{c}	0.0250^{bc}	36.00	0.041799	0.002747	3.625286
0 (Control)	0.692^{b}	0.0326^{ab}	37.70	0.028494	0.002246	1.645937
50	0.733^{a}	0.0296^{abc}	35.65	0.031231	0.002039	1.824583
25	0.698^{b}	0.0336^{a}	33.30	0.023940	0.002122	1.365813

Equation = $a * e^{e^{-b*(Time-c)}}$ where a = asymptote, b = growth rate, c = inflection point, e = exponent, time = rearing days. SEM = Standard deviation. Different superscript letters in the same column indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake.

(100), far below the recommendation proposed by Kröncke and Benning (2022). On the other hand, the substrates 25 and 50 showed good production yields and intermediate carbohydrate:protein ratios of 2.14:1 and 1.43:1, respectively, indicating flexibility for this parameter.

Most of the correlations between the larval rearing efficiency variables and the nutritional composition of the substrates were moderate, close to 0.70 (or −0.70) (Supplementary Table 1), which reinforces the importance of a balance of nutrients in the substrate of *Zophobas atratus* larvae. In addition, linseed cake contains some anti-nutritional factors, such as linatin, which can compromise the larval development of insects, depending on the amount present (Oonincx et al., 2019; Gai et al., 2023). This may have been a determining factor regarding statistical difference ($p < 0.05$) between mortality rate for treatment 100 (36.00 %) in comparison with Control (18.50 %). Linatin is an antagonist of vitamin B6 and reduces its bioavailability. Compromising the availability of this nutrient can cause delayed mass gain and increased mortality in insects (Abbas, 2020), which is in line with the findings of the present study, where the mortality rate for the larvae increases proportionally to the increase of linseed cake in the feeding substrate.

Linseed cake contains various polyphenols (20.8 mg GAE/g, (Ho et al., 2007)), substances that have insect-deterrent properties, as studied by Niveyro et al. (2023). Roth et al. (1997) found that phenolic glycosides impaired larval growth of the species *Lymantria dispar*. Therefore, the presence of these anti-nutritional factors in greater quantities in the substrate composed exclusively of linseed cake may have compromised the mass gain and increased the mortality of *Zophobas atratus* larvae.

3.2. Proximate composition

Table 4 shows the proximate composition of the larvae fed with substrates containing different percentages of linseed cake. As for moisture, the lowest value was found for the Control (61.05 %) and the highest for the 100 treatment (66.01 %). In general, the higher the percentage of linseed cake in the larvae's substrate, the greater the increase in moisture ($p < 0.05$). The larvae in the Control treatment had

Table 3
Mortality Rate (MR (%)), Conversion Efficiency of Ingested Feed (ECI), and Feed Conversion Ratio (FCR) of *Zophobas atratus* larvae fed substrates containing different percentages of linseed cake.

Parameters	Treatments				
	Control	25	50	75	100
MR (%)	$18.50^{b} \pm 6.76$	$20.00^{b} \pm 5.23$	$28.00^{ab} \pm 3.37$	$29.75^{ab} \pm 5.25$	$36.00^{a} \pm 6.22$
ECI (%)	$26.59^{a} \pm 3.73$	$24.03^{a} \pm 0.54$	$24.26^{a} \pm 1.88$	$22.22^{a} \pm 1.81$	$16.58^{b} \pm 0.85$
FCR	$3.78^{a} \pm 0.45$	$4.08^{a} \pm 0.19$	$4.07^{a} \pm 0.36$	$4.54^{a} \pm 0.35$	$6.96^{b} \pm 0.46$

Results presented as mean ± standard deviation. Different letters on the same line indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake. ECI (%) (dry basis) = (weight gained)/(weight of feed ingested) × 100. FCR (wet basis) = (weight of feed ingested)/(weight gained). MR (%) = (number of dead insects)/(number of initial insects) × 100.

Table 4
Proximate composition of *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Parameters	Treatments				
	Control	25	50	75	100
Moisture (%)	$61.05^{c} \pm 0.74$	$60.59^{c} \pm 0.58$	$62.26^{bc} \pm 0.61$	$63.12^{b} \pm 0.87$	$66.01^{a} \pm 0.40$
Proteins (%)	$41.38^{d*} \pm 0.30$	$42.36^{c*} \pm 0.27$	$42.15^{c*} \pm 0.08$	$43.21^{b*} \pm 0.03$	$46.68^{a*} \pm 0.04$
Lipids (%)	$34.74^{a} \pm 0.32$	$33.38^{a} \pm 0.15$	$33.79^{a} \pm 0.95$	$32.37^{a} \pm 0.76$	$32.12^{a} \pm 2.15$
Carbohydrates (%)	$13.07^{a} \pm 0.92$	$11.35^{ab} \pm 0.31$	$11.37^{ab} \pm 0.91$	$7.67^{c} \pm 0.68$	$10.04^{bc} \pm 1.81$
Crude fiber (%)	$7.70^{d*} \pm 0.42$	$10.06^{bc*} \pm 0.75$	$10.05^{b*} \pm 0.04$	$14.25^{a*} \pm 0.55$	$8.65^{cd*} \pm 0.39$
Ash (%)	$3.11^{a*} \pm 0.07$	$2.85^{b*} \pm 0.02$	$2.64^{c*} \pm 0.03$	$2.50^{d*} \pm 0.02$	$2.51^{d*} \pm 0.02$
Energy (Kcal)	$530.46^{a} \pm 1.78$	$515.22^{a} \pm 3.68$	$518.22^{a} \pm 4.64$	$494.82^{b} \pm 5.22$	$516.01^{a} \pm 12.31$

Results presented as mean ± standard deviation of relative percentage in dry matter, except for moisture (relative percentage of wet matter) and energy (kcal of dry matter macronutrients). Different letters in the same column indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test, except when the column shows.

* , which indicates the Mann-Whitney U test. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake.

the lowest protein content (41.38 %). The percentage of protein increased the higher the proportion of linseed cake in the substrate, reaching a maximum value of 46.68 % for treatment 100 ($p < 0.05$). The value for carbohydrates ranged from 7.67 % for treatment 75 to 13.07 % in the Control. Crude fiber was lowest for the Control (7.70 %) and highest for treatment 75 (14.25 %). Treatments 25 (10.06 %) and 50 (10.05 %) showed intermediate and statistically equal results with the Control. As for the ash content, the Control showed the highest value (3.11 %), decreasing in treatments 25 (2.85 %), 50 (2.64 %), 75 (2.50 %), and 100 (2.51 %), with a statistical difference between all samples, except for 75 and 100 treatments. Finally, the energy value was a minimum of 494.82 kcal in treatment 75 (statistically different from all samples) and a maximum of 530.46 kcal in the Control.

3.3. Protein

The 5.3 percent increase in protein content (from 41.38 % - Control to 46.68 % - 100 treatment) represents a substantial nutritional enhancement when considered in the context of protein-rich food sources and daily dietary requirements. This elevation corresponds to a relative increase of approximately 12.8 % in total protein content, which is particularly significant given that when compared to conventional foods, *Zophobas atratus* could be considered a rich source of proteins. The protein content of *Zophobas atratus* in the present study was close to that found for raw and boneless beef ribeye steak (approximately 47 %, dry basis), but was below that of raw boneless pork loin (approximately 67 %, dry basis) (USDA, 2023). Soybean flour, on the other hand, has between 40 and 42 % protein on a dry basis, similar to that found for *Zophobas atratus* (USDA, 2023).

From a nutritional and practical perspective, considering the Recommended Daily Intake (RDA) of protein for a healthy adult with a minimum level of physical activity (0.8 g of protein per kg of body weight/day), a 75 kg person would need 60 g of protein (Wu, 2016). This value can be achieved by consuming between 128.53 g (100) and 144.99 g (Control) of *Zophobas atratus* larvae throughout the day (dry basis). In comparison, approximately 127.65 g of raw and boneless beef ribeye steak (dry basis) would be required (USDA, 2023).

This result represents an important ability to convert organic material, originally disposable, into a biomass of high nutritional value. Similar result was noted by Ruschioni et al. (2020), when feeding *Tenebrio molitor* with a substrate consisting of 25 % olive oil cake and 75 % wheat bran and verifying a percentage of 47.58 % protein. In addition, the larval protein content showed strong correlations with all the nutritional characteristics of the substrate (Supplementary Table 1): protein (0.84), lipid (0.88), carbohydrate (−0.89), moisture (−0.86), crude fiber (0.88), ash (−0.88), omega-6/omega-3 (−0.85), C18:2n6 (−0.91), C18:3n3 (0.92), ∑ PUFA (0.86), ∑ MUFA (−0.80) and ∑SFA (−0.90). Paying attention to these parameters can help rear insects with a biomass richer in protein.

Some studies have reported the influence of the protein content of the substrate on larval protein. Adámková et al. (2020) carried out a study with *Tenebrio molitor*, feeding their larvae wheat bran and lentil flour. As a result, it was found that increasing the protein content of the substrate led to an increase in larval protein, ranging from approximately 54 % for larvae fed a low-protein substrate (16.20 % protein content in the substrate) to up to 65 % for larvae fed a high-protein substrate (24.10 % protein content in the substrate) (dry basis). Van Broekhoven et al. (2015) found similar results with *Zophobas atratus* larvae fed various wastes, including cookies, bread crumbles and discarded grains. As a result, a substrate rich (39.10 % protein content in the substrate) in protein enabled a maximum larval protein value of 42.50 % to be reached, compared to a substrate poor (11.90 % protein content in the substrate) in protein (34.20 %). This trend was also seen in this study and may be associated with the fact that, due to the higher protein content, the larvae are more easily able to ingest, metabolize, and transform the amino acids into larval protein.

The whole *Zophobas atratus* larva or its isolated protein have great potential as ingredients in the protein enrichment of processed foods, such as animal feed, food supplements, protein bars and products with high global consumption. As examples, cookies fortified with 30 % *Zophobas atratus* flour showed a 63 % increase in their protein content (from 9.40 % to 14.92 %) (Sriprablom et al., 2022); snacks developed with *Alphitobius diaperinus* flour, where fortification in the proportion of 30 % insect flour could result in a 99.30 % increase in protein for the snack (from 12.53 % to 24.98 %) (Roncolini et al., 2020).

3.4. Lipids and fatty acids

The lipid content remained statistically constant (from 32.12 % (100) to 34.74 % (Control)), regardless of the food source of the *Zophobas atratus* larvae. Kulma et al. (2020) found a range of 31.30 to 36.00 % lipids in *Zophobas atratus* larvae fed wheat bran between 60 and 120 days old, close to the values found in this study. Arrese and Soulages (2010) argue that the lipid content of the substrate has a positive

correlation with the lipid concentration in the larval biomass, which is the opposite of what was found in this study (Supplementary Table 1).

Nascimento et al. (2022) found that *Zophobas atratus* larvae fed substrates with higher concentrations of carbohydrates had a higher lipid content (ranging from 40.50 % to 45.58 %), which would be in line with the correlation found in this study. Lipid synthesis in insects can occur from the conversion of carbohydrates into triglycerides (Arrese and Soulages, 2010). In this way, it could be assumed that, for the present study, the lower carbohydrate content of the substrates containing linseed cake, combined with the higher lipid content of these substrates, may have been a balancing factor in the total lipids found in the larval biomasses. To modulate the lipid content of the larvae, the following strong correlations with substrate parameters should be considered: protein (−0.71); carbohydrate (0.72), and C18:2n6 (0.74) (Supplementary Table 1).

As for the percentages of fatty acids (Table 5), significant changes were observed as the content of linseed cake incorporated into the substrates of *Zophobas atratus* larvae increased. For the Control larvae, the predominant fatty acids were palmitic (C16:0), oleic (C18:1n9 *cis*), linoleic (C18:2n6 *cis*) and stearic (C18:0). These data are in agreement with the study carried out by Kulma et al. (2020), who found values for palmitic acid (C16:0) between 25.60 % and 28.34 %; oleic acid (C18:1n9 *cis*) between 27.75 % and 29.67 %; linoleic acid (C18:2n6 *cis*) between 19.91 % and 21.33 %; and stearic acid (C18:0) between 11.93 % and 12.93 %, depending on the insect's developmental stage. The high presence of alpha-linolenic acid (C18:3n3) in linseed cake (Table 1) may have been able to modulate the lipid profile of the larvae, to the extent that, for treatment 100, the predominant fatty acid was alpha-linolenic (C18:3n3) (28.66 %), which showed an increase of almost 28 times the value of the Control larvae (1.03 %). Despite the value of linoleic acid (C18:2n6) decreasing from 24.22 % in the control larvae to 16.62 % in the 100 treatment, the ∑ PUFA increased from 25.25 % in the Control to 45.28 % (100). Similar results were verified by Lawal et al. (2021) when using varying proportions of linseed in the substrate of *Tenebrio molitor*. They found that the content of alpha-linolenic acid (C18:3n3) increased from 0.36 % in the Control larvae to 6.40 % in the larvae fed 20 % linseed as a substitute for wheat bran.

The increase in ∑ PUFA was mainly at the expense of the ∑ SFA, whose value was significantly reduced from 41.71 % in the Control to 24.92 % in treatment 100. A more significant reduction was seen in palmitic acid, from 30.56 % to 17.03 %, which may be again associated with the type of substrate offered. Linseed cake had little SFA (14.43 % - Table 1) and only 9.53 % palmitic acid. Even so, the larvae still had levels of SFA and palmitic acid, which may be justified by the fact that fatty acids of 12 to 18 carbons can be biosynthesized by some insect species to fulfill important functions in their organisms (Hoc et al., 2020; Lawal et al., 2021).

Polyunsaturated fatty acids play an important role in regulating inflammatory processes in the body, where omega-3, in general terms, can play an anti-inflammatory role and omega-6, a pro-inflammatory role. Omega-3 fats play a crucial role in building cell membranes and influence the function of cell receptors within these membranes. They also serve as precursors for hormones related to blood clotting, arterial contraction and relaxation, and inflammation. Therefore, their consumption may provide protective effects against various conditions, including autoimmune diseases, heart disease, stroke, and cancer (Patted et al., 2024). On the other hand, excessive consumption of omega-6 can cause a physiological imbalance and generate a series of adverse effects, including a chronic inflammatory state and, in the long term, neoplastic processes (Orkusz, 2021). In this way, it can be considered that the shift in omega fatty acid profile of *Zophobas atratus* larvae is nutritionally significant as it aligns with recommended dietary patterns for human health.

The increase in alpha-linolenic acid (C18:3n3) resulted in the modification of the omega-6/omega-3 ratio in this study, which ranged from 23.61 in the Control larva to 0.58 in the 100 larvae. In general, insects of the Coleoptera order have a high omega-6/omega-3 ratio. As an example, *Tenebrio molitor* larvae studied by Dreassi et al. (2017) had a ratio between 21.55 and 34.27, well above the values for beef tenderloin (2.67) (Orkusz, 2021) and the values recommended for human consumption in the literature (not higher than 5) (Kulma et al., 2020; Mukhametov et al., 2022). Current Western diets typically exhibit an

Table 5
Fatty acid profile of *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Fatty acids (%)	Treatments				
	0 (Control)	25	50	75	100
Saturated					
C8:0	$0.75^{a} \pm 0.09$	$0.88^{a} \pm 0.16$	$0.66^{a} \pm 0.04$	$0.84^{a} \pm 0.02$	$0.79^{a} \pm 0.09$
C10:0	$0.11^{a*} \pm 0.01$	$0.12^{a*} \pm 0.01$	$0.09^{a*} \pm 0.00$	$0.09^{a*} \pm 0.00$	$0.09^{a*} \pm 0.00$
C12:0	$0.07^{ab*} \pm 0.02$	$0.34^{a*} \pm 0.00$	$0.06^{ab*} \pm 0.00$	$0.05^{ab*} \pm 0.00$	$0.03^{b*} \pm 0.00$
C14:0	$1.14^{b} \pm 0.03$	$1.28^{a} \pm 0.01$	$1.04^{c} \pm 0.00$	$0.95^{d} \pm 0.04$	$0.73^{e} \pm 0.04$
C15:0	$0.23^{c} \pm 0.00$	$0.27^{a} \pm 0.00$	$0.25^{b} \pm 0.00$	$0.21^{d} \pm 0.00$	$0.17^{e} \pm 0.01$
C16:0	$30.56^{a} \pm 0.29$	$27.19^{b} \pm 0.08$	$24.89^{c} \pm 0.33$	$22.20^{d} \pm 0.09$	$17.03^{e} \pm 0.17$
C17:0	$0.45^{b*} \pm 0.01$	$0.52^{ab*} \pm 0.01$	$0.79^{a*} \pm 0.02$	$0.48^{ab*} \pm 0.00$	$0.58^{ab*} \pm 0.15$
C18:0	$8.39^{a*} \pm 0.21$	$6.67^{ab*} \pm 0.04$	$6.08^{ab*} \pm 0.23$	$5.83^{ab*} \pm 0.12$	$5.51^{b*} \pm 0.26$
Monounsaturated					
C16:1	$0.85^{a*} \pm 0.01$	$0.83^{ab*} \pm 0.00$	$0.79^{ab*} \pm 0.02$	$0.69^{ab*} \pm 0.00$	$0.63^{b*} \pm 0.01$
C17:1	$0.68^{ab*} \pm 0.03$	$0.70^{a*} \pm 0.00$	$0.51^{ab*} \pm 0.01$	$0.50^{ab*} \pm 0.00$	$0.38^{b*} \pm 0.01$
C18:1n9 *cis*	$29.99^{a*} \pm 0.22$	$27.98^{ab*} \pm 0.12$	$27.59^{ab*} \pm 0.17$	$27.36^{ab*} \pm 0.15$	$27.04^{b*} \pm 0.15$
Polyunsaturated					
C18:2n6 *cis*	$24.22^{a} \pm 0.33$	$22.11^{b} \pm 0.26$	$21.14^{c} \pm 0.16$	$18.86^{d} \pm 0.03$	$16.62^{e} \pm 0.24$
C18:3n3	$1.03^{e} \pm 0.09$	$9.77^{d} \pm 0.15$	$14.43^{c} \pm 0.33$	$19.67^{b} \pm 0.07$	$28.66^{a} \pm 0.45$
Other Fatty Acids	$1.50^{ab} \pm 0.27$	$1.34^{b} \pm 0.23$	$1.82^{ab} \pm 0.14$	$2.26^{a} \pm 0.51$	$1.75^{ab} \pm 0.15$
∑ SFA	$41.71^{a} \pm 0.45$	$37.26^{b} \pm 0.31$	$33.58^{c} \pm 0.51$	$30.65^{d} \pm 0.27$	$24.92^{e} \pm 0.60$
∑ MUFA	$31.52^{a*} \pm 0.18$	$29.52^{ab*} \pm 0.13$	$29.03^{ab*} \pm 0.13$	$28.56^{ab*} \pm 0.15$	$28.05^{b*} \pm 0.15$
∑ PUFA	$25.25^{e} \pm 0.27$	$31.88^{d} \pm 0.41$	$35.57^{c} \pm 0.49$	$38.53^{b} \pm 0.10$	$45.28^{a} \pm 0.67$
omega-6/omega-3	$23.61^{a*} \pm 2.11$	$2.26^{b*} \pm 0.01$	$1.47^{b*} \pm 0.02$	$0.96^{b*} \pm 0.00$	$0.58^{b*} \pm 0.01$

Results presented as mean ± standard deviation of the relative percentages of the fatty acids, except for omega-6/omega-3 (presented as % of omega-6 / 1 % of omega-3). Different letters on the same line indicate a statistical difference between treatments ($p \leq 0.05$) according to the Tukey test, except when the line shows.
* , which indicates the Mann-Whitney U test. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake. ∑ PUFA: sum of polyunsaturated fatty acids; ∑ MUFA: sum of monounsaturated fatty acids; ∑ SFA: sum of saturated fatty acids.

omega-6/omega-3 ratio of 15:1 to 20:1, substantially higher than the recommended ratio of 1:1 to 4:1 associated with reduced risk of cardiovascular disease, improved inflammatory responses, and better neurological function (Simopoulos, 2008). The present study, however, was able to verify that modifications to the substrate of *Zophobas atratus* larvae can alter the omega-6/omega-3 ratio to values closer to the human consumption recommendation.

The European Food Safety Authority (EFSA, 2010) proposes an adequate intake of alpha-linolenic acid (C18:3n3) of 0.5 % of daily energy intake (or approximately 1.1 g for a 2000 kcal diet). The consumption of 100 g of *Zophobas atratus* larvae in this study can provide 0.34 g (Control), 3.12 g (25), 4.68 g (50), 6.09 g (75) or 8.80 g (100) of C18:3n3 (dry basis), depending on the substrate offered, thus, helping to reach the recommended daily intake of omega-3 for humans. Considering the consumption of oil extracted from larvae, the values can reach 0.97 g/100 g (Control), 9.33 g/100 g (25), 13.84 g/100 g (50), 18.80 g/100 g (75) and 27.39 g/100 g (100), demonstrating the richness in alpha-linolenic fatty acid (C18:3n3) in the larvae fed linseed cake and making it possible to use the insect in its whole form or the extracted oil as a source of this polyunsaturated fatty acid. As an example, Tzompa-Sosa et al. (2019) extracted oil from *Tenebrio molitor,* which was liquid at room temperature and had compounds related to pleasant aromas, suggesting that it could be used as a table oil as an alternative to olive oil and as a food ingredient in various preparations, such as cakes, pies and cookies.

While precise global statistics on insect consumption as a source of omega fatty acids are limited, entomophagy is practiced regularly by approximately 2 billion people, primarily in Asia, Africa, and Latin America, where insects contribute significantly to traditional diets and food security (van Huis et al., 2013). However, it's important to note that insects are rarely consumed specifically as a source of omega fatty acids; rather, they serve as a comprehensive nutrient package providing protein, fats, minerals, and vitamins (van Huis et al., 2013). Therefore, there is a potential to enhance these naturally nutritious food sources to better align with modern nutritional requirements, particularly in regions where entomophagy is culturally accepted and economically important.

Table 6
Lipid quality indices of *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Lipid Quality Parameters	Treatments				
	Control	25	50	75	100
UI	83.05^{e} ± 0.65	103.03^{d} ± 0.84	114.46^{c} ± 1.49	125.27^{b} ± 0.01	147.27^{a} ± 1.98
PI	27.02^{e} ± 0.15	42.34^{d} ± 0.55	50.68^{c} ± 0.82	58.88^{b} ± 0.10	74.61^{a} ± 1.14
COX	3.01^{e} ± 0.01	4.56^{d} ± 0.05	5.57^{c} ± 0.08	6.46^{b} ± 0.01	8.17^{a} ± 0.12
H/H	1.73^{e} ± 0.01	2.07^{d} ± 0.01	2.43^{c} ± 0.04	2.84^{b} ± 0.02	4.06^{a} ± 0.01
HPI	1.61^{e} ± 0.01	1.88^{d} ± 0.01	2.22^{c} ± 0.03	2.57^{b} ± 0.03	3.68^{a} ± 0.02
TI	1.29^{a}* ± 0.02	0.63^{b}* ± 0.01	0.46^{c}* ± 0.02	0.35^{d}* ± 0.01	0.21^{e}* ± 0.01
AI	0.62^{a} ± 0.01	0.53^{b} ± 0.01	0.45^{c} ± 0.01	0.39^{d} ± 0.01	0.27^{e} ± 0.01

Results presented as mean ± standard deviation. Different letters in the same row indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test, except when the column shows *, which indicates the Mann-Whitney U test. H/H: Hypocholesterolemic/hypercholesterolemic ratio; HPI: Health promotion index; UI: Unsaturation index; COX: Oxidizability index; PI: Peroxidizability index; AI: Atherogenicity index; TI: Thrombogenicity index. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake.

3.5. Lipid quality parameters

The AI and TI indices (Table 6) are used to assess the risks of consuming a particular source of lipids on the development of atherosclerosis or thrombosis (Orkusz, 2021). Mlček et al. (2019) found an AI of 0.70 and a TI of 1.40 for *Zophobas atratus* larvae fed wheat bran, close to the Control larvae in this study (0.62 and 1.29, respectively), and Orkusz's (2021) findings for sirloin (0.81 and 1.27, respectively). However, due to the modification of the fatty acid profile in the *Zophobas atratus larvae* in this study, it was possible to see a significant reduction in both parameters to much lower values, of up to 0.27 for AI and 0.21 for TI, indicating greater safety in the consumption of this insect regarding arterial and thrombogenic risks, even when compared to traditional foods.

The Hypocholesterolemic/Hypercholesterolemic ratio (H/H) is associated with the ability of a given fatty acid source to influence lipoprotein metabolism and evaluates the quality of the lipid profile based on the effect of different fatty acids as cholesterol-lowering or with the risk of developing cardiovascular diseases. Therefore, higher values are of greater interest, as they indicate a higher proportion of lipid-lowering fatty acids and a lower risk of developing cardiovascular diseases (Santos-Silva et al., 2002; Paszczyk and Łuczyńska, 2020). Paszczyk and Łuczyńska (2020) found considerably lower values than the present study (between 1.73 - Control and 4.06 - 100) for cow (0.55), sheep (0.55) and goat (0.52) cheeses. Hanula et al. (2022) found an H/H of 1.30 for beef burgers. Da Silva et al. (2024) found that *Zophobas atratus* larvae fed wheat bran and brewery grains showed values (1.74) close to those found for the Control in the present study (1.73), but lower compared to the other treatments. In this way, the values in this study are higher than other animal sources, reflecting greater safety in their consumption regarding the development of cardiovascular diseases, again due to their higher content in C18:3 and lower proportion of saturated fatty acids.

The Health Promotion Index (HPI) is an index used to assess the overall health benefits of the fatty acids present in a food source and is associated with the safety of its consumption in terms of the risk of atherogenesis (Chen and Liu, 2020). The higher its value, the greater its benefit for protecting cardiovascular health. Fadıloğlu et al. (2023) found a value of 1.20 for the HPI of beef burgers and Alabisoet al. (2021) of 1.83 for beef salami. Kotsou et al. (2023) found values of 2.82, 2.57, and 2.49 for *Tenebrio molitor* larvae fed wheat bran and spent coffee beans, where the HPI decreased as the coffee bean content in the substrate increased. For the present study, the results of the Control (1.61), 25 (1.88) and 50 (2.22) treatments were above those found for beef hamburger (Fadıloğlu et al., 2023), close to beef salami (Alabisoet al., 2021) and below those found for *Tenebrio molitor* (Kotsou et al., 2023). In the present study, the *Zophobas atratus* larvae fed with higher linseed contents (75 and 100) showed substantially higher values (2.57 and 3.68 respectively) than those found by Fadıloğlu et al. (2023) and Alabiso et al. (2021). This indicates that, in general, the larvae of insects from the Tenebrionidae family may have HPI with values of great nutritional interest.

The UI is an index that assesses the degree of unsaturation of the lipids present in a food source. In its formula, fatty acids with a higher number of double chains have a greater impact on its result, but it still considers fatty acids with a low degree of unsaturation. Ghassemi-Golezani and Farhangi-Abriz (2018) observed a maximum value of 155 for the UI of soybeans, while Realini et al. (2013) found a value of 111 in porcine *longissimus thoracis* muscle, part of the pork loin and one of the highly valued primal elements of pig carcasses (Szmańko et al., 2020). For the present study, it was found that the UI values for *Zophobas atratus* larvae Control (83.05), 25 (103.03) and 50 (114.46) were close to the result found for pigs, but lower than soybeans. On the other hand, as the linseed content in the substrate increased, the larvae showed an increase in UI value, mainly due to the accumulation of omega-3. Thus, treatment 100 showed the highest value (147.27), close to that found for soybeans

and above that found for porcine *longissimus thoracis*. This corroborates the positive modification of the UI of *Zophobas atratus* larvae fed with rearing substrates rich in PUFA.

The Oxidizability Index (Cox) analyzes the effect of fatty acid composition on the oxidative stability of lipids, with an emphasis on unsaturated fatty acids. Thus, lower Cox values are desirable to obtain food with greater oxidative stability (Kotsou et al., 2023). The Cox value of the Control larva (3.01), higher than that of virgin palm oil (1.29 (Mba et al., 2017)), is close to that found for olive oil (approximately 2.25 (Khaleghi et al., 2023)) and lower than canola oil (4.46 (Mba et al., 2017)). On the other hand, the Cox of larva 25 (4.56) is close to that of canola oil. This would indicate oxidative stability similar to that of commonly marketed oils. Larvae 50 (5.57), 75 (6.46) and 100 (8.17) have higher values than the other three oils presented above, again meaning that the greater presence of polyunsaturated fatty acids in larvae 25, 50, 75, and 100 is a determining factor in the increased oxidative susceptibility of the lipids. Kotsou et al. (2023) found a similar trend. In their study, *Tenebrio molitor* larvae were fed varying concentrations of wheat bran and *Moringa oleifera* leaves. The Cox values of the larvae ranged from 4.76 to 5.29, where a reduction in this index was noticed as the polyunsaturated fatty acid content of the substrate decreased.

The peroxidizability index (PI) is used to assess the susceptibility of a lipid source to undergo peroxidation reactions, and it is strictly related to the degree of unsaturation of fatty acids. Usually, the higher the UI, the more susceptible fatty acids are to oxidation, therefore, the higher the PI value should be. The peroxidation process can lead to rancidity or deterioration of the food and oxidative stress in biological systems. The lower the index, the less susceptible the food is to peroxidation (Gharibzahedi and Altintas, 2023). On the other hand, Wołoszyn et al. (2020) report that higher PI values indicate a food's greater protective potential against coronary artery disease. In the present study, the PI values ranged from 27.02 (Control), 42.34 (25), 50.68 (50), 58.88 (75) and 74.61 (100). Thus, the PI increased proportionally to the polyunsaturated fatty acid content of the larvae. Zula and Desta (2021) found a PI of 38.27 for raw Nile tilapia, close to the values found for Control (27.02) and 25 (42.34) larvae. Gruffat et al. (2020) found the PI for the *longissimus thoracis* of lambs to be between 23.40 and 38.20, close to the Control (27.02) and 25 (42.34) treatments in this study. Gharibzahedi and Altintas (2023) analyzed the oil from the larvae of *Alphitobius diaperinus* (Tenebrionidae) and found variations in PI between 34.99 and 41.69, depending on the extraction method. Thus although the increase in alpha-linolenic acid (C18:3n3) in larvae fed linseed cake is potentially beneficial from a nutritional point of view, some undesirable effects may also become more present, such as greater susceptibility to oxidation and peroxidation. It is important to note that the oxidative stability of an oil can be modulated through the incorporation of antioxidant agents such as vitamin E, plant extracts and the formation of blends with oils with a lower content of polyunsaturated fatty acids (Fadda et al., 2022).

3.6. Fibers

The crude fibers found in insects are in their exoskeleton in the form of chitin. For the present study, the results ranged from 7.70 % in the Control larva to 14.25 % in larva 75. Dragojlović et al. (2022) found values between 5.73 % and 9.12 % for *Zophobas atratus larvae* fed various substrates containing cabbage, carrots and linseed. This may strengthen the idea that chitin production in larvae is dependent on the type of substrate consumed. In addition, it has been found that oligosaccharides derived from the breakdown of chitin can promote beneficial effects in rats in the control of glucose metabolic disorders and in the suppression of regulators of lipogenesis, gluconeogenesis, adipocyte differentiation and inflammation in adipose tissues (Zheng et al., 2018).

Table 7
Metal composition in *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Metals (mg/100 g)	Treatments				
	Control	25	50	75	100
Potassium	526.14^{c} ± 9.27	531.85^{c} ± 7.83	552.64^{b} ± 9.90	533.94^{bc} ± 15.33	594.90^{a} ± 1.25
Phosphorus	527.67^{b} ± 13.78	530.38^{ab} ± 19.10	542.90^{ab} ± 23.60	561.35^{ab} ± 16.39	589.75^{a} ± 33.55
Magnesium	123.00^{ab} ± 3.00	118.82^{bc} ± 0.37	117.29 ^{cd} ± 0.36	112.95^{d} ± 1.95	124.84^{a} ± 0.29
Sodium	65.99^{d} ± 0.96	68.32^{c} ± 0.10	73.99^{b} ± 1.01	69.81^{c} ± 0.07	78.28^{a} ± 1.24
Calcium	39.81^{a} ± 0.60	36.30^{ab} ± 2.45	34.17^{c} ± 1.12	34.41^{c} ± 3.27	39.10^{ab} ± 0.32
Zinc	7.91^{c} ± 0.19	8.21^{bc} ± 0.12	8.78^{ab} ± 0.03	7.89^{c} ± 0.44	8.84^{a} ± 0.19
Iron	2.60^{a}* ± 0.69	1.32^{b}* ± 0.03	1.70^{ab}* ± 0.56	1.24^{b}* ± 0.20	1.80^{a}* ± 0.36
Manganese	1.21^{a}* ± 0.01	1.20^{a}* ± 0.00	1.21^{a}* ± 0.01	1.06^{b}* ± 0.04	1.15^{ab}* ± 0.05
Copper	0.92^{ab} ± 0.01	0.91^{b} ± 0.01	0.98^{ab} ± 0.01	0.90^{b} ± 0.07	1.01^{a} ± 0.01
Nickel	< 0025**	< 0025**	0.05^{a}* ± 0.01	0.03^{a}* ± 0.01	0.05^{a}* ± 0.01
Arsenic	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**
Cadmium	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**
Cobalt	< 0025**	< 0025**	< 0025**	< 0025**	< 0025**
Selenium	< 0025**	< 0025**	< 0025**	< 0025**	< 0025**

Results presented as mean ± standard deviation of each mineral mass (mg/100 g of sample) in dry matter. Different letters on the same line indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test, except when the line shows.
* , which indicates the Mann-Whitney U test.
** indicates results below the detection limit. Treatments: 0 (Control) = 70 % poultry growth feed + 30 % wheat bran; 25 = 75 % Control + 25 % linseed cake; 50 = 50 % Control + 50 % linseed cake; 75 = 25 % Control + 75 % linseed cake; 100 = 100 % linseed cake.

3.7. Ash and metal profile

In this study, changes in the substrates of *Zophobas atratus* larvae were not able to significantly alter the ash content, which was between 2.50 % and 3.11 %. The values were close to those described by Kuntadi et al. (2018), of 3.41 % for larvae of the same species. Insects contain a diversity of micronutrients, which play important roles in maintaining their biological processes. According to van Huis et al. (2013), iron, zinc, copper, calcium, potassium and magnesium can be considered the main minerals present in insects. In this study, (Table 7) from the control to treatment 100, the level of potassium ranged from 526.14 mg/100 g to 594.90 mg/100 g, phosphorus from 527.67 mg/100 g to 589.75 mg/100 g, sodium from 65.99 mg/100 g to 78.28 mg/100 g, and zinc from 7.91 mg/100 g to 8.84 mg/100 g. Magnesium, calcium, copper, iron, and manganese also varied between treatments. The magnesium content showed higher values ($p < 0.05$) for treatment 100 (124.84 mg/100 g) and Control (123.00 mg/100 g) and lower results for treatment 75 (112.95 mg/100 g). Calcium varied between 34.17 mg/100 g (50) and 39.81 mg/100 g (Control). Copper was present in all treatments and ranged from 0.90 mg/100 g (75) to 1.01 mg/100 g (100). Iron ranged from 1.24 mg/100 g in the 75 treatment to 2.60 mg/100 g in the Control. Manganese ranged from 1.06 mg/100 g (75) to 1.21 mg/100 g in the Control and 50 treatments. On the other hand, arsenic, cadmium and selenium were not found in any of the treatments and nickel was only found in treatments 50 (0.05 mg/100 g), 75 (0.03 mg/100 g) and 100 (0.05 mg/100 g). Similar results were found by Nascimento et al. (2022), with the exception that sodium, iron and potassium were more present in *Zophobas atratus* larvae fed different concentrations of grape residue.

Considering the reference values for daily mineral intake (INSTITUTE OF MEDICINE, 1997, 2001, 2004) (700 mg/day of phosphorus; 400 mg/day of magnesium; 11 mg/day of zinc; 8mg/day of iron and 2.3 mg/day of manganese for a young adult male between 19 and 30 years old); and the food labeling rules defined by FAO/WHO (2007), the Z. *atratus* larvae studied can be considered high in phosphorus (>210 mg/100 g), magnesium (>120 mg/100 g for the Control and 100 larvae), zinc (>3.3 mg/100 g), iron (>2.4 mg/100 g for the Control larva) and manganese (>0.69 mg/100 g); and a source of magnesium (>60 mg/100 g for larvae 25, 50 and 75) and iron (>1.2 mg/100 g for larvae 25, 50, 75 and 100).

Considering the metal contents of the substrates (Table 1), and the correlations between larval and substrate metals (Supplementary Table 2), it is possible to verify the correlation only for phosphorus (significant correlation of 0.55, $p < 0.05$). According to Oonincx and Finke (2021), because they don't have mineralized exoskeleton, mealworms aren't able to bioaccumulate minerals such as copper, zinc, lead, or cadmium. These elements can be present in greater quantities in the larvae due to their accumulation in their guts and not necessarily due to their absorption by the insects. This may explain the lack of correlation between magnesium, iron, manganese, sodium, and calcium levels in the substrates and the larvae in this study and may indicate the need for a longer fasting period to allow the complete emptying of the larval digestive tract more completely, resulting in a more reliable metal quantification.

4. Conclusion

The production and consumption of insects have become more popular in recent years. Thus, the use of an agro-industrial by-product, such as linseed cake, as a substitute for conventional substrates in their rearing is a way of reducing costs, as well as promoting more sustainable rearing, contributing to the circular economy.

The results of this study suggest that the rearing of *Zophobas atratus* larvae could overall benefit from the use of linseed cake as a rearing substrate. The larval rearing indicators showed that replacing conventional commercial substrate with up to 75 % linseed cake could statistically maintain feed conversion efficiency. On the other hand, total replacement would not be recommended, as rearing was less efficient.

In addition, the consumption of linseed cake by the larvae increases their protein content and modulates their fatty acid profile, with emphasis on the bioaccumulation of alpha-linolenic acid (C18:3n3) and the improvement of the omega-6/omega-3 ratio and lipid quality indicators. This could make *Zophobas atratus* nutritionally healthier for human consumption.

As a limitation, the linseed cake may have compromised the rearing efficiency of the larvae fed only on the by-product. Further studies are therefore suggested, focusing on the ideal conditions for supplementing agro-industrial by-products so that they are nutritionally equal to commercial substrates, as well as assessing the safety of human consumption of insects fed linseed cake.

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Ethics statement

The present study involved an invertebrate and no ethical approval was required.

CRediT authorship contribution statement

Pedro Paulo Lordelo Guimarães Tavares: Writing – review & editing, Methodology, Writing – original draft, Investigation, Conceptualization, Software, Formal analysis. **Matheus dos Santos Lima:** Writing – original draft, Investigation, Formal analysis. **Elba Santos da Boa Morte:** Investigation, Formal analysis. **Roberta Barreto de Andrade Bulos:** Formal analysis, Investigation. **Cláudio Vaz Di Mambro Ribeiro:** Validation, Supervision, Software, Visualization, Writing – review & editing. **Carolina Oliveira de Souza:** Visualization, Project administration, Writing – review & editing, Methodology, Resources, Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100743.

Data availability

No data was used for the research described in the article.

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