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# Effect of Temperature on the Nutritional Quality and Growth Parameters of Yellow Mealworm (*Tenebrio molitor* L.): A Preliminary Study

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**Abstract:** The nutritional quality of insects is related to many factors, including their rearing conditions. In this study, the effects of temperature on the contents of crude protein, lipids, ash, and amino acids and the body size and weight of *Tenebrio molitor* larvae were analysed. The larvae were reared with the occurrence of the first 20 pupae in a laboratory incubator at temperatures of 22, 25, and 28 °C. The results revealed that the weight (from 0.09 to 0.15 g), dry matter (DM) content (from 30.72 to 36.55 g/100 g), and fat concentration (from 22.46 to 36.01 g/100 g DM) of the larvae increased with increasing rearing temperature. In contrast, the crude protein content significantly decreased (from 64.33 to 54.41 g/100 g DM). Methionine was the limiting amino acid. The essential amino acid index ranged from 37% to 45%. Information about the effect of temperature on the growth and nutritional parameters of mealworms may contribute to the optimisation of mealworm-rearing technology.

**Keywords:** edible insect; lipid; protein; amino acid; rearing insect



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## 1. Introduction

Earth's population is predicted to increase to 9 billion, and the current food production needs are expected to double by 2050 [1]. Therefore, it is essential to identify sustainable, high-quality alternative food sources [2–4]. Moreover, there are currently criticisms of killing animals for meat, and makes sense to look for new sources of protein. Because some meat alternatives (e.g., tofu) require high levels of processing, it remains to be determined if these alternatives can be considered healthy replacements [5].

Entomophagy, which is the consumption of edible insects, is an interesting option in the present situation [2–4]. Ten years ago, the Food and Agriculture Organization of the United Nations (2013) published an extensive report explaining why this new type of food should be accepted [1], and the related Regulation (EU) 2015/2283 included selected insect species among the novel foods. Species recommended by the European Food Safety Authority include *Acheta domesticus*, *Tenebrio molitor*, *Alphitobius diaperinus*, and *Locusta migratoria* [6].

Edible insects are promising alternative sources of nutrients for human food and animal feed because of their good nutritional profiles, high digestibility [7,8], feasibility, and environmental benefits, as rearing insects for livestock purposes helps reduce negative climate impacts due to the low gas emissions and improved biodiversity [8]. Compared with conventional livestock, insects may also have other benefits, such as high reproductive rates and high feed conversion ratios [2,9,10].

Commonly consumed insect species are good sources of the main nutrients, including proteins, lipids, dietary fibre, and essential fatty and amino acids, as well as micronutrients [11], such as iron, zinc, manganese, and magnesium [4,12]. The yellow mealworm

(*Tenebrio molitor*) is the most promising insect species for mass production [13] and is widely used for human consumption and animal feed [10]. Yellow mealworm larvae contain even more protein and total fat than some traditional meats (chicken, pork, and beef) [11,14]. They contain approximately 50 g of protein, 35 g of lipids, and 6 g of fibre per 100 g of dry matter (DM) [9,11,15].

The nutritional value of insects is influenced by several determinants. It varies depending on the species, developmental stage, diet, environment (temperature, humidity, photoperiod) [16], killing, preparation, processing (frying, boiling, drying, etc.) [17], and analytical method [16]. Knowledge of the abiotic factors affecting the development and chemical composition of *T. molitor* is essential for the optimal farming of mealworms for future consumption by people or farm animals. Temperature is an important abiotic factor affecting rearing. Optimizing the temperature range can improve rearing efficiency and environmental sustainability. Increasing the temperature usually increases growth and production, but the correlation is not linear and can have adverse effects when the temperature increases to an excessive degree [10]. The optimal temperature range for mealworms, which are classified as poikilothermic insects, is 22–28 °C [13,18], whereas the growth slows down at temperatures below or above this range—specifically, temperatures of 10 and 35 °C have been described as unfavourable because the survival rate of mealworm larvae is reduced [18]. The growth, production, and metabolic rate are maximal within this temperature range [10]. Regarding the developmental speed of mealworms, Bjørge et al. [10] achieved the best results between 23 and 31 °C. Furthermore, Adámková et al. [13] found that while the protein content of *T. molitor* remained constant across different temperatures, there were notable variations in the fat content and fatty acid profiles. In contrast, Bjørge et al. [10] reported differences in protein content with a negative correlation with increasing temperature and, conversely, a positive correlation of fat content with increasing temperature.

As insects are becoming more common as novel foods, it is important to obtain information on the optimisation of their rearing conditions in relation to their nutritional value. Therefore, this study aimed to investigate the effects of temperature on the growth parameters and proximate nutritional value of artificially reared *T. molitor*. The above-mentioned growth and nutritional parameters were investigated at three temperatures of 22, 25, and 28 °C, which corresponded to the optimal temperature range for rearing *T. molitor* based on the literature.

## 2. Materials and Methods

### *2.1. Insect Samples*

Larvae of *T. molitor* were reared in an insectarium at the Czech University of Life Sciences in Prague. The insects were kept in a laboratory incubator (Steinberg Systems, Berlin, Germany) at temperatures of 22, 25, and 28 ± 0.1 °C with a constant humidity of 40–50% [19] and a photoperiod of 12:12 h. Approximately 500 adults were kept in a plastic box measuring 39 × 28 × 14 cm. The bottom was replaced with an aluminium anti-insect net. The entire box was then placed in a plastic box of the same size as the substrate, which consisted of a mixture of breadcrumbs and wheat bran (1:4), for 24 h. During this time, the adults laid eggs on the substrate using a net. The eggs were then placed together with the substrate in plastic trays with dimensions of 17 × 12 × 6 cm for each temperature. It was assumed that this would result in a constant number of equally aged larvae. The mealworms were reared in these boxes (4 boxes for temperatures of 22 and 28 °C, 5 boxes for 25 °C) until they reached their largest larval stages, that is, until 20 pupae were collected from the box.

The larvae were fed ad libitum and supplemented with fresh vegetables daily (apple, carrot, lettuce leaves), excluding weekends. Leftovers of fresh food were removed before each feeding to prevent the growth of mould on the substrate. The larvae were starved for 24 h, killed via freezing at −80 °C for 24 h, and stored in boxes in a freezer prior to the nutritional analysis.

### 2.2. Analytical Methods

The body parameters—the lengths and weights of 20 randomly selected larvae from each group—were measured. The length was measured in millimetres using a ruler scale, and the weight was determined in grams using analytical scales (AE 200; Mettler-Toledo GmbH, Greifensee, Switzerland) to four decimal places. Samples were then lyophilized via freezing at −80 °C for one hour, followed by freeze-drying at low pressure for 24 h. The lyophilized insect samples were homogenized in a laboratory mill (Retsch Grandprix 2008, Haan, Germany) and stored in a refrigerator (4 ± 1 °C) prior to analysis.

Nutrient analyses were performed in three analytical replicates for each group according to Kulma et al. [19] and the Commission Regulation (EC) No 152/2009. The total fat content was extracted by using the Soxhlet method (ISO 5983-1:2005) [20]. About 3 g of sample was weighed into the extraction thimble and extracted by using 70 mL of petroleum ether with a boiling point of 40–60 °C (Lach-Ner, Neratovice, Czech Republic). The samples were analysed using a Gerhardt Soxtherm SOX414 apparatus (C. Gerhardt GmbH and Co. KG, Königswinter, Germany). Then, the samples were dried, and the fat content was gravimetrically evaluated. The crude protein content was determined using the Kjeldahl method (ISO 1871:2009) [21]; 0.2 g of sample was used for one determination with a titanium dioxide Kjeldahl tablet (Kjeltabs CK AA17; Thomson & Capper Ltd., Cheshire, United Kingdom), 10 mL of 96% sulphuric acid (Penta, Prague, Czech Republic), and 10 mL of hydrogen peroxide (Lach-Ner, Neratovice, Czech Republic). The samples were then mineralised at 420 °C for 1 h. After cooling down, 10 mL of distilled water was added to each sample, and the analysis was conducted with a Kjeltec 2400 analyser (FOSS, Hilleroed, Denmark) using a nitrogen-to-protein conversion factor of 6.25.

The amino acid profile was based on ISO 13903:2005 [22] and was determined according to Kulma et al. [20]. Acid hydrolysis and oxidative hydrolysis for sulphur amino acids were used for sample preparation. In the acid hydrolysis, 0.4 g of sample with a few drops of ethanol (Penta, Prague, Czech Republic) and 50 mL of 6M hydrochloric acid (Lach-Ner, Neratovice, Czech Republic) were mixed. Air was removed from the sample with nitrogen (Linde, Prague, Czech Republic), and then the sample was hydrolysed in sealed glass tubes for 23 h at 110 °C. In oxidative hydrolysis, 0.4 g of sample was mixed with 5–15 mL of oxidizing mixture (1:9), 30% hydrogen peroxide, and 85% formic acid (Lach-Ner, Neratovice, Czech Republic). The sample was placed in a refrigerator for 16 h. Then, 100 mL of 6M hydrochloric acid (Lach-Ner, Neratovice, Czech Republic) was added, and the sample was placed on a 200 °C heating plate for 23 h.

After acidic or oxidative hydrolysis, the samples were filtered and evaporated on a vacuum evaporator (Laborota 4000 (Heidolph Instruments GmH & Co., Schwabach, Germany)) at 50 °C and analysed using an Amino Acid Analyzer 400 (INGOS, Prague, Czech Republic) with sodium citrate buffers, CYS-H/MET-S hydrolysate standards (INGOS, Prague, Czech Republic), and post-column ninhydrin derivatization. Tryptophan was not detected due to its decomposition during acid hydrolysis using 6M HCl. The amino acid score (AAS) and essential amino acid index (EAAI) were calculated according to the method described by Kulma et al. [20] using the following formulas:

$$\text{AAS} = \text{g of amino acid in 100 g of analysed protein} \times 100$$

$$\text{EAAI} = \sqrt[7]{\frac{\text{g of lysine in 100 g of analysed protein} \times 100}{\text{g of lysine in 100 g of reference protein}} + (\text{etc.for other 6 EAA})}$$

The DM content was determined by drying 2.5–3.0 g of a fresh sample at 103 ± 2 °C to a constant weight in an oven for an average of 4 h (Memmert, Schwabach, Germany). To determine the ash content, the same samples were mineralized in a muffle furnace LAC (Verkon, Praha, Czech Republic) at 550 °C overnight.

### 2.3. Statistical Analysis

The data were statistically evaluated with the Statistica 13.2 software (StatSoft, Inc., Tulsa, OK, USA) using one-way analysis of variance (ANOVA) with Scheffe's post hoc analyses at a significance level of $\alpha = 0.05$. Each analysis was carried out in triplicate, and the results are expressed as the arithmetical mean $(\bar{x}) \pm$ standard deviation (SD).

## 3. Results

The values of growth, body parameters, and proximate nutritional value of *T. molitor* larvae reared at different temperatures are presented in Table 1. Larvae emerged from eggs and grew faster at higher temperatures. While larvae in groups reared at 22 °C were harvested after ~108 days, the larvae at 28 °C reached their terminal stage in 47 days. Therefore, with increasing temperature, there was a significant decrease in the time interval from oviposition to larval emergence and harvesting.

**Table 1.** Growth parameters and proximate composition of *T. molitor* larvae reared at different temperatures.

| Parameter | 22 °C | 25 °C | 28 °C |
|---|---|---|---|
| Weight (g) | $0.09 \pm 0.03^{a}$ | $0.15 \pm 0.03^{b}$ | $0.15 \pm 0.04^{c}$ |
| Length (cm) | $1.99 \pm 0.40^{a}$ | $2.49 \pm 0.20^{c}$ | $2.26 \pm 0.30^{b}$ |
| Dry matter (g/100 g FW) | $30.72 \pm 1.29^{a}$ | $32.35 \pm 2.70^{b}$ | $36.55 \pm 0.79^{c}$ |
| Ash (g/100 g DM) | $3.47 \pm 0.44^{b}$ | $2.85 \pm 0.73^{a}$ | $5.65 \pm 0.26^{c}$ |
| Fat (g/100 g DM) | $22.46 \pm 3.54^{a}$ | $27.32 \pm 4.62^{b}$ | $36.01 \pm 3.31^{c}$ |
| Crude protein (g/100 g DM) | $64.34 \pm 2.98^{c}$ | $56.91 \pm 6.29^{b}$ | $54.41 \pm 2.47^{a}$ |
| First emergence of larvae (days) | $10.00 \pm 2.87^{a}$ | $7.50 \pm 5.54^{ab}$ | $5.78 \pm 1.69^{b}$ |
| Terminal larval stage (days) | $107.66 \pm 12.10^{a}$ | $84.41 \pm 13.18^{b}$ | $47.43 \pm 6.61^{c}$ |

DM = dry matter, FW = fresh weight. Superscripts in the same row represent a significant difference ($p \leq 0.05$).

The body weight of the larvae reached its maximum at 28 °C (increased by 67%) (Figure 1); however, the larval body length (Figure 2) was the highest at 25 °C. Statistical evaluation ($p < 0.05$) showed that there was a statistically significant difference in body weight between 22 and 25 °C and between 22 and 28 °C and in the body length at all rearing temperatures of *T. molitor* larvae.

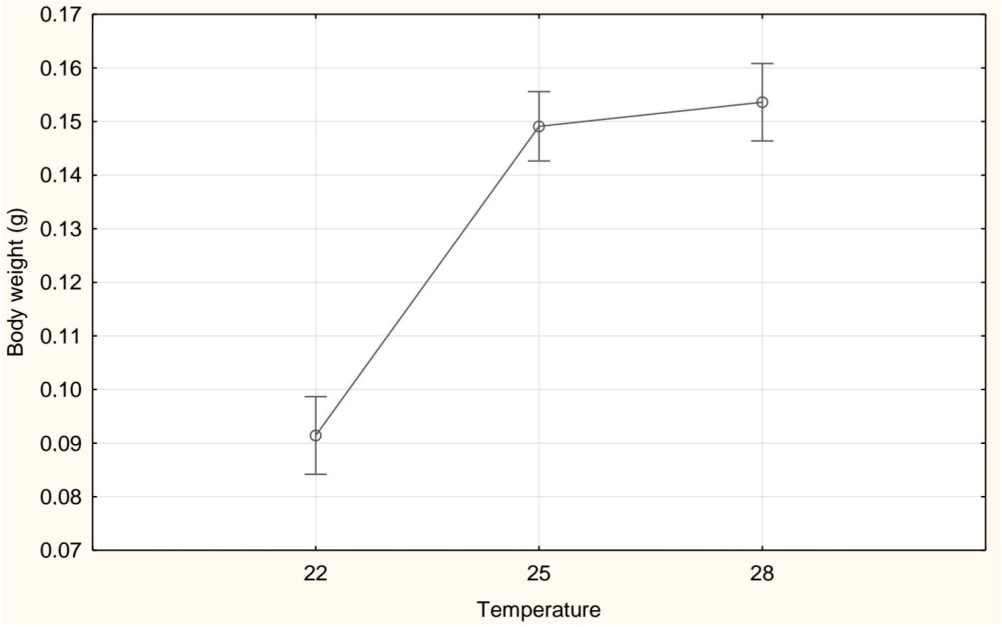


**Figure 1.** The effect of rearing temperature on the body weight of yellow mealworm larvae.

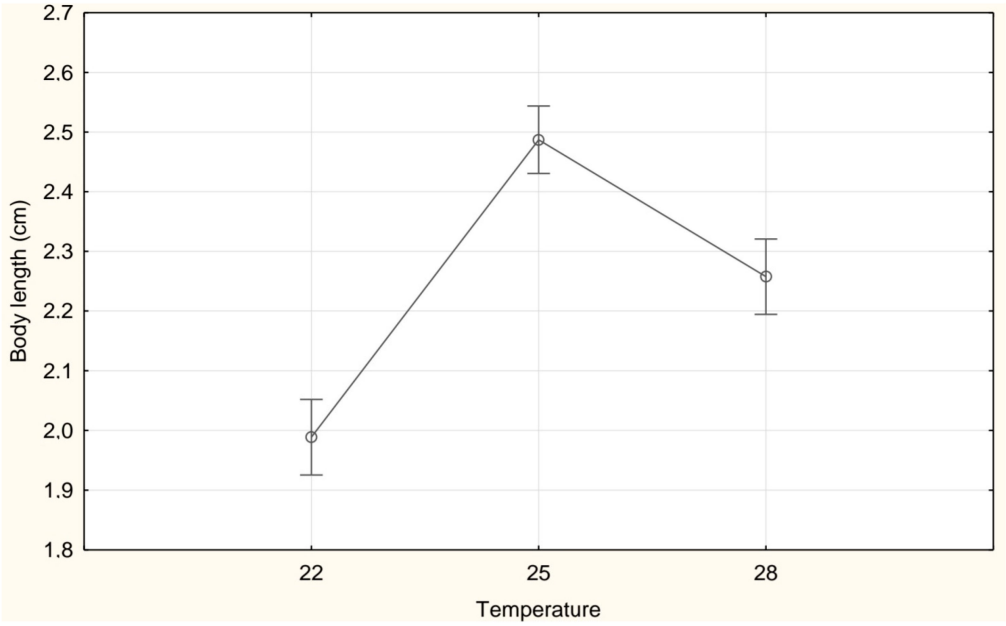


**Figure 2.** The effect of rearing temperature on the body length of yellow mealworm larvae.

The highest contents of dry matter and ash were measured for the mealworms reared at 28 °C, whereas the lowest values were obtained in those reared at 22 °C in the case of DM (Figure 3) and at 25 °C in the case of the ash content (Figure 4). The dry matter content increased by 19% based on the comparison of samples reared at the lowest and highest temperatures. There was a statistically significant difference ($p < 0.05$) in dry matter between the temperatures of 22 and 28 °C and between the temperatures of 25 and 28 °C (Figure 3). In the case of ash, there was a significant difference in the values at all rearing temperatures (Figure 4).

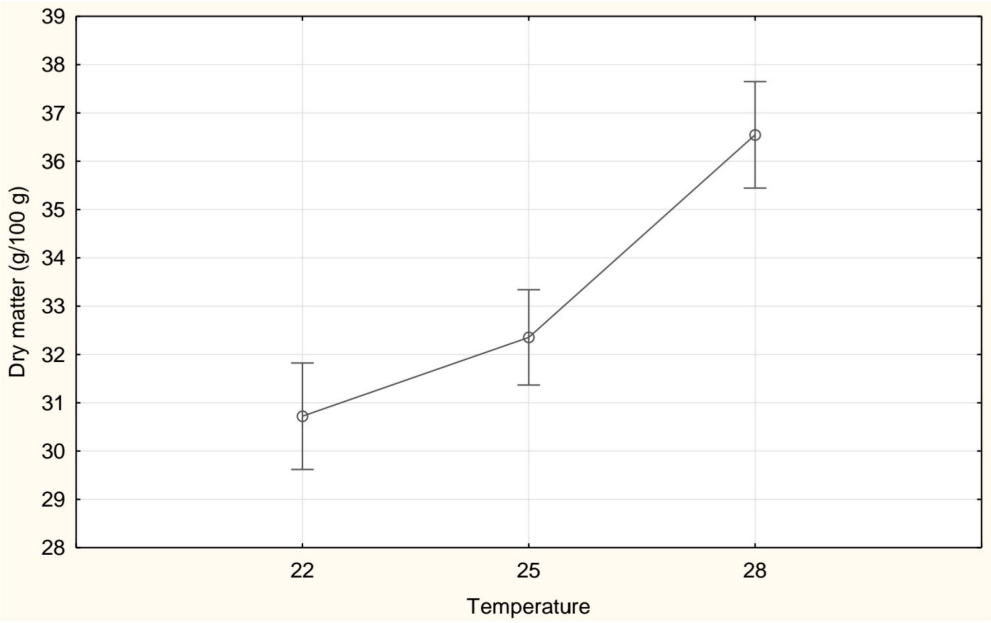


**Figure 3.** The effect of rearing temperature on the dry matter content of yellow mealworm larvae.

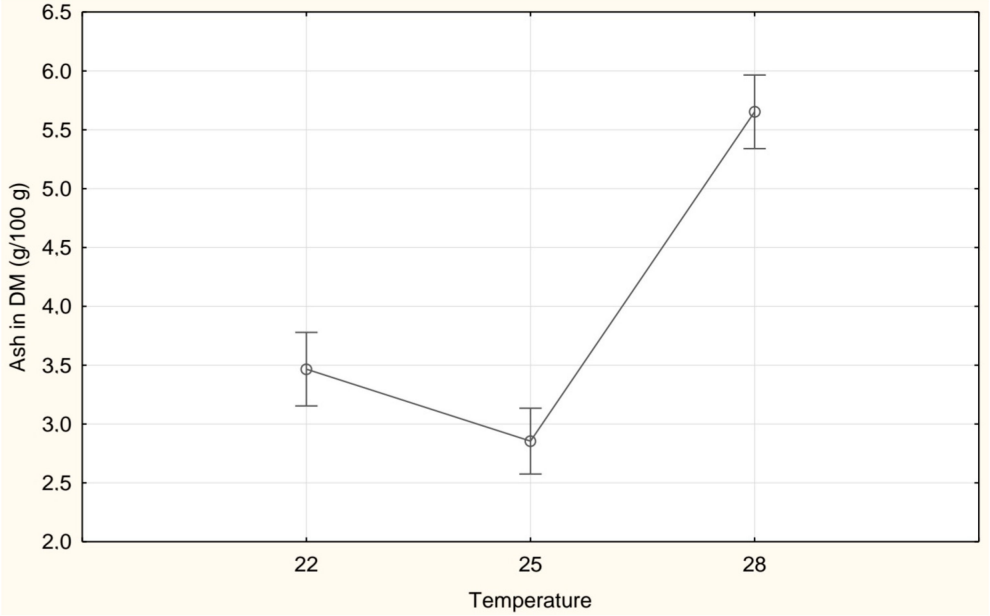


**Figure 4.** The effect of rearing temperature on the ash content of yellow mealworm larvae.

Concerning the main nutrients, the fat content increased with increasing temperature (Figure 5). The difference between the minimum and maximum average values was 9.49 g/100 g fresh weight, and there was a significant difference in fat content per unit DM ($p < 0.05$) at all rearing temperatures. A positive correlation was observed between the rearing temperature and fat content (R = 0.8413). On the other hand, the most appropriate rearing temperature for obtaining a high protein content was determined to be 22 °C (Figure 6). A negative correlation was observed between the crude protein content per unit DM and temperature (R = −0.6577). The protein content of the samples reared at 28 °C decreased by 15% compared with that of larvae reared at the lowest temperature. There was a statistically significant difference ($p < 0.05$) in dry matter between the temperatures of 22 and 25 °C and between the temperatures of 22 and 28 °C.

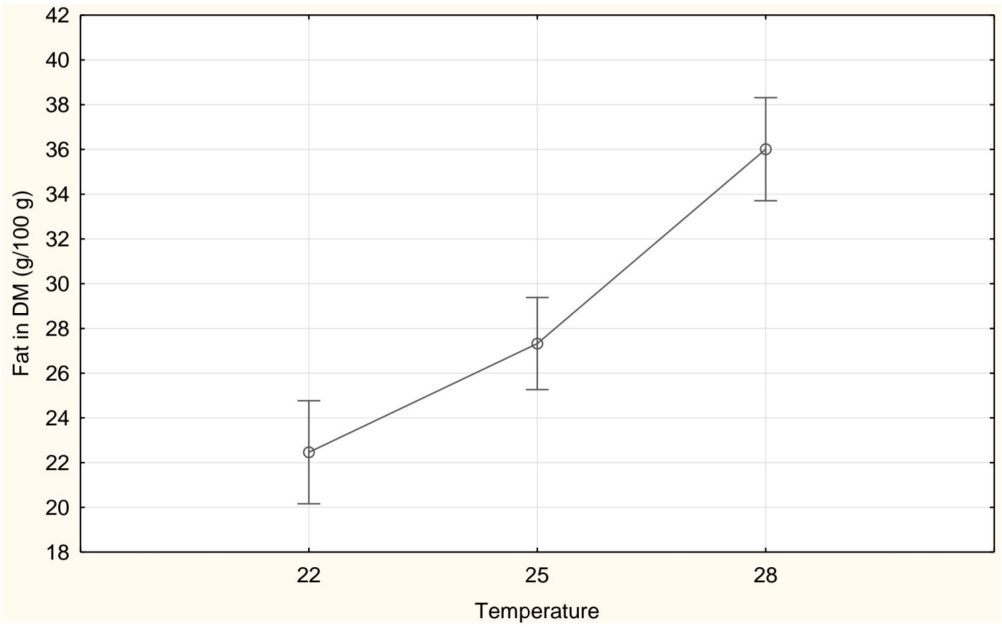


**Figure 5.** The effect of rearing temperature on the fat content of yellow mealworm larvae.

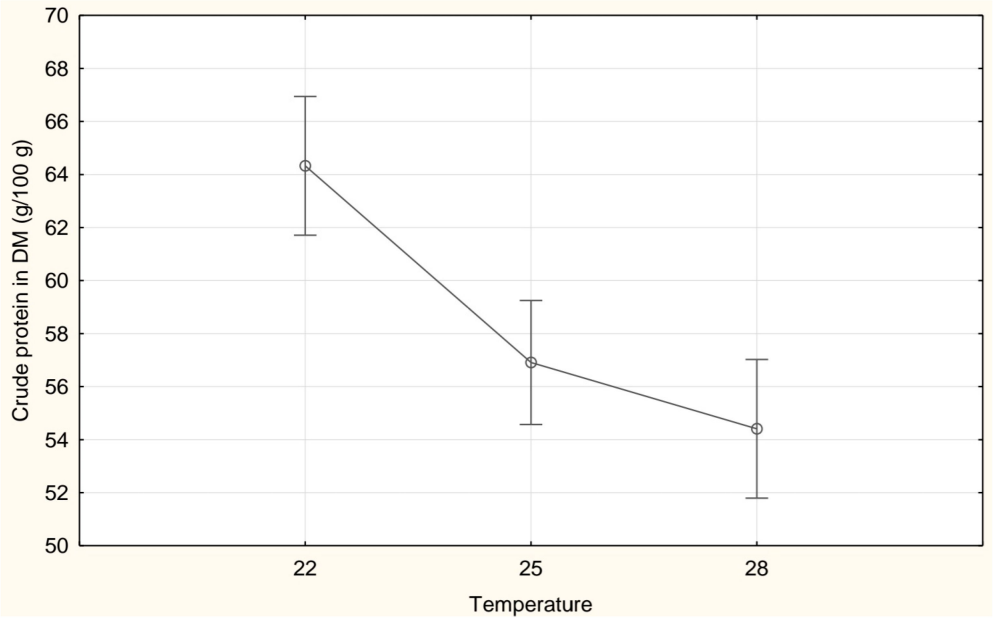


**Figure 6.** The effect of rearing temperature on the crude protein content of yellow mealworm larvae.

Cluster analysis was performed on the nutritional parameters (dry matter, ash, fat, and crude protein content) (Figure 7). The results showed that the mealworms reared at 28 °C were more different from the other two samples that were reared at lower temperatures. The differences were mainly in the higher contents of DM, ash, and fat in the larvae reared at 28 °C compared to those of larvae reared at lower temperatures.

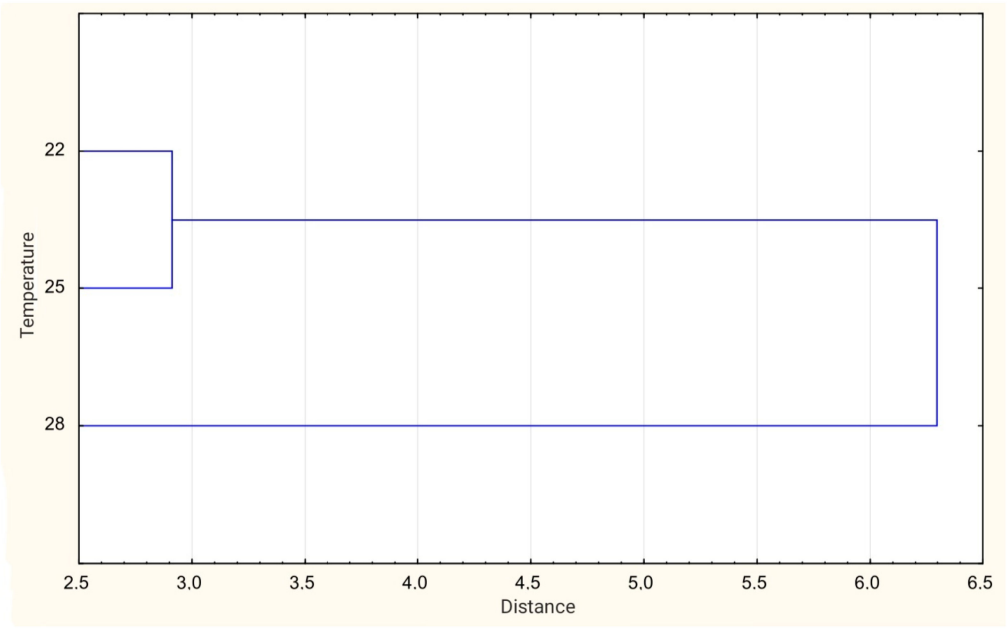


**Figure 7.** Cluster analysis based on the DM, ash, fat, and crude protein contents per unit fresh weight.

Because insects are considered to be an alternative source of proteins, the protein quality was evaluated by analysing their amino acid content (Table 2). The most abundant amino acids in *T. molitor* larvae reared at 22 °C were alanine and proline at 25 and 28 °C, followed by glutamic and aspartic acids. In contrast, the least abundant amino acids were the sulphur amino acids cysteine and methionine, followed by histidine. No statistically significant differences were found in aspartic acid, threonine, glycine, valine, leucine, phenylalanine, histidine, and methionine according to the temperature. There were signifi-

cant differences in serine, glutamic acid, proline, isoleucine, tyrosine, and arginine between samples reared at the temperatures of 25 and 28 °C, in alanine in the samples reared at 22 and 28 °C and 25 and 28 °C, and in lysine among the samples reared at all temperatures.

**Table 2.** Amino acid content in g per 100 g DM of *T. molitor* larvae reared at 22, 25, and 28 °C.

| Amino Acid | 22 °C | 25 °C | 28 °C |
|---|---|---|---|
| Aspartic acid | 3.395 ± 0.142 $^{a}$ | 3.375 ± 0.111 $^{a}$ | 3.030 ± 0.180 $^{a}$ |
| Threonine | 1.962 ± 0.073 $^{a}$ | 2.128 ± 0.229 $^{a}$ | 1.907 ± 0.025 $^{a}$ |
| Serine | 2.208 ± 0.106 $^{ab}$ | 2.425 ± 0.250 $^{a}$ | 1.940 ± 0.041 $^{b}$ |
| Glutamic acid | 3.332 ± 0.370 $^{ab}$ | 3.538 ± 0.311 $^{a}$ | 2.647 ± 0.090 $^{b}$ |
| Proline | 3.343 ± 0.031 $^{ab}$ | 3.747 ± 0.308 $^{a}$ | 3.040 ± 0.064 $^{b}$ |
| Glycine | 2.990 ± 0.062 $^{a}$ | 2.693 ± 0.263 $^{a}$ | 2.617 ± 0.050 $^{a}$ |
| Alanine | 3.568 ± 0.056 $^{a}$ | 3.638 ± 0.213 $^{a}$ | 2.523 ± 0.058 $^{b}$ |
| Valine | 2.388 ± 0.035 $^{a}$ | 2.415 ± 0.212 $^{a}$ | 2.417 ± 0.176 $^{a}$ |
| Isoleucine | 1.538 ± 0.040 $^{ab}$ | 1.790 ± 0.193 $^{a}$ | 1.453 ± 0.063 $^{b}$ |
| Leucine | 2.000 ± 0.050 $^{a}$ | 2.090 ± 0.229 $^{a}$ | 1.820 ± 0.093 $^{a}$ |
| Tyrosine | 1.768 ± 0.097 $^{ab}$ | 1.845 ± 0.100 $^{a}$ | 1.583 ± 0.061 $^{b}$ |
| Phenylalanine | 1.143 ± 0.078 $^{a}$ | 1.075 ± 0.120 $^{a}$ | 1.107 ± 0.041 $^{a}$ |
| Histidine | 1.055 ± 0.034 $^{a}$ | 1.153 ± 0.164 $^{a}$ | 0.873 ± 0.062 $^{a}$ |
| Lysine | 1.635 ± 0.030 $^{a}$ | 1.810 ± 0.068 $^{b}$ | 1.177 ± 0.033 $^{c}$ |
| Arginine | 1.668 ± 0.049 $^{ab}$ | 1.757 ± 0.197 $^{a}$ | 1.330 ± 0.022 $^{b}$ |
| Cysteine | 0.123 ± 0.004 $^{a}$ | 0.248 ± 0.068 $^{b}$ | 0.197 ± 0.037 $^{ab}$ |
| Methionine | 0.445 ± 0.018 $^{a}$ | 0.558 ± 0.080 $^{a}$ | 0.470 ± 0.051 $^{a}$ |

Superscripts on the same row represent a significant difference ($p \leq 0.05$).

The calculated amino acid score (AAS) and essential amino acid index (EAAI) values are listed in Table 3. Based on these results, methionine was the limiting amino acid for *T. molitor* larvae at all rearing temperatures. The EAAI based on the AAS was affected by the rearing temperature. The highest value was calculated to be 46 for *T. molitor* reared at 25 °C, and there was a statistically significant difference between 22 and 25 °C.

**Table 3.** Amino acid score (AAS) and essential amino acid index (EAAI).

| Essential Amino Acid | 22 °C | 25 °C | 28 °C |
|---|---|---|---|
| Threonine | 59.81 ± 2.22 $^{a}$ | 73.30 ± 7.89 $^{b}$ | 68.71 ± 0.90 $^{c}$ |
| Valine | 50.83 ± 0.74 $^{ab}$ | 58.13 ± 5.11 $^{a}$ | 60.84 ± 4.43 $^{b}$ |
| Isoleucine | 36.21 ± 0.95 $^{a}$ | 47.66 ± 5.15 $^{b}$ | 40.47 ± 1.77 $^{c}$ |
| Leucine | 35.32 ± 0.88 $^{ab}$ | 41.73 ± 4.58 $^{a}$ | 38.01 ± 1.94 $^{b}$ |
| Lysine | 39.71 ± 0.74 $^{a}$ | 49.69 ± 1.87 $^{b}$ | 33.79 ± 0.95 $^{c}$ |
| Methionine | 21.61 ± 0.88 $^{a}$ | 30.61 ± 4.38 $^{b}$ | 26.99 ± 2.93 $^{ab}$ |
| Phenylalanine | 30.62 ± 2.09 $^{a}$ | 32.57 ± 3.64 $^{a}$ | 35.07 ± 1.30 $^{b}$ |
| EAAI (%) | 37 $^{a}$ | 46 $^{b}$ | 41 $^{ab}$ |

Superscripts on the same row represent a significant difference ($p \leq 0.05$).

## 4. Discussion

The results that were obtained indicated that all observed parameters (body weight and length of yellow mealworm larvae and their DM, ash, fat, and crude protein contents) were affected by the rearing temperature. The optimal temperature range for rearing *T. molitor* is 22–30 °C [13–18,23,24], which includes the temperatures selected for this experiment.

The temperature significantly influenced the growth of larvae. Mealworms reared at 28 °C grew faster than those reared at 22 °C. This relationship between insect growth and temperature has been well described for many insects pests. An increase in temperature increases physiological activity, the metabolic rate, the efficiency of energy assimilation, and protein and lipid contents [10]. Thus, insects consume more substrate and grow faster than at lower temperatures [25]. In the case of mealworms, the positive effects of rising

temperatures on the development of larvae were previously described by Eberle et al. [18]. However, the developmental speed of the mealworm larvae in our study was shorter than that in previous studies. This may have been influenced by other factors that affect the developmental time, such as the larval density, diet, or access to water sources. Grau et al. and Rumbos et al. [26,27] demonstrated that access to water is essential for mealworms.

Concerning the proximate composition, the results for the DM content were consistent with the results of Bednářová et al. [28], who reported that *T. molitor* contained 29.41 g of DM per 100 g of FW. The ash content agreed with the values of ~3.6 g/100 g per unit DM reported by Baek et al. [29].

The fat content of the fresh sample reached an average value of 9.60 g/100 FW or 28.5 g/100 g DM. Accordingly, the average analysed fat content corresponded to the range reported in the literature of 15–50 g/100 g of fat per unit DM [30–33]. A positive effect of increasing temperature on the fat content of edible insects was found. The increase in the lipid content at higher temperatures was consistent with observations by Bjørge et al. [10], who monitored the fat content of mealworm larvae at seven different temperatures ranging from 15.2 to 39 °C. In their study, the fat content also increased with the increase in temperature up to 37 °C (maximum fat content of 47.4 g/100 g DM) and then significantly decreased to 16.0 g/100 g DM at 39 °C. Bjorge et al. [10] suggested that the fat content varied significantly with temperature and depended on the diet and life stage of insects. Another key factor may be the activity of enzymes and hormones that can affect fat metabolism [13,34]. Bjorge et al. [10] explained their results based on the possible bioconversion of carbohydrates into lipids. In addition, they found that at temperatures that caused the accumulation of more lipids, insects contained less water. Our measured results and the results of Bjorge et al. [10] disagreed with the findings published by Adámková et al. [13,34], who reported that the fat content reached its highest values at 20 °C and decreased at 15 °C and 25 °C. They justified these changes in the fat content by claiming that the insects analysed did not require larger amounts of fat to support their physiological processes as the temperature increased.

The average protein content was 19.25 g/100 g in the fresh sample and 58.42 g/100 g of DM, which was consistent with the literature [32,33,35,36]. The protein values were obtained with a nitrogen-to-protein conversion factor of 6.25, though Janssen et al. [37] suggested a conversion factor of 4.76 for the determination of protein in yellow mealworm larvae. Using this factor, the protein value was 49.00 g/100 g DM at 22 °C, 43.34 g/100 g DM at 25 °C, and 41.44 g/100 g DM at 28 °C. However, the effect of temperature on the nutritional value of *T. molitor* resulting in a decrease in protein content with the increase in temperature remains confirmed. This tendency is in line with the literature [32,33,35,36], where the values decreased from 57.8 ± 2.1 to 37.9 ± 1.4 g/100 g DM with the increase in temperature from 15 to 31 °C. The monitored decrease in the protein content with increasing temperature also agreed with Bjørge et al. [10], who analysed *T. molitor* when reared at seven different temperatures and observed a decrease in the protein content until 37 °C and a subsequent increase. At 39 °C, the protein content increased to 65.5 g/100 g DM. Bjørge et al. [10] explained the different rates of larval development at different life stages and temperatures. At lower temperatures, the rate of development decreased because of slower metabolic reactions [38]. This might explain the differences in the protein or lipid contents [10].

Insects are considered to be an alternative protein source, and it was, therefore, appropriate to determine their amino acid profiles. The tryptophan content was not determined because this amino acid was degraded during acid hydrolysis. Finke [39] reported a tryptophan content of 0.39 g/100 g DM for *T. molitor* larvae. Based on a comparison of the results obtained with the amino acid requirements issued by the World Health Organization (WHO), the insects met the requirements with respect to the essential amino acid content and can, thus, be considered to be a good source of protein. The total amino acid content measured in this study (33.80 ± 1.01 g/100 g DM at 22 °C, 36.28 ± 0.95 g/100 g DM at 25 °C, and 31.02 ± 0.84 g/100 g DM at 28 °C) was higher than that reported by Wu et al. (29.83 g/100 g DM) [40]. Higher values were reported by Ghosh et al. (44.50 g/100 g

DM) [15]. Ghosh et al. [15] reported that the larvae of *Allomyrina dichotoma* or *Protaetia brevitarsis* had a higher amino acid content (48.74 and 39.16 g/100 g DM) than that of the investigated mealworms. A higher amino acid content compared with *T. molitor* was also detected in egg white, which does not contain lipids or chitin (90.15 g/100 g DM), and chicken breast (88.25 g/100 g DM) [41]. In this study, the mealworm larvae contained the largest amounts of alanine at 22 and 25 °C and the largest amounts of proline at 28 °C. Rumpold and Schlüter [41], Zielińska et al. [42], Ghosh et al. [15], and Wu et al. [40] reported that the glutamic acid content was the highest, followed by the alanine, aspartic acid, and proline contents. However, higher values of glutamic and aspartic acids were measured at all temperatures in this study. Sulphur amino acids (cysteine + methionine) and histidine were determined to be the least abundant amino acids in mealworms, which was consistent with the results reported by Finke [39], Rumpold and Schlüter [41], Zielińska et al. [42], and Ghosh et al. [18]. With respect to the rearing temperature, no statistically significant differences were observed in aspartic acid, threonine, glycine, valine, leucine, phenylalanine, histidine, and methionine depending on the temperature. There were found significant differences in serine, glutamic acid, proline, isoleucine, tyrosine, and arginine between the temperatures of 25 and 28 °C, in alanine between 22 and 28 °C and between 25 and 28 °C, and in lysine among all temperatures. It can be concluded that the rearing temperature did not have a significant effect on the protein quality, and the decrease in protein content did not necessarily mean that the content of every amino acid was decreasing. Similar results were obtained in a previous study by Kulma et al. [19].

The calculated EAAI of *T. molitor* reared at three different temperatures using egg as a reference protein [43] was 37, 45, and 41%, respectively. Statistically significance differences were found between the of temperatures 22 and 25 °C. The differences in the calculated values were due to the different AAS values of the amino acids at different temperatures. The calculated EAAIs of *T. molitor* were close to those of lentils (41), beans (47), peas (50), corn (55), wheat (68), rice (74), rye (75), and poultry (78). Beef (82), pork (84), and milk (95) have higher EAAIs [43]. Based on the requirements recommended by the FAO and WHO, edible insects contain almost all essential amino acids at concentrations sufficient to fulfil the dietary requirements of healthy humans [15,34,36]. Thus, the obtained results support the claim that *T. molitor* larvae can be an alternative protein source with a satisfactory amino acid composition, providing almost the daily required amounts of essential amino acids according to the FAO and WHO. At all temperatures, the insects obtained adequate amounts of threonine, valine, histidine, phenylalanine, and tyrosine. Larger amounts of isoleucine were obtained at 25 °C, and sufficient amounts of cysteine and methionine were obtained at 28 °C [15]. The essential amino acid profile was comparable in quantity to that of conventional resource-intensive foods of animal origin [15].

There have already been studies dealing with the effects of temperature on fat and protein contents, but our research also provided detailed information on the effect on protein quality in terms of amino acid composition and changes in the AAS and EAAI. Even though our results bring some interesting outputs that might be useful for insect-rearing farmers, future research focused on accounting for larval density, feed effects, improved rearing methodology, and the completion of results with the fatty acid profiles of the tested larvae would be required.

## 5. Conclusions

Significant differences in the body weight and length of larvae and the DM, ash, lipid, and protein contents per unit DM were identified depending on the temperature. No statistically significant differences were found in aspartic acid, threonine, glycine, valine, leucine, phenylalanine, histidine, and methionine depending on the temperature, and a good amino acid profile was found. There was a tendency towards an increase in the weight (by 67%), dry matter content (by 19%), and fat per unit dry matter content (by 60%) of the larvae with increasing rearing temperatures. In contrast, a decreasing trend (by 15%) was observed in the case of crude protein per unit DM content. The highest mealworm

weight and body length were achieved at 28 and 25 °C, respectively. The highest lipid content per unit DM was measured in *T. molitor* that was farmed at 28 °C. In contrast, the highest crude protein content of *T. molitor* was obtained at 22 °C.

Information on the effects of temperature on larval growth and nutritional parameters is important for insect farmers to determine the optimal rearing temperature for the subsequent utilisation of insects as food or feed. However, it is important to note that the effects of temperature on the nutritional parameters of insects were not always in the same direction; therefore, the purpose for which an insect is reared needs to be considered. The growth and nutritional parameters of *T. molitor* larvae, however, can be substantially affected by many other factors, such as food composition and insect population density, and should, therefore, be taken into consideration.

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