Rocz. Nauk. Zoot., T. 53, z. 1 (2026): 155–164 *DOI: 10.58146/q5pq-0g82*

CHEMICAL COMPOSITION AND TOCOPHEROL CONTENT OF *TENEBRIO MOLITOR* LARVAE FED DIETS SUPPLEMENTED WITH EGG POWDER

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The study was financed from the statutory activities of the Department of Fur Animal Breeding and Game Management, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn.

Abstract

The study aimed to evaluate the effect of diets containing varying levels of egg powder on the chemical composition and tocopherol content of yellow mealworm (Tenebrio molitor) larvae. The control group (K) was fed pelleted wheat bran without any additives. In the first experimental group (D1), wheat bran and egg powder each accounted for 50% of the diet, whereas in the second experimental group (D2) these components accounted for 25% and 75%, respectively. Based on the results obtained, it was found that 48 hours after administration, egg powder supplementation increased the dry matter and crude fat content of the larvae. In contrast, crude ash and nitrogen-free extract levels decreased. The total tocopherol content and the α- and γ-tocopherol fractions also increased, whereas the β-tocopherol fraction decreased.

Keywords: gut loading, yellow mealworm, egg powder, chemical composition, tocopherols

Introduction

Modern animal science deals not only with typical livestock species but also with animals kept for hobby purposes. Among them are numerous species conventionally referred to as "exotic" animals, for which captive housing and feeding requirements have not yet been fully established. Properly balancing the diet to meet nutritional requirements is crucial for maintaining a high level of welfare in these animals (Cooper and Williams, 2014). Gut loading is a feeding practice in which feeder insects are given a specially prepared, enriched diet shortly before being consumed by the target animal. This allows selected nutrients, minerals or various biologically active substances to be transferred indirectly to the organisms of those animals (Oonincx and Finke, 2023). The method was initially developed in response to calcium and vitamin A deficiencies commonly found in insects and in reptiles fed on them. It is used both by hobby breeders and by commercial companies producing pet food (Pennino et al., 1991; Finke et al., 2005; Boykin et al., 2021). Most studies indicate that the gut contents of feeder insects can modify their chemical composition, which is particularly important in professional reptile and amphibian breeding for maintaining nutritional welfare (Oonincx et al., 2015).

Modern approaches to gut loading include not only minerals but also provitamins, vitamins and their derivatives. Carotenoids are of particular interest because, among other functions, they affect colour intensity and immune system function in animals (Tan et al., 2020). Vitamin E and tocopherols have been studied less frequently (Pennino et al., 1991; Oonincx and Finke, 2021). Finke (2003) suggests adding 660 mg/kg α-tocopherol, among other compounds, to mealworm diets intended to enrich their digestive tracts. Tocopherols are strong antioxidants and the most bioactive form of vitamin E; among them, α-tocopherol is the form most commonly predominant in plant tissues and is always predominant in animal tissues. However, their relatively low stability due to high sensitivity to external factors should be kept in mind (Galli and Azzi, 2010). Tocopherols occur in many plant materials, such as seeds and products derived from their processing. They are also present in egg yolk, where they constitute an important antioxidant component (Bourre and Galea, 2006; Ko et al., 2020).

Because the passage time of digesta in insects is short, the optimal supplementation period for enrichment is usually 24 to 48 hours before feeding the insects to the target animals. The effectiveness of gut loading depends on the insect species. Crickets accumulate nutrients faster than yellow mealworm (Tenebrio molitor) larvae (Gorst et al., 2015). Nevertheless, the latter species is the most commonly used feeder insect because it is easy to rear (Finke, 2003). The yellow mealworm is a ubiquitous global pest of stored grains and cereal products that has become one of the most commonly reared feeder insects (Finke, 2003; Huis and Gasco, 2023). For decades it has been used to feed reptiles in hobby collections and zoos (Valdés et al., 2022). In recent years its use has continued to increase, as it has also begun to be used in feeding companion animals such as dogs and cats, as well as in aquaculture. It was the first edible insect to be granted “Novel Food” status in the EU, paving the way for mass production of insect-based protein meals (Huis and Gasco, 2023).

In the nutrition of insectivorous reptiles, such as bearded dragons (Pogona vitticeps) and leopard geckos (Eublepharis macularius), mealworm larvae serve as a high-protein energy source providing essential amino acids (Makkar et al., 2014). Although attention has been drawn to their unfavourable calcium-to-phosphorus ratio, modern feeding methods, including gut loading, make it possible to optimize their nutritional value through calcium supplementation (Moruzzo et al., 2021). In addition, because of their mobility, mealworm larvae stimulate the natural hunting instincts of lizards, which is very important for the expression of natural behaviour in reptiles kept in captivity (Grau et al., 2017). The yellow mealworm is now regarded as one of the most sustainable alternatives for producing protein otherwise derived from livestock, because its rearing requires minimal water and land resources (Oonincx et al., 2015). The present study was conducted to evaluate the effect of diets containing 50 and 75% egg powder on the basic chemical composition and tocopherol content of yellow mealworm larvae.

Material and methods

The study was conducted in the Laboratory of the Department of Fur Animal Breeding and Game Management at the University of Warmia and Mazury in Olsztyn. Live yellow mealworm larvae in the penultimate developmental stage, purchased from a domestic company specializing in feeder insect production, were placed in 18 plastic containers measuring 30 × 14.5 × 10 cm, fitted with ventilated lids and a water reservoir to maintain adequate humidity. Each container held 200 g of larvae. The containers were divided into three feeding groups (n=6). Room temperature was maintained at 22–24°C, relative humidity at 50–70%, and the light/dark regime at 10 h/14 h. Before the experiment began, the larvae were deprived of feed for 48 h, in accordance with common practice in studies of this type (Finke, 2003; Boykin et al., 2021).

The experimental factor was the addition of egg powder to the standard feed, which consisted of wheat bran. Both feed materials were purchased from a commercial feed supplier and, according to the seller's declaration, were produced in Poland. To obtain maximum homogeneity, the feeds were mixed and then pelleted. A KL, ZLSP biomass pellet mill with a 4-mm die was used for pelleting. Pellets were produced in short time intervals so that the pellet mill body did not heat above 35°C; this was monitored with a thermometer placed on the pellet mill body.

The control group (K) was fed pelleted wheat bran without any additives. In the first experimental group (D1), wheat bran and egg powder each accounted for 50% of the diet, whereas in the feed given to the second experimental group (D2) the respective proportions were 25 and 75%. The basic chemical composition and tocopherol levels of wheat bran and egg powder are presented in Tables 1 and 2, while those of pellets K, D1 and D2 are shown in Tables 3 and 4.

The actual experiment, during which the mealworms were fed the experimental diets, lasted a further 48 h. The insects were then frozen at −25°C and transferred to the Laboratory of the Department of Animal Nutrition, Feed Science and Cattle Breeding at the University of Warmia and Mazury for chemical analyses and determination of dry matter, crude ash, crude protein, crude fat and crude fibre in the feeds, their components and the larvae. Organic matter was calculated as the difference between dry matter and crude ash, and nitrogen-free extract was calculated by subtracting crude protein, crude fat and crude fibre from organic matter. Analyses were carried out according to standard AOAC methods (2006). Tocopherol content in feeds was determined by high-performance liquid chromatography (HPLC) in accordance with Polish Standards (PN–EN ISO 14565:2002, PN–EN ISO 6867:2002).

Statistical analysis of the results was performed by one-way analysis of variance using STATSOFT INC. Statistica 12.0 (2015), and the significance of differences between means was determined with Duncan's test at $P<0.05$ and $P<0.01$.

Table 1. Chemical composition of wheat bran and egg powder (%)

Feeds	Specification						
	Dry matter	Crude ash	Organic matter	Crude protein	Crude fat	Crude fibre	NFE*
Wheat bran	86,96	4,64	82,32	15,67	3,00	10,82	52,83
Egg powder	92,54	2,65	89,89	55,33	20,16	0,50	13,90

*Nitrogen-free extract.

Table 2. Tocopherol content in wheat bran and egg powder (mg/kg)

Feeds	Specification				
	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols
Wheat bran	5,408	0,299	5,233	0,547	11,487

Table 3. Chemical composition of feed mixtures (%)

Specification	Feed mixtures		
	K	D1	D2
Sucha masa Dry matter	86,96	89,75	91,14
Popiół surowy Crude ash	4,64	3,65	3,15
Substancja organiczna Organic matter	82,32	86,10	91,14
Crude protein	15,67	35,50	45,42
Tłuszcz surowy Crude fat	3,00	11,58	15,87
Crude fibre	10,82	5,66	3,08
NFE*	52,82	33,36	26,77

*Nitrogen-free extract.

Table 4. Tocopherol content in feed mixtures (mg/kg)

Specification	Feed mixtures		
	K	D1	D2
α-tokoferol α-tocopherol	5,408	12,828	16,538
β-tokoferol β-tocopherol	0,299	0,211	0,168
γ-tocopherol	5,233	6,589	7,267
δ-tocopherol	0,547	0,745	0,845
Suma tokoferoli Total tocopherols	11,487	20,373	24,818

Results

Table 5 presents the chemical composition of yellow mealworm larvae fed wheat bran (group K) and diets D1 and D2 containing 50 and 75% egg powder, respectively. Addition of the experimental factor increased the dry matter content of the larvae. In group D2, the difference compared with group K was statistically significant. The crude ash level also decreased significantly. In addition, crude fat content increased in groups D1 and D2; in group D2 this difference was statistically significant. The level of nitrogen-free extract, however, decreased significantly in the experimental groups.

It was also found that, despite the lack of statistically significant differences, addition of egg powder to the diets increased the organic matter and protein levels and decreased crude fibre content in mealworm larvae.

The tocopherol content of mealworm larvae is presented in Table 6. Egg powder supplementation significantly differentiated the total tocopherol content and the individual tocopherol fractions, except δ-tocopherol. Inclusion of 50 and 75% egg powder in the diet increased α-tocopherol and γ-tocopherol levels in mealworm larvae compared with the control group. For α-tocopherol, the difference was highly significant not only between D2 and K but also between D2 and D1. The γ-tocopherol level was highly significantly higher in both experimental groups. In contrast, the β-tocopherol fraction decreased significantly in larvae fed diets supplemented with the experimental factor. Total tocopherol content in mealworm larvae increased with increasing egg powder inclusion in the feed mixtures. It was highly significantly higher in D2 than in K and significantly higher in D1 than in K. A statistically significant difference also occurred between the experimental groups D1 and D2.

Table 5. Chemical composition (%) of mealworm larvae (mean±SD)

Specification	Groups		
	K	D1	D2
Dry matter	$40{,}84^{b}\pm1{,}89$	43,22±1,79	$44{,}05^{a}\pm0{,}89$
Popiół surowy Crude ash	$1{,}60^{a}\pm0{,}07$	$1{,}11^{b}\pm0{,}11$	$1{,}01^{b}\pm0{,}22$
Substancja organiczna Organic matter	39,24±1,77	42,11±1,73	43,04±0,74
Białko ogólne Crude protein	21,66±1,27	22,52±1,49	23,06±1,57
Tłuszcz surowy Crude fat	$13{,}63^{b}\pm0{,}28$	15,98±2,83	$16{,}70^{a}\pm2{,}61$
Włókno surowe Crude fibre	1,72±0,07	1,57±0,05	1,35±0,10
NFE*	$2{,}23^{Aa}\pm2{,}24$	$2{,}04^{b}\pm5{,}43$	$1{,}93^{B}\pm2{,}12$

*Nitrogen-free extract.
A,B – $P<0.01$; a,b – $P<0.05$.

Table 6. Tocopherol content (mg/kg) in mealworm larvae (mean±SD)

Specification	Groups		
	K	D1	D2
α-tokoferol α-tocopherol	$0{,}306^{B}\pm0{,}092$	$0{,}371^{B}\pm0{,}052$	$0{,}466^{A}\pm0{,}030$
β-tokoferol β-tocopherol	$0{,}046^{Aa}\pm0{,}003$	$0{,}038^{b}\pm0{,}006$	$0{,}033^{B}\pm0{,}005$
γ-tokoferol γ-tocopherol	$0{,}058^{B}\pm0{,}003$	$0{,}061^{A}\pm0{,}003$	$0{,}066^{A}\pm0{,}002$
δ-tokoferol δ-tocopherol	0,013±0,001	0,016±0,001	0,017±0,001
Suma tokoferoli Total tocopherols	$0{,}423^{B}\pm0{,}008$	$0{,}486^{b}\pm0{,}010$	$0{,}582^{Aa}\pm0{,}013$

A,B – $P<0.01$; a,b – $P<0.05$.

Discussion

The chemical composition results obtained for mealworm larvae in all groups (Table 5) can be considered to fall within the ranges reported in the literature. Finke (2003) notes that the chemical composition of insects, even within the same species, can vary considerably. Such variation results from numerous factors, including the chemical composition of the diet, larval developmental stage, temperature and humidity (Siemianowska et al., 2013; Yi et al., 2013; Mattioli et al., 2024; Mancini et al., 2021; Kröncke and Benning, 2023).

The feed offered to the insects had the greatest effect on their nutrient content. The chemical composition of the wheat bran and egg powder (Tables 1 and 2) was typical of these feed materials and fell within ranges reported in the literature. The wheat bran analysed by Apprich et al. (2014) contained 87.9% dry matter, 13.2–18.4% crude protein, 3.5–3.9% crude fat, 13.8–24.9% crude fibre and 56.8% carbohydrates. By contrast, the chemical composition of egg powder reported by Edusei (2025) and Nyefene et al. (2025) ranged from 93.68 to 97.68% dry matter, 55.93 to 58.3% crude protein, 29.49 to 31.9% crude fat and 2.9 to 7.69% carbohydrates.

Mattioli et al. (2024) reported that mealworm larvae fed a mixture of stale bread and brewer's spent grain contained 32.77% dry matter, 0.81% crude ash, 18.82% crude protein and

13.82% crude fat. The composition reported by Liu et al. (2020) was similar: dry matter 35.9%, crude ash 1.5%, crude protein 17.6%, crude fat 13.8% and carbohydrates 3.1%. Siemianowska et al. (2013), in turn, reported 43.74% dry matter, 1.55% crude ash, 17.92% crude protein and 21.93% crude fat. Kröncke and Benning (2023), who fed mealworm larvae diets containing 10–80% protein and 3–11% fat, obtained larval contents of 14–15% protein and 4–20% fat, respectively. The fat content reported by Mancini et al. (2021) ranged from 6.46 to 14.82% depending on diet, while Yi et al. (2013) reported 9.9%. The proportion of dry matter in larvae also varies considerably, from 32–33% (Mancini et al., 2021), through 36% (Yi et al., 2013), to approximately 42% (Ghaly and Alkoaik, 2009), which consequently affects the final content of the other nutrients.

The differences in nutrient content observed in the present study (Table 5) undoubtedly resulted from nutrient levels in the feed materials and pellets (Tables 1 and 3). The addition of high-protein or fat-rich products is generally considered to increase the corresponding components in larvae. This concept, among others, underlies the development of the feeding enrichment technique known as gut loading (Oonincx and Finke, 2023). However, not all nutrients or minerals are reflected in larval chemical composition to the same extent. Kröncke and Benning (2023) observed a low correlation between protein content in diets and in the larvae fed those diets, and found the relationship to be stronger for fat and carbohydrates. Jajić et al. (2022), by contrast, reported correlations of 0.317 between dietary and larval protein content and 0.06 for fat. A linear relationship was observed: the greater the proportion of protein or fat in the diet, the greater the proportion of that component in larval biomass. It is worth noting that not all researchers studying gut loading confirm its usefulness. Gorst et al. (2015) found no statistically significant increase in nutrients such as protein, fat, dry matter or ash in mealworm larvae examined 12 hours after feeding. However, the diet used in that study contained only 19.34% protein and 1.32% fat.

Examples are available in the literature of vitamin supplementation of feeds to obtain feeder insects with increased vitamin content. Some authors even suggest that such enriched feeds can be regarded as nutraceuticals (Martínez-Pineda et al., 2024). The levels of individual tocopherol fractions in the larvae (Table 6), and the differences among them, resulted from the different vitamin E contents of the feed materials and pellets (Tables 2 and 4).

The total tocopherol level determined in the wheat bran fed to the mealworms (Table 2) was similar to that reported by Zhou et al. (2005), i.e. 12.71 mg/kg. In another study, these authors reported that α-tocopherol was the predominant fraction, as was also the case in the wheat bran analysed in the present study (Zhou et al., 2004). By contrast, the tocopherol level in egg powder (Table 2) was lower than values reported in the literature. Pirkwieser et al. (2022) determined a level of 39.3 mg/100 g dry matter, with α-tocopherol as the predominant fraction. The observed differences probably result from the relatively low stability of vitamin E (Jeon et al., 2016). Tocopherol levels in mealworm larvae (Table 6) differed among groups. The values obtained were nevertheless low, which corresponded to their levels in the diets. Other authors have reported higher tocopherol concentrations in mealworm larvae. For example, Zhang et al. (2023) found a vitamin E level of 218.4 mg/kg in mealworm larvae fed wheat bran. In another study described by Mattioli et al. (2024), mealworms contained 0.27 mg/kg tocopherols, of which α-tocopherol accounted for 0.21 mg/kg and β-tocopherol for 0.01 mg/kg, whereas Finke (2013) reported 13 mg/kg α-tocopherol. Mealworm oil analysed by Martínez-Pineda et al. (2024), meanwhile, contained 105.8 mg/kg tocopherols, with α- and γ-tocopherol predominating.

Summary

The results indicate that mealworm larvae can be enriched with nutrients and tocopherols using a natural feed material, namely egg powder. It was found that inclusion of egg powder at 50 and 75% in feed mixtures for mealworm larvae increased their dry matter and crude fat content after 48 hours of feeding. In contrast, crude ash and nitrogen-free extract levels decreased. The total tocopherol content and the α- and γ-tocopherol fractions also increased, whereas the β-tocopherol fraction decreased. The results are of practical importance because they may serve as a reference point for further studies aimed at optimizing the nutritional value of insects intended for animal feeding.

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Approved for publication: 8 May 2026

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SUMMARY

The study aimed to evaluate the effect of diets containing varying levels of egg powder on the chemical composition and tocopherol content of yellow mealworm (*Tenebrio molitor*) larvae. The control group (K) was fed a diet consisting of pelleted wheat bran without additives. In the first experimental group (D1), the diet comprised 50% wheat bran and 50% egg powder, while in the second experimental group (D2), these components accounted for 25% and 75% of the diet, respectively. Based on the results obtained 48 hours after administration, it was found that egg powder supplementation led to an increase in dry matter and crude fat content. Conversely, the levels of crude ash and nitrogen-free extracts decreased. Furthermore, an increase was observed in the total tocopherol content as well as the α- and ɣ-tocopherol fractions, whereas the β-tocopherol level declined.

Keywords: gut loading, Tenebrio molitor, egg powder, chemical composition, tocopherols