

Nutrient Composition, Amino Acid and Fatty Acid Profiles, and Mineral Content of Silkworm (*Bombyx mori* L.) Pupa Powder to be used as a feed ingredient in Poultry Diets

Şeniz ÖZİŞ ALTINÇEKİÇİ¹, Arda SÖZCÜ²

^{1,2}Bursa Uludağ Üniversitesi, Ziraat Fakültesi, Zootekni Bölümü, Bursa, Türkiye

¹<https://orcid.org/0000-0001-9044-8092>, ²<https://orcid.org/0000-0002-0955-4371>

✉: seniz@uludag.edu.tr

ABSTRACT

The aim of this study was to determine the nutrient composition, amino acid and fatty acid profiles, and mineral content of silkworm (*Bombyx mori* L.) pupa powder (SPP) that can be used as a feed ingredient in poultry diets. In this study, the dry matter, organic matter, crude protein (CP), ether extract (EE), crude fiber, and ash contents, amino acid and fatty acid profiles, and the contents of some minerals of SPP dried until they reached a constant weight at 130°C for 12 hours were determined. In addition, color characteristics (L*, a*, and b* values) were also determined. The CP, EE, and ash contents of SPP were determined to be %58.8±0.39, %20.8±0.93, and 5.9±0.04, respectively, on a dry matter basis. The most abundant amino acids were arginine (35.4±3.26 mg/g), aspartic acid (40.2±3.64 mg/g), glycine (104.0±6.69 mg/g), lysine (98.1±5.80 mg/g), proline (39.9±3.57 mg/g), and serine (43.1±3.38 mg/g) in SPP. The total saturated, monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids were found to be 26.7±0.05, 31.2±0.08, and 42.0±0.05, respectively. The fatty acids lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, and α-linolenic acid were found as abundant fatty acids in silkworm pupa powder. The potassium, phosphorus, magnesium, and calcium contents of SPP were 83.9±0.6±, 63.4±1.8, 26.2±0.3, and 9.8±0.5, respectively. These findings clearly show that SPP is a potential protein source that can be used in poultry nutrition.

Animal Breeding

Research Article

Article History

Received : 15.04.2025

Accepted : 14.06.2025

Keywords

Alternative feedstuff

Protein source

Insect meal

Nutrient composition

Nutritional value

Kanatlı Hayvan Yemlerinde Yem Hammaddesi Olarak Kullanılabilecek İpekböceği (*Bombyx mori* L.) Pupa Tozunun Besin Maddeleri Kompozisyonu, Amino Asit ve Yağ Asitleri Profili ve Mineral Madde İçerikleri

ÖZET

Bu çalışmanın amacı, kanatlı kümes hayvanları karma yemlerinde yem hammaddesi olarak kullanılabilecek ipekböceği (*Bombyx mori* L.) pupa tozunun (İPT) besin maddeleri kompozisyonu, amino asit ve yağ asitleri profili ve mineral maddeler içeriğinin belirlenmesidir. Bu çalışmada sabit ağırlığa ulaşıncaya kadar 130°C'de 12 saat kurutulmuş İPT'nin kuru madde, organik maddeler, ham protein (HP), eter ekstrakt (EE), crude fiber ve kül içerikleri, amino asit ve yağ asidi profilleri ve bazı mineral maddeler içerikleri belirlenmiştir. Ayrıca, renk özellikleri (L*, a* ve b* değerleri) de belirlenmiştir. İPT'nin kuru maddede %58.8±0.39, %20.8±0.93 ve 5.9±0.04 düzeylerinde HP, EE ve kül içerdiği belirlenmiştir. Pupada en bol bulunan amino asitler arginin (35.4±3.26 mg/g), aspartik asit (40.2±3.64 mg/g), glisin (104.0±6.69 mg/g), lizin (98.1±5.80 mg/g), prolin (39.9±3.57 mg/g) ve serin (43.1±3.38 mg/g) idi. Toplam doymuş, tekli doymamış (TD) ve çoklu doymamış (ÇD) yağ asitleri içeriği sırasıyla 26.7±0.05, 31.2±0.08 ve 42.0±0.05 olarak bulunmuştur. İPT'de laurik asit, miristik asit, palmitik asit, stearik asit, oleik asit ve α-linolenik asit yağ asitleri bol miktarda bulunmuştur. İPT'nin, potasyum, fosfor, magnezyum ve kalsiyum içeriği sırasıyla 83.9±0.6±, 63.4±1.8, 26.2±0.3 ve 9.8±0.5'dir. Bu bulgular, İPT'nin kanatlı

Zootekni

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 15.04.2025

Kabul Tarihi : 14.06.2025

Anahtar Kelimeler

Alternatif yem

Protein kaynağı

Böcek unu

Besin bileşeni

Besin değeri

beslemede kullanılabilecek bir protein kaynağı olduğunu göstermektedir.

- To Cite:** Öziş Altınçekiç, Ş., & Sözcü, A., (2025). Nutrient Composition, Amino Acid and Fatty Acid Profiles, and Mineral Content of Silkworm (*Bombyx mori* L.) Pupa Powder to be used as a feed ingredient in Poultry Diets. *KSU J. Agric Nat* 28 (5), 1394-1403. DOI: 10.18016/ksutarimdog.a.vi.1676565
- Atıf İçin :** Öziş Altınçekiç, Ş., & Sözcü, A., (2025). Kanatlı Hayvan Yemlerinde Yem Hammaddesi Olarak Kullanılabilecek İpekböceği (*Bombyx mori* L.) Pupa Tozunun Besin Maddeleri Kompozisyonu, Amino Asit ve Yağ Asitleri Profili ve Mineral Maddeler İçeriği. *KSÜ Tarım ve Doğa Derg* 28 (5), 1394-1403. DOI: 10.18016/ksutarimdog.a.vi.1676565.

INTRODUCTION

Bombyx mori L. (*B. mori*) is an economically and ecologically important insect, representing an ideal lepidopteran species in many scientific studies. It was domesticated in China over five thousand years ago (Gong et al., 2016). The silkworm is a type of insect characterized by a complete metamorphosis with a short and rapid life cycle consisting of four stages, including egg, larva, pupae (in the cocoon), and butterfly (adult; Sharma et al., 2022). The main purpose of silkworm breeding is to obtain silk, so after the completion of cocoon spinning by larvae, the pupae are killed through the cocoon boiling process to prevent the emergence of pupae from the cocoon. The remaining pupae after silk reeling become a waste material in large quantities, which could be accepted as an important by-product of silk production (Patil et al., 2013). On the other hand, waste pupae are often thrown out of the environment or used as fertilizer, leading to serious environmental pollution and the loss of valuable resources (Wei et al., 2009). In other words, pupae left over after silk reeling are highly nutritious waste that have to be utilized with different aims to support the sustainable agriculture production (Lamberti et al., 2019).

More than 200,000 tons of spent silkworm pupae are obtained each year throughout the world. The biggest producer countries are mainly China and India, followed by Brazil, Thailand, Indonesia, Vietnam, and South Korea (Thirupathaiah et al., 2018). In Turkey, 78 tons of fresh cocoon production were achieved in 2023 (TÜİK, 2024). However, since the moisture content of dead pupae is very high, they could easily spoil and cause a bad odor and environmental pollution (Rashmi et al., 2023). Therefore, it could be offered to dry under the sun and ground before usage, to ensure a more uniform mixture in the rations (Jintatataporn, 2012). Additionally, using such a valuable source of protein in poultry nutrition is a good way to reduce the environmental impact of the silk industry (Kowalska et al., 2020). Previous studies demonstrated that silkworm pupa could be accepted as a nutritious source of its major nutrient components and bio-active compounds (Mishra et al., 2003; Meyer-Rochow & Jung, 2020).

In poultry nutrition, traditional protein sources include plant-based protein sources such as soybean meal and sunflower seed meal, and animal-based protein sources such as meat meal, meat-bone meal, and fish meal (Sajid et al., 2023). However, since soybean production in Turkey is very insufficient, soybean meal, which is used in large quantities in poultry rations, is supplied through imports. On the other hand, when evaluating slaughterhouse by-products such as meat-bone meal and blood meal as protein source feed ingredients, issues such as the risk of Salmonella and the inability to definitively determine feed values could cause difficulties (Limeneh et al., 2022). Therefore, to comply with future tendencies by sustainable production in the agricultural production chain, it is crucial to investigate the local, available, and economic alternative protein sources to replace the traditional ingredients in poultry feed (El-Sobrout et al., 2023; Khalifah et al., 2023). Considering all these factors, it has been demonstrated through some studies that the pupa, obtained as a natural waste product, could serve as an alternative to traditional proteins, and it has gained increasing importance in recent years (Kowalska et al., 2020; Tamuly et al., 2024; Zotte et al., 2024).

In a recent study performed by Yeruva et al. (2023), dried pupae, that were harvested at 6th day of spinning process, had a balanced nutrient profile for protein (51-55%), fat (25-32%), carbohydrate, minerals and vitamins which make the silkworm pupae as good source for human food and animal feed. Also, the researchers highlighted that the dried pupa had a rich content of bioactive compounds, including essential amino acids, e.g., lysine, leucine, methionine, and tryptophan, omega-3 fatty acids, e.g., α -linolenic acid, phenols, and flavonoids. Besides, it was found that silkworm pupa had 18 different components of phenolic acids, mainly cinnamic acid and ferulic acid, and 15 different components of flavonoids, including quercetin, catechin, myricetin, luteolin, and epicatechin. Therefore, the pupae could be used as a functional food due to their potential beneficial effects, such as anti-oxidant, anti-hypertensive, anti-tumor, anti-coagulant, and anti-bacterial effects. It is noteworthy that the nutritional content of the silkworm pupa powder could show variations according to the silkworm lines, feeding conditions, harvesting time during the spinning process, drying, and grinding processes of the pupa. However, no comprehensive data exists on the nutrient profile of silkworm pupae produced under Turkish sericulture conditions. We hypothesized that Turkish silkworm pupae would exhibit a nutrient profile competitive with conventional protein sources. In this respect, determining the nutrient content of silkworm pupae raised under the

current production and feeding conditions in Turkey is an important issue for making a general comparison and evaluating their potential against traditional protein feed ingredients in animal nutrition. The objective of this study is to investigate the nutrient composition, amino acid and fatty acid profiles, and mineral content of silkworm pupa powder, which was reared in Bursa (Türkiye) and harvested at the end of the spinning process (at the 6th day of this process).

MATERIAL and METHOD

In the study, the pupae of the hybrid silkworm line obtained from the Bursa Koza Agricultural Sales Cooperatives Union were used as material. The silkworms were kept at an ambient temperature of about 250 °C and ambient humidity of 75% and fed fresh mulberry leaves for all instars. Silkworm cocoons were harvested on the 6th day after the beginning of cocoon spinning. To prevent the emergence of adult butterflies from the harvested cocoons, the cocoons were dried in an 80 °C oven (Heraeus T6120) for 30 minutes. After the cooling process, the pupae obtained by cutting cocoons were dried in an oven set at 130°C for 12 hours until they reached a constant weight, then ground in a laboratory mill (Fritsch Pulverisette14) with a 1 mm sieve, and stored in an airtight container until further analysis (Pereira et al., 2003).). Each analysis was performed with 3 samples of silkworm pupa powder for creating 3 repetitions.

Chemical Composition Analyses

The analyses of proximate composition of silkworm pupa powder, moisture, ash, fat, protein, and crude fibre content were determined according to the standard methods reported by AOAC (2005).

Moisture

To determine the moisture content of silkworm pupa, a 10 g silkworm pupa powder sample (initial weight, IW) was placed in a hot-air oven (100°C). The sample was then collected after drying for the required amount of time, and its final weight was measured. The samples were heated once again in the same oven for one hour and weighed once they had cooled to make sure the drying was finished (final weight, FW).

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{Dry weight}}{\text{Initial weight}} \times 100$$

Ash

After precisely weighing 5.0 g of the dried silkworm pupa powder sample into a crucible that previously had been heated and cooled, it was placed in a muffle furnace set at 600°C for approximately five hours, provided cooling in a desiccator, and then weighed again.

Crude protein

The crude protein content of samples was determined by multiplying the nitrogen value by 6.25 and determining the sample's nitrogen content. The standard Kjeldahl technique was used to estimate nitrogen.

$$\text{Protein content (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Ether extract

To determine the crude lipid content of samples, 10 g of dried pupa powder was put into a thimble and covered with cotton according to the procedure in the Soxhlet extraction method. Then the thimble was extracted using hexane for almost eight hours. The extract was filtered; the oil content was measured after removing the hexane content by a vacuum evaporator.

$$\text{Lipid content} \left(\frac{\text{g}}{100} \text{ g sample} \right) = \frac{\text{Weight of hexane extract}}{\text{Weight of sample}} \times 100$$

Carbohydrate

The carbohydrate content of samples was determined by using the difference method according to Egounlety and Awoh (1990). The total percentage of the moisture, crude fiber, ash content, and protein content of the samples was subtracted from 100%, thereby determining the remnant as the total carbohydrate content.

Fatty acid profile

A total of 3 g of pupae powder was placed into 50-mL screw-cap tubes. Then, 30 milliliters of Folch-I solution

(CHCl₃:CH₃OH, 2:1 v/v; Folch et al., 1957) was added. After the vortex procedure, the sample was filtered into another volumetric flask. After adding saline solution (10 mL, 0.88% NaCl) and Folch-I (2 mL) into the flask, it was vortexed. The phase separation was provided, and the bottom layer was transferred into a petri dish. After the drying process (40 °C for 2 hours), the dried lipids with an amount of 0.4 mL were mixed with 1 mL BF₃-methanol for the solubilization process (Cherian et al., 2002). Afterwards, heating (90-100°C for 60 min), cooling, and phase separation were applied to obtain the hexane layer. Gas chromatography was used to separate and quantify fatty acid methyl esters (FAME). The fatty acid methyl esters (FAME) were analyzed using a gas chromatograph (Agilent 6890N Series) fitted with a flame ionization detector (Agilent Technologies Inc., Wilmington, DE). The studies were conducted using an HP 5-MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies Inc.). At 250°C and 300°C, respectively, the injector and flame ionization detector were kept. By comparing the retention times to FAME standards (Mixture ME-100; Greyhound Chromatography and Allied Chemicals, Birkenhead, Merseyside, UK), sample FAME was identified. The fatty acid content of samples was given as percentages of total fatty acids.

Color characteristics

The color characteristics were evaluated with measuring of redness (a*), lightness (L*) and yellowness (b*) of silkworm pupa powder (n = 6; SPP (six independent samples)) by using a spectrophotometer (Konica Minolta, Osaka, Japan) as explained by (Uguz & Sozcu, 2023).

Amino acid profile

After weighing a uniform 0.5 g sample of silkworm pupa powder, 20 mL of hydrochloric acid was added, and the mixture was burned for 18 to 24 hours at 110 °C. After adding 20 milliliters of distilled water, the sample was dried at 70 degrees Celsius in an evaporator. After adding distilled water to reach 25 mL, the mixture was centrifuged for 15 minutes at 10,000 g. After that, 0.2 µL of the sample was extracted and dissolved in 0.1% formic acid-containing methanol. The supernatant was then put onto the column (Zorbax Eclipse AAA 4.6 X 150 mm, 3.5 µm) after being filtered using a nylon filter membrane. Amino acid analysis was conducted using the LC-MS conditions (Nimbalkar et al., 2012). Solvent A, which is water with 0.1% formic acid, and Solvent B, which is methanol with 0.1% formic acid, were utilized as the mobile phase.

Minerals

The samples of silkworm pupa powder with an amount of 0.25 g were mixed with 10 mL of nitric acid (HNO₃, 67% v/v). The mouths of the microwave containers were tightly sealed, and the samples were dried at 200°C for 15 minutes and then allowed to cool. Then, the samples that had turned into a solution were completed to 25 mL with ultra-pure water and analyzed for mineral nutrients according to the induction coupled plasma atomic emission spectroscopy (ICPAES) procedures (Eknayake et al., 1999). The instrument was calibrated with known standards, and samples were analyzed at corresponding wavelengths.

Statistical Analysis

All results were given as the mean of three measurements belonging to three samples of silkworm pupa powder, presented as mean ± standard deviation.

RESULTS and DISCUSSION

The silkworm pupa powder is a rich source of essential nutrients, minerals, and bioactive compounds; therefore, it has already been used for medicinal and human nutrition purposes. Likewise, current results clearly showed that the silkworm pupae had a rich content of nutrients, which could potentially be used as an alternative protein source in poultry nutrition. The chemical composition of silkworm pupa powder is presented in Table 1. The moisture, dry matter, and ash content of the silkworm pupa powder were found to be 75.50%, 24.50%, and 5.9%, respectively. The crude protein was 58.84%, and the EE content was found as 20.81%. The silkworm pupa powder had 4.80% of crude fiber, 89.93% of organic matter, and 0.35% of carbohydrates. In another study, Yeruva et al. (2023) investigated the proximate composition of silkworm pupa powder, including the moisture content (76.7%), dry matter (23.08%), crude protein (54.50%), fat (28.03%), ash (4.5%), and carbohydrates (4.7%).

Previous reports clearly highlighted huge variations in the composition of silkworm pupae. Indeed, the moisture content varied between 5.89 and 10% (Mishra et al., 2003; Nisha et al., 2014; Bhagat & Barat, 2017). Dry matter content of silkworm pupa powder was found to be 87.50% (Oso & Iwalaye, 2014) and 92.10% (Ullah, 2016). On the other hand, ash content of silkworm pupa powder ranged from 0.8% to 17.2% (Ahmad et al., 2000; Koundinya & Thangavelu, 2005; Ullah, 2016; Bhagat & Barat, 2017). Fiber content was reported as 14% (Kouřimská &

Adámkov, 2016), 11.20% (Banday et al., 2009), 5.01% (Oso & Iwalaye, 2014), 6.6% (Makkar et al., 2014), 5.55% (Ullah, 2016), and 3.5-4.7% (Shukurova et al., 2021). Observed these changes in constituents of silkworm pupa powder could probably arise from silkworm strains (Eri, Muga, Tasar, Mulberry silkworms), feeding level of larvae, harvesting location and time, seasonal and environmental conditions, storage and drying conditions, de-fattening process of pupa (Oduguwa et al., 2005; Ojewola et al., 2005; Zhou & Han, 2006; Banday et al., 2023). These facts have hypothesized that silkworm pupa powder could have a specialty as a regional product due to changes in its nutritional composition (Meyer-Rochow et al., 2021).

Table 1. Nutrient content and color characteristics of silkworm pupa powder

Çizelge 1. Ipek böceği pupa tozunun besin madde içeriği ve renk özellikleri

<i>Parameters</i>	
Chemical composition	<i>% (Dry matter basis)</i>
Moisture	75.5 ± 0.95
Dry matter	24.5 ± 0.29
Crude protein	58.8 ± 0.39
Ether Extract	20.8 ± 0.93
Crude fiber	4.8 ± 0.08
Ash	5.9 ± 0.04
Organic matter	89.9 ± 0.09
Carbohydrates	0.35 ± 0.08
Color characteristics	
L* (lightness)	44.0 ± 0.69
a* (redness)	8.0 ± 0.10
b* (yellowness)	23.5 ± 0.80

Values are mean ±SD of 3 samples of silkworm pupa powder.

Laying hens have no ability to synthesize the color pigments. Therefore, the diets should be supplemented with ingredients with a rich content of pigments. The pigment value of feed materials is crucial for the color of poultry products to attract consumers (Baiao et al., 1999). The coloration capacity of feed materials could be determined by colorimetric measurement of L^* , a^* , and b^* values. As given in Table 1, the L^* , a^* , and b^* values of silkworm pupa powder were found to be 44.0, 8.0, and 23.5, respectively. According to the color measuring scale, L^* value determines the lightness from black to white (values from 0 to 100), a^* indicates the redness from green (-120) red (+120) and b^* represents the yellowness from blue (-120) to yellow (+120) (Pujari et al., 2010). According to the findings, silkworm pupa powder was found to be a color scale between red and yellow with a mild, darker tint. Therefore, silkworm pupa powder could have potentially beneficial effects for the pigmentation of broiler meat and egg yolk. However, it requires demonstrating the effectiveness of silkworm pupa powder on the color of meat and yolk. Kongsup et al. (2022) found a darker yellowness of skin color in broilers fed with 10% supplementation of silkworm (*Samia ricini*) pupa meal. This finding has been explained by the involvement of different pigments (melanin, pterins, ommatidial chromophores, and especially carotenoids) in insect coloration (Jariyahatthakij et al., 2018), which reflects the meat color. Furthermore, Priyadharshini et al. (2017) highlighted the ameliorative effect of silkworm pupae supplementation for egg yolk color in laying hens.

As seen in Table 2, the most abundant essential amino acids were lysine with an amount of 98.14 g, followed by histidine (18.92 g), phenylalanine (15.10 g), methionine (14.62 g), and isoleucine (14.30 g) in silkworm pupa powder. The higher content of non-essential amino acids was found for glycine (104.03 g), serine (43.09 g), aspartic acid (40.22 g), proline (39.93 g) and arginine (35.36 g), compared to the alanine (15.50 g), cystine (6.66 g), glutamic acid (29.78 g), tyrosine (25.95 g) in silkworm pupa powder.

The silkworm pupa could be accepted as a good and high-quality protein source with a crude protein content between 50% and 60% on a dry matter basis, with a rich content of 17 different amino acids (Rashmi et al., 2023). This amount of crude protein is higher than many animal and plant origin feed materials. Indeed, recently edible insects, also silkworms, have been largely recommended as a protein source for both human and animal nutrition (Wendin & Nyberg, 2021). In poultry nutrition, the limiting amino acids are lysine and methionine (Zhou & Han, 2006). The findings showed that silkworm pupae powder lysine and methionine contents are satisfactory for digestibility and poultry requirements. It is consistent with previous studies performed by Chandrasekharaiah et al. (2002, 2003). Longvah et al. (2011) found the amino acid score and a protein digestibility-corrected amino acid score with values of 100 and 86, respectively, for silkworm pupae.

Table 2. The profile of essential and non-essential amino acids of silkworm pupa powder
Çizelge 2. Ipek böceği pupa tozunun esansiyel ve esansiyel olmayan amino asit profili

<i>Amino acids</i>	<i>Content (mg/g)</i>
Essential amino acids	
Histidine	18.9 ± 1.32
Isoleucine	14.3 ± 0.80
Leucine	8.3 ± 0.15
Lysine	98.1 ± 5.80
Methionine	14.6 ± 1.43
Phenylalanine	15.1 ± 1.38
Threonine	9.3 ± 0.80
Valine	21.4 ± 1.68
Non-essential amino acids	
Alanine	15.5 ± 1.95
Arginine	35.4 ± 3.26
Aspartic acid	40.2 ± 3.64
Cysteine	6.7 ± 0.50
Glutamic acid	29.8 ± 2.45
Glycine	104.0 ± 6.69
Proline	39.9 ± 3.57
Serine	43.1 ± 3.38
Tyrosine	25.9 ± 1.82

Values are mean ±SD of 3 samples of silkworm pupa powder.

When comparing the amino acid profile of silkworm pupa to conventional protein sources, such as soybean meal or fishmeal, it has been demonstrated that comparable differences exist between amino acid levels, especially for lysine and methionine. According to the previous reports, the methionine content was found to be with values of 3.88 g/100 g in silkworm pupa, 3.02 g/100 g in fishmeal and 0.52 g/100 g in soybean meal, whereas the lysine amount was 7.52 g/100 g in silkworm pupa, 4.56 g/100 g in fishmeal and 2.62 g/100 g in soybean meal (Ji et al., 2015; Ullah, 2016; Dawit, 2019). Silkworm pupa powder's lysine content (98.1 mg/g) surpasses fishmeal (45.6 mg/g) by 115%, addressing a key limitation in poultry diets. Previous reports have indicated that silkworm pupa had higher nutritional value (Khatun et al., 2003; Ullah, 2016), and it could partially replace 50% of the traditional protein feed ingredients, mainly soybean or fish meal, in poultry nutrition (Valeria et al., 2015; Heuzé et al., 2017).

In the current study, the fat content was found to be similar to previous findings reported by Yeruva et al. (2023), who found a fat content of 25-30% in dried silkworm pupa with a rich profile of essential fatty acids, including α -linolenic acid, and omega-3 fatty acids. In another study, it has been reported that silkworm pupa powder had a high number of essential oils (49.0%, including α -linolenic acid and linoleic acid), non-essential fatty acids (8.6%), and eicosatetraenoic acid (0.3%; Kwon et al., 2012). The fatty acid profile of silkworm pupa powder is presented in Table 3.

The fatty acids lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, and α -linolenic acid were found as abundant fatty acids in silkworm pupa powder. These fatty acid values were found to be similar to previous reports. The high ω -3: ω -6 ratio (4:1) may enhance egg yolk fatty acid profile and anti-inflammatory responses. The C16:0 content of silkworm pupa was reported as 21.3-28.3% by Pereira et al. (2003), 24.2% by Tomotake et al. (2010), 23.18% by Kumar et al. (2021), and 23.18% by Zhou et al. (2022). The oleic and α -linolenic acid concentrations ranged between 26.00-38.00% and 17.00-38.25%, respectively, in previous studies (Nakasone & Ito, 1967; Kotake-Nara et al., 2002; Pereira et al., 2003; Tomotake et al., 2010; Kumar et al., 2021).

Some of fatty acids are accepted as essential for poultry, due to their inability in synthesizing or converting of fatty acids one to another fatty acid in same series (Enser, 1984). Therefore, the essential fatty acids, especially linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4), have to be added to the diets to obtain optimum growth and a strong immunity. To provide an adequate amount of essential fatty acids in the poultry diet, a minimum supplementation of fat with a level of 10 g/kg is recommended by Leeson & Summers (2005). However, to ensure the confidential amount in commercial poultry nutrition, a fat level of between 20 and 50 g/kg is usually applied with different ingredients, for example, oil or cereal grains, according to their prices and availability (Ravindran et al., 2016).

Table 3. Fatty acids profile of silkworm pupa powder
Çizelge 3. Ipek böceği pupa tozunun yağ asitleri içeriği

<i>Fatty acid</i>	<i>Analyte</i>	<i>(%)</i>
C12:0	Lauric acid	0.05±0.01
C14:0	Myristic acid	0.13±0.01
C16:0	Palmitic acid	20.10±0.14
C16:1	Palmitoleic acid	0.98±0.02
C17:0	Heptadecanoic acid	0.07±0.01
C17:1	Cis-10-heptadecaenoic acid	0.05±0.01
C18:0	Stearic acid	5.92±0.08
C18:1c	Oleic acid	30.15±0.21
C18:2c	Linoleic acid	8.66±0.03
C18:3n6	Gamma-linolenic acid	0.01±0.01
C18:3n3	Alpha-linolenic acid	33.18±0.17
C22:0	Behenic acid	0.13±0.02
C22:2	Cis-13,16-docosadienoic acid	0.08±0.01
Saturated fatty acids (SFA)		26.7±0.05
Mono-unsaturated fatty acids (MUFA)		31.2±0.08
Poly-unsaturated fatty acids (PUFA)		42.0±0.05

Values are mean ±SD of 3 samples of silkworm pupa powder.

The proportion of PUFA was found to be the highest, with a value of 42.0%, then SFA (26.7%) and MUFA (31.2%) in silkworm pupa powder. Similarly, Wei et al. (2009) reported that the silkworm pupa had a higher content of PUFA, especially the C18:3t, comprising approximately 68% of total UFA. The PUFA mainly includes linoleic acid and alpha-linolenic acid, which are classified as essential fatty acids and have to be received through diets due to a lack of the required enzymes for synthesizing PUFA (Orsavova et al., 2015). Hăbeanu et al. (2023) highlighted that silkworm pupa could be accepted as a sustainable, safe, valuable, and alternative source for n-3-rich PUFA.

Table 4. Selected minerals content of silkworm pupae powder
Çizelge 4. Ipek böceği pupa tozunun bazı mineral madde içerikleri

<i>Minerals</i>	<i>Concentration (ppm)</i>
Potassium (K)	83.9±0.6
Phosphorus (P)	63.4±1.8
Magnesium (Mg)	26.2±0.3
Calcium (Ca)	9.80±0.5
Iron (Fe)	0.51±0.02
Sodium (Na)	0.65±0.03
Zinc (Zn)	0.74±0.03

Hăbeanu et al. (2023) noted that the silkworm pupa powder could include many minerals, up to 25 different minerals, which potentially have crucial physiological functions. However, Zhou et al. (2022) have emphasized that the type and amount of minerals could show variations according to the silkworm strain, location, and feeding status of larvae. Koundinya and Thangavelu (2005) indicated that silkworm pupa powder had a rich content of minerals such as calcium, phosphorus, and iron, whereas Rashmi et al. (2023) recently suggested that silkworm pupa had a low content of minerals, accounting for 3-10% minerals on a dry matter basis. Zhou et al. (2022) reported that silkworm pupae represent a substantial source of calcium, potassium, iron, and zinc. Their analysis of the mineral composition of silkworm pupae, expressed on a dry matter basis, revealed the following concentrations: phosphorus (474 mg), magnesium (207 mg), calcium (158 mg), iron (26 mg), zinc (23 mg), chromium (1.69 mg), manganese (0.71 mg), and copper (0.15 mg).

CONCLUSION

In conclusion, our results indicate that the silkworm pupa powder, a significant byproduct of the silk industry, is a nutrient-rich. Moreover, it has an excellent profile in terms of nutrients such as protein, fat, amino acids, and fatty acid composition. It is a source rich in limiting amino acids such as methionine and lysine, with an essential amino acid profile. Additionally, it is a rich source of monounsaturated and polyunsaturated fatty acids, which are

crucial for overall health functions. This nutritional profile makes the silkworm pupa powder a valuable and versatile feedstuff that could be used in animal feed as an alternative to traditional protein sources like soybean meal. Additionally, due to their sustainability, environmental friendliness, and nutritional value, they are becoming increasingly popular as an animal-based protein source. Due to this potential, the sericulture industry should continue to be developed. Thus, the use of silkworm pupa powder in animal feed will reduce dependence on traditional protein sources. At this point, it could reduce feed costs and potentially minimize the negative environmental impact. In summary, silkworm pupa powder is abundant in protein, environmentally friendly, and a low-cost animal feed ingredient.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

Authors declare that there is no conflict of interest.

REFERENCES

- Ahmad, S., Hasan, M.U., Sageer, M., & Sahi, S.T. (2020). Impact of bio-accumulated cadmium in *Bombyx mori* L. reared on mulberry plants grown in heavy metal impregnated soils in Faisalabad, Pakistan. *African Entomology*, 28, 406–414.
- AOAC, 2005. Association of Official Analytical Chemists, Official Methods of Analysis. 18th ed. AOAC, Maryland, USA.
- Baiao, N.C., Mendez, J., Mateos, J., García, M., & Mateos, G.G. (1999). Pigmenting efficacy of several oxycarotenoids on egg yolk. *Journal of Applied Poultry Research*, 8, 472–479.
- Banday, M.T., Bhat, G.A., Shahnazand, S., & Bhakat, M. (2009). Influence of feeding processed silkworm pupae meal on the performance of broiler chicken. *Indian Journal of Animal Nutrition*, 26, 292–295.
- Banday, M.T., Sheikh, A., Islam, U., Sheikh, H.H., Farhat, I., Qadri, M.E., Sahfi, H.S.A.W. S., Taia, A., Abd, E.M., Heba, M., Salem, A.E., Taha, M.T.E.S, & Mohamed, E.A.E.H. (2023). The use of silkworm pupae (*Bombyx mori*) meal as an alternative protein source for poultry. *World's Poultry Science Journal*, 79, 119-134.
- Bhagat, R.P., & Barat, S. (2017). Effect of artificial feed on survival and growth of rainbow trout, *oncorhynchusmykiss* (Walbaum) during exogenous feeding in the raceways of Kathmandu, Nepal. *Turkish Journal of Fisheries and Aquatic Sciences*, 5, 149–156.
- Chandrasekharaiah, M., Sampath, K.T., & Thulasi, A. (2002). Rumen protein degradability of certain feedstuffs in cattle determined by nylon bag technique. *Indian Journal of Dairy & Biosciences*, 13, 18–21.
- Chandrasekharaiah, M., Sampath, K.T., Thulasi, A. (2003). Essential amino acid content of commonly used feedstuffs. *Indian Journal of Animal Sciences*, 73, 305–307.
- Dawit, M.Z. (2019). Nutritional evaluation of insects pupae-larva and its utilization in poultry compound feed. *Open Agriculture Journal*, 14, 1–8.
- Egounlety, M. & Aworh, D.C. (1990). Production and physico-chemical properties of Tempeh fortified maize based weaning food. *Nigerian Food Journal*, 70, 92-102.
- Eknayake, S., Jansz, E.R., & Nair, B.M. (1999). Proximate composition, mineral and amino acid content of mature *Canavania gladiata* seeds. *Food Chemistry*, 66, 115–119.
- El-Sabrou, K., Khalifah, A., Mishra, B. (2023). Application of botanical products as nutraceutical feed additives for improving poultry health and production. *Veterinary World*, 16, 369.
- Enser, M., (1984). The chemistry, biochemistry and nutritional importance of animal fats, in: Wiseman, J. (Ed.), *Fats in Animal Nutrition*, Butterworths Publishing, London, pp. 23–51.
- Folch, J., Lees, M., & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Gong, Y., Li, L., Gong, D., Yin, H., & Zhang, J. (2016). Biomolecular evidence of silk from 8,500 years ago. *PLoS ONE*, 11, e0168042.
- Hăbeanu, M., Gheorghe, A., & Mihalcea, T. (2023). Nutritional value of silkworm pupae (*Bombyx mori*) with emphases on fatty acids profile and their potential applications for humans and animals. *Insects*, 14, 254.
- Heuzé, V., Tran, G., Giger-Reverdin, S., & Lebas, F. (2017). Silkworm pupae meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. <https://feedipedia.org/node/199>
- Jariyahatthakij, P., Chomtee, B., Poeikhampha, T., Loongyai, W., & Bunchasak, C. (2018). Effects of adding methionine in low-protein diet and subsequently fed low-energy diet on productive performance, blood chemical profile, and lipid metabolism related gene expression of broiler chickens. *Poultry Science*, 97, 2021–2033.
- Ji, H., Zhang, J.L., Huang, J.Q., Cheng, X.F., & Liu, C. (2015). Effect of replacement of dietary fishmeal with

- silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in Juvenile Carp (*Cyprinus Carpio* Var. Jain). *Aquaculture Research*, 46, 1209–1221.
- Jintasataporn, O. (2012). Production performance of broiler chickens fed with silkworm pupa (*Bombyx mori*). *Journal of Agricultural Sciences and Technology*, 2, 505–510.
- Khalifah, A., Abdalla, S., Rageb, M., Maruccio, L., Ciani, F., & El-Sabrou, K. (2023). Could insect products provide a safe and sustainable feed alternative for the poultry industry? A comprehensive review. *Animals*, 13, 1534.
- Khatun, R., Howlider, M.A.R., Rahman, M.M., & Hasanuzzaman, M. (2003). Replacement of fish meal by silkworm pupae in broiler diets. *Pakistan Journal of Biological Sciences*, 6, 955–958.
- Kongsup, P., Lertjirakul, S., Chotimanothum, B., Chundang, P., & Kovitvadhi, A. (2022). Effects of eri silkworm (*Samia ricini*) pupae inclusion in broiler diets on growth performances, health, carcass characteristics and meat quality. *Animal Bioscience*, 35, 711–720.
- Kotake-Nara, E., Yamamoto, K., Nozawa, M., Miyashita, K., & Murakami, T. (2002). Lipid profiles and oxidative stability of silkworm pupal oil. *Journal of Oleo Science*, 51, 681–690.
- Koundinya, P.R., & Thangavelu, K. (2005). Silk proteins in biomedical research. *Indian Silk*, 43, 5–8.
- Kouřimská, L., & Adámek, A. (2016). Nutritional and sensory quality of edible insects. *NFS Journal*, 4, 22–26.
- Kowalska, D., Gugolek, A., & Strychalski, J. (2020). Evaluation of slaughter parameters and meat quality of rabbits fed diets with silkworm pupae and mealworm larvae meals. *Annals of Animal Science*, 20, 551–564.
- Kumar, R.V., Srivastava, D., Kumar, U., Kumar, M., & Singh, P. (2021). Bioprospecting of omega-3 fatty acid from silkworm pupal oil: From molecular mechanism to biological activities. *Journal of Biologically Active Products from Nature*, 10, 495–506.
- Kwon, M.U.G., Kim, D.S., Lee, J.H., Park, S.W., Choo, Y.K., Han, Y.S., Kim, J.S., Hwang, K.A., Kinarm, K.O., & Kiung, K. (2012). Isolation and analysis of natural compounds from silkworm pupae and effect of its extracts on alcohol detoxification. *Entomological Research*, 42, 55–62.
- Lamberti, C., Gai, F., Cirrincione, S., Giribaldi, M., Purrotti, M., Manfredi, M., Marengo, E., Sicuro, B., Saviane, A., Cappellozza, S., Giuffrida, M.G., & Cavallarin, L. (2019). Investigation of the protein profile of silkworm (*Bombyx mori*) pupae reared on a well-calibrated artificial diet compared to mulberry leaf diet. *PeerJ*, 7, e6723.
- Leeson, S., & Summers, J.D. (2005). *Commercial Poultry Nutrition*, third ed. Publ. Univ. Books, Guelph, Ontario, Canada.
- Limeneh, D.Y., Tesfaye, T., Ayele, M., Husien, N.M., Ferede, E., Haile, A., Mengie, W., Abuhay, A., Gelebo, G.G., Gibril, M., & Kong, F. (2022). A comprehensive review on utilization of slaughterhouse by-product: current status and prospect. *Sustainability*, 14, 6469.
- Longvah, T., Mangthya, K., & Ramulu, P. (2011). Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricini*) prepupae and pupae. *Food Chemistry*, 128, 400–403.
- Makkar, H.P.S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 197, 1–33.
- Meyer-Rochow, V. B., & Jung, C. (2020). Insects used as food and feed: Isn't that what we all need? *Foods*, 9, 1003.
- Meyer-Rochow, V.B., Gahukar, R.T., Ghosh, S., & Jung, C. (2021). Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stage, gender, diet, and processing method. *Foods*, 10, 1036.
- Mishra, N., Hazarika, N.C., Narain, K., & Mahanta, J. (2003). Nutritive value of nonmulberry and mulberry Silkworm pupae and consumption pattern in Assam, India. *Nutrition Research*, 23, 1303–1311.
- Nakasone, S., & Ito, T. (1967). Fatty acid composition of the silkworm, *Bombyx mori* L. *Journal of Insect Physiology*, 13, 1237–1246.
- Nimbalkar, M.S., Pai, S.R., Pawar, N.V., Oulkar, D., & Dixit, G.B. 2012. Free amino acid profiling in grain Amaranth using LC-MS/MS. *Food Chemistry*, 134, 2565–2569.
- Nisha, S.N., Jothi, B.A., & Geetha, B. (2014). Growth performance and haematological parameters of the ornamental fish, (*Maylandiaestherae*), fed varying inclusion of silkworm pupae meal. *Advances in Biological Research*, 8, 268–273.
- Oduguwa, O.O., Fanimo, A.O., & Oso, M.J. (2005). Effect of replacing dietary fish meal or soybean with shrimp waste meal on the performance of laying hens." *Niger. Nigerian Journal of Animal Production*, 32, 224–232.
- Ojewola, G.S., Okopyo, F.C., & Ukoha, O.A. (2005). Comparative utilisation of three animal protein sources by broiler chickens. *International Journal of Poultry Science*, 4, 462–467.
- Orsavova, J., Misurcova, L., Ambrozova, J.V., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Sciences*, 16, 12871–12890.
- Oso, J.A., & Iwalaye, O.A. (2014). Growth performance and nutrient utilization efficiency of *Clarias gariepinus* juveniles fed *Bombyx mori* (mulberry silkworm) meal as a partial replacement for fishmeal. *British Journal of Applied Science & Technology*, 4, 3805–3812.

- Patil, S.R., Amena, S., Vikas, A., Rahul, P., Jagadeesh, K., & Praveen, K. (2013). Utilization of silkworm litter and pupal waste an eco-friendly approach for mass production of *Bacillus thuringiensis*. *Bioresource Technology*, *131*, 545–547.
- Pereira, N.R., Ferrarese-Filho, O., Matsushita, M., & de Souza, N.E. (2003). Proximate composition and fatty acid profile of *Bombyx mori* L. chrysalis toast. *Journal of Food Composition and Analysis*, *16*, 451–457.
- Priyadharshini, P., Joncy, M.A., & Saratha, M. (2017). Industrial utilization of silkworm pupae—A review. *International Journal of Academic Multidisciplinary Research*, *5*, 62–70.
- Pujari, J., Pushpalatha, S.N., & Desai, P.D. (2010). Content-based image retrieval using color and shape descriptors. In: Proceedings of the 2010 International Conference on Signal and Image Processing, ICSIP 2010. pp. 239–242.
- Rashmi, K.M., Chandrasekharaiah, M., Soren, N.M., Prasad, K.S., David, C.G., Thirupathaiah, Y., & Shivaprasad, V. (2023). Silkworm pupae meal: An alternative protein source for livestock. *Pharma Innovation Journal*, *12*, 3691–3696.
- Ravindran, V., Tancharoenrat, P., Zaefarian, F., & Ravindran, G. (2016). Fats in poultry nutrition: Digestive physiology and factors influencing their utilization. *Animal Feed Science and Technology*, *213*, 1–21.
- Sajid, Q.U.A., Asghar, M.U., Tariq, H., Wilk, M., & Platek, A. (2023). Insect meal as an alternative to protein concentrates in poultry nutrition with future perspectives (An updated review). *Agriculture*, *13*, 1239.
- Sharma, A., Gupta, R., Sharma, P., Duwa, A.K., & Bandral, R. (2022). Silkworm as an edible insect: A review. *Pharma Innovation Journal*, *11*, 1667–1674.
- Shukurova, Z.Y., Khalilov, Z.M., & Shukurlu, I.H. (2021). Study of the organic and mineral composition of living pupae of the wild silkworm *Saturnia pyri* for use as food additives. *International Journal of Industrial Entomology*, *43*, 52–58.
- Tamuly, B., Manimegalai, S., Chitra, P., Priyadharshini, P., & Baranidharan, K. (2024). Silkworm pupae meal: A breakthrough for conventional poultry feed. *Archives of Current Research International* *24*(7), 111–22.
- Thirupathaiah, Y., Chandel, A. K., & Sivaprasad, V. (2018). Potential applications of enzymes in sericulture, in Singh, O.V., Chandel, A.K. (Eds.), *Sustainable Biotechnology- Enzymatic Resources of Renewable Energy*. Springer Nature Publishing, pp. 463–472.
- Tomotake, H., Katagiri, M., & Yamato, M. (2010). Silkworm pupae (*Bombyx mori*) are new sources of high quality protein and lipid. *Journal of Nutritional Science and Vitaminology*, *56*, 446–448.
- TÜİK, (2024). <https://data.tuik.gov.tr/Bulten/Index?p=Animal-Production-Statistics-2023-49681>.
- Uguz, S., & Sozcu, A. (2023). Nutritional Value of Microalgae and Cyanobacteria Produced with Batch and Continuous Cultivation: Potential Use as Feed Material in Poultry Nutrition. *Animals*, *13*, 3431.
- Ullah, R. (2016). Replacement of soybean meal with silkworm meal (*Bombyx mori*) in poultry ration. PhD. thesis in poultry science, Peshawar: The University of Agriculture.
- Valerie, H., Tran, G., Giger-Reverdin, S., & Lebas, F. (2015). Silkworm Pupae Meal. Feedipedia, a Programme by INRA, CIRAD, AFZ and FAO. 2015. Available online: <http://www.feedipedia.org/node/199>.
- Wei, Z.J., Liao, A.M., Zhang, H.X., Liu, J., & Jiang, S.T. (2009). Optimization of supercritical carbon dioxide extraction of silkworm pupal oil applying the response surface methodology. *Bioresource Technology*, *100*, 4214–4219.
- Wendin, K., & Nyberg, M. (2021). Factors influencing consumer perception and acceptability of insect-based foods. *Current Opinion in Food Science*, *40*, 67–71.
- Yeruva, T., Jayaram, H., Aurade, R., Shunmugam, M.M., Shinde, V.S., Venkatesharao, S.R.B., & Azhiyakathu, M.J. (2023). Profiling of nutrients and bioactive compounds in the pupae of silkworm *Bombyx Mori*. *Food Chemistry Advances*, *3*, 100382.
- Zhou, J., & Han, D. (2006). Proximate amino acid and mineral composition of pupae of the silkworm *antheraea pernyi* in China. *Journal of Food Composition and Analysis*, *19*, 850–853.
- Zhou, Y., Zhou, S., Duan, H., Wang, J., & Yan, W. (2022). Silkworm pupae: A functional food with health benefits for humans. *Foods*, *11*, 1594.
- Zotte, A.D., Singh, Y., Zsedely, E., Contiero, B., Palumbo, B., & Cullere, M. (2024). Dietary inclusion of defatted silkworm (*Bombyx mori* L.) pupa meal in broiler chickens: phase feeding effects on nutritional and sensory meat quality. *Poultry Science*, *103*, 103812.