

Nutritional Value and Antioxidant Potential of Silkworm Pupae and Soybean in Broiler Diets

Ioannis Panitsidis¹, Panagiotis Sakkas¹, Konstantina Vasilopoulou¹,
Georgios Papadopoulos², Vasilios Tsiouris³, Zissis Tzikas⁴, Dimitrios Kyrtsoudis⁴,
Ioannis Mitsopoulos⁴, Vasileios Bampidis⁴, Ilias Giannenas^{1*}

¹ Aristotle University of Thessaloniki, School of Veterinary Medicine, Laboratory of Nutrition, 54124 Thessaloniki, Greece.

² Aristotle University of Thessaloniki, School of Veterinary Medicine, Laboratory of Animal Husbandry, 54124 Thessaloniki, Greece.

³ Aristotle University of Thessaloniki, School of Veterinary Medicine, Clinic of Farm Animals, Unit of Avian Medicine, Thessaloniki, Greece.

⁴ International Hellenic University, School of Geotechnical Sciences, Department of Agriculture, 57400 Sindos Thessaloniki, Greece.

Abstract

Efforts to reduce dependence on soy-based feed ingredients have increased the search for sustainable protein sources for poultry nutrition. Silkworm (*Bombyx mori*) pupae, a byproduct of sericulture, represent a promising alternative due to their high nutritional value. This study compared whole-fat silkworm pupae and whole-fat soybeans in terms of chemical composition, fatty acid profile, and antioxidant status as a preliminary assessment of their potential use in broiler diets. On a dry matter basis, silkworm pupae contained higher crude protein (53.5% vs. 39.2%) and crude fat (25.8% vs. 20.1%) than soybean, whereas soybean showed higher ash (5.5% vs. 4.3%) and crude fibre (5.8% vs. 3.3%). Silkworm pupae also exhibited a more favourable fatty acid profile, with higher α -linolenic acid (30.46% vs. 5.37%) and total ω 3 fatty acids (30.53% vs. 5.39%), resulting in a markedly lower ω 6/ ω 3 ratio (0.19 vs. 9.53) ($P < 0.001$). Soybean contained higher linoleic acid, ω 6 fatty acids, and total PUFA ($P < 0.001$). Water-soluble antioxidant capacity was higher in silkworm pupae, while other antioxidant parameters did not differ. Overall, silkworm pupae show strong potential as a sustainable alternative feed ingredient for broiler nutrition.

Keywords: alternative protein sources, antioxidant properties, chemical composition, insect-based feed, ω 3 fatty acids

1. Introduction

The increasing demand for sustainable and cost-effective protein sources in poultry nutrition has intensified the search for alternatives to conventional soy-based feed ingredients. Although soybean remains one of the most widely used protein sources in poultry diets due to its favourable nutritional profile, its production and

supply are associated with environmental, economic, and value-chain sustainability concerns. [1,2] These challenges have encouraged research into novel feed materials that can partially or totally replace soybean while maintaining nutritional quality and supporting animal performance. [2]

Among the alternative protein sources currently under investigation, insect-derived ingredients have attracted considerable attention because of their high protein content, favourable lipid composition, and lower environmental footprint. In poultry nutrition, insect meals have been described as promising

* Corresponding author: Ilias Giannenas, +30 2310 999937, igiannenas@vet.auth.gr

substitutes for soybean meal and fish meal, with the potential to support growth performance and product quality without adverse effects. [3] In this context, silkworm pupae (*Bombyx mori*), a byproduct of the sericulture industry, represent a promising candidate for use in poultry feeding. [4] In addition to being rich in protein and fat, silkworm pupae also contain biologically valuable fatty acids, particularly α -linolenic acid, as well as several bioactive compounds, including phenolic acids and flavonoids, which may contribute to the nutritional and functional value of poultry diets. [4,5]

Before such an ingredient can be effectively incorporated into broiler nutrition, its chemical and functional characteristics must be thoroughly evaluated in comparison with established conventional feedstuffs. Therefore, the present study was conducted to compare whole-fat silkworm pupae and whole-fat soybeans in terms of chemical composition, fatty acid profile, and antioxidant status, in order to assess the potential of silkworm pupae as a sustainable alternative feed ingredient for broiler chickens.

2. Materials and methods

2.1 Samples

Whole-fat silkworm pupae (*Bombyx mori*) and whole-fat soybeans were used as the experimental materials in the present study. Six independent samples of silkworm pupae and six independent samples of whole-fat soybeans were analysed in order to compare their chemical composition, fatty acid profile, and antioxidant properties. Prior to analysis, all samples were ground and homogenized to obtain representative and uniform analytical material.

2.2 Chemical composition analysis

The proximate composition of whole-fat silkworm pupae and whole-fat soybeans was determined on a dry matter basis according to the Weende analysis system and standard AOAC procedures [6]. Crude protein was measured by the Kjeldahl method, ether extract by Soxhlet extraction, crude fibre using a Fibertherm apparatus, and ash by combustion in a muffle furnace. Gross energy was determined using a bomb calorimeter and expressed as kcal/kg dry matter. Nitrogen-free extracts were calculated by difference as: $100 - (\text{crude protein} + \text{ether extract} + \text{crude fibre} + \text{ash})$.

2.3 Fatty acid analysis

Fatty acid methyl esters (FAMES) were prepared according to the cold methanolic potassium hydroxide transesterification method of the International Olive Council [7], with slight modifications. Total lipids were first extracted from each feed ingredient by Soxhlet extraction. Subsequently, 0.1 g of the extracted fat was mixed with 2 mL of hexane and vortexed thoroughly. Then, 0.1 mL of methanolic potassium hydroxide was added, and the mixture was vortexed again. Thereafter, 2 mL of sodium chloride solution was added to facilitate phase separation. The upper hexane phase containing the FAMES was collected and transferred into GC vials for subsequent gas chromatographic analysis. Fatty acid analysis was performed by gas chromatography equipped with a flame ionization detector (GC-FID). Individual fatty acids were identified by comparing their retention times with those of a standard FAME mixture, and the results were expressed as percentage of total identified fatty acids. In addition, the sums of saturated fatty acids (Σ SFA), monounsaturated fatty acids (Σ MUFA), polyunsaturated fatty acids (Σ PUFA), ω 3 fatty acids, and ω 6 fatty acids, as well as the ω 6/ ω 3 ratio, were calculated.

2.4 Antioxidant assays

Total antioxidant capacity (TAC) was determined following the phosphomolybdate method [8]. Briefly, 100 μ L of sample extract was mixed with 2 mL of phosphomolybdate reagent and vortexed thoroughly. The reaction mixture was incubated in a water bath at 95 °C for 90 min. After cooling to room temperature, absorbance was recorded at 695 nm. Quantification was performed using an ascorbic acid standard curve, and results were expressed as μ g ascorbic acid equivalents (AAE) per g of dry matter.

Total phenolic content (TPC) was determined for the feed additives using a Folin–Ciocalteu-based procedure according to Singleton and Rossi [9], with slight modifications. Briefly, 0.02 mL of extract was mixed with 2.5 mL of distilled water and 0.4 mL of Folin–Ciocalteu reagent, vortexed, and incubated for 8 min. Subsequently, 0.5 mL of 7% sodium carbonate (Na_2CO_3) solution was added, and the mixture was vortexed again and incubated in the dark at room temperature for 2 h. Absorbance was measured at 750 nm using a spectrophotometer, and results were expressed as

µg gallic acid equivalents (GAE) per g of dry matter.

Trolox equivalent antioxidant capacity (TEAC) of the water-soluble and liposoluble fractions was determined according to Di Mattia et al [10]. Water-soluble and liposoluble extracts were prepared separately. For the liposoluble fraction, ground samples were defatted by three cycles of hexane washing, and the combined supernatants were recovered as the liposoluble extract. The lipid-free residue was then used for the extraction of the water-soluble fraction with distilled water under dark conditions, followed by centrifugation and filtration. The radical scavenging activity was measured by the ABTS radical cation decolorization assay. The bleaching rate of ABTS^{•+} in the presence of the sample was monitored at 734 nm using a spectrophotometer. The ABTS^{•+} stock solution was diluted either in water or in ethanol to an absorbance of 0.70 ± 0.02 for the analysis of the aqueous and apolar extracts, respectively. The reaction was carried out at 30 °C, and the decolorization after 5 min was used as the measure of antioxidant activity. Results were expressed as mmol Trolox equivalents (TE) per 100 g of sample for both the water-soluble (TEAC_{aq}) and liposoluble (TEAC_{lipo}) fractions.

3. Results and discussion

3.1 Chemical composition

The proximate composition analysis (Table 1) showed that whole-fat *Bombyx mori* pupae contained higher crude protein (53.5%), crude fat (25.8%), and gross energy (6976.6 kcal/kg DM) than whole-fat soybean (39.2%, 20.1%, and 5567.4 kcal/kg DM, respectively), whereas soybean exhibited higher ash (5.5% vs. 4.3%) and crude fibre (5.8% vs. 3.3%). These results indicate that silkworm pupae are a more concentrated source of protein and energy, mainly due to their higher lipid content, while soybean contributes relatively more mineral matter and fibre. The high protein- and lipid-density observed in *B. mori* is in agreement with previous studies describing silkworm pupae as a nutritionally rich by-product, although their composition may vary according to strain, rearing medium, developmental stage, and processing conditions [11–13]. Likewise, the soybean values obtained in the present study are consistent with the literature, in which soybean is

generally described as a protein- and oil-rich feed ingredient containing approximately 35% protein and 20% oil on a dry matter basis [14]. The higher crude fibre content recorded in soybean is also consistent with previous reports showing that soybean seeds contain substantial amounts of dietary fibre, predominantly in the insoluble fraction, derived mainly from plant cell-wall polysaccharides [15]. Overall, compared with soybean, whole-fat silkworm pupae appear to be nutritionally advantageous when a dense source of protein and energy is required, whereas soybean remains relatively richer in ash and fibre.

3.2 Fatty acid analysis

The fatty acid profile differed markedly between whole-fat *Bombyx mori* pupae and whole-fat soybean (Table 2). Silkworm pupae contained significantly higher proportions of palmitic acid (22.92% vs. 12.57%), palmitoleic acid (1.18% vs. 0.02%), oleic acid (28.16% vs. 24.53%), and especially α -linolenic acid (30.46% vs. 5.37%), whereas soybean was characterized by a much higher linoleic acid content (51.23% vs. 5.81%). As a result, silkworm pupae showed higher total saturated fatty acids (Σ SFA; 28.98% vs. 18.49%) and monounsaturated fatty acids (Σ MUFA; 34.63% vs. 24.84%), while soybean had a higher total polyunsaturated fatty acid fraction (Σ PUFA; 56.67% vs. 36.39%). However, this difference in Σ PUFA was primarily due to the predominance of ω 6 fatty acids in soybean, whereas silkworm pupae were distinguished by their markedly higher ω 3 content (30.53% vs. 5.39%) and much lower ω 6/ ω 3 ratio (0.19 vs. 9.53). Minor fatty acids such as myristic, pentadecanoic, heptadecanoic, and arachidic acid were present at low levels and contributed little to the overall differences between the two feed ingredients.

The fatty acid pattern observed in silkworm pupae is consistent with previous reports showing that *B. mori* oil is mainly characterized by α -linolenic, oleic, and palmitic acids. Pereira et al. [12] reported that silkworm chrysalis toast contained 24.4% α -linolenic acid and displayed a low n-6/n-3 ratio of 0.30, while Tangsanthatkun et al. [16] found 24.85% α -linolenic acid, 26.0% palmitic acid, and only 4.25% linoleic acid in silkworm pupae oil. Likewise, Chieco et al. [13] showed that the fatty acid composition of silkworm pupae is strongly influenced by the feeding substrate, with mulberry leaf feeding promoting greater linolenic

acid deposition and a lower n-6/n-3 ratio. Therefore, the high α -linolenic acid concentration and the very low ω 6/ ω 3 ratio recorded in the present study confirm that silkworm pupae represent a distinctly ω 3-rich lipid source compared with conventional plant feed ingredients.

In contrast, the soybean fatty acid profile obtained in the present study corresponds closely to the typical composition of commodity soybean oil, in which linoleic acid is the dominant fatty acid, followed by oleic, palmitic, linolenic, and stearic acids. Clemente and Cahoon [17] described average soybean oil values of approximately 11–13% palmitic acid, 4% stearic acid, 20–23% oleic acid, 54–55% linoleic acid, and 8–13% linolenic acid, which are in close agreement with the values observed here. Similarly, Abdelghany et al. [18] confirmed that palmitic, stearic, oleic, linoleic, and linolenic acids are the principal fatty acids in soybean, although their relative proportions can vary considerably depending on genotype and growing conditions. Overall, the present results indicate that soybean provides a lipid fraction dominated by ω 6 polyunsaturated fatty acids, whereas silkworm pupae provide a more ω 3-enriched fatty acid profile, suggesting that the latter may offer additional value when the objective is to enrich poultry diets with n-3 precursor fatty acids.

3.3 Antioxidant assays

The antioxidant evaluation revealed clear differences between silkworm pupae and soybean depending on the analytical parameter considered. TEAC_{aq} was significantly higher in silkworm pupae than in soybean (24.68 vs. 21.43 mmol TE/100 g sample; $P = 0.003$), whereas no significant differences were observed in liposoluble antioxidant capacity (TEAC_{lipo}; 0.57 vs. 0.63 mmol TE/100 g sample; $P = 0.128$), total antioxidant capacity (TAC; 93.7 vs. 96.89 μ g AAE/g DM; $P = 0.070$), or total phenolic content (TPC; 72.70 vs. 64.99 μ g GAE/g DM; $P = 0.304$). These findings indicate that silkworm pupae possess a stronger antioxidant activity in the aqueous fraction, while both feed ingredients show broadly comparable antioxidant potential in the lipophilic fraction and similar overall phenolic

content. The absence of significant differences in TPC, despite the higher TEAC_{aq} value of silkworm pupae, suggests that compounds other than phenolics may also contribute substantially to the antioxidant activity of the pupal matrix, such as peptides, vitamins, or other water-soluble bioactive constituents [10,19,20].

The higher TEAC_{aq} value observed in silkworm pupae is consistent with previous reports describing *Bombyx mori* as a source of bioactive compounds with antioxidant properties. Di Mattia et al. [10] reported that silkworm showed one of the highest TEAC values among the tested edible insects in the water-soluble fraction, while also exhibiting one of the highest values in the liposoluble fraction. Likewise, Yeruva et al. [19] demonstrated that silkworm pupae contain several phenolic acids and flavonoids, whereas Zhou et al. [20] emphasized that silkworm pupae contain antioxidant peptides, unsaturated fatty acids, and phenolic compounds that may act synergistically. Therefore, the stronger aqueous antioxidant response measured in the present study may reasonably be related to the broader spectrum of hydrophilic antioxidant constituents present in the pupae [10,19,20].

In contrast, soybean is also well recognized as a source of antioxidant compounds, particularly phenolics and isoflavones. Tyug et al. [21] reported measurable TEAC and TPC in soybean-derived materials, while Król-Grzymała and Amarowicz [22] showed that soybean seed extracts possess substantial free-radical scavenging activity closely associated with their phenolic composition. In addition, Lee et al. [23] demonstrated that soybean isoflavones and their glycosides exhibit antioxidant activity, although their potency differs among compounds. Thus, the lack of significant differences between silkworm pupae and soybean in TEAC_{lipo}, TAC, and TPC suggests that both materials can be considered sources of antioxidant constituents, but with a different distribution between the water-soluble and lipid-soluble fractions. Overall, the present results support the view that silkworm pupae may offer an antioxidant advantage over soybean mainly through the hydrophilic fraction, while maintaining a broadly comparable total antioxidant and phenolic profile.

Table 1. Proximate analysis on dry matter basis

Antioxidant parameter	Units	Silkworm Pupae	Soybean
Crude Protein	%	53.5	39.2
Gross Energy	kcal/kg	6976.6	5567.4
Crude Fat	%	25.8	20.1
Ash	%	4.3	5.5
Crude Fibre	%	3.3	5.8

Table 2. Fatty acid analysis as of %

	Silkworm Pupae	Soybean	P
C14:0 (Myristic)	0.20±0.01	0.16±0.03	0.137
C15:0 (Pentadecanoic)	0.04±0.01	0.04±0.01	0.157
C16:0 (Palmitic)	22.92±0.14	12.57±0.54	<0.001
C16:1 (Palmitoleic)	1.18±0.01	0.20±0.02	<0.001
C17:0 (Heptadecanoic)	0.13±0.01	0.12±0.01	0.249
C17:1 (cis-10-Heptadecenoic)	0.22±0.05	0.09±0.01	0.014
C18:0 (Stearic)	5.51±0.06	5.22±0.17	0.054
C18:1n9t (Elaidic)	5.07±0.21	0.02±0.01	<0.001
C18:1n9c (Oleic)	28.16±0.61	24.53±0.70	0.002
C18:2n6t (Linolelaidic)	0.05±0.01	0.02±0.01	0.031
C18:2n6c (Linoleic)	5.81±0.21	51.26±0.11	<0.001
C20:0 (Arachidic)	0.20±0.01	0.37±0.12	0.065
C18:3n3 (α-Linolenic)	30.46±0.42	5.37±0.23	<0.001
C22:6n3 (cis-4,7,10,13,16,19-Docosahexaenoic)	0.07±0.01	0.02±0.01	0.001
Σ SFA	28.98±0.07	18.49±0.62	<0.001
Σ MUFA	34.63±0.35	24.84±0.68	<0.001
Σ PUFA	36.39±0.29	56.67±0.26	<0.001
ω3	30.53±0.42	5.39±0.23	<0.001
ω6	5.86±0.21	51.28±0.11	<0.001
ω6/ω3	0.19±0.01	9.53±0.40	<0.001

Table 3. Antioxidant assays

Antioxidant parameter	Units	Silkworm Pupae	Soybean	P
TEAC _{ipo}	mmol TE / 100 g sample	0.57±0.07	0.63±0.05	0.128
TEAC _{aq}	mmol TE / 100 g sample	24.68±1.56	21.43±1.25	0.003
TAC	μg AAE / g DM	93.74±2.48	96.89±2.88	0.070
TPC	μg GAE / g DM	72.70±12.79	64.99±11.86	0.304

4. Conclusions

In conclusion, whole-fat *Bombyx mori* pupae exhibited a favourable nutritional and functional profile compared with whole-fat soybean, supporting their potential as an alternative feed ingredient for broiler nutrition. Silkworm pupae provided higher crude protein, crude fat, and gross energy, together with a distinctive fatty acid composition characterized by elevated α-linolenic acid and ω3 fatty acids and a markedly lower ω6/ω3 ratio. In addition, silkworm pupae showed significantly greater water-soluble antioxidant capacity, while total antioxidant capacity, total phenolic content, and liposoluble antioxidant

capacity were comparable to those of soybean. Overall, these findings indicate that silkworm pupae combine high nutritional density with a more favorable ω3-enriched lipid profile and relevant antioxidant properties, highlighting their promise as a sustainable alternative to conventional soy-based feed ingredients. Further in vivo studies are warranted to determine their effects on broiler performance, health status, and product quality.

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