

NUTRITIONAL QUALITY AND VALORIZATION OF SILKWORM PUPAE (*BOMBYX MORI*. L) IN POULTRY DIETS – REVIEW

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Abstract

The development of the poultry industry increased the request for conventional feed ingredients (soybean meal and oil, fish meal), leading to higher costs and environmental pressure. In this context, there is an increased interest in finding sustainable alternatives such as protein-rich insect species as innovative ingredients in poultry diets. Sericulture produces a lot of by-products, including silkworm pupae (SWP), a valuable and less expensive alternative protein source. Therefore, this review firstly presents the nutritional value of full-fat and defatted SWP as potential feed ingredients for poultry. The proximate composition of SWP consists of 37.8–82.8% crude protein (with a higher digestibility ranging from 76% to 98%), 2–36.2% crude fat, 1–9.5% crude fibre, 1–10.6% ash, and 21.9–25.8 MJ/kg gross energy. SWP has high-quality protein and lipids due to their essential amino acids (40–48% of total amino acids content) and fatty acids profiles (especially alpha-linolenic, 20–38% and linoleic, 5.5–21%). The vitamins, minerals, and other bioactive compounds (i.e., 1-Deoxynojirimycin, 1-DNJ) complete their nutritional value. SWP also contains a polysaccharide, chitin (1.5–3.4% of dry matter), with structural function in organisms. Secondly, we reported the effects of using SWP in broilers, laying hens and quails diets, focusing on productive performance, health status and quality of poultry products. The findings demonstrated that SWP could partially replace conventional ingredients in poultry diets, maintaining appropriate performance and/or improving meat or egg qualities.

Keywords: *nutrients, silkworm pupae, poultry diets*

1. INTRODUCTION

Statistics show that in 2021, global poultry meat production was 135.4 million tons, approximately 38% of global meat production (FAO, 2021). The world population is expected to increase to more than 9 billion by 2050; thus, an increase in meat demand by 75% is also preconised (Kim et al., 2019).

The increase in global meat demand, on the one hand, and the development of the poultry industry, on the other hand, increase the request for conventional feed ingredients (soy grains and other oilseed sources), leading to higher costs and forward environmental pressure. It is known that protein is the most expensive and limiting component in feed formulations, and the cost of conventional protein sources has increased considerably due to productivity factors and competition between plant-based human and animal feeds (Van Huis and Oonincx, 2017; Parisi et al. 2020).

In this context, there is an increased interest in finding sustainable alternatives such as protein-rich insect species as innovative ingredients in animal feeds (Van Huis and Oonincx, 2017; Allegretti et al., 2018; Kim et al., 2019; Patel et al., 2019; Gravel and Doyen, 2020; Gorbunova and Zakharov, 2021; Lu et al., 2022).

Sericulture industry produces a lot of by-products, including larvae, pupae and excreta (Bhavsar et al., 2020), and the economic potential of this sector depends on the ability to valorize them (Sharma et al., 2022; Hăbeanu et al., 2023a). Mulberry silkworm pupae (SWP), the main by-product of the silk industry, are considered a valuable alternative protein and fat source for feed (Saviane et al., 2021; Wu et al., 2021; Herman et al., 2022), supporting the circular production systems and preventing the discharge of potentially hazardous waste into the environment (Grzeškowiak and Łochyńska, 2021;

Tassoni et al., 2022). According to Asimi et al. (2017), a small percentage of waste pupae (up to 30%) have been used directly for livestock feeding (fish, duck, and chicken).

The SWP are a rich source of nutrients with health-promoting properties (Herman et al., 2022), such as protein and essential amino acids, lipids and essential fatty acids (omega-3), minerals, vitamins, and bioactive compounds (chitin and 1-Deoxynojirimycin). Previous studies on chickens demonstrated that chitin could act as a prebiotic by enhancing the birds' immune responses and increasing the caecal production of butyric acid, the enterocytes' primary energy source (Bovera et al., 2015), or contrary, some researchers have shown that chitin can decrease hens' ability to absorb nutrients (Razdan and Pettersson, 1994; Bovera et al., 2015; Vichasilp et al., 2016; Cullere et al., 2016). Therefore, this review firstly presents the nutritional value of SWPs as potential feed ingredients for poultry and, secondly, reports the effects of using SWP in poultry diets, focusing on productive performance, nutrient digestibility, health status and quality of poultry products.

2. MATERIALS AND METHODS

For this study, databases such as PubMed, Web of Science, Google Scholar, ScienceDirect, MDPI, Springer, and Elsevier were searched for relevant research papers with full text in English. The keywords used were: "*Bombyx mori* L.", "sericulture by-products", "silkworms pupae", "chemical composition," "amino acids composition," "fatty acids profile," and "poultry feed". We searched recent scientific papers and also through the listed studies' references as additional sources.

3. RESULTS AND DISCUSSION

3.1. Nutritional value of SWP

Reviewed literature sources have emphasized that SWP chemical composition reflects the nutrient profile of mulberry leaves in transforming plant nutrients into silk. Certain factors that may impact the nutritional content of SWP include the rearing technologies used, nutrition (mulberry leaves or artificial diet), silkworm strains and gender, silkworm killing techniques, drying methods, and analyses (Lamberti et al., 2019; Herman et al., 2022; Tassoni et al., 2022; Singh et al., 2023).

As can be seen in Table 1, the full-fat SWP shown a variable chemical composition with protein content range from 37.8% (Singh et al., 2023) to 60.7% (Akande et al., 2020a), crude fat ranged from 19.21% (Kim et al., 2016a) to 36.21% (Anootthato et al., 2019), crude fibre from 1.1% (Akande et al., 2020a) to 6.38% (Kim et al., 2016b), nitrogen-free extractive ranges from 0.92% (Kuntadi et al., 2018) to 28.15% (Akande et al., 2020b), ash content from 0.9 (Akande et al., 2020a) to 7.24% (Felix et al., 2020) and gross energy from 25.2 (Singh et al., 2023) to 25.8 MJ/kg (Heuze et al., 2017).

Regarding the defatted SWP composition, the results have shown a higher protein content between 66.7% (Dalle Zotte et al., 2021) to 82.88% (Anootthato et al., 2019), lower fat that ranges less than 2% (Felix et al. 2020; Anootthato et al., 2019; Kim et al., 2016b) to 9.49% (Dalle Zotte et al., 2021), 3.24 to 9.54% crude fibre (Kim et al., 2016b), 6.65% (Dalle Zotte et al., 2021) to 10.63% ash (Felix et al., 2020) and approximately 22 MJ/kg gross energy (Heuze et al., 2017; Dalle Zotte et al., 2021).

SWP also contains chitin (N-acetyl D-glucosamine polymer), a polysaccharide ranging from 1.5 (Singh et al., 2023) to 3.4% (Dalle Zotte et al., 2021) that has a structural function in organisms being a component of the exoskeleton (Van Huis, 2013; Makkar et al., 2014). Chitosan is the active component of chitin, another significant polysaccharide in addition to cellulose. Although chitin is regarded as an indigestible fibre, it can enhance monogastric animals immune systems (Li et al., 2013; Swiatkiewicz et al., 2015). According to previous studies, chitin can reduce the digestibility of proteins and lipids from feed ingredients, which might hinder poultry ability to absorb these nutrients (Vichasilp et al., 2016).

The bioactive component of SWP 1-Deoxynojirimycin (1-DNJ), also known as moranolin, is a transition-state mimicry-based-glucosidase inhibitor that inhibits glycosidase enzymes (Dalle Zotte et

al., 2021). Therefore, the 1-DNJ inhibits enzymes involved in saccharide hydrolysis, glycoprotein processing, and glycogenolysis (Tomotake et al., 2010; Yatsunami et al., 2011), and due to this fact, the SWP use in chicken diets to be further examined (Dalle Zotte et al., 2021; Singh et al., 2023). The 1-DNJ content of SWP varies from 75 to 105 mg/100 g (Vichasilp et al., 2016).

Table 1. Chemical composition (g/100 g) of silkworm pupae (*Bombyx mori* L.)

References	SWP type	DM	CP	Fat	CF	NFE	Ash	Chitin	GE
Kim et al., 2016a	FF	n/a	52.58	19.21	n/a	23.41	4.8	n/a	n/a
Rodriguez-Ortega et al., 2016	FF	n/a	46.87	20.63	4.89	3.92	6.25	n/a	n/a
Kuntadi et al., 2018	FF	n/a	60.03	29.47	n/a	0.92	5.79	n/a	n/a
Ghosh et al., 2020	FF	n/a	55.87	23.45	3.77	1.76	5.38	n/a	n/a
Akande et al., 2020a	FF	n/a	60.7	23.5	1.1	11.3	0.9	n/a	n/a
Akande et al., 2020b	FF	n/a	47.17	32.16	1.68	28.15	2.12	n/a	n/a
Hirunyophat et al., 2021	FF	n/a	46.22- 56.00	23.95- 34.69	4.78-5.58	9.82- 12.00	3.94- 4.73	n/a	n/a
Singh et al., 2023	FF	93.5	37.8	29.2	n/a	n/a	4.89	1.46	25.2
Kim et al., 2016b	FF	n/a	47.87	30.85	6.38	n/a	6.96	n/a	n/a
	DF	n/a	67.54- 73.51	2.05- 4.75	3.24-9.54	n/a	8.04- 9.54	n/a	n/a
Heuze et al., 2017	FF	91.4	60.7	25.7	3.9	n/a	5.8	n/a	25.8
	DF	93.8	75.6	4.7	6.6	n/a	6.8	n/a	22.0
Anootthato et al., 2019	FF	n/a	52.69-56.64	34.07-36.21	n/a	n/a	n/a	n/a	n/a
	DF	n/a	82.88	1.47	n/a	n/a	n/a	n/a	n/a
Felix et al., 2020	FF	n/a	55.43	31.72	n/a	5.60	7.24	n/a	n/a
	DF	n/a	81.14	<0.2	n/a	8.23	10.63	n/a	n/a
Dalle Zotte et al., 2021	FF	94.4	53.9	29.1	n/a	n/a	5.01	2.87	25.2
	DF	94.7	66.7	9.49	n/a	n/a	6.65	3.4	21.9

Abbreviations: SWP, silkworm pupae; FF, full-fat; DF, defatted; DM, dry matter; CP, crude protein; CF, crude fibre; NFE, nitrogen-free extractive; GE, gross energy (MJ/kg).

Literature data show that the protein content of SWP has a well-balanced AA profile (Table 2). Mulberry SWPs are rich in most of the AA as compared to other silkworms and eggs of hens (Tomotake et al., 2010). The AA profile of SWP is similar but superior to soybean meal (Ji et al., 2015). The lysine and methionine contents are higher, representing 7-8% and 3-4% of the CP. The SWP have higher contents of the essential AA, namely, lysine (6.1-9.1%), leucine (5.8-8.16%), arginine (5.1-6.62%), phenylalanine (4.2-5.4%), valine (4.9-6.2%), threonine (4.6-5.08%) and methionine (2.28-4.0%). Total AA average values ranged from 74.8 (Heuze et al., 2017) to 96.2% (Tomotake et al., 2010), from which essential AA ranges from 40.6 (Heuze et al., 2017) to 48.46% (Anootthato et al., 2019) and non-essential AA from 34.2 to 51.0% (Heuze et al., 2017). The essential/non-essential AA and essential/total AA ratios range between 0.84-1.23% and 0.46-0.55%, respectively (Akande et al., 2020a; Anootthato et al., 2019).

Table 2. Amino acids composition of silkworm pupae (*Bombyx mori* L.)

Amino acids (g/100 g)	SWP type					
	FF	FF	DF	DF	FF	FF
Lysine	7.2	7.0	6.1	6.1	9.09	9.00
Methionine	4.0	3.5	3.0	3.0	n/a	2.28
Threonine	4.6	5.1	4.8	4.8	5.08	4.62
Arginine	5.5	5.6	5.1	5.1	6.61	6.62
Valine	5.6	5.5	4.9	4.9	6.18	4.94
Phenylalanine	5.4	5.2	4.4	4.4	5.25	4.21
Isoleucine	4.0	5.1	3.9	3.9	4.84	4.92
Leucine	7.3	7.5	5.8	5.8	8.16	8.16
Histidine	3.2	2.6	2.6	2.6	3.25	2.63
EAA¹	46.8	47.1	40.6	40.6	48.46	41.38
Aspartic acid	10.7	10.4	7.8	7.8	n/a	8.57
Glutamic acid	11.1	13.9	8.3	8.3	13.58	17.02
Alanine	4.6	5.8	4.4	4.4	5.80	7.85
Glycine	4.2	4.8	3.7	3.7	4.51	5.16
Proline	8.2	5.2	n/a	0.0	4.87	3.61
Serine	4.3	5.0	4.5	4.5	5.3	4.63
Tyrosine	6.3	5.9	5.5	5.5	5.35	2.62
NEAA¹	49.4	51.0	34.2	34.2	39.41	49.46
Total AA¹	96.2	98.1	74.8	74.8	87.87	90.84
EAA/NEAA ratio¹	0.95	0.92	1.18	1.18	1.23	0.84
EAA/Total AA ratio¹	0.49	0.48	0.54	0.54	0.55	0.46
References	Tomotake et al., 2010	Heuze et al., 2017	Khan et al., 2018	Anoonthato et al., 2019	Akande et al., 2020a	

Abbreviations: SWP, silkworm pupae; FF, full-fat; DF, defatted; ¹Calculated values.

The fatty acids profile of SWP (Table 3) revealed a total saturated fatty acids (SFA) content between 26.3 (Tong et al., 2011) to 30.4% of total FAME (Dalle Zotte et al., 2021), monounsaturated fatty acids (MUFA) ranged from 27.6 (Yu et al., 2018) to 34.83% of total FAME (Wang et al., 2020), polyunsaturated fatty acids (PUFA) from 35.7 (Tong et al., 2011) to 44.15 % of total FAME (Dalle Zotte et al., 2021), from which n-3 PUFA represents 20.99-38.16% (Yu et al., 2018; Hu et al., 2017), and n-6:n-3 ratio 0.14-1.00% (Saviane et al. 2021; Yu et al., 2018).

The most abundant SFA are palmitic acid (C16:0), which ranges from 16.0 to 24.45% of total FAME, and stearic acid (C18:0), which ranges from 4.5 to 12.55% of total FAME. The highest content of MUFA is oleic acid (C18:1 n-9), which ranges from 26.0 to 33.9% of total FAME. Among PUFA, the essential FAs linolenic acid (C18:3n-3) ranges from 20.99 to 38.16% of total FAME, and linoleic acid

(C18:2n-6) ranges from 4.64 to 21.19% of total FAME. It is well-documented that from linoleic and linolenic FAs are derived other long-chain PUFAs; thus, these essential FAs possess nutraceuticals and functional properties for human health (Orsavova et al., 2015; Habeanu et al., 2023b). Therefore, SWP represents a sustainable alternative source of beneficial n-3 PUFA for animal diets (Dalle Zotte et al., 2021; Habeanu et al., 2023b; Singh et al., 2023).

Table 3. Fatty acids profile of silkworm pupae (*Bombyx mori* L.)

Fatty acids (% of FAME)	SWP type							
	DF	DF	DF	DF	DF	FF	DF	FF
C14:0	0.1	n/a	0.19	n/a	n/a	n/a	n/a	0.18
C15:0	n/a	0.15	n/a	n/a	n/a	0.17	n/a	0.07
C16:0	24.2	16.03	23.11	16.67	22.03	24.2	23.7	24.45
C18:0	4.5	9.45	4.68	12.55	6.84	5.45	5.89	4.35
C20:0	n/a	0.7	0.16	0.63	n/a	n/a	n/a	0.16
C23:0	n/a	n/a	n/a	n/a	n/a	0.59	0.0	n/a
Total SFA	28.8	26.33	28.14	29.85	28.87	30.4	29.6	29.21
C16:1	1.7	0.39	1.06	0.79	0.92	1.04	1.37	1.56
C18:1 n-9	26.0	28.12	28.24	26.81	33.91	31.2	29.3	31.17
C24:1 n-9	n/a	n/a	n/a	n/a	n/a	0.54	n/a	n/a
Total MUFA	27.7	28.51	29.3	27.6	34.83	32.8	30.7	32.73
C18:2 n-6	7.3	12.24	3.87	21.19	5.48	6.16	6.18	4.64
C18:3 n-3	36.3	31.91	38.16	20.99	30.81	29.5	31.5	32.68
Total PUFA	43.6	44.15	42.03	42.18	36.29	35.7	37.7	37.32
Total n-6	7.3	12.24	3.87	21.19	5.48	6.22	6.18	4.64
Total n-3	36.3	31.91	38.16	20.99	30.81	29.5	31.5	32.68
n-6/n-3	0.20	0.38	0.10	1.00	0.18	0.21	0.20	0.14
References	Tomotake et al., 2010	Tong et al., 2011	Hu et al., 2017	Yu et al., 2018	Wang et al., 2020	Dalle Zotte et al., 2021	Saviane et al., 2021	

Abbreviations: SWP, silkworm pupae; FAME total fatty acid methyl esters; FF, full-fat; DF, defatted.

The mineral composition of SWP found in literature studies shows a variable content with higher average means of phosphorus, magnesium, calcium, zinc, iron, manganese and copper (Table 4).

Table 4. Minerals composition of silkworm pupae (*Bombyx mori* L.)

Minerals (mg/100 g)	SWP type		
	FF	FF	FF
Calcium	102.31	98.72	137.01
Phosphorus	1369.94	870.92	n/a
Magnesium	287.96	252.29	280.8
Manganese	2.08	1.68	1.08
Zinc	17.75	14.70	1.39
Copper	2.08	0.94	n/a
Iron	9.54	4.95	n/a
References	Kumar et al., 2015 Kim et al., 2016b David-Birman et al., 2019		

Abbreviations: SWP, silkworm pupae; FF, full-fat; DF, defatted.

3.2. Effect of using the SWP in poultry diets

Table 5 summarized the main results of research studies regarding the effects of using SWP in poultry diets as a partial or total replacement of soybean meal (SBM) or fish meal (FM).

Table 5. Effect of silkworm pupae (*Bombyx mori* L.) in poultry

References	Species	Breed	SWP type	Level of inclusion and/or substitution, %	Age/Period	Main results
Khatun et al., 2003	Chicken	Arbor Acres	FF	6% FM+0% SWP 4% FM+2% SWP 2% FM+4% SWP 0% FM+6% SWP	1-42 d	↑ growth rate with the ↑ in dietary SWP level. ↓ FI and ↑ FCR at 28 and 42 d. ↓ the total cost of production/ kg live broiler and ↑ profit/broiler ↑ in dietary SWP level.
Khatun et al., 2005	Chicken	Rhode Island Red (RIR)	FF	6 to 8% SWP, replacing protein concentrate	28-45 weeks	6% SWP ↑ profitability, growth and egg production performance of RIR. ↓ gradually the cost/kg of feed with ↑ the level of SWP in the diet.
Konwar et al., 2008	Chicken	Broiler	n/a	0–100% of FM, with or without enzyme	n/a	No adverse effect on replacing FM with SWP. SWP and enzymes boosted development and ↓ FI. 50% SWP substitution of FM and enzyme supplementation obtained the highest results.

Dutta et al., 2012	Chicken	RIR	FF	0 and 100% substitution of FM	3-56 d	50% replacement of FM with SWP ↑ marginal profit due to lower cost of SWP. a linear ↑ SWP level in poultry feed was corresponding to ↓ in cost/ unit of feed.
Jintasataporn, 2012	Chicken	Broiler	DF	0-20% SWP, replacing 0-100% of FM	21-42 d	10% SWP inclusion level had a little adverse effect on broiler growth but no negative effect on the percentage of muscle yield, sensory traits of breast meat and palatability. ↑ SWP inclusion level detrimentally affects FCR and muscle yield.
Rahmasari et al., 2015	Quail	-	FF	0, 25, 50, 75% substitution of FM	10 weeks	↓ FCR, ↑ egg production and did not affect the physical quality of quail eggs.
Ullah et al., 2017	Chicken	White Leghorn	FF	0, 25, 50, 100% substitution of SBM	52-58 weeks	No difference in layers performance (BW, FI, hen day production (%), egg weight, FCR), nutrient digestibility, blood profile and egg quality parameters. Up to 50% SBM substitution with SWP in laying hens diet is recommended to archive better results.
Ullah et al., 2018	Chicken	Broiler	FF	0, 2, 4, 6, 8% SWP, 0, 25, 50, 75, 100% substitution of SBM	1-28 d	No effect on broiler performance and carcass quality. ↓ gradually the cost/kg of feed with ↑ dietary inclusion level of SWP, indicating higher economic benefit. Up to 75% SBM substitution with SWP in the starter diet is recommended to archive better performance and dressing percentage without affecting meat sensory quality.
Khan et al., 2018	Chicken	Ross 308	DF	7.8% SWP	1-35 d	Improve BW and FCR with no difference in FI, mortality and dressing percentage, and sensory analysis of broiler breast meat compared to control.
Miah et al., 2020	Chicken	Rhode Island Red x Fayoumi crossbred	FF	7 and 14% SWP, 0, 25, 50% substitution of SBM and 100% of soybean oil	1-56 d	7% SWP dietary level achieves good growth performance, carcass traits and proximate composition of meat. 14% SWP dietary level favourably modifies the fatty acids profile of the chicken breast meat, decreasing the n-6/n-3 PUFAs ratio.

Rafiullah et al., 2020	Chicken	White Leghorn		0, 25, 50, 100% substitution of SBM	45-55 weeks	Substitution of SBM with SWP had no adverse effect on production performance, intestinal histomorphology and blood biochemical functions of liver and kidney.
Dalle Zotte et al., 2021	Quail	-	FF DF	12.5% SWP 12.5% SWP	16-33 d 33-46 d	<p>↑ DM intake and excreta production. ↓ apparent digestibility of DM, OM CP, EE, starch and energy due to the presence of chitin and 1-DNJ.</p> <p>↑ <i>Streptococcaceae</i>, <i>Rikenellaceae</i> and <i>Eubacteriaceae</i> families and taxa at the species level, such as <i>Lactobacillus delbrueckii</i>, <i>Aneurinibacillus thermoaerophilus</i> and <i>Bacillus thermoamylovorans</i> in excreta of quails fed FF than DF and control.</p>
Pietras et al., 2021	Chicken	Ross 308	FF	17% SWP and 20% lupin	21-42 d	SWP and lupin can totally replace SBM in the grower diet for broilers with no adverse effect on production, slaughter performances and sensory quality of the meat.
Zsedely et al., 2023	Chicken	Ross 308	DF	0, 4% SWP (starter), 4% SWP (grower-finisher)	1-42 d	<p>Comparable growth performance, carcass, and physicochemical meat quality traits.</p> <p>Based on the meat proximate analysis results, SWP use in the grower-finisher diet was the best option.</p>

Abbreviations: SWP, silkworm pupae; FF, full-fat; DF, defatted; FM, fish meal; SBM, soybean meal; DM, dry matter; OM, organic matter; EE, ether extract; 1-DNJ, 1-Deoxynojirimycin; ↑ = increased; ↓ = decreased; BW, body weight; FI, feed intake; FCR, feed conversion ratio.

4. CONCLUSIONS

The present review paper highlighted that SWPs are sericulture by-products with valuable nutritional value that can be considered cost-effective and sustainable alternative protein sources for poultry feed, especially in the current context of increasing demand for protein. The findings demonstrated that SWP could partially or replace conventional protein ingredients (SBM or FM) in poultry diets, maintaining appropriate performance, nutrient digestibility, health status and/or improving meat or egg qualities.

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