Biochemical Profiling of *Bombyx mori* L. Droppings: Insights into Protein and Carbohydrate Composition for Agricultural Implications

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Received:- 03 March 2024/ Revised:- 11 March 2024/ Accepted:- 19 March 2024/ Published: 31-03-2024

Abstract— *This study meticulously examined the protein and carbohydrate contents in aqueous and chloroform extracts of Bombyx mori L. (silkworm) droppings from six distinct silkworm breeds (CSR2, CSR4, M43, M46, SK4 and Sanish 8) using standardized protocols. The findings consistently indicated heightened protein and carbohydrate levels in aqueous extracts across both 4th and 5thinstar droppings. Particularly noteworthy was the CSR4 breed, displaying the highest protein content at 10.76% and 8.09% in 4th and5th instar, respectively, while the M46 breed showcased the lowest protein content. Conversely, carbohydrate content peaked in the droppings of the M46 breed. This investigation underscores the indispensable role played by these constituents in soil health and agricultural practices, emphasizing their potential as organic fertilizers. It advocates for further comprehensive exploration of these elements to unlock their manifold applications beyond silk production. Recognizing their significance in sustainable agriculture and environmental management, this study prompts further research endeavors to harness their potential across various sectors.*

Keywords— Bombyx mori L., Silkworm droppings, Carbohydrate, Protein.

I. INTRODUCTION

Bombyx mori L., commonly known as the mulberry silkworm, has garnered enduring scientific interest over centuries, owing to its multifaceted economic, ecological, and scientific implications (Goldsmith *et al*., 2005; Hardy *et al*., 2008). Beyond its iconic role in silk production, the silkworm serves as an invaluable model organism for unravelling diverse biological processes. A facet often overshadowed in silkworm biology pertains to the composition of its excretory byproduct, colloquially known as droppings, which offers insights into dietary habits, metabolism, and potential applications. Employing biochemical profiling, a potent analytical approach, allows for an in-depth exploration of the intricate molecular makeup of biological samples (Smith and Fieldsend, 2021).

Silkworm excrement, a consequential byproduct of sericulture, emerges as a subject of interest due to its intricate composition. Despite consuming mulberry leaves at a rate approximately ten times its body mass, only 40% of the leaves undergo digestion, with the remaining 60% expelled as droppings. Investigation reveals that some compounds in the excreta originate from mulberry leaves, while others undergo bio-transformation in the silkworm intestine (Katayama *et al*., 2007). The reported chemical constituents of silkworm excreta encompass chlorophyll and its derivatives, xanthophyll, carotenoids, and flavonoids (Park *et al*., 2003; Park et al., 2011). Complementary examinations have probed the lipid profile of silkworm excreta (Uzakova *et al*., 1987), while Chen (2003) comprehensively outlined the nutrient composition, including moisture, crude protein, crude fat, crude fiber, non-nitrogen extracts, and minerals.

In the broader context of soil nutrition and plant growth, the pivotal roles played by proteins and carbohydrates cannot be overstated (Fernandez-Carazo *et al*., 2020). Proteins, acting as nitrogen sources, enrich soil fertility by facilitating nutrient availability, supporting microbial activity, and influencing biochemical processes vital for nutrient cycling (Gupta *et al*., 2018). Simultaneously, carbohydrates function as organic matter, enhancing soil structure, aeration, water retention, and serving as a carbon source for beneficial soil microorganisms (Garcia *et al*., 2018).

This synergy between proteins and carbohydrates creates a dynamic soil environment that fosters plant growth. The ensuing interplay of nutrient availability, improved soil structure, and microbial interactions collectively contributes to enhanced plant health, development, and overall agricultural productivity. Silkworm droppings, often underestimated yet rich in proteins and carbohydrates, emerge as a valuable resource with profound implications for agriculture. Research indicates the presence of essential proteins vital for plant growth and development in silkworm excrement (Seo *et al*., 1985). Nitrogen-rich proteins act as potent fertilizers, augmenting soil fertility and promoting microbial activity for enhanced nutrient cycling (Park *et al*., 2011). Concurrently, carbohydrates in silkworm droppings act as organic matter, enhancing soil structure and providing a carbon source for beneficial soil microorganisms (Chen, 2003). This microbial activity further supports nutrient availability for plants, fostering sustainable nutrient recycling practices in agriculture.

The amalgamation of proteins and carbohydrates from silkworm droppings not only nurtures soil health but also provides a promising avenue for organic fertilization and nutrient management in crop production. This aligns seamlessly with the principles of sustainable and eco-friendly agriculture. The present study seeks to quantify protein and carbohydrate levels in the droppings of diverse mulberry silkworm breeds, contributing to the expanding understanding of the agronomic potential inherent in this often-overlooked byproduct.

II. MATERIALS AND METHODS

2.1 Collection of Material:

As shown in Table 1, 100 grams of excreta on 2nd day of 4th instar and 3rd day of 5th instar of 06 different breeds of silkworm (*Bombyx mori.* L) was collected separately during the rearing conducted at College of Temperate Sericulture, Mirgund. The samples were dried in tray dryer at 60˚C until constant weight was obtained. Then the samples were crushed into fine powder before analysis.

TABLE 1
COLLECTION OF DROPPINGS FROM SILKWORM *BOMBYX MORI* L. BREEDS

Breed	Name of Breed
B1	CSR2
B2	CSR4
B3	M43
B4	M46
B5	SK4
B6	Sanish-8

2.2 Preparation of extract:

Extraction of the samples was carried out using two solvents viz., water and chloroform by mixing 1.25 gram of excreta in 25ml of solvent filtered through Whatman filter paper 42 followed by centrifugation at 5000 rpm for 15 minutes. The extracts were pooled and collected up to a final volume of 25 ml and stored in cryo vials at -5°C till further analysis.

2.3 Bioactive compound analysis:

2.3.1 Estimation of Total Protein content

Protein content in aqueous and chloroform dropping extract of 06 different breeds of *Bombyx mori*. L, was estimated by Lowry's method (Lowry *et al*., 1951). 500mg of the sample was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer. This solution was centrifuged and the supernatant was used for protein estimation. 0.2,0.4,0.6,0.8 and 1ml of the working standards were pipetted into a series of test tubes. The aqueous and chloroform sample extracts were pipetted in other test tubes. The volume was made to 1 ml in all the test tubes. The tube with 1ml of water served as the blank. 5ml of Alkaline copper reagent was added to each tube including the blank. This was mixed well and allowed to stand for 10 min. Then 0.5ml of Folin-Ciocalteau reagent was added, mixed and incubated at room temperature in the dark for 30 min and blue colour was developed. The readings were recorded at 660 nm. Standard graph was drawn and the amount of protein in the sample was calculated. Protein percentage was calculated in relation to fresh weight and dry weight basis. The amount of protein mg/g or 100g sample was expressed.

2.3.2 Estimation of Total Carbohydrate

The total carbohydrate content in aqueous and chloroform dropping extract of 06 different breeds of *Bombyx mori*. L, was estimated by anthrone method (Hodge and Hofreiter, 1962). 100mg of the sample was weighed into a boiling tube. This was hydrolyzed by keeping it in a boiling water bath for three hours with 5ml of 2.5 N – HCl and cool to room temperature. Then, this solution was neutralized with solid sodium carbonate until the effervescence ceases and made to 100ml and centrifuged. The supernatant was collected and 0.5, 1ml aliquots were taken for analysis. Standard solution was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard 'O' served as blank. The volume is made to 1ml in all the tubes including the sample tubes by adding distilled water. Then 4ml of anthrone reagent was added and this was heated for eight minutes in a boiling water bath and made to cool. Green to dark green colour was recorded at 630nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. The amount of carbohydrate present in the tube was calculated from the graph. Carbohydrate percentage was calculated relation to fresh weight and dry weight basis. The Calculation is done by using this formula:

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \frac{mg\ of\ glucose}{\text{Volume of test sample}} \text{x} 100 \quad (1)$$

III. RESULTS AND DISCUSSION

3.1 Total Protein (%)

The comprehensive analysis of total protein content in the 4th and 5th instars of silkworm droppings across six distinct breeds and varied extracts is elucidated in Table 2 and Fig. 1. The aqueous extract exhibited significantly higher total protein content, closely followed by the chloroform extract in both instars. In the aqueous extract, the total protein content in silkworm droppings ranged from 7.32% to 10.76% in the 4th instar and 5.63% to 9.55% in the 5th instar. Notably, the highest total protein content in the 4th instar droppings was observed in the CSR4 breed (10.76%), succeeded by the CSR2 breed (10.01%), while the M46 breed exhibited the lowest total protein content at 7.32%. Similarly, in the 5th instar droppings, CSR4 recorded the highest total protein content (8.09%), followed by CSR2 (7.72%), with the lowest total protein content at 5.31% observed in the M46 breed.

The total protein content in the chloroform extract of silkworm droppings ranged from 5.31% to 8.09% in the 4th instar and 4.15% to 7.04% in the 5th instar. In the 4th instar droppings, CSR4 breed exhibited the highest total protein content (8.09%), followed by CSR2 (7.72%), while M46 breed displayed the lowest at 5.31%. In the 5th instar droppings, CSR4 again recorded the maximum total protein content (7.04%), followed by CSR2 (6.85%), with M46 breed showing the lowest total protein content of 4.15%.

The observed trends in the present study align with the findings of Murthy (2015), who reported a decrease in protein content with each passing instar, thereby highlighting the developmental dynamics of protein synthesis in silkworms. This conformity not only underscores the robustness of the methodology applied in the present study but also adds to the body of knowledge concerning the physiological changes in silkworm droppings across different breeds and developmental stages (Patil *et al*., 2013).

TABLE 2
TOTAL PROTEIN CONTENT IN 4th AND 5th INSTAR AQUEOUS AND CHLOROFORM EXTRACT OF SILKWORM DROPPINGS OF SIX BREEDS (%)

Breed \ Extraction Method	4th instar			5th instar		
	Aqueous	Chloroform	Mean	Aqueous	Chloroform	Mean
B1: CSR2	10.01	7.72	8.86	9.11	6.85	7.98
B2: CSR4	10.76	8.09	9.42	9.55	7.04	8.30
B3: M43	7.54	5.51	6.52	5.81	4.45	5.13
B4: M46	7.32	5.31	6.31	5.63	4.15	4.89
B5: SK4	8.17	6.17	7.17	8.55	5.47	7.01
B6: Sanish-8	7.55	5.79	6.67	8.30	5.01	6.65
Mean	8.56	6.43		7.82	5.49	

C.D($p \leq 0.05$)		**C.D($p \leq 0.05$)**	
Extraction Method (M)	: 0.149	Extraction Method (M)	: 0.054
Breed (B)	: 0.258	Breed (B)	: 0.094
MxB	: 0.365	MxB	: 0.133

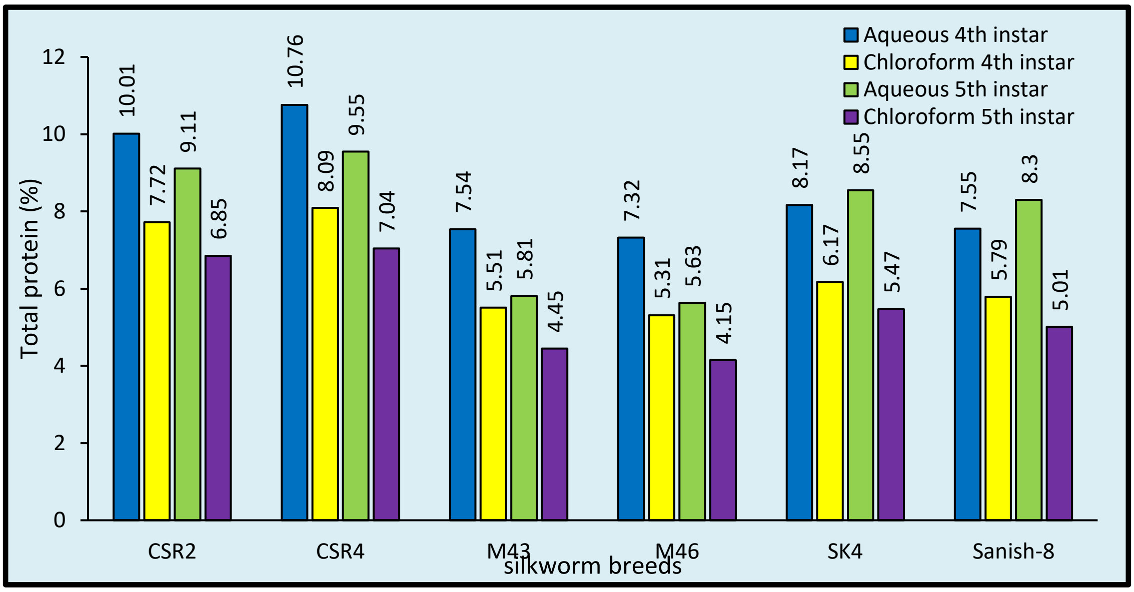

FIGURE 1: Total protein content in aqueous and chloroform extract of 4th and 5th instar silkworm droppings of 06 different breeds (%)

3.2 Total Carbohydrate (%)

The investigation into the total carbohydrate content of silkworm droppings in the 4th and 5th instars, encompassing six different breeds and various extracts, is summarized in Table 3 and Fig. 2. Notably, the aqueous extract consistently exhibited significantly higher total carbohydrate content compared to the chloroform extract in both instars. In the aqueous extract, the total carbohydrate content in silkworm droppings ranged from 4.31% to 7.15% in the 4th instar and 4.03% to 7.03% in the 5th instar. The M46 breed demonstrated the highest total carbohydrate content in the 4th instar (7.15%), followed by M43 (6.63%), while CSR4 exhibited the lowest at 4.31%. Similarly, in the 5th instar, M46 recorded the maximum total carbohydrate content (7.03%), followed by M43 (6.49%), with CSR4 presenting the lowest content at 4.03%. The chloroform extract of silkworm droppings exhibited total carbohydrate content ranging from 2.82% to 4.10% in the 4th instar and 2.12% to 3.54% in the 5th instar. In the 4th instar, M46 breed displayed the highest total carbohydrate content (4.10%), followed by M43 (3.58%), with CSR4 exhibiting the lowest at 2.82%. For the 5th instar, M46 again recorded the maximum total carbohydrate content (3.54%), followed by M43 (3.50%), while CSR4 displayed the lowest at 2.12%.

These findings are consistent with the results reported by Patil *et al*. (2013), where a similar methodology revealed a 4% carbohydrate content in silkworm droppings. The observed agreement reinforces the reliability of the methodology employed in the present study. The significant variations in total carbohydrate content across breeds and instars underscore the dynamic nature of silkworm droppings and their potential significance in agriculture. Further research could delve into the underlying factors influencing these variations and explore the implications for soil health and plant growth.

TABLE 3
TOTAL CARBOHYDRATE CONTENT IN 4th AND 5th INSTAR AQUEOUS AND CHLOROFORM EXTRACT OF SILKWORM DROPPINGS OF SIX BREEDS (%)

Breed \ Extraction Method	4th instar			5th instar		
	Aqueous	Chloroform	Mean	Aqueous	Chloroform	Mean
B1: CSR2	4.77	3.10	3.93	4.37	2.92	3.65
B2: CSR4	4.31	2.82	3.56	4.03	2.12	3.08
B3: M43	6.63	3.58	5.11	6.49	3.50	4.99
B4: M46	7.15	4.10	5.62	7.03	3.54	5.29
B5: SK4	6.30	3.51	4.91	5.60	2.33	3.97
B6: Sanish-8	6.35	3.52	4.93	6.49	3.01	4.75
Mean	5.92	3.44		5.67	2.90	

C.D(p≤0.05)
Extraction Method (M) : 0.033
Breed (B) : 0.057
MxB : 0.081

C.D(p≤0.05)
Extraction Method (M) : 0.010
Breed (B) : 0.017
MxB : 0.024

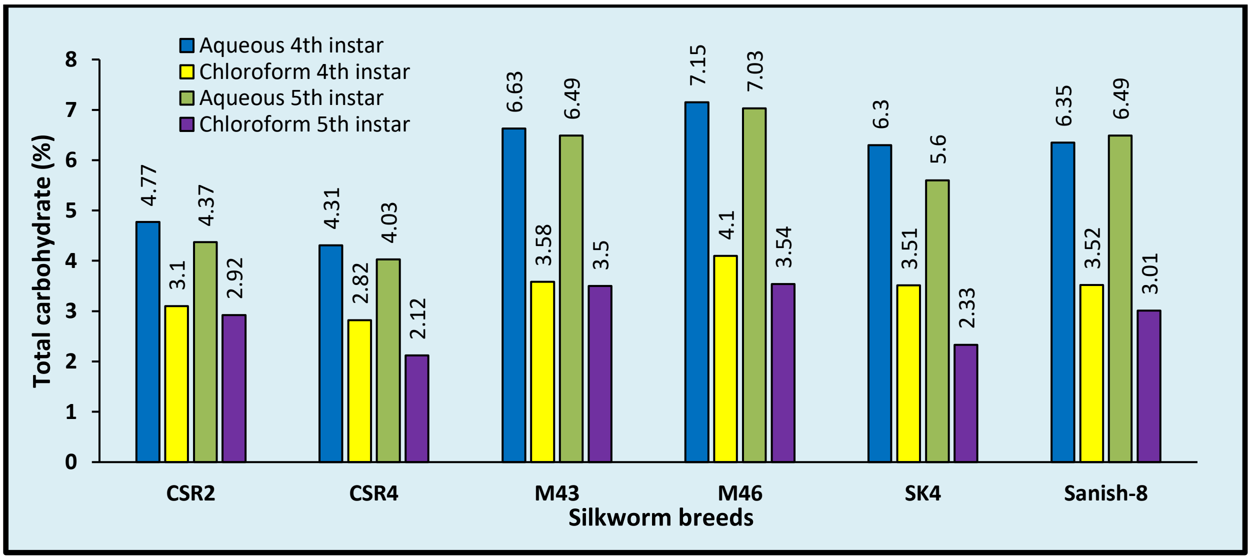

FIGURE 2: Total carbohydrate content in aqueous and chloroform extract of 4th and 5th instar silkworm droppings of 06 different breeds (%).

Understanding the factors influencing protein dynamics in silkworm droppings can provide valuable insights into soil fertility and plant growth, presenting a rich avenue for further exploration in sustainable agriculture (Gupta *et al*., 2018; Fernandez-Carazo *et al*., 2020). The intricate interplay between protein content, soil health, and plant productivity invites a nuanced investigation, with potential implications for optimizing agricultural practices. Future research endeavors in this direction may contribute to the development of eco-friendly and sustainable strategies in agriculture.

The observed variations in protein and carbohydrate content within silkworm droppings hold significant implications for their utilization in agriculture, offering potential benefits for soil health and overall productivity.

Silkworm droppings, enriched with proteins, can serve as valuable organic fertilizers due to their nitrogen-rich composition. Proteins play a crucial role in soil nutrition by acting as potent nitrogen sources. Nitrogen is an essential component for plant growth, and its availability in the soil directly influences crop productivity (Gyaneshwar *et al*., 2002). The proteins in silkworm droppings can enhance soil fertility by facilitating the availability of nitrogen to plants, promoting robust growth and development. Moreover, proteins support microbial activity in the soil, contributing to the decomposition of organic matter. This decomposition process releases essential nutrients, making them available for plant uptake. Proteins also serve as building blocks for soil enzymes, influencing biochemical processes vital for nutrient cycling (Marschner, 2011). The microbial activity supported by proteins in silkworm droppings enhances the overall nutrient cycling in the soil, fostering a nutrient-rich environment for plants.

Carbohydrates present in silkworm droppings, especially in the aqueous extract, contribute to improving soil structure and fostering better aeration and water retention (Lehmann *et al*., 2015). Carbohydrates serve as organic matter in the soil, enhancing soil fertility and creating a conducive environment for plant growth. The carbon source provided by carbohydrates supports the growth and activity of beneficial soil microorganisms. The microbial activity fueled by carbohydrates in silkworm droppings further contributes to nutrient transformation and availability for plants (Bardgett and van der Putten, 2014). Microorganisms break down complex organic matter into simpler forms, releasing nutrients that are vital for plant nutrition. This symbiotic relationship between carbohydrates, microorganisms, and nutrient availability creates a dynamic soil environment that supports plant growth.

The combination of proteins and carbohydrates in silkworm droppings offers a holistic approach to enhancing soil health and, consequently, agricultural productivity. The availability of essential nutrients, improved soil structure, and microbial interactions influenced by these biomolecules collectively contribute to enhanced plant health, development, and overall agricultural productivity.

By utilizing silkworm droppings as organic fertilizers, agricultural practices can align with principles of sustainable and eco-friendly farming. The recycling of nutrients through the incorporation of silkworm droppings into soil contributes to the development of nutrient-rich soils, reducing the dependency on chemical fertilizers and promoting environmentally sustainable agriculture.

IV. CONCLUSION AND FUTURE SCOPE

In the current study, a meticulous examination of the biochemical composition of silkworm droppings, specifically focusing on the aqueous and chloroform extracts of the 4th instar droppings of CSR4 and M46 breeds, revealed noteworthy variations in total protein and carbohydrate content.

The aqueous extract of the 4th instar droppings from the CSR4 breed exhibited the highest total protein content at 10.76%, surpassing the chloroform extract with a content of 8.09%. Similarly, concerning total carbohydrate content, the aqueous extract from the 4th instar droppings of the M46 breed displayed the highest concentration at 7.15%, outperforming the chloroform extract with a content of 4.10%. In conclusion, this comprehensive analysis provides valuable insights into the nutritional dynamics of silkworm droppings, shedding light on breed-specific variations in protein and carbohydrate content. The higher protein content in the CSR4 breed suggests potential genetic influences on protein synthesis, while the elevated carbohydrate content in the M46 breed implies variations in dietary habits or metabolic processes. The unraveling of the intricate biochemical profile of silkworm droppings not only contributes to our understanding of silkworm biology but also presents potential applications in various fields. These applications could range from the development of organic fertilizers enriched with specific nutrients to biotechnological processes leveraging silkworm-derived biomolecules.

As we delve deeper into the biochemical nuances of silkworm droppings, the avenues for future research become increasingly promising. Exploring the genetic and environmental factors influencing the observed variations, optimizing the extraction processes, and assessing the practical applications of these findings in agriculture are directions that merit further exploration. In essence, this study marks a crucial step in decoding the biochemical intricacies of silkworm droppings, laying the groundwork for future research endeavors and practical applications that could contribute to advancements in agriculture and related fields.

Overall, the protein and carbohydrate content in silkworm droppings represents a reservoir of untapped potential with implications spanning agriculture, biotechnology, medicine and environmental sustainability. Continued research efforts in this area promise to unlock further insights and innovations for the benefit of society and the ecosystem alike.

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