

Article

On the Digestibility of Mulberry Leaf Fed to *Bombyx mori* Larvae

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Abstract: Considering that sericulture is an important branch of animal husbandry, not only for the production of silk but also as a valuable source of protein, it is necessary to constantly study the possibilities for its improvement as a branch of this domain. Therefore, the purpose of this paper is to assess the nutritional value and digestibility of mulberry leaves from the Kokuso 21 and Eforie varieties, as consumed by silkworms (*Bombyx mori* L., *Bombicidae* Family, *Lepidoptera* Order) during a summer study, in 2021. The Japanese variety (Kokuso 21) and the Romanian variety (Eforie) were used as food sources for the Triumf hybrid, developed in Romania; the larvae were divided in two batches of 300 larvae and each set was subdivided into six groups of 50 larvae, which were raised in paper trays based on their age and size. The research indicated that mulberry leaves have an average digestibility value of 54.46%; the aging process of the leaves altered their chemical composition, with most nutrients showing a decreasing trend in digestibility throughout the larval growth period, except for crude fiber, which remained unchanged in the early larval stages and increased to 26.78% towards the end of the experiment. Overall, the Kokuso 21 variety demonstrated superior nutrient digestibility compared to Eforie. An important finding from this study is the need for future research to determine the degree of nutrient metabolism and conversion into silk.

Keywords: silkworm; sericulture; mulberry; leaves; nutrition; silk



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1. Introduction

These days, sericulture extends beyond merely producing silk; it now serves as a significant protein source for a diverse range of animal species, including fish, birds, mammals, and even humans [1–5]. Longvah et al., 2011, highlight the rich nutritional content provided by silkworm species, boasting 16% protein, 8% fat, and essential minerals, with an impressive high protein digestibility score (amino acid score of 86—PDCAAS) [2]. Consequently, *B. mori* L. (*Bombicidae* Family, *Lepidoptera* Order), among other insects, is frequently considered a valuable nutrient source for humanity [6–14]. Moreover, insect farming for this purpose carries a significantly lower environmental impact compared to activities like cattle ranching [9].

Furthermore, the nutritional quality of mulberry leaves has led to their increased use in feeding various farm animals, such as sheep, goats, cattle, pigs, rabbits, chicken, quail, and fish. These leaves can reduce dependence on other food sources, such as soybeans for laying hens. For instance, in an experiment involving Leghorn breed hens, the incorporation of silkworm meal showcased beneficial effects on their health and pro-duction, establishing it as a viable alternative protein source to soybean meal [10]. The age of mulberry trees has been observed to impact digestibility, as evidenced in goat farming [11–13]. Additionally, mulberry leaves have been shown to positively influence protein intake for swine when

constituting up to 0.3% of a sow's diet [14,15]. Similarly, research on sheep has indicated favorable limits in mulberry digestibility [16], as they exhibit a preference for these leaves due to their palatability [17].

The Kokuso 21 mulberry (*Morus alba* L.) is a cultivar known for its highly appreciated characteristics such as the leaf yield, the nutritional content, the resistance to harsh environmental conditions and diseases, the growth rate, and palatability for the leaves. Kokuso 21 is renowned for its high leaf yield, which provides ample food for silkworms, rich in nutrients, particularly protein and essential amino acids, which are vital for the growth and health of silkworms, as well as for silk production. This variety is known for its resilience in arid and semi-arid conditions, making it suitable for cultivation in regions with limited water availability, being also highly resistant to common mulberry diseases (it helps ensure a very good quality and stable leaf supply. It also has a fast growth rate, allowing for multiple harvests of leaves within a growing season (which is beneficial for continuous sericulture operations)). Furthermore, Kokuso 21 is preferred by the silkworms because of its high palatability (it encourages better feeding and growth rates, ultimately leading to higher silk yields). The listed characteristics make this variety a popular choice among sericulturists, particularly in regions where environmental conditions can be challenging [14–16].

The Eforie mulberry is a specific variety known for certain distinct characteristics, particularly in silkworm farming. It is appreciated for the high leaf yield (it is known for producing a significant amount of leaves, which are rich in nutrients, especially in protein content, crucial for the health and development of *B. mori*, L., *Bombicidae* Family, *Lepidoptera* Order) and high resilience to cold and diseases. Eforie mulberry trees are noted for their resistance to cold weather, making them suitable for cultivation in regions with cooler climates, and they tend to have good resistance to common mulberry diseases, helping to maintain a healthy crop and ensuring a consistent supply of quality leaves. This variety also has a great growth rate (it typically exhibits a rapid growth rate, which is beneficial for producing leaves throughout the growing season) and a high adaptability (it is adaptable to different soil types and climatic conditions, which makes it a versatile choice for various geographic regions). All these traits make Eforie preferred in certain sericulture practices, especially in areas where climate and disease resilience are critical factors [14,15]. Irrespective of the intended purpose of sericultural activities, whether for silk production or other advantages, the primary focus of research in this field revolves around assessing the nutritional value of mulberry leaves, the factors influencing this value, and how *B. mori* larvae utilize these nutrients. Additionally, it is noteworthy to mention the use of various foliar additives aimed at enhancing the nutritional value of mulberry leaves [18–20].

Sericulture specialists are highly focused on producing premium biological materials, developing superior mulberry varieties or hybrids and silkworm new breeds, bloodlines, and hybrids. Also, the breeding of biological material, as well as microclimate conditions (temperature, humidity, brightness), hygiene, nutrition, and the use of specific equipment, are part of the mulberry hybrids' enhancement. Over the years, different types of mulberry varieties and hybrids have been developed to produce high-quality leaf crops adapted with resilience against environmental factors such as drought, frost, diseases, and pests. Larvae of *B. mori* originating from these varieties exhibit enhanced productivity and an increased ability to withstand environmental factors and diseases, effectively utilizing the nutrients provided by mulberry leaves.

To optimize the peak productivity, especially in larvae used within intensive silk production systems, it is crucial to fine-tune every aspect of the growth process, where nutrition plays a pivotal role. Both the quantity and quality of mulberry leaves fed to the larvae substantially impact their growth rate, overall health, vitality, and silk production. Leaf quality is affected by diverse factors such as soil conditions, climate, seasonal changes, mulberry variety, harvesting methods, and storage [15–18,20,21].

Mulberry leaves furnish silkworm larvae with essential proteins, carbohydrates, fats, minerals, vitamins, and water necessary for growth and silk yield. The quality of leaves is determined by their chemical composition, which is influenced by various factors like leaf

maturity, their position on the branch, harvesting time and method, mulberry type, climate, use of fertilizers, and maintenance techniques applied to the mulberry plantations [16,17].

Young leaves, abundant in protein, possess lower energy due to fewer carbohydrates, leading to increased protein consumption. Leaves at branch tips contain more water and protein, while those at the base contain higher cellulose and minerals. Typically, feeding silkworms with leaves harvested 30–40 days from the middle of branches yields consistent results. However, feeding leaves harvested 90–100 days from the base prolongs the larval period, raises mortality rates, and diminishes cocoon quality by reducing silk content [20].

Leaves collected in the morning exhibit different chemical compositions compared to those collected in the evening, primarily due to the influence of direct sunlight synthesizing organic compounds [20,21]. Fertilizer application post-pruning for leaf production compensates for substantial organic matter loss. Enhancing soil fertility via proper fertilizer application remains crucial in increasing leaf yield. Mulberry leaf nutritional value can be enhanced by applying organic and mineral fertilizers, resulting in leaves with higher protein and ascorbic acid content [17–19,21].

Mulberry variety also affects leaf quality, showcasing variations in yield and nutritional value among different varieties. High temperatures and low humidity can impact leaf quality, potentially causing drought during summer heat, compromising leaf harvests to varying degrees [21].

Fresh leaves yield the best results when fed to the larvae. Chopped leaves are more efficiently consumed during the early larval stages as they provide more edges for consumption. In the final larval stages, whole leaves or leaves on branches are typically administered [22].

Hence, this study aims to contribute to the understanding of mulberry leaf quality from Romanian varieties and their utilization by *B. mori* larvae. Romania historically played a significant role in European sericulture, developing valuable mulberry varieties and hybrids, along with *B. mori* breeds and hybrids through extensive research.

This paper further aims to unveil the value of the Romanian Eforie mulberry variety, particularly its digestibility compared to the Japanese Kokuso 21 variety, in a summer experiment. These findings hold significant importance in the field of sericulture due to the uniqueness of the selected mulberry variety and the digestibility-related results obtained.

2. Materials and Methods

2.1. The Animal Material

The animal material involved two sets, designated as L1 and L2, each consisting of 300 *B. mori* larvae of the Romanian hybrid called Triumf. This hybrid demonstrates consistent productive traits and a significant degree of heterosis. To enable tracking, each set was subdivided into six groups of 50 larvae and raised in paper trays based on their age and size. Additionally, a backup group was established, comprising larvae raised separately but under identical conditions, ready to substitute any deceased individuals in the main experimental group. These larvae were reared throughout August in a controlled environment, meticulously monitoring all microclimate factors within an air-conditioned room.

For each subgroup, the quantities of administered leaves, unconsumed residues, and excreta were recorded separately for each of the five larval stages. Samples were collected from these and subsequently subjected to chemical analysis. Thus, each subgroup was given the same amounts of mulberry leaves, from which samples were collected in advance for chemical analysis. Daily, and at the same time, the uneaten mulberry leaf residues and excreta were collected, weighed, and recorded from the sublots. Additionally, samples of residues and excreta were collected from each subplot and then subjected to chemical analysis. To separate the larvae from the residues and excreta, nets with mesh sizes appropriate for the larvae's age were used. These were placed above the larvae, with fresh leaves scattered over them. To reach the fresh leaves above, the larvae had to cross the net. The uneaten leaf residues and excreta remained below the net, where they were collected, separated, weighed, and recorded. By knowing the quantities of administered

mulberry leaves, uneaten residues, and excreta, as well as their chemical composition, the digestibility coefficients of the mulberry leaves could be calculated for each variety and period.

After the donuts were formed, those harvested from each batch were sectioned and emptied of content (such as chrysalis and the leftovers from the last shedding of the larvae). This is how the silk wrap was separated and then the dry matter content was determined. In the following calculation regarding the food conversion, the average content of dry matter of the silk wrap, determined in the harvested donuts of each subgroup, was utilized.

The efficiency of the use of nutrients from leaf by the larvae was expressed by the amount of ingested/digested dry matter required for increasing 1 g of body mass/weight (silk wrap), respectively, by the efficiency of conversion of ingested substances (ECIS%)/digested (ECDS%) in weight [22–25].

The efficiency of nutrient utilization from mulberry leaves [26–28] was expressed by the following:

- grams of dry matter (DM) ingested necessary to produce one gram of growth (grams of dry matter of ingested leaf/grams of dry matter of silk wrap); this ratio provides insight into the feed efficiency of the larvae, indicating how much of the leaf's dry matter is needed to produce a specific amount of silk (a lower ratio indicates higher efficiency, as less feed is required per unit of silk produced).
- grams of dry matter (DM) digested necessary to produce one gram of growth (grams of dry matter of digested leaf/grams of dry matter of silk wrap); this ratio reflects the efficiency of the larvae's digestive system, highlighting how much of the ingested dry matter is actually utilized (absorbed and metabolized) for silk production (a more efficient digestive system results in a lower DM digested value).
- efficiency of conversion of ingested substances (ECIS) into silk wrap ($ECIS = \text{dry matter of silk wrap} / \text{dry matter of ingested leaf} \times 100$); a higher ECIS value indicates better utilization of the ingested nutrients for silk production, demonstrating the larvae's ability to convert the feed into the desired product effectively.
- efficiency of conversion of digested substances (ECDS) into silk wrap ($ECDS = \text{silk wrap} / \text{digested leaf} \times 100$); this ratio provides an understanding of the efficiency with which the digested nutrients are converted into silk, accounting for the absorption and metabolic processes (a higher ECDS value suggests that a larger proportion of the digested nutrients contribute to silk synthesis).

The analysis of the nutritional content and digestibility of the mulberry leaves is crucial for interpreting these efficiency metrics. This study involved assessing the protein, carbohydrate, lipid, and fiber content of the leaves, as these components are vital for the growth and silk production of the larvae. Additionally, understanding the digestibility of these nutrients helps in determining how much of the ingested food can be metabolically converted into silk.

By integrating these detailed metrics and analyses, this study provides a thorough evaluation of the silkworms' efficiency in converting mulberry leaf nutrients into silk, which is essential for optimizing feeding strategies and improving silk yield.

2.2. The Vegetal Material

The vegetal material comprised Kokuso 21 mulberry leaves (adapted well to Romania's conditions, obtained through a crossbreeding of the Naganua, Gariin, and Shiso varieties) provided to the L1 set. The Eforie variety (a high-yielding Romanian strain) was given to the L2 group. Each subgroup within the larger sets received identical amounts of leaves (15.5 g at stage I, 26 g at stage II, 77 g at stage III, 242 g at stage IV, and ultimately reaching 1000 g at stage V, accumulating a total weight of 1254.5 g over the entire growth period). Samples were extracted beforehand from these leaves and subjected to chemical analyses. The mulberry leaf leftovers and excreta were collected daily and recorded at the same time. The unconsumed portions were weighed and recorded for each subgroup. Samples were also collected and subjected to chemical analysis (the excreta for age I was 0.147 g in

Kokuso 21 and 0.155 g in Eforie; for age II, 1.019 g in Kokuso 21 and 0.622 g in Eforie; for age III, 4.125 g in Kokuso 21 and 3.629 g in Eforie; for age IV, 19.858 g in Kokuso 21 and 22.204 g in Eforie; and for age V, 122.39 g in Kokuso 21 and 126.96 g in Eforie).

2.3. The Analytical Techniques

The analytical techniques were aimed at evaluating the nutritional content of mulberry leaves and relied on examining their chemical composition and digestibility of individual elements.

The chemical composition was determined using the “proximate analysis” method, which involved samples that had been previously dried at 65 °C and subsequently ground [29].

To ascertain the moisture content, collected samples underwent drying in a Matest oven at 105 °C for 4–5 h (Treviolo, Italy) [30]. The samples have to be prepared as follows: initially, samples of mulberry leaves were collected and subjected to drying at a temperature of 65 °C; this drying step is crucial to remove any free moisture content and stabilize the samples, preventing microbial growth or enzymatic activity that could alter the composition. After drying, the samples were ground into a fine powder; grinding increases the surface area and homogenizes the sample, ensuring that subsequent analyses accurately reflect the overall composition. Each sample was weighed and the results recorded (the samples of leaf leftovers consisted in the following: for larval age 1, Kokuso 21 had 5.007 g and Eforie 5.295 g; for age II, Kokuso 21 had 8.221 g and Eforie 9.039 g; for age III, Kokuso 21 had 23.146 g and Eforie 24.090 g; for age IV, Kokuso 21 had 67.940 g and Eforie 65.503 g; for age V, Kokuso 21 had 269.055 g and Eforie 269.837 g); these samples were then dried in a controlled temperature and airflow environment. The temperature in the first two larval stages ranged between 26 and 27 °C, in the third stage between 24 and 25 °C, and in the last two stages between 23 and 24 °C. Humidity levels were maintained at 85–90% during the first stage, 80–85% during the second and third stages, 70–75% during the fourth stage, and 65–70% during the fifth stage.

To determine the moisture content, a specific portion of the ground sample was weighed and then placed in a Matest oven. The oven temperature was set to 105 °C, and the samples were dried for 4–5 h. This temperature is chosen because it is sufficient to evaporate all free water without decomposing other components of the sample. After drying, the samples were reweighed. The difference in weight before and after drying represents the amount of moisture lost from the sample. The moisture content was then calculated using the following formula: $(\text{initial weight} - \text{final weight} / \text{initial weight}) \times 100$. This percentage indicates the proportion of the sample that was water. The higher the moisture content, the more water was present in the original sample. The dry matter content, which represents the portion of the material excluding water, was calculated by subtracting the moisture content percentage from 100%. This step is essential because dry matter includes all the nutritional components of the leaves, such as proteins, fats, carbohydrates, and fiber: $\text{dry matter content (\%)} = 100\% - \text{moisture content (\%)}$. The DM value is crucial for subsequent nutrient analysis as it allows for the quantification of nutrients relative to the dry weight of the material, rather than the fresh weight, which can vary significantly due to moisture fluctuations. The determination of Crude Ash (CA) was carried out by incinerating leaf samples in a 40-Segment Muffle Furnace (Bayswater, NSW, Australia) [30]. The samples were weighed, incinerated at 550 °C to eliminate organic components, leaving behind inorganic mineral content (ash). The ash content was calculated as a percentage of the initial wet weight using a specific formula.

The Kjeldahl method (Cole-Parmer equipment, Vernon Hills, IL, USA) was employed to measure total nitrogen content and subsequently determine crude protein (CP) [30]. This process involved heating the samples with concentrated sulfuric acid, along with catalysts, to break down organic compounds containing nitrogen into ammonium sulfate. Subsequent steps included neutralizing the acidic solution, collecting liberated ammonia gas through distillation, and then titrating it to quantify nitrogen content.

The determination of crude fat (CFa) employed the direct Randall method (Dolphin Labware 6 Test apparatus, Mumbai, India). This method relies on the property of fats to dissolve in organic solvents (petroleum ether) [30]. The process involved solvent extraction through the sample, vaporization, condensation, and refluxing, resulting in the concentration and quantification of extracted lipids based on the difference in initial and final flask masses.

Crude fiber (CFi) determination involved sample preparation and initial weighing (samples of dried and ground mulberry leaves were accurately weighed to record their initial dry weight. This initial weight serves as the baseline for calculating the crude fiber content), acid hydrolysis (the first step in the hydrolysis process involved treating the samples with a sulfuric acid solution. This step is critical for breaking down complex carbohydrates like cellulose and lignin into simpler monosaccharides and lignin residues. During the acid hydrolysis, cellulose is partially broken down, while lignin remains largely unaffected. The acid treatment dissolves other polysaccharides, such as hemicelluloses, leaving behind a solid residue primarily composed of lignin and any unhydrolyzed cellulose. The mixture was then filtered to separate the solid residues from the liquid phase. The solid residues, containing the indigestible fibers, were collected on the filter paper), known as alkaline hydrolysis. The solid residues obtained from the acid hydrolysis were then subjected to alkaline hydrolysis using a sodium hydroxide solution. This step further breaks down any remaining cellulose into simpler sugars, which are soluble in the alkaline solution. The purpose of this step is to ensure that any remaining digestible carbohydrates are removed, leaving behind only the lignin and any unhydrolyzed cellulose. After alkaline hydrolysis, the mixture was again filtered to separate the liquid phase, containing the dissolved sugars, from the solid residues. The solid residues after this step were primarily composed of lignin and any remaining unhydrolyzed cellulose, which are considered indigestible, then the drying and weighing of residues took place (the solid residues from both the acid and alkaline hydrolyses were subjected to a drying process. This drying step is crucial for removing any remaining moisture, ensuring that the weight recorded corresponds to the dry matter content of the residues. The dry weights of these residues were carefully recorded. These weights are critical for calculating the crude fiber content), and then the calculation of crude fiber content was conducted. The crude fiber content was calculated as the difference between the initial dry weight of the sample and the combined dry weight of the solid residues after both hydrolyses. This calculation excludes other processed components such as sugars and soluble fibers that were removed during the hydrolysis steps. This value represents the amount of indigestible fiber present in the sample, which consists primarily of lignin and unhydrolyzed cellulose. The hydrolysable portion was removed, leaving crude fiber and mineral salts on the filter. Crude fiber was calculated by calcinating the mineral substances, starting with accurate weighing of samples and recording their dry weights. Acid hydrolysis with sulfuric acid broke down cellulose and lignin into constituent monosaccharides and lignin residues. Filtration separated solid residues, consisting of unhydrolyzed cellulose and lignin. Alkaline hydrolysis with sodium hydroxide (Missouri, USA) further broke down cellulose into sugars. Filtration separated the liquid phase (sugars) from solid residues (lignin). Solid residues from both acid and alkaline hydrolyses underwent a drying step, and their dry weights were recorded. Crude fiber content was calculated as the difference between the initial dry weight and the combined dry weight of solid residues after both hydrolyses, excluding other processed components [31].

The nitrogen-free extract (NFE) was calculated by subtracting the percentages of water, crude protein, crude fat, crude fiber, and crude ash from 100% [32].

The digestibility of mulberry leaf nutrients was measured using the “in vivo” method, specifically simple digestibility, with a single control period. Digestibility coefficients (DC%) were determined by considering the amounts of fed leaves, leftovers, excreta, and chemical analyses data. Ingesta (difference between fed and unconsumed leftovers) and digesta (difference between intake and excretion) were calculated. Digestibility coefficients,

expressed as a percentage, indicated what portion of substances in mulberry leaves were digested by the larvae [32]. To calculate the digestibility coefficients for each nutritional substance, we analyzed the quantities of each nutrient intake, as well as those that were excreted and digested. The data were compiled separately for each mulberry variety, based on the larvae's age. In the Kokuso 21 variety, both the age of the larvae and the degree of leaf maturity significantly influenced the digestibility of organic matter; a gradual decrease from age I to age V was identified ($p < 0.001$). For the Eforie variety, the digestibility coefficients of dry matter (DM) significantly decreased ($p < 0.001$) as the larvae aged or the mulberry leaves also aged (age I vs. III, IV, and V, as well as ages II vs. IV and V, and ages III to V). A similar pattern was observed for other organic nutrients. All analytical assessments and computations were conducted in 18 repetitions (the total number of larvae was divided into 2 groups, each consisting of 300 larvae (Triumf hybrid); each group was further subdivided into 6 subgroups, with 50 larvae in each subgroup, resulting in 6 repetitions).

2.4. Statistical Data Processing

Statistical data processing involved calculating the main descriptors, such as arithmetic mean, variance, standard deviation, standard error of the mean, and coefficient of variation [33]. Graph Pad Prism 9.4.1 software was used to treat the database, in order to achieve (a) main descriptors (mean, standard deviation, standard error of mean, variation coefficient); (b) level of significance for one to one comparisons, using the unpaired Student (t) test, with Welch's correction between the means of the two varieties, within the same age period; (c) ANOVA single factor with Tukey post-hoc testing to run multiple comparisons between means (within the same variety, between more than two ages); (d) Pearson correlation coefficients when crude fiber values and the digestibility coefficients of the other organic matters were associated.

3. Results

3.1. The Proximate Composition of the Mulberry Leaves

Table 1 compiles the chemical composition data of mulberry leaves from both studied varieties based on larval age. Significant modifications in dry matter (DM) content were noted for Kokuso 21 across larval age groups (I vs. III, IV, V; II vs. IV, V; III vs. V), demonstrating notable differences ($p < 0.001$). Regarding crude protein (CP) content, substantial differences emerged between age groups: I vs. III, V, and II vs. III, IV, V ($p < 0.001$). Furthermore, significant variations were observed for crude fat (CFa) and crude fiber (CFi) contents between younger age groups I vs. II and older age groups IV vs. V for Kokuso 21.

Table 1. Proximate composition of mulberry leaves (mean $\pm$ standard deviation), based on the degree of maturity in relation to larvae age.

Larval Age	Mulberry Variety	Dry Matter % (Mean $\pm$ Standard Deviation)	Crude Protein % (Mean $\pm$ Standard Deviation)	Crude Fat % (Mean $\pm$ Standard Deviation)	Crude Fiber % (Mean $\pm$ Standard Deviation)	Nitrogen-Free Extract % (Mean $\pm$ Standard Deviation)	Ash % (Mean $\pm$ Standard Deviation)
I	Kokuso 21	27.91 $\pm$ 0.25	6.31 $\pm$ 0.09	0.79 $\pm$ 0.09	4.74 $\pm$ 0.14	12.33 $\pm$ 0.18	3.75 $\pm$ 0.45
	Eforie	28.14 $\pm$ 0.90	6.23 $\pm$ 0.40	0.85 $\pm$ 0.20	4.79 $\pm$ 0.42	12.43 $\pm$ 0.50	3.84 $\pm$ 0.88
II	Kokuso 21	28.34 $\pm$ 0.54	6.28 $\pm$ 0.18	0.88 $\pm$ 0.13	4.88 $\pm$ 0.17	12.34 $\pm$ 0.22	3.96 $\pm$ 0.67
	Eforie	28.03 $\pm$ 0.81	6.21 $\pm$ 0.33	0.88 $\pm$ 0.20	4.76 $\pm$ 0.49	12.24 $\pm$ 0.63	3.94 $\pm$ 1.27

Table 1. Cont.

Larval Age	Mulberry Variety	Dry Matter % (Mean $\pm$ Standard Deviation)	Crude Protein % (Mean $\pm$ Standard Deviation)	Crude Fat % (Mean $\pm$ Standard Deviation)	Crude Fiber % (Mean $\pm$ Standard Deviation)	Nitrogen-Free Extract % (Mean $\pm$ Standard Deviation)	Ash % (Mean $\pm$ Standard Deviation)
III	Kokuso 21	29.70 $\pm$ 0.41	6.23 $\pm$ 0.15	1.14 $\pm$ 0.14	5.31 $\pm$ 0.62	12.64 $\pm$ 0.50	4.38 $\pm$ 1.22
	Eforie	29.32 $\pm$ 0.53	6.41 $\pm$ 0.39	1.17 $\pm$ 0.25	5.26 $\pm$ 0.31	12.30 $\pm$ 0.40	4.18 $\pm$ 0.94
IV	Kokuso 21	29.87 $\pm$ 0.45	6.04 $\pm$ 0.10	1.16 $\pm$ 0.14	5.44 $\pm$ 0.42	13.09 $\pm$ 0.67	4.14 $\pm$ 1.28
	Eforie	30.47 $\pm$ 0.80	6.00 $\pm$ 0.39	1.22 $\pm$ 0.22	5.58 $\pm$ 0.50	13.37 $\pm$ 0.39	4.30 $\pm$ 1.13
V	Kokuso 21	31.14 $\pm$ 0.91	6.15 $\pm$ 0.41	1.25 $\pm$ 0.33	5.93 $\pm$ 0.75	13.41 $\pm$ 0.61	4.41 $\pm$ 0.71
	Eforie	31.85 $\pm$ 0.69	6.06 $\pm$ 0.34	1.38 $\pm$ 0.36	6.15 $\pm$ 0.28	13.58 $\pm$ 0.44	4.69 $\pm$ 0.45
I–V	Kokuso 21	29.39 $\pm$ 0.14	6.20 $\pm$ 0.10	1.04 $\pm$ 0.09	5.26 $\pm$ 0.27	12.76 $\pm$ 0.25	4.13 $\pm$ 0.50
	Eforie	29.56 $\pm$ 0.16	6.18 $\pm$ 0.08	1.10 $\pm$ 0.10	5.31 $\pm$ 0.23	12.78 $\pm$ 0.30	4.19 $\pm$ 0.34

Ash = inorganic mineral content.

In the case of the Eforie variety, the dynamics of DM content exhibited several distinctions when comparing age groups: I vs. IV ($p < 0.05$), I vs. V ($p < 0.001$), II vs. IV ($p < 0.05$), II vs. V ($p < 0.001$), and III vs. V ($p < 0.05$).

Comparisons between varieties revealed no significant differences ($p > 0.05$), regardless of the analyzed nutrients or age.

3.2. Mulberry Leaves Digestibility

The data detailing the quantities of fed leaves, residues, excreta, and their respective chemical compositions, crucial for calculating ingesta and digesta, have been separately organized for each group in Tables 2–4 which present the chemical composition of the excreta, vital for computing digestibility coefficients.

Table 2. Digesta calculation for Kokuso 21 and Eforie leaves.

Larval Age	Variety	F (g)	R (g)	I = F – R (g)	E (g)	D = I – E (g)
I	Kokuso 21	15.5	5.007	10.493	0.147	24.01
	Eforie	15.5	5.295	10.205	0.155	10.050
II	Kokuso 21	26	8.221	17.779	1.019	16.760
	Eforie	26	9.039	16.961	0.622	16.339
III	Kokuso 21	77	23.146	53.885	4.125	49.730
	Eforie	77	24.090	52.910	3.629	49.282
IV	Kokuso 21	242	67.940	174.143	19.858	154.286
	Eforie	242	65.503	176.497	22.204	154.294
V	Kokuso 21	1000	269.055	730.945	122.390	608.555
	Eforie	1000	269.837	730.163	126.960	603.203
I–V	Kokuso 21	1365.5	373.368	987.215	147.539	839.676
	Eforie	1365.5	373.763	986.737	153.570	833.167

F = fed leaves quantity; R = residues; I = ingesta; E = excreta; D = digesta.

Table 3. Proximate composition of residues of Kokuso 21 and Eforie mulberry leaves (mean $\pm$ standard deviation), based on larvae age.

Larval Age	Mulberry Variety	Dry Matter % (Mean $\pm$ Standard Deviation)	Crude Protein % (Mean $\pm$ Standard Deviation)	Crude Fat % (Mean $\pm$ Standard Deviation)	Crude Fiber % (Mean $\pm$ Standard Deviation)	Nitrogen-Free Extract % (Mean $\pm$ Standard Deviation)	Ash % (Mean $\pm$ Standard Deviation)
I	Kokuso 21	63.82 $\pm$ 0.41	14.04 $\pm$ 0.26	2.01 $\pm$ 0.37	14.59 $\pm$ 0.37	25.97 $\pm$ 0.44	7.23 $\pm$ 1.43
	Eforie	63.61 $\pm$ 0.41	15.01 $\pm$ 0.44	1.68 $\pm$ 0.43	13.92 $\pm$ 0.41	24.01 $\pm$ 0.42	9.00 $\pm$ 1.09
II	Kokuso 21	60.03 $\pm$ 0.36	12.01 $\pm$ 0.81	2.11 $\pm$ 0.39	14.88 $\pm$ 0.43	21.01 $\pm$ 0.67	10.02 $\pm$ 1.24
	Eforie	59.16 $\pm$ 0.43	13.01 $\pm$ 0.32	2.01 $\pm$ 0.44	13.51 $\pm$ 0.45	22.61 $\pm$ 0.39	8.03 $\pm$ 1.13
III	Kokuso 21	58.39 $\pm$ 0.33	10.78 $\pm$ 0.19	2.31 $\pm$ 0.25	15.99 $\pm$ 0.38	22.12 $\pm$ 0.31	7.20 $\pm$ 0.43
	Eforie	58.82 $\pm$ 0.63	12.33 $\pm$ 0.47	2.36 $\pm$ 0.32	15.77 $\pm$ 0.45	24.02 $\pm$ 0.42	4.33 $\pm$ 1.01
IV	Kokuso 21	59.53 $\pm$ 0.40	12.62 $\pm$ 0.34	2.45 $\pm$ 0.42	14.49 $\pm$ 0.35	24.72 $\pm$ 0.54	5.27 $\pm$ 1.01
	Eforie	58.06 $\pm$ 0.36	12.06 $\pm$ 0.48	1.72 $\pm$ 0.45	15.71 $\pm$ 0.38	24.66 $\pm$ 0.41	3.91 $\pm$ 0.79
V	Kokuso 21	56.73 $\pm$ 0.46	11.46 $\pm$ 0.41	1.88 $\pm$ 0.37	13.66 $\pm$ 0.39	23.97 $\pm$ 0.55	5.75 $\pm$ 1.12
	Eforie	58.56 $\pm$ 0.45	11.06 $\pm$ 0.55	2.61 $\pm$ 0.40	11.99 $\pm$ 0.72	24.88 $\pm$ 0.63	8.03 $\pm$ 1.88
I–V	Kokuso 21	59.70 $\pm$ 0.17	12.18 $\pm$ 0.17	2.15 $\pm$ 0.11	14.72 $\pm$ 0.27	23.56 $\pm$ 0.24	7.09 $\pm$ 0.76
	Eforie	59.64 $\pm$ 0.12	12.69 $\pm$ 0.20	2.08 $\pm$ 0.28	14.18 $\pm$ 0.26	24.04 $\pm$ 0.32	6.66 $\pm$ 0.98

Table 4. Chemical composition of excreta from the Kokuso 21 and Eforie varieties (mean $\pm$ standard deviation), in relation to larvae age.

Larval Age	Mulberry Variety	Dry Matter % (Mean $\pm$ Standard Deviation)	Crude Protein % (Mean $\pm$ Standard Deviation)	Crude Fat % (Mean $\pm$ Standard Deviation)	Crude Fiber % (Mean $\pm$ Standard Deviation)	Nitrogen-Free Extract % (Mean $\pm$ Standard Deviation)	Ash % (Mean $\pm$ Standard Deviation)
I	Kokuso 21	66.15 $\pm$ 0.41	21.87 $\pm$ 0.42	5.67 $\pm$ 0.41	2.33 $\pm$ 0.38	27.21 $\pm$ 0.48	9.07 $\pm$ 0.138
	Eforie	69.82 $\pm$ 0.42	14.33 $\pm$ 0.44	15.02 $\pm$ 0.44	3.35 $\pm$ 0.51	27.09 $\pm$ 0.70	9.13 $\pm$ 2.06
<i>p</i> value		0.9999	0.9999	>0.9999	>0.9999	0.9999	0.9999
II	Kokuso 21	64.69 $\pm$ 0.53	14.93 $\pm$ 0.62	3.64 $\pm$ 0.39	4.31 $\pm$ 0.35	27.85 $\pm$ 0.70	13.96 $\pm$ 1.87
	Eforie	63.22 $\pm$ 0.62	11.01 $\pm$ 0.59	4.01 $\pm$ 0.52	2.43 $\pm$ 0.43	29.78 $\pm$ 0.55	15.99 $\pm$ 1.91
<i>p</i> value		0.9999	>0.9999	>0.9999	0.9999	0.9999	>0.9999
III	Kokuso 21	65.15 $\pm$ 0.44	14.99 $\pm$ 0.40	2.63 $\pm$ 0.43	7.99 $\pm$ 0.38	25.53 $\pm$ 0.53	14.00 $\pm$ 1.04
	Eforie	64.53 $\pm$ 0.64	16.29 $\pm$ 0.43	2.01 $\pm$ 0.20	6.09 $\pm$ 0.70	28.02 $\pm$ 0.52	12.12 $\pm$ 1.82
<i>p</i> value		0.9999	0.9999	>0.9999	>0.9999	0.9999	0.9999
IV	Kokuso 21	63.52 $\pm$ 0.40	11.01 $\pm$ 0.47	2.02 $\pm$ 0.41	14.09 $\pm$ 0.29	24.99 $\pm$ 0.48	11.41 $\pm$ 1.35
	Eforie	64.06 $\pm$ 0.46	11.01 $\pm$ 0.50	2.66 $\pm$ 0.34	12.04 $\pm$ 0.44	27.29 $\pm$ 0.42	11.06 $\pm$ 1.78
<i>p</i> value		0.9985	>0.9999	>0.9999	0.9999	0.9999	0.9999
V	Kokuso 21	61.03 $\pm$ 0.49	9.98 $\pm$ 0.70	3.22 $\pm$ 0.24	13.99 $\pm$ 0.45	25.01 $\pm$ 0.54	8.83 $\pm$ 1.53
	Eforie	62.58 $\pm$ 0.42	10.38 $\pm$ 0.43	3.01 $\pm$ 0.48	16.06 $\pm$ 0.23	24.02 $\pm$ 0.32	9.11 $\pm$ 1.15
<i>p</i> value		0.9973	>0.9999	0.9999	0.9999	0.9999	0.9999
I–V	Kokuso 21	64.11 $\pm$ 0.25	14.56 $\pm$ 0.31	3.43 $\pm$ 0.26	8.54 $\pm$ 0.20	26.12 $\pm$ 0.44	11.46 $\pm$ 1.31
	Eforie	64.84 $\pm$ 0.26	12.60 $\pm$ 0.30	5.34 $\pm$ 0.18	8.00 $\pm$ 0.23	27.42 $\pm$ 0.45	11.48 $\pm$ 1.33
<i>p</i> value		0.9999	>0.9999	0.9979	>0.9999	>0.9999	0.9999

The average values of the digestibility coefficients of the mulberry leaves were calculated for each variety and stage of growth; the results are presented in Table 5.

Table 5. Digestibility coefficients of mulberry leaves.

Larval Age	Mulberry Variety	Dry Matter % (Mean ± Standard Deviation)	Crude Protein % (Mean ± Standard Deviation)	Crude Fat % (Mean ± Standard Deviation)	Crude Fiber % (Mean ± Standard Deviation)	Nitrogen-Free Extract % (Mean ± Standard Deviation)
I	Kokuso 21	91.38 ± 1.66	88.30 ± 2.25	61.30 ± 7.36	0.17 ± 0.08	93.45 ± 1.26
	Eforie	89.08 ± 1.42	87.02 ± 1.67	46.01 ± 7.07	0.53 ± 0.21	93.36 ± 0.87
<i>p</i> value		>0.9999	>0.9999	6.1×10^{-6}	>0.9999	>0.9999
II	Kokuso 21	72.91 ± 1.10	76.46 ± 0.97	31.80 ± 2.68	4.40 ± 1.92	80.85 ± 0.79
	Eforie	79.87 ± 10.18	84.51 ± 7.84	47.50 ± 26.33	3.62 ± 1.10	83.81 ± 8.25
<i>p</i> value		0.8709	0.5441	2.5×10^{-6}	>0.9999	>0.9999
III	Kokuso 21	71.26 ± 0.10	73.09 ± 0.10	68.15 ± 0.12	15.44 ± 0.56	77.17 ± 0.08
	Eforie	72.20 ± 3.88	70.02 ± 4.23	77.94 ± 3.07	10.21 ± 2.19	72.47 ± 3.87
<i>p</i> value		>0.9999	0.9999	0.1042	0.9992	0.9999
IV	Kokuso 21	60.42 ± 0.08	63.86 ± 0.08	65.25 ± 0.08	15.66 ± 0.29	66.68 ± 0.07
	Eforie	60.16 ± 0.87	63.13 ± 0.77	67.58 ± 0.83	16.76 ± 1.88	62.61 ± 0.83
<i>p</i> value		>0.9999	>0.9999	>0.9999	>0.9999	0.9999
V	Kokuso 21	52.96 ± 0.02	60.10 ± 0.02	46.62 ± 0.02	24.15 ± 0.03	56.01 ± 0.02
	Eforie	50.49 ± 0.89	57.14 ± 0.77	43.51 ± 1.03	30.09 ± 1.30	55.55 ± 0.80
<i>p</i> value		>0.9999	>0.9999	0.9999	0.9882	>0.9999
I–V	Kokuso 21	55.42 ± 0.02	61.88 ± 0.03	49.79 ± 0.03	22.91 ± 0.06	59.48 ± 0.02
	Eforie	53.49 ± 0.74	59.19 ± 0.65	49.67 ± 0.84	28.62 ± 1.26	58.14 ± 0.67
<i>p</i> value		0.0001	6.5×10^{-8}	0.9999	7.1×10^{-15}	0.0122
I–V	Average values per larval period	54.45 ± 0.38	60.53 ± 0.34	49.73 ± 0.43	25.67 ± 0.66	58.81 ± 0.69

In Table 6, the correlations between the crude fiber content of leaves and the digestibility of the organic matter achieved by larvae, for the entire period (ages I–V), revealed negative values in both Kokuso 21 and Eforie hybrids, but yet not significant in both situations ($p > 0.05$) and with stronger correlations in Eforie samples.

Table 6. Correlations between CFI and the digestibility of organic matter compounds in larvae (overall ages I–V).

Variety	Organic Matter Compounds and Digestibility			
	Dry Matter %	Crude Protein %	Crude Fat %	Nitrogen-Free Extract %
Crude fiber in Kokuso 21	$r = -0.33$	$r = -0.39$	$r = -0.36$	$r = -0.30$
<i>p</i> value	0.5288	0.4422	0.4875	0.5100
Crude fiber in Eforie	$r = -0.66$	$r = -0.65$	$r = -0.63$	$r = -0.65$
<i>p</i> value	0.1502	0.1608	0.1785	0.1641

r = correlations between the digestibility of organic matter compounds and the dietary crude fiber content.

Based on the determinations made on the cocoons collected from each subgroup, it appears that the silk wrap obtained from a single larva weighed approximately 0.4048 g DM (20.24 ± 0.23 g DM/subgroup) in the case of the batch fed with leaves from the Kokuso 21 variety, and 0.4096 g DM (20.48 ± 0.14 g DM/subgroup) in the case of the batch fed with leaves from the Eforie variety. From this perspective, no statistically significant differences were observed between the two batches (p value = 0.060736728). The data regarding the conversion of mulberry leaves into silk have been summarized in Table 7.

Table 7. The efficiency of utilizing mulberry leaves by *B. mori* (mean $\pm$ standard deviation).

Mulberry Variety	Ingested Dry Matter/Silk Wrap Dry Matter (Mean $\pm$ Standard Deviation)	Digested Dry Matter/Silk Wrap Dry Matter (Mean $\pm$ Standard Deviation)	ECIS Silk Wrap (%) (Mean $\pm$ Standard Deviation)	ECDS Silk Wrap (%) (Mean $\pm$ Standard Deviation)
Kokuso 21	10.06 ± 0.11	5.57 ± 0.06	9.90 ± 0.11	17.94 ± 0.04
Eforie	10.14 ± 0.08	5.42 ± 0.10	9.87 ± 0.08	18.45 ± 0.35
p value	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$

ECIS = efficiency of conversion of ingested substances; ECDS = efficiency of conversion of digested substances.

Based on the data in Table 7, it can be observed that for each gram of silk dry matter (DM) in the cocoon, the larvae ingested an average of 10.06 g of leaf DM in the case of the Kokuso 21 variety and 10.14 g of leaf DM in the case of the Eforie variety. In other words, the efficiency of the conversion of ingested matter into silken cocoon (ECIS) was on average 9.9% for the Kokuso 21 variety and 9.87% for the Eforie variety, with the differences not being significant.

When the comparison was made based on digested matter, it was observed that for each gram of silk DM in the cocoon, an average of 5.57 g of digested leaf DM was necessary for the Kokuso 21 variety and 5.42 g of digested leaf DM for the Eforie variety. In this way, the efficiency of the conversion of digested matter into silken cocoon (ECDS) was on average 17.94% for the Kokuso 21 variety and 18.45% for the Eforie variety, with the differences being significant this time.

Thus, although there were no significant differences in food conversion into silk based on ingested matter between the two varieties, the complex phenomena occurring during digestion make these differences significant, with the Eforie variety showing better leaf utilization by the larvae.

Therefore, it can be stated that, at least for summer rearing, the Eforie variety performs at least as well as the Kokuso 21 variety, which is why it can be recommended for the specific pedoclimatic conditions of Romania.

Regarding the efficiency of the use of the mulberry leaf by the *B. mori* larvae, the data obtained from the experiment are similar to those presented in the literature [33–37].

4. Discussion

4.1. The Proximate Composition of Mulberry Leaves

The intake of mulberry leaves significantly impacts the growth of silkworm larvae, especially during their initial stages when they prefer tender and well-hydrated leaves. Chemical analyses conducted throughout the larvae's growth period on the mulberry leaves used as feed (Tables 1 and 2) revealed an average leaf moisture content of 70.53% and a total solids (dry matter) content of 29.47%. Over the larvae's growth period, the leaf moisture decreased by an average of 3.78%.

In the literature, relative humidity values for mulberry leaves typically range from 65% to 75% [16–20,22,23,31,32]. Compared to common mulberry leaves, which have a moisture content ranging from 69.80% to 73%, the studied varieties had higher water content. In the larvae's bodies, water is found in larger quantities in the digestive tract and hemolymph, and in smaller proportions in tissues and muscles. The water content in larvae varies by age, with younger larvae having a higher percentage of water compared to adults. The water

balance is maintained through its consumption with mulberry leaves and its elimination through processes such as excretion, respiration, and transpiration. The intensity of water metabolism is influenced by factors such as the water content, temperature, and humidity.

In the studied variety, the water content varies from 71.50% in the initial two phases to less than 65% in the later phases. The best results in silkworm growth are achieved when feeding starts with the appearance of the third or fifth leaf. Initiating growth when the seventh leaf appears leads to an extended larval period, and starting with the ninth leaf results in lower outcomes in terms of cocoon weight, silk cocoon shell, and silk's technological properties. Feeding larvae with young leaves results in better consumption, digestion, and utilization of protein substances compared to feeding them with mature leaves. As leaves age, their composition changes, with a decrease in water content, protein, and easily soluble carbohydrates, while the quantity of cellulose and ash increases, making the leaves coarse. Leaf maturity should largely correspond to larval development; thus, first- and second-stage larvae feed exclusively on young leaves. Young leaves, rich in protein, have lower energy value and are less rich in carbohydrates, leading to higher protein consumption. If the leaves are old, their nutritional efficiency is lower due to poor water content, which slows down biological reactions in the digestive tract. The same occurs if the leaves begin to wither. Leaf age significantly influences silk composition—to obtain leaves of suitable quality throughout the mulberry's entire growing period, intensive cultivation plantations employ harvesting practices that promote the growth of new leaves for repeated silkworm rearing cycles [38].

Fresh mulberry leaves have a dry matter content of 24.86%, of which 84.33% is organic matter [36]. The dry matter content of mulberry leaves harvested during the same period can vary depending on the variety or hybrid, with values ranging from 23.61% to 27.56%. Mulberry leaf humidity is influenced by the season, ranging from 71.85% to 77.81% in spring, 68.42% to 75.64% in summer, and 64.10% to 73.64% in autumn [39].

The protein content of mulberry leaves significantly influences the growth, development, and cocoon formation of silkworm larvae, as well as the overall silk yield. Throughout the study period, the average crude protein content in the mulberry leaves was 6.19%. In terms of dry matter, crude protein values ranged from 22.38% in the first larval stage to 19.39% in the fifth stage.

In the literature, the crude protein content of mulberry leaves is reported as 6.16% in fresh leaves [21], 16.57% [26], 16.67–21.62% [36], 19.60% [37], 20.34% [38], 20.97% [39], and 29.80% in dry matter, with 24.36% in organic matter [40]. Crude protein values in leaves can vary based on the season, time of day, mulberry variety/hybrid, with reported values ranging from 32.40% in spring to 24.53% in autumn [21], 26.80% in the morning to 29.10% in the evening [26], and between 20.20% and 26.72% depending on the variety [26–28] (these values are expressed as percent of crude protein in leaves' dry matter).

Protein metabolism is crucial in the nutrition of silkworms. The concept of crude protein used in experimentation encompasses both proteinaceous and non-proteinaceous nitrogenous substances. During digestion, proteins are broken down into peptides and amino acids, which are absorbed by the larva's body. After the cleavage of proteins from mulberry leaves, the resulting amino acids are re-synthesized into specific proteins within the organism. Some amino acids are synthesized by the organism. An increase in leucine, proline, and tryptophan is observed, along with a decrease in glycine, tyrosine, and other amino acids, in the stages of pupa and butterfly, during which the insect no longer feeds. Adding tryptophan and tyrosine to the silkworm's diet increases larval weight and shortens the larval period. Tryptophan and cysteine influence larval development, while alanine and tyrosine impact silk formation. Globulins are synthesized throughout the entire larval period (with an intensification in the fourth and fifth stages), while albumin synthesis occurs only in the last two stages. During the larval stage, various nitrogenous substances from mulberry leaves stored in the silkworm's body, such as globulins, prolamins, glutenin, and albumins, are particularly active in metabolism. Amino acids, besides their structural role, also serve as energy sources, degrading into CO₂, H₂O, and NH₃. The resulting

nitrogen is eliminated as uric acid, which requires energy consumption. Depending on the larva's health, uric acid can also form from the nitrogen resulting from tissue cell breakdown. Additionally, amino acids not contributing to silk formation are either eliminated or degraded and converted into uric acid. Alongside uric acid, urates, small quantities of urea, ammonia, and oxalates, are found. The nitrogen balance in the silkworm's body during the larval period is positive [41].

Lipids are the most concentrated form of energy storage in any organism, including *B. mori*. In silkworms, fats serve as an energy source and play a structural role in cells. Lipid metabolism, though under-studied, involves the breakdown of fats into fatty acids and glycerol by alkaline gastric juice. These are absorbed in the midgut, where neutral fats are resynthesized and stored in adipose tissue and other cells. Lipids can also pass directly into tissues through the hemolymph. Larvae synthesize lipids from carbohydrates, with lipid metabolism becoming crucial during metamorphosis, especially after feeding ceases. In larvae, energy is primarily derived from carbohydrates, while in pupal and adult stages, lipids become the main energy source. The synthesized lipids contain both saturated and unsaturated fatty acids, with 75% being unsaturated, including oleic, linoleic, and linolenic acids [42].

The quality of mulberry leaves, which are essential for silkworm nutrition, is influenced by their crude fiber content. Higher cellulose content indicates leaf aging, making them tougher and harder to consume. The average crude fiber content in the analyzed samples was 5.29% (17.9% in DM), increasing by 1.28% over the study period. Literature values for crude fiber range from 11.63% to 15.50% in DM [43], with common mulberry varieties showing 10.43% to 13.70% [44]. The nitrogen-free extract had an average proportion of 12.77% (43.38% in DM), increasing by 1.12% during the period, similar to literature findings [45].

Carbohydrates are the primary energy source, degraded enzymatically into sugars like glucose, which are directly used by the larvae's tissues. Carbohydrates can also be resynthesized into fats due to the mulberry leaves' low lipid content. Glycogen, stored in the adipose tissue, is mobilized as needed, with its degradation controlled by a hormone from the prothoracic glands. Glycogen breakdown in muscles, primarily anaerobic, forms lactic acid, which is partly oxidized for energy and partly resynthesized into glycogen. Carbohydrate metabolism varies with muscular activity, temperature, humidity, and leaf water content, also protecting proteins from excessive energy consumption [46].

The ash content (total minerals) had an overall average of 4.16%, with an increase of approximately 0.76% recorded during the study period. In terms of dry matter, ash represented 14.06%. Total mineral values are in line with those found in the literature, such as 6.04–8.56% [21], 8.7–13.15% [26], 9.13–17.38% [27], 10.00–12.30% [29], 11.52–12.80% [33], and 13.37% [39]. Calcium accounted for 2.10–2.94%, and phosphorus was at 0.20–0.23% [24,25].

Chemical composition differences among mulberry varieties were minimal, except at the 5th larval age. The Eforie variety, comparable to the globally recognized Kokuso 21, demonstrated valuable potential and significant contributions to sericulture, matching the standard set by Kokuso 21 in leaf chemical composition. This highlights the Eforie variety's relevance and importance in scientific research.

4.2. Mulberry Leaf Digestibility

Throughout the intricate process of digestion, nutrients undergo breakdown into simpler compounds, which are subsequently absorbed across various segments of the digestive tract epithelium in silkworms. These compounds are utilized by silkworms for their growth and cocoon formation during their metamorphic phases. The quantification of substances digested showcases the difference between the ingested substances from their feed and those present in excretions. However, as not all substances in excretions originate directly from the diet (some are endogenous), the outcome derived from this distinction is essentially termed as apparent digestibility. The term “apparent digestibility” becomes particularly relevant when considering the presence of excreted products in

B. mori's feces, which complicates the accurate evaluation of nutrient digestibility in mulberry leaves [31,33,36,39,40].

Measuring the digestibility of nutrients in mulberry leaves by silkworms holds significance due to several reasons: enhancing silk production—assessing how efficiently silkworms process nutrients from mulberry leaves aids silk producers in optimizing rearing conditions, potentially improving cocoon quality and silk production yields; nutrient balance and growth—understanding which nutrients silkworms readily absorb enables the formulation of diets that provide essential nutrients, fostering healthier and faster-growing silkworms; economic impact—maximizing nutrient digestibility can boost silk production's economic viability by enhancing cocoon quality and silk yield, benefiting both producers and local economies; resource efficiency—higher nutrient extraction by silkworms means less leftover material, potentially valuable for other uses like organic fertilizers; ecosystem sustainability—assessing nutrient digestibility contributes to understanding silkworm–mulberry tree interactions within ecosystems, impacting nutrient flow dynamics; research and innovation—insights into silkworm nutrition and digestibility can drive innovations in sericulture practices, guiding the development of specialized diets or enhanced digestibility in silkworm strains, significantly influencing the silk industry's future [39,40].

Our experiments revealed that, on average, the digestibility of mulberry leaf as a whole is 54.46%. This value represents the mean between the recorded digestibility coefficients for the two varieties during the larval period. The digestibility of dry matter decreased by an average of 38.54% throughout the larval growth period. As larvae develop, their enzymatic apparatus evolves, resulting in alterations in how they utilize nutrients from leaves. These alterations correlate with qualitative changes in the chemical composition of the leaves [21].

On average, mulberry leaf digestibility varies between 46.40% and 58.90% depending on the variety [21]. Some studies suggest that mulberry leaves fed to the 5th larval age exhibit an approximate digestibility ranging from 27.99% to 32.44% [26]. This parameter decreases from 71.07% in the first age to 39.99% for male larvae and 48.26% for female larvae in the 5th age [28]. The digestibility of mulberry leaves can reach a maximum of 70% [30].

The average digestibility of crude protein in mulberry leaves was around 60.54%. Throughout the larval growth period, digestibility coefficients showed an average decrease of 28.87%. The high digestibility in the 1st age may be attributed to the rich amide content present in young leaves, comprising simpler nitrogenous substances easier to digest compared to the complex protein-structured nitrogenous substances prevalent in older leaves. Reported digestibility coefficients of crude protein in the literature range from 69.21% to 78.92 [33], 60.06% to 74.69% [38], and 71.62% to 93.48% [41].

The average digestibility of crude fat in mulberry leaves was approximately 49.73% over the larval growth phase, displaying a sinusoidal pattern between 31.80% and 77.49%. It is crucial to note that interpreting crude fat digestibility test results from mulberry leaves can be inconclusive due to the possibility that many lipid compounds found in the excreta might originate from the larvae's digestive tract rather than solely from the leaves. This complexity underscores the importance of evaluating 'ether extract', which may contain significant pigment amounts. The observed fluctuations in crude fat digestibility during the study period might be attributed to these complexities.

In the larval digestive system, lipases break down fats from the leaves into glycerol and fatty acids. However, the presence of these lipases in gastric juice remains a subject of debate. Most authors agree that *B. mori* larvae digest and assimilate lipids, storing reserves for the chrysalis (cocoon) and butterfly stages. The mechanisms through which this occurs have sparked debates. In 1917, Hiratsuka observed a difference in the fat content of leaves and excrement, indicating larval utilization [42]. Some sources suggested that the lipase exists in the cells that make up the absorptive epithelium in the silkworm larval digestive tract [21,43,44]. Proteins, lipids, and carbohydrates (glycogen) are stored in the body tissues of *B. mori* larvae, especially in the fats [44]. Reported digestibility coefficient values for crude fat in the literature range between 63.28% and 74.19% [45].

The digestibility of crude fiber obtained from mulberry leaves showed a decline throughout the larval growth period, averaging 25.77% across both hybrids during the entire larval phase. It commenced at nearly negligible levels (almost zero) in young larvae, progressively increasing significantly towards the end (26.78%). This pattern in crude fiber digestibility aligned closely with the development of enzymatic equipment in the larval digestive tract. At the onset, enzymes responsible for cellulose digestion were barely present, gradually increasing and reaching their peak in the 5th age, coinciding with higher crude fiber content in mulberry leaves. According to certain sources, crude fiber was not digestible in the first two larval stages but reached 8% in the 3rd age and 21.13% in the 5th age [44].

The nitrogen-free extract (NFE) derived from mulberry leaves exhibited an average digestibility of 58.81% and encountered an average decrease of 37.62% throughout the studied period. These values align with existing literature, where NFE digestibility coefficients range between 63.40% and 94.97% depending on mulberry variety and larval age [46].

While no significant differences were noted in the proximate composition between the varieties, significant variations were observed within each variety across different larval ages ($p < 0.001$). When comparing the average digestibility coefficient values for leaf nutrients across the entire larval growth period, significant differences emerged between the varieties ($p < 0.001$ or $p < 0.05$), except for the digestibility coefficients of crude fat ($p > 0.05$). Conversely, comparing values for each larval age, except for the digestibility coefficients of crude fat in ages 1 and 2, the differences were insignificant, underscoring the consistent quality of Eforie variety leaves. Additionally, higher negative correlation coefficients in Eforie suggest a relatively lower capacity of silkworms to digest organic matter (as shown in Table 6), potentially due to the dietary crude fiber content in leaves, in contrast to Kokuso 21 leaves. This trend is similarly evident in the data from Table 5, observed over the entire period (ages I–V).

5. Conclusions

Throughout the developmental stages of the larvae, mulberry leaves undergo a dynamic shift in their components. This includes a decrease in certain nutrients alongside an increase in crude fiber content. This fluctuation in nutrients reflects an adaptive response by the larvae, which develop a specialized enzymatic complex, specifically geared toward breaking down fiber compounds. This adaptation enables them to more effectively utilize the cellulose richness in the leaves as they progress through their growth stages, contrasting with their initial developmental phases.

In general, most nutrients experience a decrease in digestibility as both the leaves and the larvae mature, with the notable exception of crude fiber, which displays an increase in digestibility.

In summary, we conclude that the nutritional value of the two mulberry varieties (Eforie and Kokuso 21) is comparable, making them suitable for utilization under similar conditions. The nutritional value of the leaves evolves throughout the larval growth period, indicating a reduction in the digestibility of all substances, except for crude fiber. Further research endeavors should involve the inclusion of additional silkworm hybrids from Romania, such as Băneasa Super, Zefir, Select, Miraj, or Record, as a follow-up. These hybrids showcase superior technological characteristics concerning cocoons and silk fibers. Additionally, it is crucial to investigate whether the nutrients in the leaves, whose digestibility we have studied, are effectively metabolized and transformed into the pupal silk wrap, both quantitatively and qualitatively.

On average, ECIS values for the two varieties were similar, with $9.90 \pm 0.11\%$ for Kokuso 21 and $9.87 \pm 0.08\%$ for Eforie. However, ECDS was significantly better for the Eforie variety ($18.45 \pm 0.35\%$) compared to the Kokuso 21 variety ($17.94 \pm 0.04\%$).

We consider the main novelty to be the comparison of the performance of the Romanian variety (Eforie) with that of a highly valuable and well-known variety (Kokuso 21), in a manner that has not been previously researched.

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