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CONTRIBUTIONS TO STUDY OF MULBERRY LEAF USE BY *BOMBYX MORI* LARVAE

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Abstract

In order to assess the efficiency of use of mulberry leaf by the hybrid Băneasa Super of Bombyx mori larvae, during a series of growth, some determinations were made related to the nutritional value and digestibility of the three varieties of leaf. The results showed that the advanced vegetation stage and during each period of growth of silkworm larvae, the mulberry leaf undergoes an aging process, translated by the quality decrease regarding the chemical composition. According to this aspect, in most mulberry leaf nutrients other than cellulose, a continuous decrease in digestibility was observed throughout the growing period. The results show that for the Bombyx mori larvae, from mulberry leaf, an average of 10.38 grams of dry matter is ingested and 5.92 grams of digested dry matter is required for each gram of silky coating, which indicates an efficient conversion into silk of 9.65% of the intake (ECI), and 17% of digest (ECI).

Key words: *capitalization, energy, larvae, leaf, mulberry.*

INTRODUCTION

Besides the continuous improvement of the growth technologies, one of the main concerns of the specialists in sericulture is to produce biological material of high genetic value; thus, *Bombyx mori* larvae will have an increased productive potential, it will be more resilient to the environmental factors and also to diseases, and it will be capable of using nutrients offered by the mulberry leaf to the best of their advantage.
Regarding these aspects, the performances of used larvae in intensive breeding systems have greatly increased, but at the same time, in order for them to be able to reach their full potential, it is necessary to improve all the factors involved in the breeding process. From the multitude of factors that directly influence the growth process of the larvae and the economic results obtained, it is encountered also nutrition.
The quantity and especially the quality of the leaf used in feeding of larvae, directly influence the growth rate, their health and vitality, but also the quantitative and qualitative production of silk. In turn, the quality of the leaf is also influenced by many factors related to the pedo-climate conditions, season, variety of the mulberry, harvesting style and type of storage etc.
The knowledge of the nutritional value and how some factors influence the growth process, as well *Bombyx mori* larvae are harnessed to kill the nutrients in the wormwood, has attracted the attention of a significant number of researchers.
At the end of the last century, Romania was considered an important point on the map of European sericulture. Thus, in its record, Romania can boast in this field with a quite complex literature, as well as with creating new varieties and valuable hybrids of worm, as *Bombyx mori*, all considered as being the result of some decade research work of Romanian specialists.
Thus, we consider appropriate to bring a modest contribution to the study of using the mulberry leaf, derived from indigenous varieties, by larvae of breeds or hybrids created in Romania.

MATERIALS AND METHODS

The biological material used in the experiments was represented by three groups (L1, L2, and L3) of 150 larvae of *Bombyx mori* belonging to the Romanian hybrid Băneasa Super, obtained through simple crossing between the maternal breed of Japanese type and the paternal one of Chinese type. To make it easier to track, in each batch, the 150 larvae were grouped into three subgroups (repeats) of 50 larvae each, which were grown in paper trays sized according to the age and size of the larvae, in addition, a reserve group was also formed, with 50 larvae raised separately, but under the same conditions, which served to replace the dead larvae in the experimental group.

The growth of the larvae took place in August, in an air-conditioned room, respecting all the microclimate factors.

The 1st group received leaves from selected mulberry hybrid, 2nd group of Eforie variety and 3rd group leaves from the Kokuso 21 variety. Each subgroup, within each batch, was given the same amount of leaf, of which samples were previously collected and subjected to chemical analysis.

Daily and at the same time from each subgroup (repartition) were collected, weighted, and registered the leftover mulberry leaves and the excreta.

The number of leftovers mulberry leaves from each subgroup were summed, the result being than divided to 3, obtaining the average quantity of leftover leaves from the 50 larvae; the value was representative for the entire batch. This value was used to calculate the digestibility coefficients of the nutrients from mulberry leaves. The same system was used to determine the excreta.

From each subgroup were collected samples of leftover, as excreta, which were homogenized in order to obtain an average sample for each batch; those samples were chemically analyzed. Also, three subgroups of 50 larvae were raised separately, under the same environmental conditions.

During the experiment the larval mortality was observed from each batch and, if was necessary, the dead larvae were immediately replaced with ones from repartition batch.

Also, the groups were weighed at the beginning of growth (after hatching) and at the end (before budding), the difference between the two weights, divided by the number of larvae in each group, representing the body mass accumulated by larvae.

From the separated batch were extracted 10 larvae, whose content was determined in dry matter; thus, multiplying the average dry substance content of larvae, calculated from the separated batches, with the increasing body mass in the experimental batches, the average increasing of body mass of a larva was determined.

After gobbling, 15 cocoons were harvested, from which the silk wrapper was separated, weighed and its dry matter content determined, thus obtaining the average dry wool content of the silk wrapper.

Working methods aimed to determine the nutritive value of the mulberry leaves taking into account the chemical composition and the digestibility of its components.

The chemical composition was determined using the "Proximate Analysis" scheme and the digestibility (approximate digestibility) through *in vivo* method - simple digestibility with a single period control (FAO).

The chemical analyses were done on samples previously dried to 65ºC and grinded. The obtained results were processed, noted, and expressed in both fresh and dried leaves. The collected samples moisture determination was done by drying them into the hot air oven for 4-5 hours at 105ºC (Halga et al., 2005; Regulation EU, 2009).

The ashes content was determined using the incineration method of the samples (Stan & Simeanu, 2005).

To determine the protein content (RP), the Kjeldhal method was used (Stan & Simeanu, 2005).

The fat content (EE) was determined using Soxhlet method; its principle is based on the fat property of dissolving in the organic solvent (such as, petroleum ether) (Stan & Simeanu, 2005).

The raw fiber (RF) was determined by the sample acid-basic hydrolyze, then from the leaf the hydrolysable part is removed, on the filter paper remaining only the cellulose and minerals; the minerals are determined through calcination, so the raw cellulose is calculated by difference (Stan & Simeanu, 2005).

Nitrogen free extract was calculated through difference from fresh leaf or dried one. In the first case, from 100% was decreased the water, protein, fat, cellulose, and ashes percentage. In the second case, from the dry matter percentage was obtained the raw protein, extract etherate, raw fiber, and ash percentage.

In order to determine the nutritive matter digestibility from mulberry leaves which were administered in silk larvae feeding, it was respected the digestibility principle “in vivo”, with a single control period. There were calculated the digestibility coefficients (DC% = $\frac{Digest}{Intake}$ x 100) (Al-Kirshi et al., 2013; Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017; Halga et al., 2005; Hirano et al., 1993).

Based on the quantity of administered leaves, the leftover waste, excrete and on the data obtained from chemical analyses firstly, were found out the intake nutrients or ingest (the difference between administered quantities and the leftover) and finally the intake of the nutritive substances or digest (difference between ingest and feces). Expressing in percentage the digest from ingesta, were obtained the digestibility coefficients, which shows how much from the leaves nutrients are digested into the digestive system of the larvae.

Based on the digestible coefficients, there was calculated the digestible content for each nutrient which represents the result between the raw chemical content and the digestible coefficient which was divided to 100.

The obtained values were summed obtaining in the end the total digestible nutrients (TDN) from the mulberry leaves. The fat content was multiplied by 2.25, because it is considered that the fat has 2.25 times more energy than the others intake nutrients.

Also, because the nutritive value was expressed in TDN/kg, and the calculated values were reported to 100 g, it was multiplied with 10 (Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017; Halga et al., 2005; Matei, 1995; Stan & Simeanu, 2005).

Regarding the energy value, the working methods were mainly the specific ones used to determine the raw energy (use of specific computation equations and regression coefficients recommended by the OKIT system), digestible (calculation equation recommended for monogastric species), and metabolic (equations recommended for monogastric animals and birds) contain (Halga et al., 2005; Pop et al., 2006; Stan & Simeanu, 2005).

The efficiency of the use of nutrients in the worm leaf by the larvae was expressed by the amount of ingested/digested dry matter required for increasing 1 gram of body mass/weight (silk wrap), respectively by the efficiency of conversion of ingested substances (ECI%)/digested (ECD%) in body mass/weight (Matei, 1995; Rahmathulla et al., 2002; Sarkar, 1993).

The main experimental data obtained were statistically processed through the arithmetic average, variance, the average standard deviation, and the variability coefficient (Cucu et al., 2004; Maciuc et al., 2015; Sandu, 1995).

RESULTS AND DISCUSSIONS

The values regarding the mulberry chemical composition evolution throughout growth period of the silkworm larvae, were centralized and statistically processed in the Table 1a and Table 1b.

For the selected Hybrid (L1), according to these data it is observed that during the experiment the values of the relative humidity of the mulberry leaf had a descending evolution, registering average values between 71.96%, in the period corresponding to the first age of the larvae and 68.24%, in the period when the larvae were in the fifth age. During the entire studied period, the mulberry leaf had a composition, on average, of 29.38 ± 0.676% dry matter and 70.62% water.

For the Eforie hybrid (L2), the average relative humidity of the mulberry leaves during the research was 70.44%, and a decreasing evolution was registered average values between 71.86% (at the first determination corresponding to the first age of the silkworm larvae) and 68.15% (to the last determination when the silkworm larvae are in the fifth age). The dry matter represented 29.56 ± 0.725%.

For the Kokuso 21 hybrid, the average relative humidity of the mulberry leaves during the research was 70.63%, and a decreasing evolution was registered average values between 72.09% (at the first determination corresponding to the first age of the silkworm larvae) and 68.86% (to the last determination when the silkworm larvae are in the V^{th} age). The dry matter represented 29.37±0.575%.

Table 1a. Chemical composition of mulberry leaf in relation to the age of the silkworm larvae and the mulberry variety (%)

Analysis	water	DM	RP		EE	
			F*	DM**	F	DM
Selected hybrid						
I	71.96	28.04	6.13	21.86	0.83	2.96
II	71.75	28.25	6.23	22.05	0.87	3.08
III	71.03	28.97	6.21	21.44	1.12	3.87
IV	70.13	29.87	5.85	19.58	1.23	4.12
V	68.24	31.76	6.03	18.99	1.42	4.47
$\overline{X}$	70.62	29.38	6.09	20.78	1.09	3.70
$S_{\overline{x}}$	-	0.676	-	0.627	-	0.294
Cv%	-	5.147	-	6.744	-	17.775
Eforie						
I	71.86	28.14	6.23	22.14	0.85	3.02
II	71.98	28.02	6.21	22.16	0.88	3.14
III	70.68	29.32	6.41	21.86	1.17	3.99
IV	69.53	30.47	6.00	19.69	1.22	4.00
V	68.15	31.85	6.06	19.03	1.38	4.33
$\overline{X}$	70.44	29.56	6.18	20.98	1.10	3.70
$S_{\overline{x}}$	-	0.725	-	0.670	-	0.260
Cv%	-	5.486	-	7.143	-	15.700
Kokuso 21						
I	72.09	27.91	6.31	22.61	0.79	2.83
II	71.66	28.34	6.28	22.16	0.88	3.11
III	70.31	29.59	6.23	21.05	1.14	3.85
IV	70.13	29.87	6.04	20.22	1.16	3.88
V	68.86	31.14	6.15	19.75	1.25	4.01
$\overline{X}$	70.63	29.37	6.20	21.16	1.04	3.54
$S_{\overline{x}}$	-	0.575	-	0.547	-	0.237
Cv%	-	4.381	-	5.782	-	14.975

* fresh leaves; ** dry matter

Table 1b. Chemical composition of mulberry leaf in relation to the age of the silkworm larvae and the mulberry variety (%)

Analysis	RF		NEF		Ash	
	F	DM	F	DM	F	DM
Selected hybrid						
I	4.92	17.55	12.23	43.61	3.93	14.02
II	4.99	17.66	12.18	43.12	3.98	14.09
III	5.18	17.88	12.43	42.90	4.03	13.91
IV	5.49	18.38	13.05	43.69	4.25	14.23
V	6.04	19.02	13.59	42.79	4.68	14.73
$\overline{X}$	5.32	18.10	12.71	43.22	4.17	14.20
$s_{\overline{x}}$	-	0.271	-	0.183	-	0.143
Cv%	-	3.349	-	0.947	-	2.256
Eforie						
I	4.79	17.02	12.43	44.17	3.84	13.65
II	4.76	16.99	12.24	43.68	3.93	14.03
III	5.26	17.94	12.30	41.95	4.18	14.26
IV	5.58	18.31	13.37	43.89	4.30	14.11
V	6.15	19.31	13.58	42.64	4.68	14.69
$\overline{X}$	5.31	17.88	12.78	43.29	4.19	14.15
$S_{\overline{x}}$	-	0.434	-	0.418	-	0.169
Cv%	-	5.412	-	2.163	-	2.667
Kokuso 21						
I	4.74	16.98	12.33	44.18	3.74	13.40
II	4.88	17.22	12.34	43.54	3.96	13.97
III	5.31	17.95	12.64	42.72	4.27	14.43
IV	5.44	18.21	13.09	43.83	4.14	13.86
V	5.93	19.04	13.41	43.07	4.40	14.13
$\overline{X}$	5.26	17.88	12.77	43.46	4.10	13.96
$S_{\overline{x}}$	-	0.368	-	0.261	-	0.169
Cv%	-	4.599	-	1.342	-	2.713

The mulberry leaves humidity influences the silkworm larvae consumption, which, especially in the early stages of life, prefers young leaves with a high percentage of water. In the data presented by different authors, the average humidity of the mulberry leaves varies between 65-75% (Ifrim, 1998; Lazăr & Vornicu, 2013).

Depending on the variety, the dry matter of the mulberry leaves varies between 23.61-27.56% (Matei, 1995).

Compared to the common mulberry (69.80-73%), the selected varieties have a higher water content (Miranda & Takahashi, 1998). The dry matter of the mulberry leaf, harvested in the same period, may register, depending on the variety / hybrid, different values, for example, between 23.61% and 27.56% (Craiciu, 1966).

Also, if in spring the humidity of the mulberry leaf is 71.85-77.81%, then it decreases to 68.42-75.64% in the summer time, and to 64.10-73.64% in autumn (Petkov, 1980).

In group L1, the raw mulberry leaf protein, related to the dry matter, had values between 21.86%, in the age I of the larvae and 18.99%, in the Vth age; for the entire studied period the average value for this indicator was 20.78 ± 0.627%. At the end of the growth, the raw protein content of the leaf decreased by 2.86%.

In the second group L2, the raw protein had an average value of 6.18% (20.98± 0.670% from DM). It was noticed a progressive decreasing of the protein content throughout the studied period, which showed that the content was reduced by 3.11% (from 22.14% to 19.03%).

In the third group L3, the raw protein had an average value of 6.20% (21.16±0.547% from DM). It was noticed a progressive decreasing of the protein content throughout the studied period, the content decreasing by 2.86% (from 22.61% to 19.75%).

The protein content in the mulberry leaves may be considered a real indicator of the leaf's quality. The protein intake from mulberry leaves strongly influences both the silkworm larvae growth and development and, especially, the silk production of the larvae. The protein content of mulberry leaves strongly influences both the growth and development of silk larvae and, especially, their silk production.

In the literature, the raw protein from mulberry leaves has the following average values: 32.40% in the spring, 28.21% in the summer, and 24.53% in the autumn (Borcescu, 1966), during the morning 26.80% and evening 29.10% (Mărghitaş, 1995); it also varies between 22.55 and 25.73% depending on the mulberry variety (Matei, 1995).

In the literature, the raw protein from mulberry leaf is estimated to average 6.16% in fresh leaf, 20.97% in dry matter and 24.36% in its organic matter (Tzenov, 1993). The values of the raw leaf protein can vary depending on the season, the time of day, the mulberry variety / hybrid: 32.40% in spring, 28.21% in summer and 24.53% in autumn (Sarkar, 1993), 26.80% in the morning and 29.10% in the evening (Sarkar, 1993), between 22.55% and 25.73% depending on the variety (Craiciu, 1966).

The raw fat of the mulberry leaf recorded an average value of 3.70 ± 0.294%, during the larval growth period registering an increase from 2.96% (in the period corresponding to age I) to 4.47% (in the Vth age).

For the second group L2, the fat content from the mulberry leaves had an average value of 1.10% in the fresh leaves, and 3.70% ±0.260 in DM. It is the only nutrient with a high variability, of 15.700%. The fat content increased constantly throughout the silkworm larval growth, from 0.85% to 1.38% when it was expressed in fresh leaves, or 3.2% to 4.33%, when it was reported to dry matter.

For the third group L3, the fat content from the mulberry leaves had an average value of 1.04% in fresh leaves, and 3.54% ± 0.237 in DM. It is the only nutrient with a high variability, of 14.975%.

The fat content increased uniformly throughout the silkworm larval growth, from 0.79% to 1.25% when it was expressed in fresh leaves, or 2.83% to 4.01%, when it was reported to the dry matter.

The limits presented by specific literature regarding the fat content in mulberry leaves are 2.85-6.07% (Pop, 1967).

In the selected hybrid, the raw cellulose, related to the dry matter, had an average value of 18.10 ± 0.271%, registering during the larval growth an increase of 1.47% (from 17.55% to 19.02%).

For the Eforie hybrid, the raw cellulose had an average value of 5.31% in fresh leaves (17.91 ± 0.434%, when in was reported to DM). Throughout the research, for a month, the raw cellulose increased by 2.29%, from 17.02% to 19.31%.

For the Kokuso 21 hybrid, the raw cellulose had an average value of 5.26% in fresh leaves, 17.88 ± 0.368%, when it was reported to DM. Throughout the research, for a month, the crude cellulose increased by 2.06%, from 16.98% to 19.04%.

The cellulose is highly responsible for aging processes of the mulberry leaves. As the cellulose content grows, the leaf becomes tougher, being more difficult to be consumed by the silkworm larvae. Regarding this aspect, in the silkworm larvae's growth are considered the most valuable mulberry varieties, the ones that have a lower cellulose content.

The values obtained for raw cellulose from mulberry leaves were comparable with the ones from literature. The raw cellulose quota varies between 12.33-14.38% to the common mulberry tree and between 10.43-13.70% to different selected varieties (Rahmathulla et al., 2004). Throughout the mulberry vegetation period, the cellulose content from leaves increased from 14.47 to 21.16% (Maciuc et al., 2015).

In group L1, the non-nitrogen extractive substances, in relation to the dry matter, had an average weight of 43.22 ± 0.183%, during the period of larval growth first registering a decrease, from the first determination to the third or from 43, 61% to 42.90%, followed by an increase until the fourth determination, reaching 43.69%, then decreasing again until the last determination, to 42.79%.

In the second batch L2 the NEF was represented by an average of 43.27 ± 0.418% from the dry matter of the mulberry leaves; the average values decreased from the first determination to the third, from 44.17% to 41.95%, then it increased to the fourth determination, being 43.89%, decreasing to the last analyses to 42.64%.

In the third batch L3, the NEF represented on average 43.46 ± 0.261% from the dry matter of the mulberry leaves; the average values decreased from the first determination to the third, from 44.18% to 42.72%, then was an increasing to the fourth determination, being 43.83%, decreasing to the last analyses to 43.07%.

In the first group L1, the raw ash accounted an average value of 14.20 ± 0.114% of the dry matter in the leaf. During the study, mulberry leaf minerals generally increased progressively (by 0.71%) from the first determination to the fifth, respectively from 14.02% to 14.73%, except for the third analysis, where a lower value was recorded even than in the first determination.

In the second group L2, the raw ash represented in average 4.19% in the fresh leaves and 14.15 ± 0.169% from dry matter. The minerals from the mulberry leaves throughout the research registered a continuous increase from one analysis to another. The average values varied from 3.84% to 4.68% to fresh leaves and from 13.65% to 14.69% from dry matter. An exception was registered to the third determination which had a higher value than the fourth one. The increasing in mineral content from mulberry leaves throughout the research was 1.04%.

In the third batch L3, the ash represented in average 4.10% in the fresh leaves and 13.96 ± 0.169% from dry matter.

The minerals from the mulberry leaves throughout the research registered a continuous increase from one analysis to another. The average values varied from 3.74% to 4.40% in fresh leaves and from 13.40% to 14.13% from dry matter. An exception was registered to the third determination which had a higher value than the fourth one. The increasing in mineral content from mulberry leaves throughout the research was 0.90%.

The data regarding the mineral content, obtained through burning the mulberry leaves are similar to those from specific literature, 9.13-17.38% (Miranda & Takahashi, 1998), 11.52-12.80% (Craiciu, 1966), 8.7-13.15% (Miranda & Takahashi, 1998).

Knowing the raw chemical composition of the mulberry leaf, using the specific calculation equations, it was possible to assess the nutritional value of the mulberry leaf based on its content of raw energy, which was, on average, over the entire studied period, of 1238 Kcal/kg, in fresh leaf, respectively 4216 Kcal/kg, in the dry matter (Table 2).

By recording the quantities of mulberry leaf administered, of unconsumed residues and excreted and determining their chemical composition (Table 3), subsequently it was possible to calculate its digestibility coefficients (Table 4), as the content of digestible substances in the leaf (Table 5).

Table 2. Raw energy of mulberry leaf

Specification	%		Caloric equivalent	Kcal/100 g		Kcal/kg	
	*	**		*	**	*	**
L1							
RP	6.09	20.78	5.72	34.83	118.86	348.3	1188.6
EE	1.09	3.70	9.50	10.36	35.15	103.6	351.5
CF	5.32	18.10	4.79	25.48	86.70	254.8	867.0
NEF	12.71	43.22	4.17	53.00	180.23	530.0	1802.3
						1237	4209
L2							
RP	6.18	20.98	5.72	35.35	120.01	353.5	1200.1
EE	1.10	3.70	9.50	10.45	35.15	104.5	351.5
CF	5.31	17.88	4.79	25.43	85.65	254.3	856.5
NEF	12.78	43.29	4.17	53.29	180.52	532.9	1805.2
						1245	4213
L3							
RP	6.20	21.16	5.72	35.46	121.04	354.6	1210.4
EE	1.04	3.54	9.50	9.88	33.63	98.8	336.3
CF	5.26	17.88	4.79	25.20	85.65	252.0	856.5
NEF	12.77	43.46	4.17	53.25	181.23	532.5	1812.3
						1238	4216

Table 3. Data necessary for digestibility coefficient calculation

Larvae age	Specification	Quantity (g)	Chemical composition (%)					
			DM	RP	EE	CF	NEF	Ash
L1								
I	Leaves	15.50	28.04	6.13	0.83	4.92	12.23	3.93
	Leftovers	5.29	62.78	13.85	2.11	14.22	25.15	7.45
	Excreta	0.14	69.81	18.42	10.15	7.36	26.99	6.89
II	Leaves	26	28.25	6.23	0.87	4.99	12.18	3.98
	Leftovers	8.76	59.01	13.95	2.56	14.00	24.92	3.58
	Excreta	0.88	63.00	14.14	0.15	7.57	26.84	14.30
III	Leaves	77.00	28.97	6.21	1.12	5.18	12.43	4.03
	Leftovers	23.55	59.94	12.86	1.94	16.02	24.92	4.20
	Excreta	4.02	61.12	14.89	2.83	5.08	25.47	12.85
IV	Leaves	242.00	29.87	5.85	1.23	5.49	13.05	4.25
	Leftovers	66.02	56.21	12.09	2.66	17.03	21.05	3.38
	Excreta	18.98	60.85	9.77	2.34	9.22	25.18	14.34
V	Leaves	1000	31.76	6.03	1.42	6.04	13.59	4.68
	Leftovers	267.00	56.10	10.21	1.88	14.30	25.01	4.7
	Excreta	121.00	60.45	10.00	4.05	14.95	24.33	7.12
L2								
I	Leaves	15.5	28.14	6.23	0.85	4.79	12.43	3.84
	Leftovers	5.11	62.58	13.91	2.01	14.02	24.48	8.16
	Excreta	0.17	78.25	14.01	14.68	15.11	26.12	8.33
II	Leaves	26	28.02	6.21	0.88	4.76	12.24	3.93
	Leftovers	8.01	58.85	14.33	2.16	13.89	22.06	6.41
	Excreta	0.88	74.68	12.57	3.97	14.01	29.01	15.12
III	Leaves	77	29.32	6.41	1.17	5.26	12.30	4.18
	Leftovers	22.65	61.54	12.34	2.62	15.86	25.66	5.06
	Excreta	4.07	64.06	15.92	2.08	9.91	24.12	12.03
IV	Leaves	242	30.47	6.00	1.22	5.58	13.37	4.30
	Leftovers	65.94	56.49	12.05	2.02	15.93	25.67	0.82
	Excreta	19.99	64.44	11.98	2.18	12.01	26.21	12.06
V	Leaves	1000	31.85	6.06	1.38	6.15	13.58	4.68
	Leftovers	269.01	57.92	9.96	2.97	11.92	24.82	8.25
	Excreta	119.82	60.46	10.06	3.08	14.89	24.02	8.41
L3								
I	Leaves	15.5	27.91	6.31	0.79	4.74	12.33	3.74
	Leftovers	4.98	67.99	15.87	2.03	14.62	26.44	9.03
	Excreta	0.16	70.02	20.97	8.88	4.12	27.91	8.14
II	Leaves	26	28.34	6.28	0.88	4.88	12.34	3.96
	Leftovers	8.01	62.18	13.01	2.02	15.09	20.98	11.08
	Excreta	1.02	65.52	15.42	2.11	5.81	29.01	13.17
III	Leaves	77	29.59	6.23	1.14	5.31	12.64	4.27
	Leftovers	23.82	62.07	9.85	3.01	16.08	25.12	8.01
	Excreta	3.97	63.11	14.98	2.02	6.01	25.93	14.17
IV	Leaves	242	29.87	6.04	1.16	5.44	13.09	4.14
	Leftovers	66.94	60.01	10.91	1.91	16.68	23.55	6.96
	Excreta	20.31	62.73	13.18	2.91	8.19	28.44	10.01
V	Leaves	1000	31.14	6.15	1.25	5.93	13.41	4.4
	Leftovers	269.01	59.09	11.51	1.65	14.01	24.89	7.03
	Excreta	121.94	61.12	9.81	3.83	13.49	25.18	8.81

Table 4. Specific digestibility coefficients of Băneasa Super hybrid

The larvae age	DM	RP	EE	CF	NEF
L1					
I	90.47	88.14	16.96	0.96	92.99
II	74.52	68.73	31.58	6.20	75.99
III	70.00	65.86	71.94	5.42	72.34
IV	71.95	69.97	63.61	14.32	72.97
V	56.41	63.38	46.62	18.59	57.41
I-V	59.04	64.64	49.57	18.07	61.42
L2					
I	88.57	90.66	14.09	1.53	93.43
II	75.81	76.31	37.46	1.36	81.96
III	70.29	69.73	72.46	11.92	73.17
IV	66.15	63.57	73.11	19.96	66.04
V	56.81	64.34	36.48	39.39	58.31
I-V	58.06	64.78	45.41	37.07	60.87
L3					
I	88.09	82.11	33.64	0.00	92.48
II	72.01	73.37	67.91	1.33	80.63
III	68.68	75.73	50.12	7.66	72.54
IV	60.33	63.40	61.34	16.80	63.70
V	51.11	60.83	42.07	23.89	54.27
I-V	53.77	62.45	45.35	23.05	57.44

By recording the quantities of the worm leaf administered, the non-consumed and excreted residues and also determining their chemical composition (Table 3), its digestibility coefficients could subsequently be calculated (Table 4) and also the content of digestible substances in the leaf (Table 5).

Following the complex phenomenon of digestion, nutrients are transformed into simple substances, which can thus be absorbed through the epithelium of the digestive tract, at different levels, thus being retained in the organism of silk larvae, representing the difference between the number of substances ingested through food and the number of appropriate substances found in droppings. Because not all the substances found in excrement are of dietary origin, some of them are of endogenous origin, which can be obtained by this difference, indicating only apparent digestibility. If you admit the fact that at *Bombyx mori* excretions are also found in their excrement, which complicates the establishment of the digestibility of nutrients in the wormwood even more accurately, the use of the approximate digestibility term seems to be more correct ((Al-Kirshi et al., 2013; Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017; Halga et al., 2005; Hirano et al., 1993; Sabhat et al., 2011).

Throughout the study period, the dry matter digestibility of the mulberry leaf had a digestibility between 53.77%, and 59.04%, the highest values were recorded in larvae from age I (88.09 - 90.47%), after which, by the end of the larval period, there was a decrease between 31.76 and 36.98 percent.

In the literature, the main explanation for reducing the digestibility of nutrients from the worm leaf as a whole, during the growth period of the silk larvae, would be as seen from the data in Table 1, precisely the qualitative degradation of the leaf, in terms of chemical composition (Sarkar, 1993). Digestibility of the dry substance from the worm leaf decreases from 71.07% in age I, to 39.99% (for male larvae), 48.26% (for female larvae) in age V (Rath et al., 2003). The worm leaf administered to the larvae of the fifth age has an approximate digestibility between 27.99% and 32.44% (Rahmathulla et al., 2002).

The raw protein had a digestibility coefficient for the entire period studied between 60.83 and 64.64%. The digestibility of the raw protein was progressively reduced during the studied period, by 21.28 - 26.32%, from 82.11 - 90.66%, in the first larval age, to 60.83 - 64.34%, in the last one. The high digestibility of age I could be explained by the rich content in amides, simple nitrogenous substances, which are found in the young leaf and which are digested much easier than the protein nitrogenous substances, which have the weight in the old leaf.

In the literature, for raw leaf protein, the value of digestibility coefficients is between 69.21% and 78.92% (Borcescu, 1966), 60.06% and 74.69% (Petkov, 1980), 71.62% and 93.48% (Matei, 1995).

In group 1, the gross fat of the mulberry leaf had the minimum value of digestibility, of 16.96%, in the larvae of age I, and maximum, of 71.94%, in the larvae of age III.

For the second batch L2, the raw fat from the worm leaf had the minimum digestibility value of 14.09%, in the larvae of the first age and maximum of 73.11%, in the larvae of the fourth age.

For the third batch, the raw fat from the worm leaf had the minimum digestibility value of 33.64%, in the larvae of the first age and maximum of 67.91%, in the larvae of the second age.

The results of the digestibility tests regarding the raw fat in the worm leaf are generally inconclusive, as many of these can come from the intestine of the larvae and not from the leaf, which is why, we cannot speak of a determination of the digestibility of the fat itself but of the "ethereal extract", which also contains very large quantities of pigments. Thus, the big differences regarding the evolution of the digestibility of the raw fat during the studied period could be explained.

In the literature, the values of the digestibility coefficient for raw fat between 63.28% and 74.19% (Mărghitaş, 1995).

At the first batch L1, throughout the larvae period, the digestibility of raw mulberry leaf cellulose was 18.07%, the mean value being 9.10 ± 3.206%, being very low in age I, 0.96%, after which it increased progressively, with over 17%, reaching the end of the studied period up to the value of 18.07%.

For the second batch L2, during the whole larval period, the digestibility of the raw cellulose from the mulberry leaf was 37.07%, being very low in age I, 1.53%, after which it increased progressively, by over 17%, reaching the end of the period studied up to the value of 39.39%.

For the third batch L3, during the whole larval period, the digestibility of the raw cellulose from the mulberry leaf was 23.05%, being null in age I, after which it increased progressively, reaching the end of the period studied up to the value of 23.89%.

This increase in the digestibility of raw cellulose, as the larvae grow older, is in line with the development of the enzymatic equipment in their digestive tract. Thus, if at age I, in the digestive tract of the larvae, the enzymes involved in the process of cellulose digestion are as non-existent, then they gradually increase, reaching the peak at age V, at which point the weight of raw cellulose from the worm leaf it is also bigger. This aspect, however, negatively influences the digestibility of the raw leaf protein, which during the same period, is experiencing a reduction.

At the beginning of the last century, some authors found that the leaf cellulose passes undigested through the digestive tract of the larvae and later it was concluded that this substance has a digestibility of approx. 20% (Doliș, 2008; Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017). Recently, some authors state that in the first two ages, raw cellulose would not be digested, but only from the third (8%), its digestibility reaches 21.13% in the third period (Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017; Matei, 1995).

In the first batch L1, the non-nitrogenous extractive substances from the mulberry leaf had a digestibility throughout the studied period of 61.42%, with an average of 74.34 ± 5.670%, the digestibility coefficients registering decreasing values, from 92.99%, in the case of age larvae I, at 57.41%, in the case of those of the Vth age.

For the second batch L2, the NEF from the leaf had a digestibility over the entire studied period of 60.87%, the digestibility coefficients registering decreasing values, from 93.43%, for the age I of larvae, at 58.31%, for the fifth age.

For the third batch L3, the NEF from the leaf had a digestibility over the entire studied period of 57.44%, the digestibility coefficients registering decreasing values, from 92.48%, for the larvae of age I, at 54.27%, in the case of those of fifth age.

According to Matei (1995), for the extracts not recorded from the leaves, the digestibility coefficients for the whole larval period record average values between 63.40% and 94.97%.

From the data in Table 3 it can be observed that the digestibility of the nutrients of the worm leaf showed a medium variability for dry matter and raw protein, high for raw fat and raw cellulose.

Knowing the value of digestibility coefficients, it was possible to calculate the digestible content for each nutrient separately, then the content of digestible substances in the leaf (Table 5).

Thus, in first group L1, when the report was made on fresh leaves, 139 g TSD/kg were obtained and when the report was made on dry matter from the mulberry leaf, its nutritional value was 474 g TSD/kg.

For the second batch L2, when the report was made for the fresh leaf, 134.81 g of Total Digestive Substance/kg were obtained, and when the report was made over the dry substance from the leaf of the mulberry, its nutritional value was 459.11 g TDS/kg.

For the third batch L3, the content of digestible substances of the leaf, so when the report was made to the fresh leaf, 148.75 g of Total Digestive Substance/kg were obtained, and when the report was made on the dried substance from the leaf of the mulberry, its nutritional value was 503.50 g TDS/kg.

The determination of the digestible energy content of the worm leaf administered in the feed of silk larvae was made based on the relative digestible content of the nutrients contained in it, using the calorific equivalents recommended for monogastric animal species (Table 6).

For the first group L1, the digestible energy from fresh leaf content was 639 Kcal / kg, and 2173 Kcal / kg for dry matter.

For the second group L2, the digestible energy content was 639 Kcal/kg in fresh leaf and 2173 Kcal/kg for dry matter.

For the third group L3, the digestible energy content was 619.94 Kcal/kg in fresh leaf and 2112.35 Kcal/kg for dry matter.

Table 5. Digestive content of mulberry leaf

Specification	Raw chemical composition %		Digestibility coefficients	Digestive content %		G Total Digestive substance /kg	
	*	**		*	**	*	**
L1							
RP	6.09	20.78	64.64	3.94	13.43	39.40	134.30
EE	1.09	3.70	49.57	0.54	1.83	12.15	41.18
CF	5.32	18.10	18.07	0.96	3.27	9.60	32.70
NEF	12.71	43.22	61.42	7.81	26.55	78.10	265.50
Total						139	474
L2							
RP	6.18	20.98	64.78	4.00	13.59	40.03	135.91
EE	1.10	3.70	45.41	0.50	1.68	11.24	37.80
CF	5.31	17.88	37.07	1.97	6.63	19.68	66.28
NEF	12.78	43.29	60.87	7.78	26.35	77.79	263.51
Total						149	504
L3							
RP	6.20	21.16	62.45	3.87	13.21	38.72	132.14
EE	1.04	3.54	45.35	0.47	1.61	10.61	36.12
CF	5.26	17.88	23.05	1.21	4.12	12.12	41.21
NEF	12.77	43.46	57.44	7.34	24.96	73.35	249.63
Total						135	459

* Reported to the fresh leaves; ** reported to DM

Table 6. Digestive energy of mulberry leaf

Specification	Digestive content %		Caloric equivalent (Kcal/g)	Kcal/kg	
	*	**		*	**
L1					
RP	3.94	13.43	5.78	227.73	776.25
EE	0.54	1.83	9.42	50.87	172.39
CF	0.96	3.27	4.40	42.24	143.88
NEF	7.81	26.55	4.07	317.87	1080.59
				639	2173
L2					
RP	4.00	13.59	5.78	231.20	785.50
EE	0.50	1.68	9.42	47.10	158.26
CF	1.97	6.63	4.40	86.68	291.72
NEF	7.78	26.35	4.07	316.65	1072.45
				682	2308
L3					
RP	3.87	13.21	5.78	223.69	763.54
EE	0.47	1.61	9.42	44.27	151.66
CF	1.21	4.12	4.40	53.24	181.28
NEF	7.34	24.96	4.07	298.74	1015.87
				620	2112

* Reported to the fresh leaves; ** reported to DM

The calculation of the metabolic energy of leaf administered in the feed of silk larvae was done by multiplying the digestible content of each nutrient with the energy equivalents recommended for monogastric (pigs) animal species. Considering, however, the specificity of the silkworm's digestion, and also the similarity with the digestion of the birds, for the estimation of the metabolic energy from the leaf, the energetic equivalents recommended for the birds were used (Table 7).

For the first group L1, the average metabolic energy content of fresh mulberry leaf was 597 kcal/kg, when the recommended energy coefficients for pigs were used, and 590 kcal/kg, when the recommended coefficients for birds were used. In relation to the dry matter in the leaf, the metabolic energy content was on average 2044 kcal/kg, when the recommended energy coefficients were used for pigs and 2007 kcal/kg, when the recommended coefficients for birds were used.

For the second group L2, the average content in metabolic energy from the fresh mulberry leaf was 630.24 kcal/kg, when the recommended energy ratios for pigs were used, as 630.33 kcal/kg, when the recommended coefficients for birds were used. In relation to the dry matter of the leaf, the content in metabolic energy was on average 2134.04 kcal/kg, when the recommended energy coefficients for pigs were used and 2133.59 kcal/kg, when the recommended coefficients for birds were used.

For the third group L3, the average content in metabolic energy from the fresh mulberry leaf was 576.95 kcal/kg, when the recommended energy ratios for pigs were used, respectively 571.18 kcal/kg, when the recommended coefficients for birds were used. In relation to the dry matter of the leaf, the content in metabolic energy was on average 1965.69 kcal/kg, when the recommended energy coefficients for pigs were used and 1945.78 kcal/kg, when the recommended coefficients for birds were used.

In order to determine the efficiency of use of the nutrients in worm leaf by the silk larvae, except for the intake and digestion, which were calculated during the course of the digestibility tests, it was necessary to determine the average growth rate of the larvae and the mass of the silk shell. The data necessary for calculating the efficiency of the use of the worm leaf by the larvae, as well as the results obtained in this respect, were centralized in Table 8. The data showed in this table indicate the fact that for *Bombyx mori* larvae, 10.38 grams of dry matter ingested from mulberry fruit and 5.92 grams of dry matter digested are required for each gram of silky coating, resulting an efficient conversion of the intake (ECI) in silk of 9.65% and of digestion (ECI) of 17%.

Table 7. Metabolic energy of mulberry leaf depending on the hybrid

Specification	Digestive content %		Caloric equivalent (Kcal/g)		Kcal/kg			
	*	**			*		**	
			swine	birds	swine	birds	swine	birds
L1								
RP	3.94	13.43	5.01	4.26	197.39	167.84	684.93	572.12
EE	0.54	1.83	8.93	9.50	48.22	51.30	163.42	173.85
CF	0.96	3.27	3.44	4.23	33.02	40.61	112.49	138.32
NEF	7.81	26.55	4.08	4.23	318.65	330.36	1083.24	1123.07
					597	590	2044	2007
L2								
RP	4.00	13.59	5.01	4.26	200.40	170.40	680.86	578.93
EE	0.50	1.68	8.93	9.50	44.65	47.50	150.02	159.60
CF	1.97	6.63	3.44	4.23	67.77	83.33	228.07	280.45
NEF	7.78	26.35	4.08	4.23	317.42	329.09	1075.08	1114.61
					630	630	2134	2139
L3								
RP	3.87	13.21	5.01	4.26	193.89	164.86	661.82	562.75
EE	0.47	1.61	8.93	9.50	41.97	44.65	143.77	152.95
CF	1.21	4.12	3.44	4.23	41.62	51.18	141.73	174.28
NEF	7.34	24.96	4.08	4.23	299.47	310.48	1018.37	1055.81
					577	571	1966	1946

Table 8. Efficiency use of mulberry leaf by the Băneasa Super hybrid

Specification	L1		L2	L3	Average
Average body mass gained during the whole larvae stage (g)	Living larvae	5.099	5.039	5.075	5.071
	Dry matter	0.925	0.919	0.919	0.921
Silky shell mass (g Dry Matter)	0.399		0.398	0.401	0.399
Dry Matter of ingested leaf (g)	4.288		4.231	3.918	4.146
Dry Matter of digested leaf (g)	2.532		2.457	2.107	2.365
Ingested Dry Matter/Body mass Dry Matter (g)	4.633		4.607	4.265	4.502
Dry matter ingested/ Body mass Dry Matter (g)	2.736		2.675	2.293	2.568
Dry matter ingested/Silky shell Dry Matter (g)	10.749		10.636	9.767	10.384
Dry matter digested/ Silky shell Dry Matter (g)	6.347		6.175	5.252	5.925
CEI body mass %	21.580		21.708	23.450	22.246
CED body mass %	36.550		37.387	43.610	39.182
CEI silky shell %	9.300		9.402	10.240	9.647
CED silky shell %	15.760		16.193	19.040	16.998

The data obtained from the experience performed, regarding the efficiency of the use of the mulberry leaf by the *Bombyx mori* larvae, are similar with those presented in the literature (Matei, 1995; Rahmathulla et al., 2002; Rath et al., 2003; Sarkar, 1993; Tzenov, 1993).

CONCLUSIONS

From the aspects mentioned in the paper, the following conclusion may be drawn:

Regarding the dry matter from the mulberry leaves, depending on the type, the average values were: RP = 20.78 - 21.16%; EE = 3.54 - 3.70%; RF = 17.88 - 18.10%; NEF = 43.22 - 43.42% and ash = 13.96 - 14.20%.

While vegetation degree advanced during each growth period of silkworm larvae, the mulberry leaf ages and its quality regarding the chemical composition is decreasing. During the period of 30 days of research, it was noticed a moisture decreasing by 3.23 - 3.72% and of the RP with 2.86 - 3.11% and at the same time a CF increasing by 1.12% - 2.29%.

The leaves nutrients digestibility had an average of 53.77 - 58.06%. The dry matter digestibility decreased by 31.76 - 36.98%.

Digestibility coefficients of the RP (62.45 - 71.22%) and of the NEF (57.44 - 74.34%) from the mulberry leaves decreased during the study by 21.28 - 26.03% (RP) and 35.12 - 38.21% (NEF).

The CF digestibility, very low at the beginning, increased progressively till the fifth larvae stage when it was 18.59–39.39%.

Nutritional value of the mulberry leaves was 459.11 - 474 g TDN/ kg DM.

Throughout the studied period, the energy of leaf was on average 1237 - 1238 kcal/kg, in the fresh leaf, and 4209 - 4216 kcal/kg, in dry substance. For the fresh leaf, the content of digestible energy was 620 - 682 kcal/kg, and in the case of dry matter, 2112 - 2308 kcal/kg. In relation to the dry matter of the leaf, the content of metabolic energy was on average 1966 - 2134 kcal/kg, when the recommended energy coefficients for pigs were used, and 1946 - 2139 kcal/kg, when the recommended coefficients for birds were used.

For each gram of silk wrap, 9.77 - 10.75 grams of dry matter ingested from the mulberry tree are required, respectively 5.25 - 6.35 grams of digested dry substance, resulting an efficiency of conversion of ingestion (CEI) into silk of 9.30 - 10.24%, and also of digestion (CEI) of 15.76 - 19.04%.

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