

STUDY REGARDING THE USE OF MULBERRY LEAVES (KOKUSO 21 VARIETY) BY *BOMBYX MORI* (TRIUMF HYBRID)

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Abstract

In order to assess how efficient is the use of Mulberry leaf by the *Bombyx mori* Triumph larvae hybrid, some determinations were made regarding the nutritional value and digestibility of the worm leaf administered as food (leaves from a Japanese variety, Kokuso 21), during a series of summer growth. The results showed that ongoing vegetation and growth process of this hybrid, the Mulberry leaves suffer an aging phenomenon, revealed by diminishing its chemical composition quality. According to this, most of the nutritional substances from Mulberry leaves, except cellulose, manifest a continuous decline during the growth period. The digestibility of these nutritional components registered a value of 55.4%, the raw energy value was 4216 kcal/kg dry substance, the digestive energy was 2156 kcal/kg (DS), while the metabolic energy was 1993-2010 kcal/kg (DS). The efficiency of converting ingestion into silk had a value of 10.1% and the digestion was 17.88%.

Key words: leaves, Mulberry, larvae, energy, use

Besides the continuous improvement of the growth technologies, one of the main concerns of the specialists in sericulture is represented by the production of biological material of high genetic value as the *Bombyx mori* larvae with an increasing productive potential, more resistant to the environmental factors and to diseases and to use nutrients offered by the Mulberry to the best of their advantage.

Thus, from this point of view, the performances of the 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 worm 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 pedoclimatic conditions, season, variety of the mulberry, the way of harvesting and storage etc.

In the specific literature, depending on different factors, the relative humidity values of the Mulberry leaf vary between 65-75% (Doliș M., 2008).

Compared with the common Mulberry (69.80-73%), the selected varieties have more water content (Bura M. *et al.*, 1995). The dried substance from the worm leaf, harvested in the same period, can record, depending on the variety/hybrid, different values, for example, between 23.61% and 27.56% (Matei A., 1995).

Also, if the spring moisture of the mulberry leaf is 71.85-77.81%, then it decreases to 68.42-75.64%, in the summer period, respectively to 64.10-73.64%, in the fall (Ifrim S., 1998).

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 S.S. *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 V.K. *et al.*, 2002).

The raw leaf protein is estimated to have an average value of 6.16% in the fresh leaf, 20.97% in the dry substance and 24.36% in its organic substance (Doliș M., 2008). The raw leaf protein values can vary depending on the season, the time of day, the variety/hybrid of the dude: 32.40% in spring, 28.21% in summer and 24.53% in autumn (Borcescu A., 1966), 26.80% in the morning and 29.10% in the evening (Mărghitaș L.A., 1995), between 22.55% and 25.73% depending on the variety (Matei A., 1995). In the specialty literature, for raw leaf protein, the value of digestibility

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coefficients is between 69.21% and 78.92 (Borcescu A., 1966), 60.06% and 74.69% (Petkov N., 1980), 71.62% and 93.48% (Matei A., 1995).

The limits presented by specific literature regarding the fat content in mulberry leaves are 2.85- 6.07% (Pop E.C., 1967) The values of the digestibility coefficient for raw fat are between 63.28% and 74.19% (Petkov N., 1980).

According to the data from the specialized literature, in the common Mulberry the weight of the raw cellulose ranges between 12.33-14,38%, while in the different varieties selected oscillates between 10.43-13,70% (Craiciu E., 1966). In the vegetation period of the mulberry the content in raw cellulose from the leaves increases from 14.47% to 21.16% (Pop E.C., 1967). Increased cellulose content causes aging of the worm leaf, which becomes harder and harsher, therefore harder to consume by, which is why those varieties whose leaves have less cellulose content are considered more valuable. At the beginning of the last century, some authors (Acqua, 1930 – cited by Dolis M., 2008) 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% (Legay, 1955 - cited by Dolis M., 2008). 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 (Matei A., 1995).

The values regarding the mineral substances, offered by the specialized literature, ranges between: 9.13-17.38% (Pop E.C., 1967), 11.52-12.80% (Matei A., 1995) and 8.7-13.15% (Bura M. *et al.*, 1995).

At the end of the last century, Romania could be considered an important point on the map of European sericulture. Thus, in her record, Romania can boast in this field with a quite complex literature, as well as with the creation of new varieties and valuable hybrids of worm, as *Bombyx mori*, all being the result of some decade research work of Romanian specialists (Dolis M., 2008; Lazăr S. and Vornicu O.C., 2013; Pătruică S., 2013).

For this reason, 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.

MATERIAL AND METHOD

The biological material used in the experiments was represented by a batch of 150 larvae of *Bombyx mori* from Romanian simple

hybrid *Triumf*. To be easier to follow, the group was divided into three sub-lots (repetitions) of 50 larvae each, which were raised in paper trays sized according to the age and size of the larvae; in addition, it was also made up a separate lot, with 50 larvae reared separately, but under the same conditions, which served to replace the dead larvae from the experimental group.

The growth of the larvae was in August, in an air-conditioned room, in compliance with all the microclimate factors. Each divided group received the same amount of leaf, from the same variety of worm, Kokuso 21, from where samples were previously collected, for chemical analysis.

The variety of mulberry *Kokuso 21* a mulberry variety which derives from the crossing between *Naganua*, *Gariin* and *Shiso* varieties. It is a variety of Japanese origin with whole leaf which has adapted fairly well to the climatic conditions from the south of Romania. In plantations of the intensive type may be produced from the first years a high quantity of leaves with a high protein content.

Daily and at the same time, from each group were collected, weighed and recorded what was not consumed from the Mulberry leaves and what was excreted by the larvae.

The quantities of residues, respectively of excrements, obtained from each group were summed, the result being divided into three, thus obtaining the average quantity of residues from each 50 larvae. The values obtained were subsequently used in the calculation relationships to find the digestibility coefficients. Also, from each group were collected samples of excrements, which were mixed in order to obtain medium samples for analyze.

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 increase in body mass accumulated by a larva.

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

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.

The working methods used were mainly the specific ones used to determine the nutritional value of the worm leaf and they were based on the chemical composition (the "proximate analysis" scheme), the digestibility of its components (the "in vivo" method - simple digestibility, with a single control period) and raw energy (use of specific computation equations and regression coefficients

recommended by the OKIT system), digestible (calculation equation recommended for monogastric species) and metabolizable (equations recommended for monogastric animals and birds) contained (Halga P. *et al*, 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 A., 1995; Rahmathulla V.K. *et al*, 2002; Sarkar A., 1993).

RESULTS AND DISCUSSION

Table 1 shows the data of the chemical composition evolution of larvae in relation to their age.

Table 1

The chemical composition evolution of the Kokuso 21 variety mulberry tree leaves during the silkworm larvae growth (%)

The larvae age	Water	DM	CP		EE		CF		NFE		Ash	
			F*	DM**	F*	DM**	F*	DM**	F*	DM**	F*	DM**
I	72.09	27.91	6.31	22.61	0.79	2.83	4.74	16.98	12.33	44.18	3.74	13.40
II	71.66	28.34	6.28	22.16	0.88	3.11	4.88	17.22	12.34	43.54	3.96	13.97
III	70.31	29.59	6.23	21.05	1.14	3.85	5.31	17.95	12.64	42.72	4.27	14.43
IV	70.13	29.87	6.04	20.22	1.16	3.88	5.44	18.21	13.09	43.83	4.14	13.86
V	68.86	31.14	6.15	19.75	1.25	4.01	5.93	19.04	13.41	43.07	4.40	14.13
\bar{x}	70.63	29.37	6.20	21.16	1.04	3.54	5.26	17.88	12.77	43.46	4.10	13.96
$S_{\bar{x}}$	-	0.575	-	0.547	-	0.237	-	0.368	-	0.261	-	0.169
Cv%	-	4.381	-	5.782	-	14.975	-	4.599	-	1.342	-	2.713

* fresh leaves; ** dry matter

The crude protein had an average value of 6.18% ($20.98 \pm 0.670\%$ from DM). It is noticed a progressive decreasing of the protein content throughout the studied period, the content decreasing being with 3.11 percentage points, from 22.14% to 19.03%, respectively.

The fat content from the mulberry leaves was in average 1.10% in the fresh leaves, and $3.70\% \pm 0.260$ in DM. It is the only nutrient with a high variability, of 15.700%. The fat content increased uniformly throughout the silkworm larval growth, from 0.85% to 1.38% when it was expressed in fresh leaves, or 3.2 % to 4.33% respectively, when it was reported to the dry matter.

The crude cellulose was in average 5.31% in fresh leaves, $17.91 \pm 0.434\%$, respectively when in was reported to DM. Throughout the research, for a month, the crude cellulose increased with 2.29 percentage points, from 17.02% to 19.31%, respectively.

The average values obtained for each nutrient separately are set in the limits presented by specific literature, where the data regarding the crude chemical composition of the mulberry leaves varies according to each author, to the research period, to the varieties of mulberry, etc. The average relative humidity of the mulberry leaves during the research was 70.44%, and an decreasing evolution being 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 age V-th). The dry matter represented $29.56 \pm 0.725\%$.

Nitrogen free extract represented in average $43.27 \pm 0.418\%$ 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 was an increasing to the fourth determination, being 43.89%, decreasing to the last analyses to 42.64%.

The ash represented in average 4.19% in the fresh leaves and $14.15 \pm 0.169\%$ from dry matter. The minerals from the mulberry leaves throughout the research registered a continuous increase from analyse 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%.

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 1245 Kcal/kg, in fresh leaf, respectively 4213 Kcal/kg, in the dry matter (*table 2*).

Table 2

Specification	%		Caloric equivalent	Kcal/100g		Kcal/100g	
	*	**		*	**	*	**
	CP	6.20		21.16	5.72	35.46	121.04
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

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 practically the difference between the amount of substances ingested through food and the amount 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 (Miranda J.E. and Takahashi R., 1998; Rahmathulla V.K. *et al*, 2004; Rath S.S. *et al*, 2003; Sabhat A. *et al*, 2011; Tzenov P., 1993).

Table 3

Data needed to calculate digestibility coefficients

The larvae age	Specifi-cation	Quantity (g)	Chemical composition (%/g)					
			DM	CP	EE	CF	NEF	Ash
I	Frunză	15.5	27.91	6.31	0.79	4.74	12.33	3.74
	Resturi	5.01	63.82	14.04	2.01	14.59	25.97	7.21
	Excretă	0.16	66.15	21.87	5.67	2.33	27.21	9.07
II	Frunză	26	28.34	6.28	0.88	4.88	12.34	3.96
	Resturi	8.22	60.02	12.01	2.11	14.88	21.01	10.01
	Excretă	0.98	64.69	14.93	3.64	4.31	27.85	13.96
III	Frunză	77	29.59	6.23	1.14	5.31	12.64	4.27
	Resturi	23.14	58.39	10.78	2.31	15.99	22.12	7.19
	Excretă	4.12	65.15	14.99	2.63	7.99	25.53	14.01
IV	Frunză	242	29.87	6.04	1.16	5.44	13.09	4.14
	Resturi	67.93	59.53	12.62	2.45	14.49	24.72	5.25
	Excretă	19.88	63.52	11.01	2.02	14.09	24.99	11.41
V	Frunză	1000	31.14	6.15	1.25	5.93	13.41	4.4
	Resturi	269.02	56.73	11.46	1.88	13.66	23.97	5.76
	Excretă	122.39	61.03	9.98	3.22	13.99	25.01	10.19

During the whole period studied, the digestibility of the dried substance from the worm leaf had a digestibility of 55.40%. The highest digestibility was recorded in larvae of age I

(90.63%), after which, by the end of the larval period, there was a decrease of 37.67percent.

The raw protein had a digestibility coefficient for the entire studied period of 61.93%. The raw protein digestibility decreased

progressively during the studied period, with 27.09%, respectively from 87.26%, in the first larval age, to 60.17%, 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.

Table 4

The larvae age	DM	CP	EE	CF	NEF
I	90.63	87.26	58.26	-	92.87
II	73.96	77.34	35.56	7.66	81.58
III	71.05	73.18	68.42	15.29	77.21
IV	60.35	63.79	64.86	15.67	66.63
V	52.96	60.17	47.05	24.08	56.03
I-V	55.40	61.93	50.08	22.85	59.49

The raw fat from the worm leaf had the minimum digestibility value of 35.56%, in the larvae of the second age and maximum of 68.42%, in the larvae of the third 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.

During the whole larval period, the digestibility of the raw cellulose from the mulberry leaf was 22.85%, null in age I, after which it increased progressively, reaching the end of the period studied up to the value of 24.08%. 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.

Unclaimed extractive substances from the worm leaf had a digestibility over the entire studied period of 59.49%, the digestibility coefficients registering decreasing values, from 92.87%, in the case of the larvae of age I, at 56.03%, in the case of those of fifth age.

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, so when the report was made to the fresh leaf, 138.1 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 470.33 g TDS/kg (table 5).

Table 5

Specification	Raw chemical composition %		Digestibility coefficients	Digestive content %		g Total Digestive Substance /kg		Caloric equivalent (Kcal/g)	Kcal/kg	
	*	**		*	**	*	**		*	**
CP	6.2	21.16	61.93	3.84	13.10	38.40	131.04	5.78	221.95	757.18
EE	1.04	3.54	50.08	0.52	1.77	11.72	39.89	9.42	48.98	166.73
CF	5.26	17.88	22.85	1.20	4.09	12.02	40.86	4.40	52.80	179.96
NEF	12.77	43.46	59.49	7.60	25.85	75.97	258.54	4.07	309.32	1052.10
Total						138.10	470.33		633.06	2155.97

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

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 5). In the case of the fresh leaf, the digestible energy content was 633 Kcal/kg, and in the case of the dry substance, 2156 Kcal/kg.

The calculation of the metabolic energy from the worm 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 (pig) animal species. Considering, however, the specificity of the silkworm's digestion, respectively

the similarity with the digestion of the birds, for the estimation of the metabolic energy from the worm leaf, the energetic equivalents recommended for the birds were used (table 6).

The average content in metabolic energy from the fresh mulberry leaf was 590 Kcal/kg, when the recommended energy ratios for pigs were used, respectively 585 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 2010 Kcal/kg, when the recommended energy coefficients for pigs were used, and 1993 Kcal/kg, when the recommended coefficients for birds were used.

Table 6

Metabolic energy of Mulberry leaf

Specification	Digestive content %		Caloric equivalent (Kcal/g)		Kcal/kg			
	*	**			*		**	
			swine	birds	swine	birds	swine	birds
CP	3.84	13.1	5.01	4.26	192.38	163.58	656.31	558.06
EE	0.52	1.77	8.93	9.50	46.44	49.40	158.06	168.15
CF	1.2	4.09	3.44	4.23	41.28	50.76	140.70	173.01
NEF	7.6	25.85	4.08	4.23	310.08	321.48	1054.68	1093.46
					590.18	585.22	2009.75	1992.67

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

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 7.

From the data of this table it is observed that in the case of the *Bombyx mori* Triunf larvae hybrid, for every gram of silk wrap is required 10.1 grams of dry matter ingested from the wormwood, respectively 5.59 grams of digested dry matter, resulting in an efficiency of conversion of silk intake (CEI) of 9.9%, respectively of digestion (CED) of 17.88%

Table 7

 Efficiency of using Mulberry leaf by *Bombyx mori* Triunf larvae hybrid

Average body mass gained during the whole larvae stage (g)	Living larvae	5.222
	Dry matter	0.921
Silky shell mass (g Dry Matter)		0.403
Dry Matter of ingested leaf (g)		4.069
Dry Matter of digested leaf (g)		2.254
Ingested Dry Matter/Body mass Dry Matter (g)		4.417
Dry matter ingested/ Body mass Dry Matter (g)		2.447
Dry matter ingested/Silky shell Dry Matter (g)		10.097
Dry matter digested/ Silky shell Dry Matter (g)		5.593
CEI body mass %		22.64
CED body mass %		40.87
CEI silky shell %		9.90
CED silky shell %		17.88

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

CONCLUSIONS

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

- Expressed to dry matter from the mulberry leaves, Kokuso 21 variety the average values were: CP – 21.16%, EE – 3.54%, CF – 17.88%, NEF – 43.42% and ash – 13.96%.

- At once with vegetation advancement and implicitly during each growth period of silkworm larvae, the mulberry leaf ages and its quality from the chemical composition point of view is decreasing.

- During the 30 days of the research, was noticed a decreasing of the moisture with 3.23% and of the CP with 2.86 % and in the same time an increasing of the CF with 2.06%.

- During the whole period studied, the digestibility of the dried substance from the worm leaf had a digestibility of 55.40%. The dry matter digestibility decreased with 37.67%.

- Digestibility coefficients of the CP (61.93%) and of the NFE (59.49%) from the mulberry leaves decreased during the study with 27.09% and 36.84%, respectively.

- The CF digestibility, null at the beginning, increased progressively till the fifth larval stage when it was 24.08%.

- Nutritional value of the mulberry leaves was 470 g TDN/ kg DM.

- Throughout the studied period, the gross enrichment of the worm leaf was on average 4216 Kcal/kg, in the dry substance

- In the leaf, the content of digestible energy was, in the case of dry matter, 2156 Kcal/kg.

In relation to the dry matter of the leaf, the content in metabolic energy was on average 1993-2010 Kcal/kg.

In the case of the Triumf hybrid, for each gram of silk wrap, 10.1 grams of dry matter ingested from the mulberry tree are required, respectively 5.59 grams of digested dry substance, resulting an efficiency of conversion of ingestion (CEI) into silk of 9.90%, respectively of the digestion (CEI) of 17.88%

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