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STUDY REGARDING THE USE OF MULBERRY LEAVES BY *BOMBYX MORI* - ZEFIR HYBRID

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Abstract

In order to assess how efficient is the use of Mulberry leaf by the Bombyx mori Zefir 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 55.22%, the raw energy value was 4216 kcal/kg dry substance, the digestive energy was 2103 kcal/kg (DS), while the metabolic energy was 1935-1959 kcal/kg (DS). The efficiency of converting ingestion into silk had a value of 10.33% and the digestion was 16.37%.

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

INTRODUCTION

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.

The knowledge of the nutritional value and of the influence factors, as well as the way in which 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 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 research was done during the growth period of the silkworm larvae from summer series, the biologic material being represented both by silkworm larvae and mulberry leaves which were administrated.

The animal biologic material was the Romanian simple hybrid of silkworm *Zefir*.

The vegetal biologic material used in the research was represented by *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.

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 (*http://www.fao.org*).

The chemical analyses were done on samples previously dried to 65ºC and grinded. The obtained results were processed and noted in tables being expressed in both fresh and dried leaves (*Halga P. et al., 2005*). The collected samples moisture determination was done by drying them into the hot air oven for 4-5 hours at 105ºC *(Regulation (EC) no. 152/2009 SR ISO 6496:2001)*.

The ashes content was determined using the incineration of the samples method *(Regulation (EC) no. 152/2009 SR EN ISO 2171: 2010).*

To determine the protein content (CP), the Kjeldhal method was used *(Regulation (EC) no. 152/2009 SR EN ISO 5983-2: 2009 AOAC 2001.11).*

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) *(Regulation (EC) no. 152/2009 SR ISO 6492: 2001).*

The crude fiber (CF) was determined by the sample acid-basic hydrolyze, after which from the leaf is removed the hydrolysable part, on the filter paper remaining only the cellulose and minerals; by calcination are determined the minerals and the crude cellulose is calculated through difference *(Regulation (EC) no. 152/2009 SR EN ISO 6865: 2002).*

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

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% = $\frac{\text{Digest}}{\text{Intake}}$ x 100).

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 ones leftover) and finally the intake of the nutritive substances or digest (difference between ingest and faeces). Expressing in percentage the digest from ingest, 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 it represents the result between the crude 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 with 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.

For energy value, The working methods used 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 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)*.

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

During the silkworm larvae growth, the research objective was to establish the nutritive values of the mulberry leaves depending on its maturity and silkworm larvae age, respectively. This was accomplished through digestibility trials.

There were organized an experimental lot formed from 150 larvae, which were grouped in three repetitions of 50 larvae each. In the calculations during the research were used the average values obtained in all three repetitions, the data being extrapolated to all 50 silkworm larvae.

In each repetition were used trays with paper sized accordingly with the larvae's age and size.

To each repetition had been administered the same quantity of mulberry leaves from which previously were collected samples for chemical analyses.

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

The number of leftovers mulberry leaves from each repetition were summed, the result being than divided to 3, obtaining the average quantity of leftover leaves from the 50 larvae; the value being representative for the entire lot. This value was used to calculate the digestibility coefficients of the nutrients from mulberry leaves. Similarly, was done in the case of the excreta.

From each repetition were collected samples of leftover, excreta respectively, which were homogenized in order to obtain an average sample for each lot; those samples were chemically analyzed.

Also, there were organized three reserve repetition with 50 larvae raised separately, but under the same conditions.

During the experiment was watched the larval mortality from each lot and if necessary the dead larvae were immediately replaced with ones from reserve lot.

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 larvae growth was held during 31st of July and 31st of August, respecting the breeding technology recommended by the specific literature.

RESULTS AND DISCUSSION

The values regarding the mulberry leaves chemical composition evolution throughout growth period of the silkworm larvae were centralized and statistically processed (table 1).

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.

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
$\overline{X}$	70.63	29.37	6.20	21.16	1.04	3.54	5.26	17.88	12.77	43.46	4.10	13.96
$S_{\overline{x}}$	-	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
Min	-	27.91	-	19.75	-	2.83	-	16.98	-	42.72	-	13.40
Max	-	31.14	-	22.61	-	4.01	-	19.04	-	44.18	-	14.43

* fresh leaves; ** dry matter

The average relative humidity of the mulberry leaves during the research was 70.63%, and a decreasing evolution being 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 age V^{th}). The dry matter represented 29.37±0.575%.

The mulberry leaves humidity influences its consumption by the silkworm larvae. The larvae, 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% *(Doliş 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)*.

The crude protein had an average value of 6.20% (21.16±0.547% from DM). It is noticed a progressive decreasing of the protein content throughout the studied period, the content decreasing being with 2.86 percentage points, from 22.61% to 19.75%, respectively.

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 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 *(Doliş 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 *(Mărghitaş L. A., 1995)*, between 22.55% and 25.73% depending on the variety *(Matei A., 1995)*.

The fat content from the mulberry leaves was in average 1.04% in the 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% respectively, 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 E.C., 1967)*.

The crude cellulose was in average 5.26% in fresh leaves, 17.88±0.368%, respectively when in was reported to DM. Throughout the research, for a month, the crude cellulose increased with 2.06 percentage points, from 16.98% to 19.04%, respectively.

The cellulose is highly responsible for aging processes of the mulberry leaves. As the cellulose content grows, the leaf becomes tougher and rougher, being more difficult to be consumed by the silkworm larvae.

For this reason, 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 crude cellulose from mulberry leaves were comparable with the ones from specific literature. The crude cellulose quota varies between 12.33-14.38% to the common mulberry tree and between 10.43-13.70% to different selected varieties *(Craiciu E., 1966).* Throughout the mulberry vegetation period, the cellulose content from leaves increase from 14.47 to 21.16% *(Pop E.C., 1967).*

Nitrogen free extract represented in 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%.

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 analyze to another. The average values varied from 3.74% to 4.40% to 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 obtained data regarding the mineral content are in conformity with the ones from specific literature, 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)*.

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).

Table 2

Raw average energy of Mulberry leaf

Specification	**%**		**Caloric equivalent**	**Kcal/100g**		**Kcal/100g**	
	*	**		*	**	*	**
CP	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**

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).

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	66.9	14.32	1.99	14.62	27.04	8.93
	Excretă	0.16	67.96	20.69	8.78	1.39	28.11	8.99
II	Frunză	26	28.34	6.28	0.88	4.88	12.34	3.96
	Resturi	8.78	60.51	13.61	1.92	13.97	21.03	9.98
	Excretă	0.79	65.95	14.08	3.71	5.21	29.98	12.97
III	Frunză	77	29.59	6.23	1.14	5.31	12.64	4.27
	Resturi	24.01	61.73	8.75	3.22	15.97	25.01	8.78
	Excretă	3.91	66.56	17.99	1.41	5.82	26.01	15.33
IV	Frunză	242	29.87	6.04	1.16	5.44	13.09	4.14
	Resturi	68.17	59.06	11.61	1.66	15.22	23.88	6.69
	Excretă	20.31	62.28	12.88	3.08	11.56	26.64	8.12
V	Frunză	1000	31.14	6.15	1.25	5.93	13.41	4.4
	Resturi	269.41	58.87	12.16	1.88	14.67	25.03	5.13
	Excretă	120.41	59.63	9.03	3.81	12.92	24.99	8.88

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).*

During the whole period studied, the digestibility of the dried substance from the worm leaf had a digestibility of 55.22%. The highest digestibility was recorded in larvae of age I (88.84%), after which, by the end of the larval period, there was a decrease of 35.83 percent.

Table 4

Digestibility coefficients of *Zefir* hybrid

The larvae age	Specification	DM	CP	EE	CF	NEF
I	Leaves	4.3261	0.9781	0.1225	0.7347	1.9112
	Leftovers	3.3517	0.7174	0.0997	0.7325	1.3547
	Ingest	0.9744	0.2607	0.0228	0.0022	0.5565
	Excreta	0.1087	0.0331	0.014	0.0022	0.045
	Digest	0.8657	0.2276	0.0088	0.0000	0.5115
	DC%	**88.84**	**87.30**	**38.60**	**0.00**	**91.91**
II	Leaves	7.3684	1.6328	0.2288	1.2688	3.2084
	Leftovers	5.3128	1.195	0.1686	1.2266	1.8464
	Ingest	2.0556	0.4378	0.0602	0.0422	1.3620
	Excreta	0.521	0.1112	0.0293	0.0412	0.2368
	Digest	1.5346	0.3266	0.0309	0.0010	1.1252
	DC%	**74.65**	**74.60**	**51.33**	**2.37**	**82.61**
III	Leaves	22.7843	4.7971	0.8778	4.0887	9.7328
	Leftovers	14.8214	2.1009	0.7731	3.8344	6.0049
	Ingest	7.9629	2.6962	0.1047	0.2543	3.7279
	Excreta	2.6025	0.7034	0.0551	0.2276	1.017
	Digest	5.3604	1.9928	0.0496	0.0267	2.7109
	DC%	**67.32**	**73.91**	**47.37**	**10.50**	**72.72**
IV	Leaves	72.2854	14.6168	2.8072	13.1648	31.6778
	Leftovers	40.2612	7.9145	1.1316	10.3755	16.279
	Ingest	32.0242	6.7023	1.6756	2.7893	15.3988
	Excreta	12.6491	2.6159	0.6255	2.3478	5.4106
	Digest	19.3751	4.0864	1.0501	0.4415	9.9882
	DC%	**60.50**	**60.97**	**62.67**	**15.83**	**64.86**
V	Leaves	311.4000	61.5000	12.5000	59.3000	134.1000
	Leftovers	158.6017	32.7603	5.0649	39.5224	67.4333
	Ingest	152.7983	28.7397	7.4351	19.7776	66.6667
	Excreta	71.8005	10.8730	4.5876	15.5570	30.0905
	Digest	80.9978	17.8667	2.8475	4.2206	36.5762
	DC%	**53.01**	**62.17**	**38.30**	**21.34**	**54.86**
I-V	Leaves	418.1642	83.5248	16.5363	78.557	180.6302
	Leftovers	222.3488	44.6881	7.2379	55.6914	92.9183
	Ingest	195.8154	38.8367	9.2984	22.8656	87.7119
	Excreta	87.6818	14.3366	5.3115	18.1758	36.7999
	Digest	108.1336	24.5001	3.9869	4.6898	50.9120
	DC%	**55.22**	**63.08**	**42.88**	**20.51**	**58.04**

In the specialty 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 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 protein had a digestibility coefficient for the entire studied period of 63.08%. The raw protein digestibility decreased progressively during the studied period, with 25.13%, respectively from 87.30%, in the first larval age, to 62.17%, 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.

In the specialty literature, for raw leaf protein, the value of digestibility 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 raw fat from the worm leaf had the minimum digestibility value of 38.30%, in the larvae of the fifth age and maximum of 62.67%, in the larvae of the fourth 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.

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)*.

During the whole larval period, the digestibility of the raw cellulose from the mulberry leaf was 20.51%, being null in age I, after which it increased progressively, reaching the end of the period studied up to the value of 21.34%. 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 *(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)*.

Unclaimed extractive substances from the worm leaf had a digestibility over the entire studied period of 58.04%, the digestibility coefficients registering decreasing values, from 91.91%, in the case of the larvae of age I, at 54.86%, in the case of those of fifth age.

According to Matei, 1995, for the extracts not recorded from the leaf of the worm, 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, raw protein and 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, so when the report was made to the fresh leaf, 134.05 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 456.54 g TDS/kg (table 5).

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). In the case of the fresh leaf, the digestible energy content was 617.50 Kcal/kg, and in the case of the dry substance, 2102.75 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 7).

Table 5

The nutritional value calculation of the mulberry leaves (g TDN/kg)

Specification	Raw chemical composition %		Digestibility coefficients	Digestive content %		G Total Digestive substance /kg	
	*	**		*	**	*	**
CP	6.20	21.16	63.08	3.91	13.35	39.11	133.48
EE	1.04	3.54	42.88	0.45	1.52	10.03	34.15
CF	5.26	17.88	20.51	1.08	3.67	10.79	36.67
NEF	12.77	43.46	58.04	7.41	25.22	74.12	252.24
Total						**134.05**	**456.54**

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

Table 6

Digestive energy of Mulberry leaf

Specification	Digestive content %		Caloric equivalent (Kcal/g)	Kcal/kg	
	*	**		*	**
CP	3.91	13.35	5.78	226.00	771.63
EE	0.45	1.52	9.42	42.39	143.18
CF	1.08	3.67	4.40	47.52	161.48
NEF	7.41	25.22	4.07	301.59	1026.45
Total				**617.50**	**2102.75**

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

Table 7

Metabolic energy of Mulberry leaf

Specification	Digestive content %		Caloric equivalent (Kcal/g)		Kcal/kg			
					*		**	
	*	**	swine	birds	swine	birds	swine	birds
CP	3.91	13.35	5.01	4.26	195.89	166.57	668.84	568.71
EE	0.45	1.52	8.93	9.50	40.19	42.75	135.74	144.40
CF	1.08	3.67	3.44	4.23	37.15	45.68	126.25	155.24
NEF	7.41	25.22	4.08	4.23	302.33	313.44	1028.98	1066.81
					575.56	**568.44**	**1959.80**	**1935.16**

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

The average content in metabolic energy from the fresh mulberry leaf was 575.56 Kcal/kg, when the recommended energy ratios for pigs were used, respectively 568.44 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 1959.80 Kcal/kg, when the recommended energy coefficients for pigs were used, and 1935.16 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.

Table 8

Efficiency of using Mulberry leaf by *Bombix mori* Zefir larvae hybrid

Average body mass gained during the whole larvae stage (g)	**Living larvae**	5.089
	Dry matter	0.921
Silky shell mass (g Dry Matter)		0.401
Dry Matter of ingested leaf (g)		3.884
Dry Matter of digested leaf (g)		2.450
Ingested Dry Matter/Body mass Dry Matter (g)		4.217
Dry matter ingested/ Body mass Dry Matter (g)		2.660
Dry matter ingested/Silky shell Dry Matter (g)		9.685
Dry matter digested/ Silky shell Dry Matter (g)		6.110
CEI body mass %		23.71
CED body mass %		37.59
CEI silky shell %		10.33
CED silky shell %		16.37

From the data of this table it is observed that in the case of the *Bombyx mori* Zefir larvae hybrid, for every gram of silk wrap is required 9.69 grams of dry matter ingested from the wormwood, respectively 6.11 grams of digested dry matter, resulting in an efficiency of conversion of silk intake (CEI) of 10.33%, respectively of digestion (CED) of 16.37%

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 55.22%. The dry matter digestibility decreased with 35.83%.

- Digestibility coefficients of the CP (63.08%) and of the NFE (58.04%) from the mulberry leaves decreased during the study with 25.13% and 37.05%, respectively.

- The CF digestibility, null at the beginning, increased progressively till the fifth larval stage when it was 21.34%.

- Nutritional value of the mulberry leaves was 456.54 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, 2102.75 Kcal/kg.

In relation to the dry matter of the leaf, the content in metabolic energy was on average 1935.16 – 1959.80 Kcal/kg.

In the case of the Zefir hybrid, for each gram of silk wrap, 9.69 grams of dry matter ingested from the mulberry tree are required, respectively 6.11 grams of digested dry substance, resulting an efficiency of conversion of ingestion (CEI) into silk of 10.33%, respectively of the digestion (CEI) of 16.37%

REFERENCES

1. Borcescu A., 1966 - Hrănirea viermilor de mătase în creşterile de vară şi toamnă. Sericicultura, nr. 3, p. 6-9.
2. Borcescu A., 1966 - Particularităţile digestiei la viermele de mătase, Sericicultura, no. 1, pp. 25-30.
3. Bura M.; Acatincăi S.; Pădeanu I., 1995 - Viermii de mătase - biologie şi creştere, Ed. Helicon, Timişoara.
4. Craiciu E., 1966 – Contribuţii la identificarea şi descrierea soiurilor de dud din Republica Socialistă România. Lucrări ştiinţifice S.C.A.S., vol. VI, p. 245-285, Redacţia Revistelor Agricole, Bucureşti.
5. Cucu G.I., Maciuc V., Maciuc D., 2004: Cercetarea ştiinţifică şi elemente de tehnică experimentală în zootehnie. Editura Alfa, Iaşi.
6. Doliş M., 2008 - Sericicultură. Ed. Alfa, Iaşi.
7. Halga P.; Pop I.M.; Bădeliţă C.; Popa V.; Man D., 2005 – Nutriţie şi alimentație animală. Ed. Alfa, Iaşi.
8. Ifrim S., 1998 – Mătasea naturală, Ed. Ceres, Bucureşti.
9. Lazăr S., Vornicu O.C., 2013: Bazele biologice şi tehnice ale producției sericicole. Ed. Ion Ionescu de la Brad, Iași.
10. Maciuc V., Creangă S, Maciuc Domnica, Vidu Livia, 2015 – A New Programe for Data Management in Dairy Farms. ''ST26733". International Conference"Agriculture for Life, Life for Agriculture". Agriculture and Agricultural Science Procedia 6 (2015) 226-231.
11. Matei A., 1995 – Determinarea valorii nutritive a principalelor soiuri şi hibrizi de dud cu utilizare în creşterea industrială a lui Bombyx mori. Rezumat Teză de doctorat, U.Ş.A. Bucureşti.

12. Mărghitaş L. A., 1995 – Creşterea viermilor de mătase, Ed. Ceres, Bucureşti.
13. Miranda J.E., Takahashi R., 1998 - Efficiency of food utilization by the silkworm, Bombyx mori L. (Lepidoptera: Bombycidae) feed on artificial diets. Sericologia, 38, no. 4, pp. 601-610.
14. Pătruică S., 2013 - Apicultură și Sericicultură, Ed. EUROBIT, Timișoara.
15. Petkov, N., 1980 - Studies on the utilization of mulberry leaves of some varieties at different norms and seasons of silkworm feeding. II Expenditure of mulberry leaves per unit of production, and their repayment by spring feeding of silkworms. Sericologia, 20, no. 1-2, p. 32.
16. Pop E. C., 1967 – Compoziţia chimică a frunzelor de dud în diferite perioade şi consumabilitatea ei. Sericicultura, nr. 4, p. 12-18.
17. Rahmathulla V.K., Suresh H.M., Mathur V.B., Geetha Devi, R.G., 2002 - Feed conversion efficiency of elite bivoltine CSR hibrids silkworm *Bombyx mori* L. Reared under different environ mintal conditions. Sericologia, 42, no. 2, pp. 197-203.
18. Rahmathulla V.K., Suresh H.M., Mathur V.B., Geetha Devi, R.G., 2004 - Growth and Dietary Efficiency of Mulberry Silkworm (Bombyx mori L.) Under Various Nutritional and Environmental Stress Conditions. Philippine Journal of Science 133, no. 1, pp. 39-43.
19. Rath S.S., Narain R., Prasad B.C., Roy G.C., Sinha B.R.R.P., 2003 - Food allocation budgeting in tropical tasar, *Antheraea mylitta* feed on *Terminalia tomentosa*. Sericologia, 43, no. 4, pp. 557-564.
20. Sabhat A., Malik M.A., Malik F.A., Sofi A.M., Mir M.R., 2011 - Nutritional efficiency of selected silkworm breeds of Bombyx mori L. reared on different varieties of mulberry under temperate climate of Kashmir. African Journal of Agricultural Research, 6, no.1, pp. 120-126.
21. Sandu G., 1995 – Modele experimentale în zootehnie. Ed. Coral Sanivet, București.
22. Sarkar, A., 1993 - Effect of feeding different races of silkworm (*Bombyx mori* L.) with Mulberry (Morus indica L.) leaves varying in ploidy level. Sericologia, 33, no. 1, pp. 25-34.
23. Tzenov, P., 1993 - Study on the food utilization in genetic sex- limited breeds for egg and larval traits of the silk worm, *Bombyx mori* L., at moderate, reduced and excess feeding amounts. Sericologia, 33, no. 2, pp. 247-256.
24. http://www.fao.org/docrep/field/003/ab479e/ab479e03.htm