Research on Digestibility of the Mulberry Leaf from the Kokuso 21 Variety in Relation to the Dynamics of the Chemical Content

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During the growth of silkworm larvae study, also was done a research which aimed to determine the chemical composition and the digestibility of mulberry leaves from a Japanese variety, Kokuso 21. The results showed that advancing in the vegetation stage at the same time with different periods of the silkworm larvae's growth, the mulberry leaves experience an aging process being noticed through its quality decreasing from chemical composition point of view. Accordingly, with this, to the majority of nutrients from mulberry leaves, except the crude fiber was noticed a continuous digestibility decreasing throughout larval growth period So, for example, digestibility of crude protein decreased gradually throughout the studied period, with 27.11%, respectively from 84.42% at the first chemical determination to 61.53% at the last one. The nutrients digestibility from the leaves registered in average a value of 55.87%.

Keywords: chemical composition, leaves, mulberry, silkworm larvae, digestibility

The success in the silkworm larvae's growth is influenced by multiple factors. Among them, nutrition plays a decisive role. The quality of the mulberry leaves administered in larvae's feeding directly influences their growth, health and vitality, but also the quantitative and qualitative silk production.

The leaves quality is also influenced by several factors related to pedoclimatic conditions, season, mulberry variety and so on.

Therein have been done and still are presently done studies on nutritional value of the mulberry leaves administered in *Bombyx mori* larvae feeding and their influencing factors. Those studies use fairly complex methods which among other things, in addition to leaves chemical composition determining implies also digestibility tests [1-15].

Studies regarding the nutritive value of the mulberry leaves have not only targeted their usage for the *Bombyx mori* species but also for swine, sheep, goats, cattle, rabbit and poultry [16-28].

In Romania, apart from imported and acclimated mulberry varieties were also created local ones, some quite valuable [29-33], but studies done to determine the leaves nutritive value of mulberry varieties are quite rare, obsolete and incomplete.

For this reason, it was considered useful, that this paper brings a small contribution to the studies regarding the leaves nutritive value estimation from the local mulberry varieties.

For this reason, this research is useful for that area of interest, namely to determine the nutritional value of the mulberry leaves of imported and acclimated varieties, as is the case for the Kokuso 21 variety.

Experimental part

Materials and methods

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 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.

The animal biologic material was the simple hybrid of silkworm called *Record*, which is a cross between Japanese and Chinese breeds. It presents stable and uniform characteristics and a pronounced level of heterosis.

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 [34-46].

The chemical analyses were done on samples previously dried to 65°C and grinded. The obtained results were processed and noted in tables being 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 h at 105°C [47].

The ashes content was determined using the incineration of the samples method [48].

To determine the protein content (CP), the Kjeldhal method was used [49].

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) [50].

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 [51].

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

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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 $\left(DC\% = \frac{\text{Digest}}{\text{Intake}} \times 100\right)$ [2, 45, 46].

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 [45, 46].

The main experimental data obtained were statistically processed being calculated the arithmetic average, variance, the average standard deviation and the variability coefficient [52, 53].

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.

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

Results and discussions

The values regarding the mulberry leaves chemical composition evolution throughout growth period of the silkworm larvae were centralized (table 1) and statistically processed (table 2).

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 70.63%, 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 $29.37 \pm 0.575\%$.

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

Table 1

THE CHEMICAL COMPOSITION EVOLUTION OF THE KOKUSO 21 VARIETY MULBERRY TREE LEAVES DURING THE SILKWORM LARVAE GROWTH (%)

Determination	water	DM	CP		EE		CC		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
Average	70.63	29.37	6.20	21.16	1.04	3.54	5.26	17.88	12.77	43.46	4.10	13.96

* fresh leaves; ** dry matter

	n	$\sum x$	$\sum x^2$	s^2	$\bar{x}$	$s_{\bar{x}}$	s	Cv	Min.	Max.
DM	5	146.85	4319.61	1.656	29.37	0.575	1.287	4.381	27.91	31.14
CP	5	105.79	2244.29	1.497	21.16	0.547	1.223	5.782	19.75	22.61
EE	5	17.68	63.64	0.280	3.54	0.237	0.530	14.975	2.83	4.01
CC	5	89.40	1601.18	0.676	17.88	0.368	0.822	4.599	16.98	19.04
NFE	5	217.34	9448.70	0.340	43.47	0.261	0.583	1.342	42.72	44.18
Ash	5	69.79	974.70	0.143	13.96	0.169	0.379	2.713	13.40	14.43

Table 2

STATISTICAL INDEXES REGARDING THE CHEMICAL COMPOSITION OF THE MULBERRY TREE LEAF (EXPRESSED IN DM-DRY MATTER) percentage of water. In the data presented by different authors, the average humidity of the mulberry leaves varies between 65-75%.

Depending on the variety, the dry matter of the mulberry leaves varies between 23.61-27.56% [4].

In the specific literature, the crude 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 [54], during the morning 26.80% and evening 29.10% [55]; it also varies between 22.55 and 25.73% depending on the mulberry variety [4].

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 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% [56] and 3.4-6% [57].

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 [58]. Throughout the mulberry vegetation period, the cellulose content from leaves increase from 14.47 to 21.16% [56].

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% [54], 11.52-12.80% [4], 8.7-13.15% [57].

Through digestion processes, under the action of a complex biochemical and biophysical factors, the nutrients from the fodders are converted in simple substances which may be absorbed through the epithelium of the digestive tract, to the different levels. There are considered digested the substances which are not found in the egestion (faeces). In other words, digestible nutrients from the mulberry leaves retained in the body of the silkworm larvae, represent the difference between the quantities of ingested nutrients from fodder and the quantity of nutrients found in excrement.

In reality, not all the nutrients which are eliminated in the feces have a food origin, some of them have an endogenous origin, which is why the most accurate term is apparent digestibility.

In the case of the species Bombyx mori, as well as all species of insects, the term of apparent digestibility presents also another aspect which is that, besides the substances of endogenous origin, in the faeces are found as well the excretion products, removed by Malpighi tubes to the pylorus level. This further complicates to determine precisely the nutrients digestibility from the mulberry leaves, especially the proteins. For this reason, several authors use the term of the approximate digestibility [5, 7, 13, 59, 60].

Knowing the administered quantities of mulberry leaves, the leftovers and the excreta, as well as their chemical composition (table 3) was possible to calculate the digestibility coefficients of the mulberry leaves, depending on the period (table 4), and the obtained data were then statistically analyzed (table 5).

The dry matter from the leaves had an average digestibility throughout the studied period of 55.87%. From the first to the last chemical determination, decreased digestibility of dry matter from mulberry leaves was of 39.88%.

In the specific literature, digestibility of the dry matter from the mulberry leaves decreases from 71.07% in the age I to 39.99% (for the male silkworms' larvae) and 48.26% (for the female silkworm larvae) the fifth age [60].

The mulberry leaves administered to the silkworm larvae of V^{th} age had an approximate digestibility between 27.99% and 32.44% [6].

Overall decreasing of the nutrients digestibility from the mulberry leaves, throughout the growth period of the silkworm larvae is understandable, taking into account that during this period occurs also a qualitative reduction of the leaves, in terms of chemical composition [3].

The crude protein had a digestibility coefficient throughout the studied period of 62.72%.

Digestibility of crude protein decreased gradually throughout the studied period, with 27.11%, respectively from 84.42% at the first chemical determination to 61.53% at the last one.

The high digestibility at the I^{st} age could be explained by the high content in amides and simple nitrogenous substances, which are found in young leaves and are more easily digested than the protein nitrogenous substances which are found preponderantly in the aged leaves.

In the specific literature, the crude protein from the mulberry leaves, the value of the digestibility coefficient varies between 69.21 and 78.92% [61], 60.06 and 74.69% [62], 71.62 and 93.48% [4], 64.36 and 87.15% [45], 64.15 and 89.60% [46].

The larvae age	Specification	Quantity (g)	Chemical composition (%)					
			DM	CP	EE	CF	NEF	Ash
I	Leaves	15.50	27.91	6.31	0.79	4.74	12.33	3.74
	Leftovers	5.23	65.56	14.64	1.98	13.98	26.13	8.83
	Excreta	0.15	69.35	22.07	8.67	2.53	28.21	8.07
II	Leaves	26	28.34	6.28	0.88	4.88	12.34	3.96
	Leftovers	8.56	59.89	12.66	1.92	14.39	20.12	10.80
	Excreta	0.91	65.96	15.30	3.74	3.96	28.50	14.46
III	Leaves	77.00	29.59	6.23	1.14	5.31	12.64	4.27
	Leftovers	24.00	60.02	10.05	2.11	15.77	25.00	7.09
	Excreta	3.85	65.69	15.22	2.84	7.18	26.03	14.42
IV	Leaves	242.00	29.87	6.04	1.16	5.44	13.09	4.14
	Leftovers	68.00	55.31	13.41	2.96	13.58	25.11	0.25
	Excreta	19.21	66.78	10.86	1.81	6.82	26.88	20.41
V	Leaves	1000	31.14	6.15	1.25	5.93	13.41	4.40
	Leftovers	270.21	56.36	11.01	1.36	13.19	24.07	6.73
	Excreta	122.14	61.03	10.00	3.14	14.72	24.98	8.19

Table 3
THE MULBERRY LEAVES QUANTITIES ADMINISTERED TO SILKWORM LARVAE AND THEIR CHEMICAL COMPOSITION, THE LEFTOVERS AND THE EXCRETA

The larvae age	DM	CP	EE	CF	NEF
I	88.40	84.42	31.22	-	92.23
II	73.23	74.65	47.20	2.70	82.54
III	69.82	75.43	70.57	9.05	73.15
IV	63.00	62.06	56.23	13.99	64.65
V	53.15	61.53	56.54	24.01	55.82
I-V	55.87	62.72	56.93	23.19	58.65

Table 4
THE DIGESTIBILITY COEFFICIENTS CALCULATION OF THE MULBERRY LEAVES KOKUSO 21 VARIETY

Table 5
STATISTICAL INDEXES REGARDING THE NUTRIENTS DIGESTIBILITY FROM THE MULBERRY LEAVES

Specification	n	$\sum x$	$\sum x^2$	s^2	$\bar{x}$	$s_{\bar{x}}$	s	VC	Min.	Max.
DM	5	347.60	24845.95	170.199	69.52	5.834	13.046	18.766	53.15	88.40
CP	5	358.09	26062.43	95.185	71.62	4.363	9.756	13.623	61.53	84.42
EE	5	261.76	14541.24	209.395	52.35	6.471	14.470	27.641	31.22	70.57
CF	5	49.75	861.39	80.876	12.44	4.497	8.993	72.306	2.70	24.01
NEF	5	368.39	27965.64	205.851	73.68	6.416	14.348	19.473	55.82	92.23

The values of the fat digestibility from the mulberry leaves varied between 31.22% at the first chemical determination and 70.57% at the third one.

Regarding the raw fat from the mulberry leaves, the digestibility values were generally inconclusive due primarily to their origin; many of them may be derived from the silkworm larvae's gut of larvae and not from the leaves.

In fact, in case of the fat content was not determined the digestible fat itself, but the *extract etherate*, which, as is well known, contains very high amounts of pigment.

Thus, it could be explained the high differences registered regarding the evolution of the fat digestibility during the studied period.

The specific literature present a variation of the fat digestibility coefficient between 63.28% and 74.19% [62].

It was noticed during the research that the CF digestibility from mulberry leaves had an average value of 23.19%, being very low at the first larval age (under 1%) then progressively increasing till the fifth larval age when the value was 24.01%.

The average CF digestibility had increased by over 24 percentage points, which in relative values represent an increasing of 2000%.

Regarding the cellulose digestion, the main component of the leaves cell walls, at the beginning of the last century it was thought that the cellulose passes through the digestive tract without being subjected to the digestion process. Afterwards, many researchers attribute to this substance a digestibility of almost 20%. The crude cellulose would not be digested in the first two larval ages; only in the third age may be digested (8%), reaching to the fifth age to 21.13% [4].

The continuous increasing of the CF digestibility from the mulberry leaves, at the same time with advancing in age of the silkworm larvae, may be ascribed to the development of enzymatic equipment from their digestive tract.

The enzymes involved in the cellulose digestion, almost non-existent in the gut of the silkworm larvae at first age grow gradually, reaching its peak in the fifth age; at which point the crude fiber content is also higher. This aspect, however, negatively influences the CP digestibility of the leaf, which in the same period, has a downward curve.

The digestibility of the nitrogen free extract from mulberry leaves throughout the research was in average 58.65%.

Throughout the study, the digestibility coefficient of the nitrogen free extract from mulberry leaves registered decreasing values from 92.23% for the leaves administered to first larval age to 55.82% if the ones administered in the fifth larval stage.

In the specific literature, the NFE from mulberry leaves registered throughout studied period, the average values which varied between 63.40 and 94.97% [4].

Observing the nutrients digestibility from the mulberry leaves throughout the research was noticed that it presented an average variability for DM, CP, and NEF and a higher one for EE and CF.

Based on the digestibility coefficients were calculated the digestible content for each nutrient. The sum of their values represents the total digestible nutrients (table 6).

The data obtained in the research were 139 g TDN/kg when reported to the fresh leaves or 475 g TDN/kg when expressed in dry mater.

Table 6
THE NUTRITIONAL VALUE CALCULATION OF THE MULBERRY LEAVES (g TDN/kg)

Specification	Chemical composition %		Digestibility coefficients %	Digestible content %		g TDN/kg	
	*	**		*	**	*	**
CP	6.20	21.16	62.72	3.89	13.27	38.90	132.70
EE	1.04	3.54	56.93	0.59	2.02	13.28	45.45
CF	5.26	17.88	23.19	1.22	4.15	12.20	41.50
NEF	12.77	43.46	58.65	7.49	25.49	74.90	254.90
Total						139	475

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

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%.

The leaves nutrients digestibility was in average 69.52±5.046%. The dry matter digestibility decreased with 35.25%.

The digestibility coefficients for CP (71.62±4.363%) and NEF (73.68±6.416%) from the mulberry leaves decreased throughout research.

The CF digestibility, very low at the beginning, increased progressively till the fifth larval stage when it was 24%.

Nutritional value of the mulberry leaves was 475 g TDN/kg DM.

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