COMPARATIVE STUDY REGARDING THE ENERGETIC VALUE OF TWO MULBERRY VARIETIES

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Abstract

In order to assess the nutritional value, as the chemical composition and the crude energy of mulberry leaves, during a summertime growth series, several determinations were made, which concerned two mulberry varieties (Kokuso 21 - imported and Eforie – Romanian variety); these were administered to Triumf hybrid, a Bombyx mori larvae created in Romania. The results showed that advanced vegetation and during each growth period of silkworm, the mulberry leaf undergoes an aging process, translated by diminishing its quality regarding the chemical composition. According to this fact, throughout the studied period the crude energy of the leaves generally registered an average constant increase of 144.85 Kcal/kg; thus, between varieties but also within each variety there were significant differences, depending on the larvae's age. During the entire studied period, the difference recorded between varieties was insignificant regarding the crude energy content of the mulberry leaf and the average value was 1241.6 Kcal/kg.

Key words: *leaf, mulberry, nutritive, energy*

INTRODUCTION

Nowadays, in addition to silk production, sericulture has acquired new values/utilities. Thus, it should be emphasized the purpose that pupae have as important protein sources for some species food (fish, birds, mammals) (Salem et. al., 2008; Longvah et. al., 2011; Bandlamori et. al., 2012; Tran et. al., 2015; Ullah et. al., 2017; Khan, 2018; Dalle Zotte et. al., 2021) and even for humans. Regarding this aspect, *Bombyx mori* represents one of the species that is often mentioned among the insects providing food for mankind. (Bukkens, 1997; Siriamornpun et. al., 2008; Longvah et. al., 2012; Tom et. al, 2013; Mlček et. al., 2014; Kouřimská et. al., 2016).

On the other hand, the nutritional qualities of the mulberry leaf make it increasingly used in raising different species of animals (sheep, goats, cattle, pigs, rabbits, chicken, quail, fish, etc.). (Shrivastava et. al., 2018; Lokeshwari, 2019; Omar et. al., 1999)

Over time, mulberry varieties with high quality leaf productions, with increased adaptability, resilient to environmental conditions (drought or frost), diseases and pests. At the same time, for expressing their productive potential, especially for larvae used in intensive breeding systems, it is necessary to improve all the involved factors in the growth process, among which nutrition plays a very important part.

At the end of the last century, Romania could be considered an important sericulture point on the map of Europe (Romanian specialists could be proud of obtaining a lot of valuable mulberry varieties and hybrids, as well as breeds/hybrids of *Bombyx mori*, which were the result of research over several decades). (Mărghitaș, 1995; Doliș, 2008; Lazar et.al., 2013; Păturică, 2013; Pop et. al., 2006).

This paper aimed to be a comparative study between the energy value of the mulberry leaf from an imported variety, well-known worldwide (Kokuso 21) and a variety created in Romania (Eforie). Given these aspects we consider appropriate to have a small contribution to the study of mulberry leaf quality of varieties created in Romania.

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MATERIAL AND METHOD

The biological material used in the experiment was represented by the silkworms subjected to growth and the mulberry leaf that was administered to them.

The biological material was represented by two batches (L1, L2) of 300 *Bombyx mori* larvae belonging to the Romanian Triumf hybrid (characterized by uniform productive characteristics and a pronounced degree of heterosis). To make it easier to follow, in each batch, the 300 larvae were grouped into 6 smaller batches of 50 larvae each, which were raised in paper trays sized according to the age and size of larvae; in addition, there was also a secondary batch, made of larvae grown separately, but under the same conditions, which served to replace the dead larvae from the experimental batch.

The working methods were applied to determine the nutritional value of the mulberry leaf based on the chemical composition and the digestibility of its components.

The chemical composition was determined based on the "proximate analysis" scheme. The chemical analyzes were done on samples previously dried at 65^0C and then prepared by grinding. (Pop I. M. et. al., 2006)

The moisture content of the collected samples was determined by drying them in an oven at a temperature of 105^0C for 4-5 hours. (Regulation EC)

Crude ash (Ash) was determined by calcining leaf samples. (Regulation EC)

The Kjeldahl method was used to determine the crude protein (PB). (Regulation EC)

Crude fat (EE) was determined by the Soxhlet method, based on the property of organic solvents to dissolve fats (petroleum ether). (Regulation EC)

Crude fiber (CF) was determined by acid-base hydrolysis of the samples, leaving only crude fiber and mineral salts on the filter, by calcination the mineral substances are found. (Regulation EC)

The nitrogen free extract (NFE) was calculated by the difference (the percentages of water, crude protein, crude fat, crude fiber, and ash were subtracted from 100%). (Regulation EC)

The determination of the crude energy in the leaf was achieved by using the specific calculation equations and the regression coefficients recommended by the OKIT system. (Regulation EC)

The statistical processing of the data was carried out by calculating: the arithmetic mean, the variance, the standard deviation of the mean, the coefficient of variability (Regulation EC; Diniţă et. al., 2004; Cucu, 2004), and the ANOVA test was used to interpret the statistical differences.

RESULTS AND DISCUSSIONS

The data on chemical composition of mulberry leaf from the two varieties, in relation to the age of the larvae, were illustrated in table 1, and the results obtained through the ANOVA program, regarding the differences obtained, were centralized in tables 2 and 3.

Humidity directly influences the rate of consumption by the larvae, which, especially in the first ages, prefer it tender, with a high percentage of water.

Following the chemical analyzes carried out throughout the larval growth period (table 1 and table 2), it appears that the mulberry leaf had an average humidity of 70.53%, which means a content of 29.47% dry matter. During the growth period, leaf moisture decreased on average by 3.78%.

In the literature, depending on different factors, the relative humidity values of the mulberry leaf vary between 65-75%. Compared to Common Mulberry (69.80-73%), the selected varieties have a higher water content.

Fresh mulberry leaves have a content of 24.86% dry matter and 84.33% of this percent is organic matter. The dry substance of the mulberry leaf, harvested in the same period, can register, depending on the variety/hybrid, different values, for example, between 23.61% and 27.56%. Also, if in the spring the humidity of the mulberry leaf is 71.85-77.81%, later it drops to 68.42-75.64%, in the summer and to 64.10-73.64%, in the autumn.

The protein content of the mulberry leaf strongly influences both the growth and development of silkworms and, above all, their silk production. Over the entire studied period, the crude protein from the mulberry leaf had an average weight of 6.19%. The crude protein, related to dry matter, had values between 22.38% (related to the first age of the larvae) and 19.39% (in the fifth age).

In literature, the crude protein of mulberry leaf is estimated on average of 6.16% in fresh leaf, 16.57%, 16.67-21.62%, 19.60%, 20.34, 20.97 %, 29.80% in dry matter, and 24.36% in the organic substance.

Crude leaf protein values can fluctuate depending on the season, time of day, mulberry variety/hybrid: 32.40% on spring, 28.21% on summer and 24.53% on autumn, 26.80% in the morning and 29.10% in the evening, between 20.20% and 26.72% depending on the variety (the quoted values are references to the dry substance of the leaf).

Crude fat of the mulberry leaf registered an average value of 1.07% (3.62% compared to DM), during the entire studied period, registering an average increase of 0.5%. Related to the dry substance of the leaf, the average values were 1.44%, 2.85-6.07%, 3.4-6%, 5.57%, and 7.38%.

The cellulose content directly influences the quality of the mulberry leaf. The increase in the cellulose content causes the aging of the mulberry leaf, which becomes harder and rougher, therefore more difficult to consume, which is why those varieties whose leaves have a lower cellulose content are considered more valuable.

Table 1 The chemical composition of mulberry leaf, depending on variety and degree of maturity related to age of the larvae

%	Mulberry variety	KOKUSO 21					EFORIE				
	Larvae age	I	II	III	IV	V	I	II	III	IV	V
Water	$\overline{X}$	72.09	71.66	70.31	70.13	68.86	71.86	71.98	70.68	69.53	68.15
	$\pm s_{\overline{X}}$	0.06	0.13	0.10	0.11	0.21	0.21	0.19	0.12	0.19	0.16
	CV	0.35	0.75	0.58	0.64	1.32	1.26	1.13	0.75	1.16	1.01
	Min	71.75	70.89	69.91	69.80	67.79	70.41	70.59	69.98	68.88	67.41
	Max	72.45	72.14	71.01	71.05	70.18	72.68	72.58	71.33	71.02	69.56
CP	$\overline{X}$	6.31	6.28	6.23	6.04	6.15	6.23	6.21	6.41	6.00	6.06
	$\pm s_{\overline{X}}$	0.02	0.04	0.04	0.02	0.10	0.09	0.08	0.09	0.09	0.08
	CV	1.50	2.94	2.48	1.73	6.68	6.38	5.29	6.03	6.58	5.61
	Min	6.19	5.99	5.99	5.89	5.86	5.88	5.91	5.99	5.68	5.81
	Max	6.45	6.51	6.46	6.20	7.02	7.02	6.86	7.02	6.81	6.78
EE	$\overline{X}$	0.79	0.88	1.14	1.16	1.25	0.85	0.88	1.17	1.22	1.38
	$\pm s_{\overline{X}}$	0.02	0.03	0.03	0.03	0.08	0.05	0.05	0.06	0.05	0.09
	CV	11.91	14.43	12.14	12.45	26.38	23.31	22.28	21.52	18.29	26.37
	Min	0.61	0.69	0.97	0.95	0.77	0.61	0.63	0.88	0.87	0.88
	Max	0.89	1.02	1.31	1.33	1.56	1.14	1.12	1.58	1.43	1.88
CF	$\overline{X}$	4.74	4.88	5.31	5.44	5.93	4.79	4.76	5.26	5.58	6.15
	$\pm s_{\overline{X}}$	0.03	0.04	0.15	0.10	0.18	0.10	0.12	0.07	0.12	0.07
	CV	2.92	3.53	11.61	7.81	12.67	8.76	10.28	5.87	9.02	4.54
	Min	4.58	4.62	4.18	4.91	4.95	3.89	3.78	4.83	5.02	5.88
	Max	4.91	5.09	5.78	6.11	6.79	5.09	5.18	5.66	6.14	6.61
NFE	$\overline{X}$	12.33	12.34	12.64	13.09	13.41	12.43	12.24	12.30	13.37	13.58
	$\pm s_{\overline{X}}$	0.04	0.05	0.12	0.16	0.14	0.12	0.15	0.10	0.09	0.10
	CV	1.48	1.76	3.96	5.13	4.56	4.00	5.11	3.29	2.90	3.23
	Min	12.02	11.99	11.92	11.99	12.37	11.69	11.52	11.88	12.96	12.88
	Max	12.52	12.66	13.02	13.80	14.12	13.08	13.11	13.02	14.02	14.22
Ash	$\overline{X}$	3.75	3.96	4.38	4.14	4.41	3.84	3.94	4.18	4.30	4.69
	$\pm s_{\overline{X}}$	0.11	0.16	0.29	0.30	0.17	0.21	0.30	0.22	0.27	0.11
	CV	11.93	16.82	27.79	30.92	16.04	22.92	32.15	22.53	26.33	9.53
	Min	3.06	2.77	3.30	2.76	3.06	3.11	2.51	2.92	2.90	3.84
	Max	4.43	4.83	6.78	6.14	5.11	5.65	6.40	5.62	5.78	5.13

CP=crude protein; EE= etherated extract; CF = crude cellulose; NFE = nitrogen free extract.

The content of mulberry leaves in crude fiber averaged over the entire studied period of 5.29% (17.9% relative to dry matter), which recorded an increase over the period of 1.28%.

According to data from the specialized literature, crude fiber, relative to dry matter, has values of 11.63%, 13.11%, 15.50% (23.04% cellulose, 10.06% hemicellulose). In common mulberry, the percentage of crude fiber ranges between 12.33-14.38%, while in different selected varieties it oscillates between 10.43-13.70%. During the mulberry vegetation period, the crude fiber content of the leaves increases from 14.47% to 21.16%.

Table 2 Results regarding the significance of differences recorded between the values of the chemical composition of leaf according to the degree of maturity related to the larvae's age

Batch	Comparisons	DM		CP		EE		CF		NFE		Ash	
		Signif.	p values	Signif.	p values	Signif.	p values	Signif.	p values	Signif.	p values	Signif.	p values
L1	I vs. II	ns	0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999
	I vs. III	ns	0.1945	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	>0.9999	ns	0.9999
	I vs. IV	ns	0.0744	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	0.9999	ns	>0.9999
	I vs. V	***	9.12×10^{-7}	ns	>0.9999	ns	0.9999	ns	0.9411	ns	0.9857	ns	0.9999
	II vs. III	ns	0.7947	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	>0.9999	ns	0.9999
	II vs. IV	ns	0.5343	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	0.9999	ns	>0.9999
	II vs. V	***	8.30×10^{-5}	ns	>0.9999	ns	>0.9999	ns	0.9897	ns	0.9876	ns	0.9999
	III vs. IV	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	>>0.9999
	III vs. V	ns	0.6679	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	0.9999	ns	>0.9999
	IV vs. V	ns	0.8871	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	>0.9999	ns	>0.9999
L2	I vs. II	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999
	I vs. III	ns	0.9911	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	>0.9999	ns	>0.9999
	I vs. IV	*	0.0400	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	0.9998	ns	>0.9999
	I vs. V	***	5.10×10^{-7}	ns	>0.9999	ns	0.9999	ns	0.9374	ns	0.9943	ns	0.9999
	II vs. III	ns	0.9669	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	>0.9999	ns	>0.9999
	II vs. IV	*	0.0201	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	0.9953	ns	>0.9999
	II vs. V	***	1.61×10^{-7}	ns	>0.9999	ns	0.9999	ns	0.9188	ns	0.9483	ns	0.9999
	III vs. IV	ns	0.9942	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	0.9983	ns	>0.9999
	III vs. V	*	0.0111	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	0.9726	ns	0.9999
	IV vs. V	ns	0.9241	ns	>0.9999	ns	>0.9999	ns	0.9999	ns	>0.9999	ns	>0.9999

*ns = nonsignificant ($p > 0.05$); * significant ($p < 0.05$); ** distinctly significant ($p < 0.01$); *** very significant ($p < 0.001$)*
DM = dry matter; CP = crude protein; EE= etherated extract; CF = crude cellulose; NFE = nitrogen free extract.

Table 3 Results regarding the significance of differences recorded between the values of chemical composition of the leaf from the Kokuso 21 (L1) and Eforie (L2) varieties, related to the age of the larvae (ANOVA)

Larval age	**I**	**II**	**III**	**IV**	**V**	**I-V**
DM	0.9999	0.9999	0.9999	0.9985	0.9973	0.9999
CP	0.9999	>0.9999	0.9999	>0.9999	>0.9999	>0.9999
EE	>0.9999	>0.9999	>0.9999	>0.9999	0.9999	0.9979
CF	>0.9999	0.9999	>0.9999	0.9999	0.9999	>0.9999
NFE	0.9999	0.9999	0.9999	0.9999	0.9999	>0.9999
Ash	0.9999	>0.9999	0.9999	0.9999	0.9999	0.9999

DM = dry matter; CP = crude protein; EE= etherated extract; CF = crude cellulose; NFE = nitrogen free extract.

The nitrogen free extract had an average weight over the entire studied period of 12.77% (43.38% compared to dry substance), which represented a growth of 1.12%. The data obtained are comparable to those found in literature (44.88%).

The crude ash, obtained from mulberry leaf calcination, had an overall average value of 4.16%, increased by about 0.76% throughout the studied period. Related to the dry substance of leaves, the crude ash was 14.06%; the obtained values were within the

limits offered by the literature: 6.04-8.56%, 8.7-13.15%, 9.13-17.38%, 10.00-12.30%, 11.52-12.80%, 13.37%. Calcium represents 2.10-2.94%% and phosphorus 0.20-0.23%. Within each studied mulberry variety, the recorded differences regarding the evolution of the chemical composition of leaf throughout the larval growth period were generally, insignificant (significant differences were observed only in the case of dry matter values recorded at young ages compared to those in the 5th age). This could be explained by the fact that, being the summertime growth series, carried out in August, the leaf is generally mature and generally more stable in terms of chemical composition, compared to younger leaf, used in spring series, when more obvious changes are observed.

Between varieties, the differences were totally insignificant ($p>0.05$), regardless of whether the obtained values for each age separately or the average values over the entire studied period were compared (table 3). This demonstrates that, in terms of chemical composition, the mulberry leaf from Eforie variety, created by Romanian specialists, rises to Kokuso 21 level, an international known variety.

Knowing the gross chemical composition of the mulberry leaf, using the specific calculation equations, as CE(Kcal/kg) = (5.72 CP% + 9.50 EE% + 4.79 Ash% +4.17 NFE%) x 10, it was possible to appreciate the nutritional value of the mulberry leaf based on its gross energy content, which was on average, over the entire studied period, 1237.7 Kcal/kg, for the Kokuso 21 variety and 1245.4 Kcal/kg, in the Eforie variety; the difference recorded between the varieties was insignificant (table 4).

Table 4 The crude energy of the mulberry leaf, depending on variety and maturity degree related to larvae's age (Kcal/100g)

Batch	Larve's age	I	II	III	IV	V	I-V
L1	$\overline{X}$	117.67	119.11	124.56	126.21	131.31	123.77
	$\pm s_{\overline{X}}$	0.21	0.54	1.27	1.16	1.05	0.53
	CV	0.75	1.93	4.33	3.89	3.38	1.83
	Min	116.19	116.37	114.64	117.93	123.89	119.10
	Max	118.42	123.14	129.75	132.87	138.25	125.28
L2	$\overline{X}$	118.52	117.69	124.25	128.39	133.85	124.54
	$\pm s_{\overline{X}}$	0.97	0.84	1.40	0.76	1.20	0.57
	CV	3.47	3.04	4.77	2.50	3.82	1.94
	Min	114.53	112.75	117.30	124.79	124.34	122.40
	Max	126.77	122.70	134.86	133.71	140.80	128.69
	L1 vs L2	ns 0.6845	* 0.0463	ns 0.9999	** 0.0090	** 0.0054	ns 0.4046
ANOVA signif./*p* values							
I vs. II	ns	0.6780	II vs. IV		***	4.74×10^{-10}	
I vs. III	***	4.74×10^{-10}	II vs. V		***	4.75×10^{-10}	
I vs. IV	***	4.74×10^{-10}	III vs. IV		ns	0.3510	
I vs. V	***	4.74×10^{-10}	III vs. V		***	4.74×10^{-10}	
II vs. III	***	4.74×10^{-10}	IV vs. V		***	4.75×10^{-10}	
I vs. II	ns	0.9999	II vs. IV		***	4.74×10^{-10}	
I vs. III	***	4.74×10^{-10}	II vs. V		***	4.75×10^{-10}	
I vs. IV	***	4.74×10^{-10}	III vs. IV		***	6.21×10^{-9}	
I vs. V	***	4.75×10^{-10}	III vs. V		***	4.74×10^{-10}	
II vs. III	***	4.74×10^{-10}	IV vs. V		***	4.76×10^{-10}	

*ns = nonsignificant ($p > 0.05$); * significant ($p < 0.05$); ** distinctly significant ($p < 0.01$); *** very significant ($p < 0.001$)*

Throughout the studied period, the crude energy of the leaf generally recorded a progressive increase of 144.85 Kcal/kg on average (136.4 Kcal/kg for Kokuso 21 variety and 153.3 Kcal/kg for the Euforie variety (differences were significant in the 2nd age and distinctly significant in the 4th and 5th ages).

Within each series, there were highly significant differences between ages (except for differences between Ist and IInd ages and IIIrd and IVth in Kokuso 21).

CONCLUSIONS

The crude energy content of the mulberry leaf was on average, over the entire studied period 1241.6 Kcal/kg, and the difference recorded between varieties was insignificant.

Throughout the studied period, the crude energy of the leaf generally recorded a progressive growth, on average of 144.85 Kcal/kg; between varieties the differences were generally significant, depending on the larvae's age. Within each variety, there were very significant differences between crude energy values as a function of larvae's age.

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