RESEARCH REGARDING CHEMICAL COMPOSITION OF THE MULBERRY LEAVES FROM KOKUSO 21 VARIETY

Doliş M.G.*, Donose Raluca*, Simeanu Cristina*, Usturoi A*, Raţu Roxana*

*University of Agricultural Science and Veterinary Medicine "Ion Ionescu de la Brad" from Iaşi , 3, Mihail Sadoveanu Alley, Iaşi, 700490, Romania, e-mail: dsimeanu @uaiasi.ro

Abstract

During the growth of silkworm larvae study, also was done a research which aimed to determine the chemical composition 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. 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%.

Key words: leaves, mulberry, silkworm larvae, chemical composition.

INTRODUCTION

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. For this reason, this research is useful for that area of interest, namely to determine the nutritional value of the mulberry leaves.

MATERIAL AND METHOD

The research was done during the growth period of the silkworm larvae from summer series (July- August), 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.

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 65^{o}C and grinded. The obtained results were processed and noted in tables being expressed in both fresh and dried leaves (*Halga et al., 2005*).

The collected samples moisture determination was done by drying them into the hot air oven for 4- 5 hours at 105^{0}C (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: 2,001).

The crude fibre (CF) was determined by the sample acid-basic hydrolyse, 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 (*Stan & Simeanu*, 2005).

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

There were organised an experimental lot formed from 150 larvae, which were grouped in three repetitions of 50 larvae each.

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.

The larvae growth was held during 31st of July and 31st of August, respecting the breeding technology recommended by the specific literature. For the young silkworm larvae, the mulberry leaves were administered chopped (strips of 1 cm for first larval stage, of 2 cm for second larval stage, of 3 cm for third larval stage) and for the adult ones whole leaves (larval stages fourth and fifth).

RESULTS AND DISCUSSION

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

Table 1

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

Determi-nation	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
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

Table 2

Statistical indexes regarding the chemical composition of the mulberry tree leaf (expressed in DM-dry matter)

	n	$\sum x$	$\sum x^2$	s^2	$\overline{X}$	$\pm s_{\overline{x}}$	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
CF	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

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 an 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 % (*Lazăr* & *Vornicu, 2013; Phiny et al, 2010*).

The crude protein from mulberry leaves was estimated around 6.16% in the fresh leaves, 20.97% when it was expressed in DM and 24.36% in OM *(Doliș, 2008).*

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

The mulberry leaves humidity is lower to the common mulberry variety (69.80- 73%) compared with the selection varieties (*Bura et al., 1995*).

Throughout vegetation period, the humidity of the mulberry leaves decrease from 71.85- 77.81% in the spring, to 68.42- 75.64% in the summer and to 64.10- 73.64% in the autumn (*Ifrim, 1998*).

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 (*Borcescu, 1966*), during the morning 26.80% and evening 29.10% (*Mărghitaș, 1995*); it also varies between 22.55 and 25.73% depending on the mulberry variety (*Matei, 1995*).

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.

After Yao et al. *(2000)*, average CP contents are 21.1 and 20.9 (% in DM) in spring and autumn, respectively.

The protein content in the mulberry leaves may be considered a real indicator of the leaf's quality *(Al-Kirshi et al.).* 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% (*Pop, 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. For example, after Craiciu (*1966*), 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. After Pop (*1967*) throughout the mulberry vegetation period, the cellulose content from leaves increase from 14.47 to 21.16%.

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 analyse 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, 1967*), 11.52- 12.80% (*Matei, 1995*), 8.7- 13.15% (*Bura et al., 1995*).

CONCLUSIONS

- 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%.

REFERENCES

1. Al-Kirshi R.A., Alimon A., Zulkifli I., Atefeh S., Wan Zahari M., Ivan M., 2013 - Nutrient Digestibility of Mulberry Leaves (*Morus Alba*). Italian Journal of Animal Science, Volume 12: e36, 2013, p. 219-221.
2. Borcescu A., 1966 - Hrănirea viermilor de mătase în creşterile de vară şi toamnă. Sericicultura, nr. 3, p. 6-9.
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. Doliș M., 2008 – Sericicultură. Editura Alfa, Iaşi.
7. Halga P.; Pop I.M.; Bădeliţă C.; Popa V.; Man D., 2005 – Nutriţie și alimentație animală. Ed. Alfa, Iaşi.
8. Ifrim S., 1998 – Mătasea naturală, Ed. Ceres, Bucureşti.
9. Lazăr Ș., Vornicu O.C., 2013: Bazele biologice și tehnice ale producției sericicole. Ed. Ion Ionescu de la Brad, Iași.
10. Maciuc V., Creangă Ș., 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. Mărghitaş L. A., 1995 – Creşterea viermilor de mătase, Ed. Ceres, Bucureşti.
12. 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.
13. Pop E. C., 1967 – Compoziţia chimică a frunzelor de dud în diferite perioade şi consumabilitatea ei. Sericicultura, nr. 4, p. 12-18.
14. Phiny C., Preston T.R., Borin K., 2010 - Effect of fresh mulberry leaves and sweet potato vines on growth performance of pigs fed a basal diet of broken rice. Livestock Research for Rural Development 22 (3) 2010. http://www.lrrd.org/lrrd22/3/phiny22044.htm.
15. Sandu G., 1995 – Modele experimentale în zootehnie. Ed. Coral Sanivet, București.
16. Stan G., Simeanu D., 2005 – Nutriție animală. Ed. Alfa, Iaşi.
17. Yao J., Yan B., Wang X.Q., Liu J.X., 2000 - Nutritional evaluation of mulberry leaves as feeds for ruminants. Livestock Research for Rural Development 12 (2) 2000 (http://www.fao.org/livestock/agap/frg/lrrd/lrrd12/2/yao122.htm).
18. *http://www.fao.org/docrep/field/003/ab479e/ab479e03.htm.*