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RESEARCH ARTICLE

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Introducing of the indices 'Clarc of energy distribution' and 'Clarc of protein transformation' in silkworm production and methods of their calculation

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ABSTRACT

The study aims to introduce three new universal (sectional, technological and environmental) indexes in the silkworm (*Bombyx mory L.*) biology and production – "Clarc of energy distribution" (CED), "Clarc of protein transformation" (CPT) and "Index of effective cocoon shell ratio" (IECSR), so as the methodology of their calculation. On base real experiments, the follow indexes were calculated: CED - 0.2090 (20.90%); CPT – 0.3248 (32.48%) and IECSR – 4.726%. For calculation of the new indexes could be used data from standard experimental methods and standard statistical programs.

Key words: Clarc of energy distribution, Clarc of protein transformation, Index of effective shell ratio, silkworm production

Introduction

Silkworm breeding is a specific, ecologically sound sub-branch of animal breeding, which provides additional income to producers and supplies customers with the expensive product in high demand – the natural silk.

In all the silkworm-rearing countries, cocoon production is a subsidiary agricultural activity carried out on a small scale with one to four boxes of silkworm eggs. Industrial-scale silkworm rearing failed because it proved to be unprofitable compared to small-scale production, as the former requires more investment in premises and mulberry plantations, expensive equipment, and a large labour force. In that aspect, small-scale silkworm rearing is more adaptable because it can be maintained with the labour of all the family members, using the available buildings and the mulberry leaves from their single trees or plantations. It is a low-risk business and the income is more secure.

Nevertheless, market principles and competition in that agricultural sector require a low cost and high-quality production of natural silk. Therefore, silkworm breeding should be aimed not only at creating highly adaptable breeds and hybrids, producing the highest possible cocoon yield per box of eggs but also at maximal utilization of the nutrients from their natural feed, the mulberry leaves (Grekov & Tsenov, 2019). Feeding is among the main factors, on which silkworm productivity depends in large- and small-scale rearing (Tzenov & Petkov, 1996a). It was established that the low rates of nutrition and the low energy value of mulberry leaves during silkworm rearing in summer and autumn led to a decrease in the values of the inheritance coefficients and a decrease in the breeding effect (Petkov, 1995).

There is a mechanism that regulates metabolism in the silkworm body so that the smaller amount of consumed and assimilated feed is compensated by its better utilization (Tzenov & Petkov, 1996b).

According to Naomi et al. (1992), the cocoon yield per box of silkworm eggs was negatively correlated with the utilization of the mulberry leaf meal.

The correlations between the traits characterizing food utilization and the biological, technological, and reproductive traits of silkworms have been studied by many authors (Petkov et al., 2006).

In a study on a group of hybrids, Liw (1992) found that the amount of food consumed and digested was highly and positively correlated with cocoon weight, silk shell weight, and the weight of the silk filament. In its turn, the weight of the digested food was positively and highly correlated with the weight of the consumed food and with food digestibility. The efficiency of conversion of ingested food (ECI) for the production of silk shell and raw silk was highly and positively correlated with the fresh cocoon yield per box and with digestibility. ECI was also positively correlated with the weight of the silk shell, shell percentage, weight and length of the silk filament, and raw silk ratio.

It is no coincidence that the quantitative and qualitative characteristics of the silk filament, even of the same breed or hybrid, differ depending on the silkworm rearing season, which is mainly related to the chemical composition of the mulberry leaves (Dolis et al., 2019). The problem of energy and protein net utilization is becoming more and more relevant to the increasing use ofartificial feed' for silkworms, which is a consequence of the large-scale research aiming at accurately establishing the requirements of silkworm larvae for energy and specific nutrient substances. According to Cappellozza & Saviane (2011) hormone-based preparations, such as Methoprene (Manta), could be also attributed to artificial feeds having a stimulating effect on mulberry silkworm, causing an increase in the accumulation of protein in the body, which can be used for biomedical purposes. Different foods could also have a negative effect on the accumulation of protein in the larval body, on the immune system, digestion, nutrient intake, metabolism, and silk synthesis (Zhou et al., 2008).

In this regard, we believe that finding the proper indices of net utilization of energy and protein from the primary unit of the food chain (in the present case it is the mulberry leaf or artificial food) to the secondary (in the present case the accumulation of energy and protein in the body of different larval instars) would be valuable from the scientific and practical point of view, especially if those indices are related to objective mathematical dependencies with the quantitative and qualitative characteristics of silk filament.

The indicators 'Clarc of energy distribution' and 'Clarc of protein transformation' were introduced in animal husbandry by Penkov & Genchev (2018) in the production of poultry meat and later methodologies were developed for reporting those indicators in egg production (Penkov & Genchev, 2019; Penkov & Grigorova, 2020) and in goat breeding for meat production (Penkov & Vuchkov, 2020). The idea for their development was borrowed from the indicators for reporting the mobility of the chemical elements (heavy metals) along with the chain 'soil → plant → animal → man' (Baykov, 1994; Dobrovolskiy, 1998).

The present study aimed to introduce the indicators 'Clarc of energy distribution' and 'Clarc of protein transformation' in sericulture and to propose a methodology for their calculation.

Materials and Methods

In 2018-2019 in the Training-and-Experimental Silkworm Breeding Site of the Agricultural University of Plovdiv, an experiment was carried out with feeding silkworm larvae of Plovdiv 14 breed.

The silkworm eggs were stored at a temperature of 2 to 5°C until the second half of March, after which a temperature shock was applied, raising the temperature to 10-12°C for several days. After taking the silkworm eggs out of the refrigerating chamber, they were immediately placed at a temperature of 24-25ºC. From the third day, the temperature was raised to 26-27°C. The relative humidity was 50-85%. During the whole period of incubation, the experiment was conducted under natural daylight, i.e., no artificial lighting was used, except when necessary for the person performing the incubation. Mass hatching of the silkworm larvae was reported on the 12th day from the beginning of the incubation and at that time they were given the first food.

The major parameters of silkworm rearing are presented in Table 1. Three replications of 200 larvae each were maintained.

Table 1. *Major parameters of silkworm rearing.*

Instar	Temperature, ºC	Humidity, %	Rearing space, m^2	Mulberry leaves consump., kg
I	26-27	85-90	0.8-1.0	3.0-3.5
II	26-27	85-90	1.5-2.0	8.0-10.0
III	25-26	80-85	4.0-5.0	28.0-30.0
IV	23-25	70-75	9.0-10.0	70.0-80.0
V	20-25	65-70	20.0-22.0	350-380
At the stage of cocooning	24-26	70-75	22.0	-
Cocoon still stored attached to twigs	20-26	65-70	-	-

A chemical analysis of the mulberry leaves, cultivars Kokuso 20 and Kinriu, which were fed to the larvae – according to AOAC (2007), was performed. For more accurate calculations, the chemical analyses of the leaves were performed three times in the process of silkworm feeding to follow up eventual changes in their chemical composition.

The exact amount of mulberry leaves (together with the leftover), consumed by the silkworms hatched from a box of eggs, was measured for each instar and in total for the rearing period.

The average content of gross energy and crude protein (A) consumed by the larvae was determined by the formula:

A = (Σ of the number of leaves consumed by each instar * content of the substance in 1 kg/total amount of leaves consumed by all larval instars.

The total silkworm weight (M) at the end of the 5th instar was determined by the formula:

M = N*V*MM; where N – number of eggs in a box; V – the percentage of vitality at the end of 5th instar of all the hatched eggs; MM – mean weight of a larva from 100 weighed larvae from a box at the end of the 5^{th} instar (the initial weight of an egg was neglected in the calculations).

The gross energy of one kg of mulberry leaves and one kg of the larval bodies per box of silkworm eggs was determined by their chemical composition using the formula of Schiemann et al. (1971):

Table 2. *Chemical composition of mulberry leaves (Kokuso 20 and Kinriu cultivars) during the periods of larvae feeding (mean of 4 samples) – spring silkworm rearing:*

Indices – %	Beginning (15.05)		Middle (30.05)		End (15.06)	
	X mean	SD	X mean	SD	X mean	SD
Dry matter (105^{0}C)	26.86	0.84	27.15	0.92	28.42	1.11
Crude protein (in native substance)	5.85	0.41	5.99	0.28	6.15	0.17
Crude fats (in natural substance)	1.12	0.02	1.09	0.01	0.92	0.05
Crude fiber (in natural substance)	4.99	0.11	5.18	0.14	6.08	0.17
Ash (in natural substance)	3.93	0,07	4.13	0.04	4.15	0,05
NPE (in natural substance)	10.86	0.94	10.74	0.71	10.98	0.83
Gross energy – MJ*kg^{-1}	4.56	0.14	4.54	0.12	4.77	0.18

GE (MJ*kg^{-1}) = 0.0242*CP + 0.0366*CFats + 0.0177*CFiber + 0.0170*NPE

The 'Clarcs of distribution/transformation' were calculated according to the formula (Penkov & Genchev, 2018):

CED = Gross energy input (from consumed forage)/gross energy obtained in the pupae

CPT = Crude protein input (from consumed forage)/crude protein obtained in the pupae

The indicator 'gross energy' was chosen in the present study for the calculations at the input of the eco-technical chain, due to the insufficient information about the energy transformation from food in the digestive system of silkworms.

Results and Discussion

The chemical composition and gross energy content in three periods of the experimental feeding of the larvae are presented in Table 2. For the calculations below, the crude protein and gross energy contents were recalculated as average weights (A) according to the formula described in the section 'Material and Methods'.

The variations in the chemical composition within one feeding period from 15 May to 15 June were, as follows: dry matter – from 26.86 (beginning) to 28.42% (end of feeding); crude protein – from 5.85 to 6.15%; crude fats – from 1.12 to 0.92%; crude fiber – from 4.99 to 6.08%; non-protein extract (NPE) – from 10.86 to 10.98%; gross energy (GE) – from 4.54 to 4.77 MJ*kg-1. Data obtained in the present study were close to those cited by Yao et al. (2000), Wang et al. (2012) and lower than those cited by Dolis et al. (2019) and Guven (2012), the differences being explained, on the one hand, by the climatic conditions in the different countries and the fact that the leaves were studied both in spring and autumn and, on the other hand – by the different mulberry cultivars included in studies.

Table 3 presents the chemical composition and the gross energy content in 1 kg of silkworms, collected for analysis immediately after the end of the 5th instar.

Table 3. *Chemical composition and gross energy content of silkworms of the 5th instar, immediately before entering the pupal stage:*

No	Indices – %	X mean	SD
1	Moisture (105°C)	74.85	3.22
2	Crude protein (in natural substance)	13.87	1.12
3	Crude fats (in natural substance)	9.27	0.94
4	Crude fiber (in natural substance)	0.98	0.08
5	Ash (in natural substance)	1.14	0.03
6	NPE (in natural substance)	0.02	0.001
7	Gross energy in natural substance – MJ*kg^{-1}	6.95	0.84

Data in the present study showed differences in the chemical composition of silkworms compared to the information in the available literature (Pereira et al., 2003; Rao, 1994; Chieco et al., 2019; Kipriotis et al., 2000). The differences are because most of the authors performed the analyses after entering the pupal stage and the spent silkworm pupae were studied to feed other farm animals with them. In the process of pupation, the body undergoes certain chemical transformations. To calculate accurately the newly introduced indices, the chemical composition must be performed just before entering the pupal stage.

Table 4 presents the main data from the conducted experiment, needed for the calculation of the newly introduced indices.

Table 4. *Consumed amounts of mulberry leaves (with the leftover), gross energy and crude protein, and the total weight of the silkworms from a box of eggs for the period from hatching until the end of the 5th instar, immediately before entering the pupal stage.*

No	Indices	X mean	SD
1	Consumed mulberry leaves – kg	458.85	2.43
2	Consumed crude protein – kg	27.990	0.15
3	Consumed gross energy – MJ	2179.54	148.25
4	Weight of the silkworms from a box of eggs at the end of the 5th instar – kg	65.55	1.34

Throughout the rearing period, the silkworm larvae hatched from a box of eggs consumed 458.85 kg of mulberry leaves. The value did not differ significantly from data obtained under the same conditions in Bulgaria. The survival rate of the silkworms from hatching to cocooning was 95% on average and the mean weight of a silkworm before cocooning was 3.49 grams, which confirmed the results of other authors.

Thus, the calculated accumulated weight of silkworms hatched from a box of eggs was 65.55 kg.

The ‘input’ of the eco-technical system (gross energy and crude protein consumed) was 2179.54 MJ and 27.99 kg, respectively.

Table 5 shows the summarized data on the output of the eco-technical system (gross energy and crude protein accumulated in the silkworm bodies from a box of eggs at the end of the 5th instar) and the calculated Clarc of energy distribution and Clarc of (crude) protein transformation. Compared to the production of other livestock, silkworms utilize the mulberry leaf energy almost as efficiently as the transformation of the substances along the ‘feed – hen eggs’ chain (Penkov & Grigorova, 2020). The real transformation is calculated by taking into account that the input of the ‘feed – hen eggs’ chain is reported by the indicator ‘metabolizable energy (ME)’. Decreasing the value according to ME/GE ratio, it can be foreseen that the net energy efficiency will be closer to that for the production of quail eggs (Penkov & Genchev, 2019).

Table 5. *Accumulated gross energy and crude protein in silkworm bodies hatched from a box of eggs and Clarcs of energy distribution/protein transformation (CED/CPT):*

Indices	X mean	SD
Gross energy accumulated in silkworms from a box of eggs until the end of the 5th instar – MJ (= line 4 in Table 3*line 7 in Table 2)	455.57	9.03
Crude protein accumulated in silkworms from a box of eggs until the end of the 5th instar – kg (=line 4 in Table 3*line 2 in Table 2)	9.092	0.24
Clarc of energy distribution (CED)	0.2090 (20.90%)	-
Clarc of (crude) protein transformation (CPT)	0.3248 (32.48%)	-

Regarding the net transformation of crude protein, it was found that the result in the present study was the highest of all the other net protein transformations studied so far by us for the different species and categories of livestock animals.

Based on the data in Table 4, we would like to suggest one more indicator for objective measurement of the transformation of energy and protein in the major product, i.e., the silk filament.

One of the most important indicators is the cocoon shell ratio, calculated by the following formula:

Cocoon shell ratio (%) = Cocoon shell weight/ Fresh cocoon weight×100

When introducing the objective criteria for the transformation of nutrients from feed to silkworm body weight, we suggest introducing an ‘Index of effective cocoon shell ratio (IES)’, which is the product of cocoon shell ratio and Clarc of energy distribution:

IES = cocoon shell ratio (%) * Clarc of energy distribution/100

In the present study, the cocoon shell ratio was 22.614 ± 0.11 on average, therefore

IES = 22.614 * 20.90/100 = 4.726%.

Conclusion

The following net utilization of energy and protein was established in the present study, expressed through the newly introduced indices:

– Clarc of energy distribution (CED) – consumed gross energy → gross energy in the silkworm bodies – 0.2090 (20.90%).

– Clarc of protein transformation (CPT) – consumed crude protein → crude protein in the silkworm bodies – 0.3248 (32.48%).

– Index of effective cocoon shell ratio – Clarc of energy distribution * shell ratio (%) – 4.726%.

The newly introduced indices could be used at least in 2 directions:

– Selection (breeding) of races having higher indices;

– Optimization of the components in the artificial feed for silkworm rearing to obtain higher indices.

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