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Rearing of monovoltine strains of *Bombyx mori* by alternating artificial diet and mulberry leaf accelerates selection for higher food conversion efficiency and silk productivity

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Abstract

Availability of mulberry leaf of high nutritional quality, under temperate climatic conditions, is restricted to the spring-summer season, a situation that represents a limiting factor in selecting *Bombyx mori* L. strains. Therefore, we coupled traditional rearing on mulberry leaf (the natural food of the monophagous silkworm) with rearing on an artificial diet, therefore obtaining increased larval efficiency in converting food and high silk production. Nineteen nutritional indexes were recorded on both foods by using the gravimetric method, and a computer-assisted calculation. Results suggest that this strategy can be used to select highly performing strains adapted to both foods and that selection on artificial diet indirectly ameliorates food conversion efficiency by larvae. Obtained pure lines can also be used to produce hybrids suitable for rearing on both leaves and diet.

Key words: artificial diet, selection, *Bombyx mori*, conversion efficiency, gravimetric method.

Introduction

One of the most important characteristics of the silkworm (*Bombyx mori* L.) (Lepidoptera Bombycidae) is its ability to convert plant proteins to produce silk. The unique natural nourishment of *B. mori* is the mulberry leaf while, under controlled laboratory conditions, larvae can be reared also on artificial diets. In 2004 an original diet recipe was developed and patented by CRA-API (Cappellozza *et al.*, 2005). In contrast to mulberry leaf, artificial diet does not encounter any seasonal changing in its quality (Scriber and Slansky, 1981) and it is also exploitable in germ-free rearing systems (Sumida and Ueda, 2007). However, diet employment is currently limited to high-tech applications because it is quite expensive in comparison to mulberry leaf. Artificial diet, which represents a pathogen-free pabulum, is preferred when the silkworm is used as a bioreactor to obtain recombinant proteins (Kato *et al.*, 2010; Tatemastu *et al.*, 2012) or as a biological model (Hamamoto *et al.*, 2005; 2009; Kaito and Sekimizu, 2007). Therefore, in order to widen artificial diet utilization it is necessary to obtain silkworm strains which are able to produce a large amount of silk with a high efficiency of food transformation into textile fibre, so that diet rearing can be cheap enough to be affordable. Furthermore this insect's characteristic should be retained when the selected *B. mori* strains are reared again on the mulberry leaf, so that multiple-purpose silkworms can be developed through one effort of selection only.

To date selection processes based on nutritional indexes have been carried out on tropical polyvoltine strains (Ramesha *et al.*, 2010; 2012), while attempts in this direction have not been recently devoted to monovoltine strains, due to the progressive loss of the importance of sericulture in temperate countries in the last century.

Another reason for the scarce application of this technique of selection is because it is a very time-demanding process which employs a lot of manual labour; it necessary to weigh the larvae, the administered leaf or the diet, the remaining food, and the excreta individually and on a daily basis. In addition, low heritability of nutritional indexes and lack of application of appropriate statistical tools for analysis of phenotypic data are further constraints (Seidavi, 2009). In fact, sericulture key-characters are under the concerted action of several genes (polygenic or quantitative traits) and non-hereditary factors. Up-to-date advances in genetic marker-assisted selection have been made mostly for characters ruled by single genes (Hou *et al.*, 2013), while for quantitative traits results are still at a very early stage (Zhan *et al.*, 2009; Esfandiari *et al.*, 2011).

Therefore, we attempted a new approach, by thinking that administration of a limited amount of food in addition to selection for quantitative characters (silk production traits, with a high heritability as demonstrated by Gamo and Hirabayashi, 1983) might result in the amelioration of food conversion of silkworm strains. Nutritional indexes were calculated at the beginning and at the end of the experiment, to validate our hypothesis.

Materials and methods

Silkworm strains

Six productive monovoltine strains belonging to the CRA-API's germplasm collection and characterized by different morphological features were chosen to be reared on mulberry leaves and artificial diet. A preliminary rearing experiment was established in springtime (data not shown) both on mulberry leaves and diets. Strains that showed no adaptability to artificial diet (scarce acceptance and slow growth of larvae, low conversion indices on diet, low fertility of adults) or scarce silk production were discarded. Strains 129 and 124 (according to germplasm collection progressive numeration) were chosen for selection, because they showed good diet acceptance (higher for strain 129, relatively lower for strain 124). The former has a Japanese phenotype (larval body with markings, and a white peanut-shaped cocoon) and it is more adaptable to artificial diet, the latter has a Chinese phenotype (plain larval body and a white oval cocoon) and a lower but acceptable adaptability to artificial diet.

Selection strategy

Larvae were repeatedly selected for silk production throughout 6 generations over a three-year period and, at the end, evaluated for their nutritional efficiency. Each strain and strain selection were reared three times on artificial diet over the three years of the experiment duration, in the autumn-winter season. Acid treatment of silkworm eggs was carried out to disrupt the diapause of the spring laying. On the other hand, each strain and strain selection coming from diet rearing were reared three times on mulberry leaf over the three years of the experiment duration, in the spring-summer season. Larvae were reared as a bulk according to the best practical procedures both on mulberry leaves and artificial diet (Cappellozza *et al.*, 2005; Grekov *et al.*, 2005) until the end of the fourth instar. After the last moult, 3 batches of 100 healthy larvae for each strain were reared until cocoon spinning. On diet, larvae ate a daily fixed and weighed amount of food sufficient to achieve the wandering stage (Cappellozza and Saviane, 2009). This phase of selection was established to individuate the best silk producers, able to convert a measured amount of food into silk, without wasting a large amount of food for their body metabolism. On the other hand, on leaf, larvae ate *ad libitum* until the wandering stage. Leaf selection was alternated with diet selection, in order not to weaken strains by continuous diet rearing; this also permitted a slow adaptation to diet rearing avoiding a quick decrease in silk production capacity.

Production assessment

Five to six days after spinning, cocoons were collected and individually weighed. They were then cut and the silk shell individually weighed after pupa extraction; eventually the silk ratio was calculated (silk ratio = weight of silk shell/weight of cocoon) according to the pupa sex (Sakaguchi, 1978). Pupae were numbered in order to maintain their production parameters identifiable. Obtained data were processed by designing an Excel data sheet, with a filter fixing a threshold for cocoon and shell weight in addition to silk ratio. The threshold was repeatedly adjusted to achieve identification of the ten best performing individuals for each sex, which were then backcrossed (Greiss, 2003). The highest stringency was applied to shell weight and silk ratio respectively in comparison to other production parameters. Remaining pupae were discarded. Eggs laid by selected moths originated new selections (named 129 top 10 = 129T10 and 124 top 10 = 124T10). After each generation silk productivity selection was reiterated for the larvae hatched from each respective egg laying, while cocoon and shell weight and silk ratio were compared for females and males separately. The original strains 129 and 124 were reared on leaf and diet under the same conditions as 129T10 and 124T10 without any selection pressure to maintain the population of origin as comparison (control).

Nutritional indexes

At the end of the experiment, after three years, the different strains were assessed for their nutritional efficiency in the fifth instar. Efficiency analysis was limited to this period when larvae consume 80-85% of the total amount of food (Rahmathulla and Suresh, 2008). After rearing in bulk until the end of the last moult, larvae were divided into males and females (Rahmathulla and Suresh, 2008) according to abdominal sexual markings (Sakaguchi, 1978). For each strain (129T10, 129, 124T10 and 124) 8 males and 8 females were individually reared in covered plastic cups both on mulberry leaf and artificial diet until cocoon spinning. Cups were covered to maintain proper humidity, fundamental for correct larval development (Paul *et al.*, 1992; Esfandarani *et al.*, 2002). After the beginning of the wandering stage larvae were left in the cups without food where they completed their spinning process. In accordance with the standard gravimetric method (Waldbauer, 1968) the fresh weight of larvae, newly added food (leaf or diet), faeces and left over food were recorded on a daily basis and used to calculate nutritional indexes (Ramesha *et al.*, 2010; 2012). Furthermore, we introduced the correction proposed by Candy and Baker (2002), which is the most appropriate, in our experimental setup, for the calculation of ingested food. Formulae, which were utilized in index calculation for consumption traits are as follows:

- $Ingesta\ (I) = \frac{w_{t0} - w_{t1}F}{\{(F-1)[ln(F)]^{-1}\}}$

where w_{t0} and w_{t1} represent the weight of freshly added food and left over food respectively; F is a weight correction factor for natural moisture loss and is calculated as the ratio between initial and final weight (after 24 hours) of an amount of control food.

- $Digesta\ (D) = Ingesta - fresh\ weight\ of\ litter$
- $Excreta\ (E) = Ingesta - Digesta$
- $Approximate\ Digestibility\ (AD) = \left(\frac{Ingesta}{Excreta}\right) * 100$
- $Reference\ Ratio\ (RR) = \frac{Ingesta}{Excreta}$
- $Consumption\ Index\ (CI) =$

$$\frac{Ingesta}{V\ instar\ mean\ fresh\ larval\ weight * V\ instar\ duration\ (days)}$$

- $Relative\ Growth\ Rate\ (RGR) =$

$$\frac{V\ instar\ larval\ weight\ gain}{V\ instar\ mean\ fresh\ larval\ weight * V\ instar\ duration\ (days)}$$

- $Respiration\ (R) = Digesta - larval\ maximum\ weight$
- $Metabolic\ Rate\ (MR) =$

$$\frac{Respiration}{V\ instar\ mean\ fresh\ larval\ weight * V\ instar\ duration\ (days)}$$

Formulae for conversion efficiency of ingesta (ECI) and digesta (ECD) are as follows:

- $ECI\ to\ larva\ (ECI_L) = \frac{V\ instar\ larval\ weight\ gain}{Ingesta} * 100$
- $ECD\ to\ larva\ (ECD_L) = \frac{V\ instar\ larval\ weight\ gain}{Digesta} * 100$

- $ECI\ to\ cocoon\ (ECI_C) = \frac{cocoon\ weight}{Ingesta} * 100$
- $ECD\ to\ cocoon\ (ECD_C) = \frac{cocoon\ weight}{Digesta} * 100$
- $ECI\ to\ shell\ (ECI_S) = \frac{shell\ weight}{Ingesta} * 100$
- $ECD\ to\ shell\ (ECD_S) = \frac{shell\ weight}{Digesta} * 100$

Formulae to calculate ingesta and digesta per gram of cocoon and cocoon shell are as follows:

- $Ingesta\ per\ cocoon\ gram\ (I/g_C) = \frac{Ingesta}{cocoon\ weight}$
- $Digesta\ per\ cocoon\ gram\ (D/g_C) = \frac{Digesta}{cocoon\ weight}$
- $Ingesta\ per\ shell\ gram\ (I/g_S) = \frac{Ingesta}{shell\ weight}$
- $Digesta\ per\ shell\ gram\ (D/g_S) = \frac{Digesta}{shell\ weight}$

During the experiments, a reserve batch was reared for each thesis to possibly replace larvae with abnormal development.

Statistical analysis

After three years and six generations of selection nutritional indexes data were analyzed by means of ANOVA and significant differences explored by Tukey's post-hoc test.

Results

Selection for silk production

As explained in the previous paragraph, selection based on main productive traits was carried out in alternation on mulberry leaf and on artificial diet; T10 subpopulations started to split themselves from parental strains throughout generations. Our experiment confirmed the high heritability of characters like cocoon weight (73.60%), shell weight (80.20%) and shell ratio (72.40%) (Gamo and Hirabayashi, 1983). However, some differences in heritability were observed between strains 129 and 124, proving that gene frequencies, which differ among different populations, affect the selection process (Grekov *et al.*, 2005). In particular strain 129 increased its performances more than strain 124. 129T10 males were superior to 129 males (parental strain) with respect to shell (0.351 g ± 0.034 vs. 0.321 g ± 0.027) and cocoon weights (1.463 g ± 0.091 vs. 1.337 g ± 0.108) without decreasing their cocoon silk ratios (23.97 ± 1.83 vs. 24.03 ± 1.52) (table 1) and the same behaviour was recorded for females for the same parameters (shell weight: 0.369 g ± 0.034 vs. 0.342 g ± 0.031; cocoon weight: 1.793 g ± 0.121 vs. 1.677 g ± 0.146; cocoon silk ratio: 20.64 ± 1.94 vs. 20.45 ± 1.17) (table 2). An identical trend was assessed when 124T10-124 male silk shells (0.309 g ± 0.034 vs. 0.296 g ± 0.029) were compared (table 1) even though the differences between them were not significant; on the other hand, silk ratios showed significant differences (23.75 ± 2.45 vs. 22.50 ± 2.02). Difference was also significant for shells in the female group (0.349 g ± 0.030 vs. 0.314 g ± 0.024) (table 2).

Consumption traits

Following the gravimetric method we calculated 9 indexes concerning consumption traits in the 4 strains under examination, separately for males and females, and on both foods. Ingesta (I), digesta (D), excreta (E), approximate digestibility (AD), reference ratio (RR), consumption index (CI), relative growth rate (RGR), respiration (R) and metabolic rate (MR) values were analyzed using factorial ANOVA and post-hoc Tukey's test. The analyzed factors were "Strain" (4 levels), "Diet" (2 levels) and "Gender" (2 levels). Observing table 3 it is possible to see that the highest order interaction was neither significant for any consumption trait index nor for the Diet*Gender interaction. The Strain*Gender interaction was significant only for I and RGR values.

Table 1. Data on male raw productivity recorded on mulberry leaves during the 3rd year of selection, spring-summer season. Silk ratio is calculated as the ratio between cocoon weight and shell weight in grams.

Strain	Cocoon ± SD (g)	Shell ± SD (g)	Silk ratio ± SD (%)
129T10	1.463 ± 0.091 a	0.351 ± 0.034 a	23.97 ± 1.83 a
129	1.337 ± 0.108 b	0.321 ± 0.027 b	24.03 ± 1.52 a
124T10	1.303 ± 0.099 b	0.309 ± 0.034 bc	23.75 ± 2.45 a
124	1.322 ± 0.133 b	0.296 ± 0.029 c	22.50 ± 2.02 b

Data are sorted according to descending shell weights. As the omnibus test was significant for all parameters (p-value < 0.001) post-hoc test was carried out. Different letters indicate significant differences among strains according to Tukey's test. Data were recorded on 50 individuals per strain.

Table 2. Data on female raw productivity recorded on mulberry leaves during the 3rd year of selection, spring-summer season. Silk ratio is calculated as the ratio between cocoon weight and shell weight in grams.

Strain	Cocoon ± SD (g)	Shell ± SD (g)	Silk ratio ± SD (%)
129T10	1.793 ± 0.121 a	0.369 ± 0.034 a	20.64 ± 1.94 a
124T10	1.660 ± 0.150 b	0.349 ± 0.030 b	21.11 ± 1.64 a
129	1.677 ± 0.146 b	0.342 ± 0.031 b	20.45 ± 1.17 a
124	1.621 ± 0.148 b	0.314 ± 0.024 c	19.44 ± 1.33 b

As in table 1 different letters indicate significant differences according to ANOVA and Tukey's post-hoc test. Data were recorded on 50 individuals per strain.

Table 3. ANOVA results for consumption traits. P-values for main effects and interactions are reported and marked in accordance to significance.

Factor	I (p)	D (p)	E (p)	AD (p)	RR (p)	CI (p)	RGR (p)	R (p)	MR (p)
Strain	<0.001	0.262	<0.001	0.001	<0.001	<0.001	<0.001	0.008	<0.001
Diet	<0.001	<0.001	<0.001	<0.001	0.092	<0.001	<0.001	<0.001	0.035
Gender	<0.001	<0.001	<0.001	0.002	0.003	0.040	0.251	0.397	0.576
Strain*Diet	<0.001	<0.001	<0.001	0.015	0.025	<0.001	<0.001	<0.001	<0.001
Strain*Gender	0.035	0.214	0.333	0.715	0.993	0.759	<0.001	0.704	0.680
Diet*Gender	0.321	0.133	0.724	0.942	0.343	0.333	0.510	0.863	0.580
Strain*Diet*Gender	0.963	0.475	0.350	0.146	0.155	0.769	0.325	0.193	0.081

I, ingesta; D, digesta; E, excreta; AD, approximate digestibility; RR, reference ratio; CI, consumption index; RGR, relative growth rate; R, respiration; MR, metabolic rate.

Table 4. ANOVA results efficiency traits. P-values for main effects and interactions are reported and marked in accordance to significance.

Factor/Interaction	ECI$_L$ (p)	ECD$_L$ (p)	ECI$_C$ (p)	ECD$_C$ (p)	ECI$_S$ (p)	ECD$_S$ (p)	I/g$_C$ (p)	D/g$_C$ (p)	I/g$_S$ (p)	D/g$_S$ (p)
Strain	<0.001	<0.001	<0.001	0.093	<0.001	0.053	<0.001	0.043	<0.001	0.085
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Gender	0.015	0.316	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.001	<0.001
Strain*Diet	<0.001	<0.001	0.194	0.285	<0.001	<0.001	0.122	0.178	<0.001	0.001
Strain*Gender	0.188	0.135	0.448	0.633	0.004	0.084	0.414	0.764	0.044	0.199
Diet*Gender	0.198	0.351	0.428	0.801	0.646	0.674	0.310	0.650	0.477	0.280
Strain*Diet*Gender	0.974	0.239	0.512	0.083	0.872	0.889	0.323	0.053	0.822	0.942

ECI$_L$-ECD$_L$, respectively efficiency of conversion of ingesta and digesta to larva; ECI$_C$-ECD$_C$, respectively efficiency of conversion of ingesta and digesta to cocoon; ECI$_S$-ECD$_S$, respectively efficiency of conversion of ingesta and digesta to shell; I/g$_C$-D/g$_C$, respectively ingesta and digesta per gram of cocoon; I/g$_S$-D/g$_S$, respectively ingesta and digesta per gram of shell.

Table 5. A summary of the most important indexes used to evaluate the cost-benefit ratio for males and females. Tukey's post-hoc tests were carried out after significant ANOVA omnibus test. Different letters indicate significant differences among genders according to Tukey's test.

Gender	I ± SD (g)	CI ± SD	ECI$_L$ ± SD (%)	ECI$_C$ ± SD (%)	ECI$_S$ ± SD (%)	I/g$_C$ ± SD (g)	I/g$_S$ ± SD (g)
Males	8.436±1.324 a	0.696±0.097 b	29.366±3.736 b	15.501±1.481 b	3.461±0.425 a	6.509±0.618 b	29.326±3.626 a
Females	9.385±1.476 b	0.678±0.090 a	30.411±4.260 a	16.694±1.542 a	3.291±0.369 b	6.042±0.584 a	30.775±3.573 b

I, ingesta; CI, consumption index; ECI$_L$ efficiency of conversion of ingesta to larva; ECI$_C$ efficiency of conversion of ingesta to cocoon; ECI$_S$ efficiency of conversion of ingesta to shell; I/g$_C$ ingesta per gram of cocoon; I/g$_S$ ingesta per gram of shell.

On the other hand, the Strain*Diet interaction was significant for all the indexes, the Gender main effect was significant for all the indexes except for RGR, R, and MR, and the main effects Strain and Diet were significant for all the indexes except for D and RR respectively. On the whole, by evaluating the consumption indexes and particularly those involved in optimizing the cost-benefit ratio (Ramesha *et al*., 2012) as I and CI, it was clearly shown that males have the lowest consumption (table 5 and 9) which principally occurred on mulberry leaf (table 6). Moreover, among the strains, 129T10 exhibited the best performances if compared to its parental strain (129) and to the other 2 strains (table 7); in particular, these showed a significantly different behaviour in relation to their interaction with the factor Diet (table 8). Results of 129T10 were, on the other hand, the most consistent, independently from the food, while 124T10 was very sensitive to the kind of nutritional source, even when compared to 124, with reasonably good performances on mulberry leaf and very negative outputs on artificial diet.

Efficiency traits

Efficiency of conversion of ingesta (ECI) and digesta (ECD) to larva, cocoon and shell for males and females was calculated on both mulberry leaves and artificial diet. The same was done for ingesta and digesta per gram (respectively I/g and D/g) of cocoon and silk shell. Factorial ANOVA was carried out in the same way as consumption traits and the results are summarized in table 4. Three-way interaction was neither significant for any index nor for the Diet*Gender interaction, whereas the Strain*Gender interaction was significant but only for ECI to shell and I/g of shell. The Strain*Diet interaction was significant for all indexes except for those related to the cocoon (table 4) and the

Table 6. A summary of the most important indexes used to evaluate the cost-benefit ratio on artificial diet and mulberry leaf. Tukey's post-hoc tests were carried out after significant ANOVA omnibus test. Different letters indicate significant differences among foods.

Diet	I ± SD (g)	CI ± SD	ECI$_{L}$ ±SD (%)	ECI$_{C}$ ± SD (%)	ECI$_{S}$ ± SD (%)	I/g$_{C}$ ± SD (g)	I/g$_{S}$ ± SD (g)
Mulberry leaf	7.970±0.890 a	0.672±0.057 a	27.931±2.553 b	16.544±1.388 a	3.514±0.336 a	6.086±0.507 a	28.711 ±2.694 a
Artificial diet	9.851±1.341 b	0.702±0.118 b	31.846±4.284 a	15.651±1.722 b	3.238±0.424 b	6.465±0.710 b	31.390±4.009 b

I, ingesta; CI, consumption index; ECI$_{L}$ efficiency of conversion of ingesta to larva; ECI$_{C}$ efficiency of conversion of ingesta to cocoon; ECI$_{S}$ efficiency of conversion of ingesta to shell; I/g$_{C}$ ingesta per gram of cocoon; I/g$_{S}$ ingesta per gram of shell.

Table 7. A summary of the most important indexes used to evaluate the cost-benefit ratio for parental strains and their breeds. Tukey's post-hoc tests were carried out after significant ANOVA omnibus test. Different letters indicate significant differences among strains.

Strain	I ± SD (g)	CI ± SD	ECI$_{L}$ ±SD (%)	ECI$_{C}$ ± SD (%)	ECI$_{S}$ ± SD (%)	I/g$_{C}$ ± SD (g)	I/g$_{S}$ ± SD (g)
129T10	8.114±1.056 a	0.579±0.055 a	33.376±4.895 a	17.644±1.365 a	3.786±0.335 a	5.702±0.458 a	26.622±2.476 a
129	9.657±1.379 c	0.685±0.051 b	27.241±3.197 b	15.680±1.166 b	3.148±0.262 b	6.413±0.493 b	31.978±2.697 b
124T10	9.154±1.856 b	0.747±0.093 c	29.643±2.146 c	15.489±1.362 b	3.304±0.431 b	6.507±0.606 b	30.799±4.282 b
124	8.715±1.060 b	0.736±0.056 c	29.294±2.717 c	15.576±1.540 b	3.265±0.249 b	6.481±0.645 b	30.802±2.441 b

I, ingesta; CI, consumption index; ECI$_{L}$ efficiency of conversion of ingesta to larva; ECI$_{C}$ efficiency of conversion of ingesta to cocoon; ECI$_{S}$ efficiency of conversion of ingesta to shell; I/g$_{C}$ ingesta per gram of cocoon; I/g$_{S}$ ingesta per gram of shell.

Table 8. Consumption and conversion efficiency indexes used to evaluate the cost-benefit ratio for strains on different foods. Tukey's post-hoc tests were carried out after significant ANOVA omnibus test. Different letters indicate significant differences. Results are ordered according to descending ECI to shell values.

Strain*Diet	I ± SD (g)	CI ± SD	ECI$_{L}$ ± SD (%)	ECI$_{C}$ ± SD (%)	ECI$_{S}$ ± SD (%)	I/g$_{C}$ ± SD (g)	I/g$_{S}$ ± SD (g)
129T10*Leaf	7.537±0.754 a	0.612±0.044 b	29.429±2.388 bc	17.924±1.272	3.832±0.304 a	5.606±0.404	26.253±2.135 a
129T10*Diet	8.692±1.012 bc	0.547±0.046 a	37.322±3.252 a	17.365±1.438	3.740±0.367 a	5.797±0.501	26.992±2.796 a
124T10*Leaf	7.509±0.803 a	0.671±0.047 c	29.478±1.745 bc	16.290±0.917	3.638±0.239 a	6.158±0.357	27.602±1.861 ae
124*Leaf	8.060±0.426 ab	0.704±0.039 c	27.500±1.798 cd	15.817±1.320	3.337±0.191 b	6.362±0.516	30.060±1.691 be
129*Leaf	8.773±0.898 bc	0.700±0.047 c	25.315±1.719 d	16.144±1.027	3.249±0.235 bc	6.219±0.411	30.928±2.158 bc
124*Diet	9.371±1.107 c	0.769±0.052 d	31.087±2.273 b	15.335±1.741	3.194±0.284 bc	6.600±0.750	31.545±2.878 bcd
129*Diet	10.541±1.207 d	0.670±0.053 c	29.167±3.202 bc	15.217±1.139	3.048±0.255 c	6.607±0.503	33.028±2.833 cd
124T10*Diet	10.800±0.833 d	0.823±0.058 e	29.809±2.532 bc	14.689±1.274	2.971±0.298 c	6.857±0.608	33.996±3.553 d

I, ingesta; CI, consumption index; ECI$_{L}$ efficiency of conversion of ingesta to larva; ECI$_{C}$ efficiency of conversion of ingesta to cocoon; ECI$_{S}$ efficiency of conversion of ingesta to shell; I/g$_{C}$ ingesta per gram of cocoon; I/g$_{S}$ ingesta per gram of shell.

Table 9. Consumption and conversion efficiency indexes used to evaluate the cost benefit-ratio for males and females of different strains. Tukey's post-hoc tests were carried out after significant ANOVA omnibus test. Different letters indicate significant differences. Results are ordered according to descending ECI to shell values.

Strain*Gender	I ± SD (g)	CI ± SD	ECI$_{L}$ ± SD (%)	ECI$_{C}$ ± SD (%)	ECI$_{S}$ ± SD (%)	I/g$_{C}$ ± SD (g)	I/g$_{S}$ ± SD (g)
129T10*Male	7.552±0.893 a	0.588±0.050	32.270±4.447	17.053±1.117	3.994±0.257 a	5.888±0.392	25.140±1.695 a
129T10*Female	8.677±0.911 b	0.571±0.060	34.481±5.208	18.236±1.363	3.579±0.271 b	5.515±0.454	28.104±2.261 b
124T10*Male	8.692±1.824 b	0.763±0.106	29.167±2.203	14.796±1.465	3.355±0.394 bc	6.821±0.673	30.222±3.787 bd
124*Female	8.879±1.260 bc	0.729±0.057	29.112±2.944	16.346±1.762	3.288±0.270 cd	6.194±0.759	30.605±2.504 bcd
124T10*Female	9.617±1.826 cd	0.730±0.078	30.119±2.043	16.183±0.812	3.254±0.472 cd	6.194±0.310	31.376±4.779 cd
129*Male	8.949±1.165 bc	0.689±0.052	26.551±3.154	15.348±1.287	3.252±0.264 cd	6.559±0.550	30.943±2.596 cd
124*Male	8.551±0.823 b	0.743±0.055	29.476±2.554	14.806±0.727	3.243±0.232 cd	6.769±0.327	31.000±2.442 cd
129*Female	10.366±1.226 d	0.681±0.052	27.931±3.186	16.013±0.958	3.044±0.220 d	6.267±0.392	33.013±2.452 c

I, ingesta; CI, consumption index; ECI$_{L}$ efficiency of conversion of ingesta to larva; ECI$_{C}$ efficiency of conversion of ingesta to cocoon; ECI$_{S}$ efficiency of conversion of ingesta to shell; I/g$_{C}$ ingesta per gram of cocoon; I/g$_{S}$ ingesta per gram of shell.

Gender effect was significant for all indexes except for ECI and ECD to larva. The factor Diet was highly significant (p-level < 0.001) for all indexes, while the Strain effect was significant for all indexes except for ECD to cocoon and shell and for D/g to shell. As in the previously-mentioned case of the consumption traits, 129T10 selected individuals appeared to be excellent performers with the highest ECI values and the lowest I/g of cocoon and shell values (table 7). Females were better at converting food matter into larval mass and cocoons but inferior to males in converting it to silk shells. It is noteworthy that this result makes sense as females have to allocate resources for egg production too; however, this datum disagrees with the findings of Rahmathulla and Suresh (2008). Once again the Food factor showed an interaction with the Strain factor producing a strong effect principally on the 124-124T10 strains and particularly on the ECI to shell, which is one of the ultimate indexes to evaluate nutritional efficiency. While 124T10 on mulberry leaf was not significantly different from 129T10 (the best on both foods; table 8), if fed with artificial diet, this selected strain had the worst ECI to shell value. Moreover, the same pattern could be individuated by looking at the ingesta required to produce one gram of silk shell (I/g to shell; table 8) whereas this difference is less extreme by looking at ECI to cocoon and to larva.

Discussion and conclusions

Silkworm conversion efficiency connected to a low level of food consumption is one of the chief factors in optimizing the benefit-cost ratio in the field of sericulture. Experiments have been recently performed in this direction and improvements have been achieved using strains adapted to tropical climates, which are characterized by a low conversion efficiency and which, therefore, obtain large benefits from selection processes based on nutritional indexes. In the present work the same target was pursued on monovoltine strains, well-adapted to temperate climates, and already capable of a good conversion of proteins which they obtain from food. Since the goal was to enhance silk production on both mulberry leaf and artificial diet but not on a "power component" basis (Scriber and Lederhouse, 1983) i.e., not by increasing the quantity of ingested food, our strategy was focused on consecutive rearing cycles by alternating high-low stringency. The low stringency step was performed on mulberry leaf, when larvae were selected only according to their productivity (cocoon and shell weight and silk ratio), while the high stringency step was represented by rearing cycles on artificial diet. In this case, larvae were not fed *ad libitum* during the last instar, as they were fed on mulberry leaf, but with a fixed amount of diet; therefore, on this basis, it was possible to identify those individuals which were able "to produce more with less". On the other hand, artificial diet represented an additional constraint for the larvae, since adaptability to this mulberry succedaneum is not equal for all strains, even though this trait is selectable. In this study we used artificial diet, because of its possibility to be standardised and because its nutritional value is similar to that of leaf for strains that adapt to it. Therefore, we used only the quantity of diet as a limiting factor. For this research we did not use autumn leaf, which usually shows a lower nutritional value than spring leaf, because its quality changes according to different years, depending on the summer season (i.e. quantity of rain, average temperatures, insect pest damages to plants...). Our aim was not to select for a poor nutritional value of leaf, but for a limited quantity of leaf, in order to save labour and material in a commercial rearing of newly-selected strains or their hybrids. Therefore, we think that new selections and hybrids will be efficient with regard to food consumption, but not necessarily resistant to nutritional constraints due to the bad quality of food.

After three years of selection the evaluation of nutritional indexes confirmed that the adopted strategy worked, although with variable efficacy. According to Ramesha *et al.* (2012), CI and ECI are the driving indexes in deciding efficiency of strains and thus, in our conditions, 129T10 expressed the best performances. On the whole, it showed the lowest I and CI values on both foods consistently for males and females. The conversion efficiency of ingesta to larva, cocoon and shell were the highest and the grams of ingesta necessary to produce one gram of cocoon and silk shell were the lowest, indicating that the larval enzymatic apparatus used to convert nutrients to body matter and, more specifically, to silk proteins was finely tuned (Rahmathulla *et al.*, 2004). On the other hand, 124T10 showed contrasting results on the two different foods. The ingesta value was the highest on artificial diet. However, when switched to mulberry leaves, its performances clearly changed and 124T10 showed the best I value and results related to CI were very similar to those of 129T10 (table 8). Moreover, ECI to shell had also the same pattern: similar to 129 and 124 on the diet, to 129T10 on the mulberry leaf, as pointed out in table 8.

Therefore, the implemented selection strategy worked well for 129T10, which showed the best performances and an important improvement compared to its parental strain in all our conditions. Due to low values of I and CI this achievement seems to be linked to the capability of larvae to maintain their metabolism working properly in spite of a low intake of nutrients indicating a high conversion efficiency, which is confirmed by ECI, ECD and I/g values. In the case of 124T10, the adopted selection process seems only partly appropriate since desired results were achieved merely on mulberry leaf and were not always statistically significant. Looking at the contrasting performances of the 124-124T10 couple on diet and leaf, we suppose that in our selection strategy there were two forces acting against each other: the first one to ameliorate performances, the other to increase adaptability to artificial diet. The final effect was that 124T10 enhanced its performances slightly on leaf as a weak result of the selection process, but worsened on diet since it was not able to fully exploit this food matrix. Probably adaptation represented a stronger constraint than selection for improved performances. This led 124, which did not undergo any imposed pressure, to be better than its selected offspring on artificial diet (see for instance I and ECI to shell even though the latter without significant differences).

On the other hand, a weak improvement of production performances of 124T10 on mulberry leaf and high values of RGR (data not shown) and ECI to larva on diet indicated that larvae were consuming a lot of food in order to reach the selection objectives, but without succeeding. This phenomenon might be due to differences in amylase activity of different strains or to the scarce development of a symbiotic intestinal flora in the diet rearing (Moon and Seol, 1983; Chatterjee *et al.*, 1993; Manjula *et al.*, 2010).

In conclusion, our strategy proved to work on 129 and to a certain extent also on 124, even though with a lower efficiency; these experimental data suggest that in case of strains with low adaptability to the artificial diet (for example, Chinese strains) a preliminary period of adaptation to diet should be established before starting with a strict selection process. The evaluation by means of nutritional indexes can thus be used as a measure of adaptability to diet and as a tool to decide whether to select both foods or just mulberry leaves. In addition, obtained breeds can represent a starting point for the production of hybrids, whose performances can be further improved through heterosis (Singh *et al.*, 2012).

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References

CANDY S. G., BAKER S. C., 2002.- Calculating food consumption in the laboratory: A formula to adjust for natural weight loss.- *Australian Journal of Entomology*, 41 (2): 170-173.

CAPPELLOZZA S., SAVIANE A., 2009.- La dieta artificiale per il baco da seta: metodica per l’allevamento su piccola scala.- *Apoidea*, 6: 46-57.

CAPPELLOZZA L., CAPPELLOZZA S., SAVIANE A., SBRENNA G., 2005.- Artificial diet rearing system for the silkworm *Bombyx mori* (Lepidoptera: Bombycidae): effect of vitamin C deprivation on larval growth and cocoon production.- *Applied Entomology and Zoology*, 40 (3): 405-412.

CHATTERJEE S. N., RAO C. G. P., CHATTERJEE G. K., ASHWATH S. K., PATNAIK A. K., 1993.- Correlation between yield and biochemical parameters in the mulberry silkworm, *Bombyx mori* L.- *Theoretical and Applied Genetics*, 87 (3): 385-391.

ESFANDARANI M. T., BAHREINI R., TAJABADI N., 2002.- Effect of mulberry leaves moisture on some traits of the silkworm (*Bombyx mori* L.).- *Sericologia*, 42 (2): 285-289.

GAMO T., HIRABAYASHI T., 1983.- Genetic analysis of growth rate, pupation rate and some quantitative characters by diallel cross in the silkworm, *Bombyx mori* L.- *Japanese Journal of Breeding*, 33 (2): 178-190.

GREISS H., 2003.- Genetic-selection studies on silkworm *Bombyx mori* L. under different environmental conditions. 63 pp. *PhD Thesis*, University of Sofia, Bulgaria.

GREKOV D., KIPRIOTIS E., TZENOV P., 2005.- *Sericulture training manual*.- National Agricultural Research Foundation, Komotini, Greece.

HAMAMOTO H., KAMURA K., RAZANAJATOVO I. M., MURAKAMI K., SANTA T., SEKIMIZU K., 2005.- Effects of molecular mass and hydrophobicity on transport rates through non-specific pathways of the silkworm larva midgut.- *International Journal of Antimicrobial Agents*, 26 (1): 38-42.

HAMAMOTO H., TONOIKE A., NARUSHIMA K., HORIE R., SEKIMIZU K., 2009.- Silkworm as a model animal to evaluate drug candidate toxicity and metabolism.- *Comparative Biochemistry and Physiology. C*, 149 (3): 334-339.

KAITO C., SEKIMIZU K., 2007.- A silkworm model of pathogenic bacterial infection.- *Drug discoveries & therapeutics*, 1 (2): 89-93.

KATO T., KAJIKAWA M., MAENAKA K., PARK E. Y., 2010.- Silkworm expression system as a platform technology in life science.- *Applied microbiology and biotechnology*, 85 (3): 459-470.

MANJULA S., SABHANAYAKAM S., MATHIVANAN V., SARAVANAN N., 2010.- Studies on the changes in the activities of digestive enzymes in the midgut of silkworm *Bombyx mori* (L.). (Lepidoptera: Bombycidae) fed with mulberry leaves supplemented with Indian bean (*Dolichos lablab*).- *International Journal of Biological & Medical Research*, 1 (4): 168-171.

MOON J. Y., SEOL G. Y., 1983.- Varietal difference in amylase activity of larval digestive fluid of the silkworm bombyx mori, reared on artificial diet.- *The sericultural journal of Korea*, 24 (2): 73-80.

PAUL D. C. C., SUBBA RAO G., DEB D. C. C., 1992.- Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* and concommitant larval duration.- *Journal of Insect Physiology*, 38 (3): 229-235.

RAHMATHULLA V. K., SURESH H. M., 2008.- Feed consumption and conversion efficiency in male and female bivoltine silkworms (*Bombyx mori* L.) - a comparative study.- *Journal of the Entomological Research Society*, 10 (1): 59-65.

RAHMATHULLA V., MATHUR V., GEETHA DEVI R., 2004.- Growth and dietary efficiency of mulberry silkworm (*Bombyx mori* L.) under various nutritional and environmental stress conditions.- *Philippine Journal of Science*, 133 (1): 39-43.

RAMESHA C., ANURADHA C. M., LAKSHMI H., SUGNANA K. S., SESHAGIRI S. V., GOEL A. K., SURESH K. C., KUMARI S. S., 2010.- Nutrigenetic traits analysis for identification of nutritionally efficient silkworm germplasm breeds.- *Biotechnology*, 9 (2): 131-140.

RAMESHA C., LAKSHMI H., KUMARI S. S., ANURADHA C. M., KUMAR C. S., 2012.- Nutrigenetic screening strains of the mulberry silkworm, *Bombyx mori*, for nutritional efficiency.- *Journal of Insect Science*, 12 (15): 1-17.

SAKAGUCHI B., 1978.- Morphology, pp 32-39. In: *The silkworm: an important laboratory tool* (TAZIMA Y., Ed.).- Kodansha Ltd, Tokyo, Japan.

SCRIBER J. M., LEDERHOUSE R. C., 1983.- Temperature as a factor in the development and feeding ecology of tiger swallowtail caterpillars, *Papilio glaucus* (Lepidoptera).- *Oikos*, 40: 95-102.

SCRIBER J. M., SLANSKY JR. F., 1981.- The nutritional ecology of immature insects.- *Annual Review of Entomology*, 26 (1): 183-211.

SEIDAVI A., 2009.- Determination and comparison of nutritional indices in commercial silkworm hybrids during various instars.- *Asian Journal of Animal and Veterinary Advances*, 4 (3): 104-113.

SINGH T., SINGH P. K., SAHAF K. A., 2012.- The heterosis phenomenon in mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae).- *Annals of Biological Research*, 3 (9): 4330-4336.

SUMIDA M., UEDA H., 2007.- Dietary sucrose suppresses midgut sucrase activity in germfree, fifth instar larvae of the silkworm, *Bombyx mori*.- *Journal of Insect Biotechnology and Sericology*, 37: 31-37.

TATEMASTU K., SEZUTSU H., TAMURA T., 2012.- Utilization of transgenic silkworms for recombinant protein production.- *Journal of Biotechnology & Biomaterials*, S9:004.

WALDBAUER G. P., 1968.- The consumption and utilization of food by insects.- *Advances in Insect Physiology*, 5: 229-288.

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