Table 4. The codon bias indices (CBI) for the unicellular and filamentous forms with respect to the preferred codons selected for the different species. The cut-off limit used for selection in standard deviation units is given in parantheses

CBI	SYN 0	ANA –	FDI –
Gram negative bacteria			
E. coli (low) (2)*	0.31	0 10	0 14
E coli (high) (2)**	0 17	0 17	0 21
E. coli (2)	0.34	0.20	0 22
Pseudomonas sp (2)	0 22	–0 09	–0 09
A tumefaciens (2)	0 28	–0 06	–0 04
A vinelandii (2)	0 21	–0 06	–0 04
K pneumoniae (2)	0 21	–0 07	–0 09
S marcescens (2)	0 21	–0 05	–0 05
S. typhimurium (2)	0 26	–0 01	–0 07
R capsulatus (2)	0 20	–0 10	–0 12
B japonicum (2)	0 22	–0 13	–0 13
Rhizobium (2)	0 22	–0 14	–0 15
Clostridium sp (2)	–0 18	0 20	0 22
Gram positive bacteria			
B. subtilis (2)	0 09	0 28	0 29
Bacillus sp (2 5)	0 13	0 30	0 29
B megaterium (2 5)	–0 03	0 20	0 17
B sphaerieus (2 0)	–0.19	0 22	0 23
Streptococcus sp (2 0)	–0 05	0 27	0.33
S aureus (2 0)	–0 12	0 24	0 26
B stearothermophilus (2 5)	0 16	0.00	–0 04
Streptomyces sp (2 0)	0 17	–0 08	–0 07
Eukaryotes			
S. pombe (2)	–0 03	0 20	0 26
S cerevisiae (2)	0 01	0 28	0.32
Kluyveromyces (2)	–0 12	0 21	0 23
Wheat chloroplast (2)	–0 17	0 25	0.30
M phymorpha (2)	–0 04	0 19	0 22
Z. mays (2)	–0 17	0 26	0 29
P sativum (2)	–0 14	0 24	0.26
Spinach (2)	–0 22	0 24	0 27
N tabaccum (2)	–0 09	0 26	0.29
Chlamydomonas (2)	–0 02	0 27	0 28

*Genes with low expression and **genes with high expression

the cyanobacterial genes with respect to other organisms (Table 4) shows that the codon usage of unicellular genes is biased towards *E. coli* (low expression) and *A. tumefaciens*, whereas the filamentous genes are biased towards *S. cerevisiae*, *Bacillus* sp., *B. subtilis* and chloroplasts. In as far as the biased codon usage is a determinant of the levels of gene expression, it is seen through this analysis that *A. tumefaciens* could form an alternate cloning host apart from *E. coli* for unicellular strains and filamentous strains may be better expressed in *S. cerevisiae*, *Bacillus* sp. or *B. subtilis*. These conclusions are being made to form a basis for molecular biology experiments involving cyanobacteria.

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Effects of feeding mulberry (*Morus* sp.) leaves supplemented with different nutrients to silkworm (*Bombyx mori* L.)

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The growth of silkworm larvae improved significantly on feeding them mulberry leaves supplemented with different nutrients. The total protein content of silk gland increased, while the water, total lipid and carbohydrate contents decreased. The larval weight, silk gland weight and total protein in silk gland were found to be highest for treatment TS_8 (soymilk + sugar + vitamins + potassium iodide salt), followed by TS_7 (glycine + alanine + sugar + vitamins + potassium iodide salt) and TS_9 (milk powder + sugar + vitamins + potassium iodide salt) treatments. The highest values of single-cocoon weight, single-shell weight and filament length were observed for treatment TS_8 (1.34 g, 0.26 g and 799.42 m/cocoon, respectively), whereas cocoon yield was found to be highest for treatment TS_7 (110.17 g/100 larvae). No significant difference was observed between the treatments TS_7, TS_8 and TS_9, their influence on silk gland and cocoon characteristics being more or less similar. The cocoon yield/100 larvae was increased by 20.61%, 20.38% and 19.06% in treatments TS_7, TS_8 and TS_9, respectively, over the control treatment.

It has been well established that the silkworm (*Bombyx mori* L.) requires certain essential sugars, proteins, amino acids, fatty acids and vitamins for its normal growth, survival and also for the growth of silk gland and higher production of good-quality silk. The nutritional status of the mulberry leaves can be improved by enriching them with extra nutrients. Fortification of mulberry leaves with extra nutrients like glucose, glycine, egg albumin, molasses, etc., was found to increase the larval growth and improve cocoon characteristics[1,2].

Feeding the silkworms mulberry leaves fortified with 2% solution of casein or jaggery or urea in tap water did not improve any of the economic cocoon characteristics significantly[3]. Gridhar and Radha[4] reported that supplementation with 10 ppm glycine resulted in higher larval weight, shell weight, filament length compared to control. Alagumalai *et al.*[5] observed that fortification of mulberry leaves with the flours of black gram and red gram bran improved the larval growth and cocoon characteristics. Young larvae reared on 1.5% ascorbic acid enriched mulberry leaves resulted in higher silk length, weight and values[6].

The work was undertaken to study the biochemical composition of silk gland as well as the growth and cocoon production of silkworm *Bombyx mori* L. on feeding mulberry leaves supplemented with different nutrients.

The silkworm race BSRI-85/3 was selected for the study. Normal mulberry leaves were supplemented with different nutrient chemicals (variety BM-3) in the form of aqueous solution as follows:

TS_0 (control): Without supplement
TS_1 : Glycine (0.5%) + alanine (0.5%)
TS_2 : Soymilk
TS_3 : Milk powder (10%)
TS_4 : Sugar (10%)
TS_5 : Vitamin B (0.5%) + vitamin C (1%)
TS_6 : Potassium iodide salt (1%)
TS_7 : $TS_1 + TS_4 + TS_5 + TS_6$
TS_8 : $TS_2 + TS_4 + TS_5 + TS_6$
TS_9 : $TS_3 + TS_4 + TS_5 + TS_6$

Mulberry leaves supplemented with these nutrients in stated concentrations were fed to silkworms from fourth instar of larval stage till spinning since during this period the worms have a more vigorous growth with larger assimilation of nutrients. Worms were reared together in mass beds up to the second larval stage and after second-moult worms were separated in four batches (replication) of 100 worms for each treatment. Worms were fed with mulberry leaves smeared with aqueous solutions of above nutrients just before feeding, at the rate of 5–10 ml solution per 30–50 g mulberry leaves.

The weights of mature larvae were determined by taking the weight of 10 larvae just before spinning, and the other individual quantitative and qualitative cocoon characteristics were determined after the spinning following standard techniques as applied for the assessment of silk cocoons.

Silk gland was isolated in the fifth instar of larval stage. The fourth batch (replication) was used for this experiment. The fresh weight and dry weight of silk gland were estimated gravimetrically. The carbohydrate content[7], total lipids[8] and total proteins[9] were estimated.

Soymilk was prepared according to the methods of Khaleque *et al.*[10] and Sarker and Khaleque[11]. The rearing was conducted (in 1992) following the method of Krishnaswami[12] during the seasons S_1 (February–March), S_2 (May–June), S_3 (August–September) and S_4 (November–December). The temperature and relative humidity of the rearing room were, respectively, 27.30 ± 3.50°C and 67.60 ± 4.90%, 29.60 ± 3.70°C and 73.50 ± 7.30%, 28.40 ± 0.60°C and 84.30 ± 5.20%, 23.65 ± 3.20°C and 69.30 ± 4.10% in different seasons.

The chemical composition of silk gland and the growth of *Bombyx mori* larvae fed mulberry leaves supplemented with different nutrients were studied in four seasons. Table 1 presents the mean larval weight, silk gland weight and the chemical composition of silk gland under different treatments of supplementation over the four seasons. The highest larval weight (3.62 g/individual) and silk gland weight (778.75 mg/larva)

Table 1. Mean weight and chemical composition of silk gland fed on mulberry leaves supplemented with different nutrients

Treatment	Average larval weight (g)	Silk gland weight (mg/larva)	Chemical component of silk gland: Water (mg/g wt)	Total protein (mg/g dry wt)	Total lipid (mg/g dry wt)	Carbohydrate (mg/g dry wt)
TS_0	2 89e	572 50d	736 85a	269 73d	634.35a	6 02a
TS_1	3.30bc	695 00bc	719 26bc	313 13b	620 22abc	5 83ab
TS_2	3 37b	708 25b	718 17bc	314 71b	615 00bcde	5 81ab
TS_3	3 27bcd	681 25bcd	720 13bc	313 24b	616 01bcd	5 77ab
TS_4	3 09de	656.25bcd	725 45bc	282 34c	617 16bcd	5 84ab
TS_5	3 02e	649 50bcd	724 78bc	287 19c	621 22ab	5 72ab
TS_6	2.99e	627 50cd	726 42b	286 03c	619 41bc	5 71ab
TS_7	3 60a	760.75a	704 38d	342 20a	604 13de	5 42b
TS_8	3 62a	778 75a	700 48d	343 21a	606 27cde	5 39b
TS_9	3 60a	741 50a	701.08d	340 87a	601 82e	5 35b

Figure(s) followed by the same letter(s) are not significantly different at 1% level as per Duncan's new multiple range test

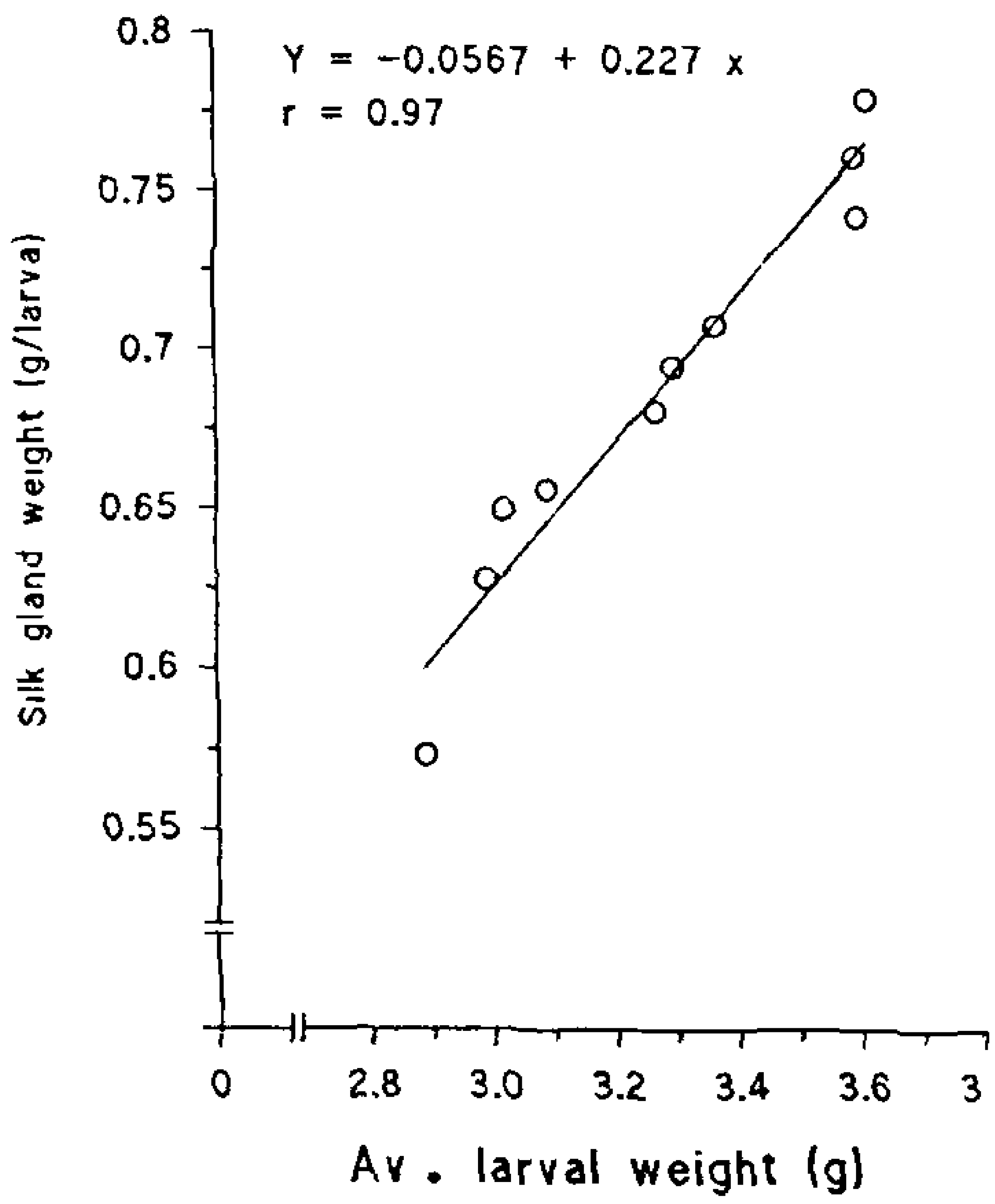

Figure 1. Mean larval weight as a function of silk gland weights

were observed on feeding mulberry leaves subjected to treatment TS_8, followed by TS_7 (3.60 g and 760.75 mg/larva, respectively) and TS_9 (3.60 g and 741.50 mg/larva, respectively). The protein content of silk gland increased while the water, total lipid and carbohydrate contents of silk gland decreased significantly due to supplementation of mulberry leaves with different nutrients. The highest total protein (343.21 mg/g) content of silk gland was noticed on feeding mulberry leaves subjected to treatment TS_8, while the water (736.85 mg/g), total lipid (634.35 mg/g) and carbohydrate (6.02 mg/g) contents silk gland were found to be highest in the control treatment (TS_0). It may be seen from the Table 1 that the values for larval weight, silk gland weight and chemical components of silk gland on feeding mulberry leaves subjected to treatments TS_7, TS_8 and TS_9 are more or less similar and did not vary significantly.

The mean larval weight was plotted against silk gland weight and the regression line was fitted by the method of least squares (Figure 1). From statistical analysis the correlation coefficient was found to be positive ($r = 0.97$), which indicates that as the larval weight increases the silk gland weight increases.

Mean cocoon characteristics under different treatments of supplementation over four seasons are presented in Table 2. It is seen from the table that single-cocoon weight, single-shell weight, cocoon yield and filament length increased significantly by feeding mulberry leaves supplemented with different nutrients. All the cocoon characteristics were found to show the

Table 2. Mean cocoon characteristics of BSRI-85/3 race fed on mulberry leaves supplemented with different nutrients

Treatment	Single-cocoon weight (g)	Single-shell weight (g)	Cocoon yield/100 larvae (g)	Filament length (m/cocoon)
TS_0	1 27f	0 19e	91 34f	684 00h
TS_1	1 30cde	0.22cd	103 16bcd	754 16cde
TS_2	1 31bcd	0 23bc	104 79b	758 50bcd
TS_3	1 30cde	0 22cd	103 45bc	745 75de
TS_4	1 28ef	0 21cde	99 99bcd	724 08f
TS_5	1 27f	0 20de	97.31de	713 08fg
TS_6	1 27f	0 20de	94 92def	700 58gh
TS_7	1 33ab	0 25ab	110 17a	776 00ab
TS_8	1 34a	0 26a	109 96a	779 42a
TS_9	1 32abc	0.25ab	108 75a	771.58abc

Figure(s) followed by the same letter(s) are not significantly different at 1% level as per Duncan's new multiple range test.

maximum improvement on feeding mulberry leaves subjected to treatment TS_8 except for cocoon yield, which was found to be highest for treatment TS_7. The lower cocoon yield in treatment TS_8 might be due to the higher mortality. However, no significant differences were observed between the treatments TS_7, TS_8 and TS_9 in respect of the cocoon characteristics of silkworm *Bombyx mori* L.

Supplementation with amino acids like tyrosine, phenylalanine and alanine improved the leaf quality of mulberry[13]. Sengupta *et al.*[1] reported that the silk content was more with 1% ascorbic acid in the diet of the silkworms. Babu *et al.*[6] observed that the first and second instar larvae reared on 1.5% ascorbic acid enriched mulberry leaves resulted in higher silk length, weight and denier values. Salts of potassium and calcium have been found to have significant effect on the growth of the silkworm. The addition of amino acids in low concentrations gave the best results regarding weights of larvae, percentage of silk gland, crude protein, cocoons and cocoon shells[14]. Beneficial effect of feeding sugars to larvae of the *Bombyx mori* silkworm was noted by Ito[15]. Fortification of mulberry leaves with the flours of black gram bran and red gram bran increased the larval weight, silk gland weight and improved cocoon characteristics like cocoon weight, shell length, cocoon length,cocoon width, shell ratio and also the reproductive parameters[5]. Observations made during this experiment corroborates these findings to a great extent. The supplementation with glycine + alanine + sugar + potassium iodide salt + vitamins (treatment TS_7), soymilk + sugar + potassium iodide salt + vitamins (treatment TS_8) and milk powder + sugar + potassium iodide salt + vitamins (treatment TS_9) significantly improved the larval weight, silk gland weight, chemical composition of silk gland and cocoon characteristics of *Bombyx mori* (Tables 1 and 2). Cocoon yield/100 larvae was increased by 20.61%, 20.38% and 19.06% with treatments TS_7, TS_8 and TS_9, respectively, over the control (no supplementation). These might be due to the presence of increased amounts of proteins/amino acids and other nutrients in mulberry leaves which contributed to the extra growth of the silkworm, thereby increasing the weights of larvae and silk gland and improving the cocoon characteristics of *Bombyx mori*.

There existed a positive correlation ($r = 0.77$) between silk gland weight and larval weight in the fifth instar larvae fed on mulberry leaves supplemented with different nutrients. Opender *et al.*[16] and Devaiah *et al.*[17] observed that the rate of increase in silk gland weight is proportional to the rate of increase in the body weight of silkworm. It has previously been reported that the ratio of the two rates was constant throughout the fifth instar[18]. The present study also confirms these results.

The significant decrease in the water, total lipid and carbohydrate contents and the increase in the total protein content in silk gland indicate the qualitative and quantitative improvement of silk gland due to feeding mulberry leaves supplemented with different nutrients, subsequently reflected in the improved cocoon characteristics of *Bombyx mori* L. However, further studies are needed on large-scale level before transfer of technology to the field.

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