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EFFECT OF PUPAL SOLUTIONS ON FEEDING BUDGET NITROGEN BUDGET AND WATER BUDGET OF MULBERRY SILKWORM, *BOMBYX MORI* L.

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Abstract

The purpose of this study is to investigate the effect of mulberry leaves enriched with pupal solutions of mulberry silkworm *Bombyx mori* L. (BM) and *Eligmma narcisus indica* (EN)on feeding budget, nitrogen budget and water budget of mulberry silkworm *Bombyx mori* L. Among the fortification agents (1% BM solution; 1% EN solution) 1% pupal solution of *B. mori* appears to have a comparatively a greater stimulatory effect on growth rate and protein efficiency ratio. Assimilation efficiency (ASe) increased 13%; 18%; 20%; 10% and 14% increase in 1% BM fortification over control. Efficiency of conversion of ingested food (ECI) increased by 84% in fifth instar larvae. Maximum increase in Efficiency of conversion of digested food or net growth efficiency by 66% and 54% was observed in 1% BM and 1% EN fortification. When compared to control 1% EN solution showed 11% increase in PEE. Significant increase in nitrogen content in body tissue by 89% and 68% was observed in 1% BM and 1% EN solutions respectively.

Key words: Pupal solution, 1% BM, 1% EN and Growth efficiencies.

Introduction

The pupae of silkworm *Bombyx mori* L. are mostly discarded after reeling as a waste; this bio waste is a reusable resource since it contains 48% proteins, necessary amino acid and vitamins. The larvae and pupae of *Eligmma narcisus* Cram cause damage to the Lucifer tree, *Ailanthus malabarica* DC a raw material for match industries. The pupal powders of these insects were supplemented with M5 mulberry leaves and fed to silkworm, *Bombyx mori* L. L x NB4D2. The efficacy of pupal powder supplementation on various parameters of feeding, nitrogen and water content was investigated.

Efficiency of conversion of digested food and Assimilation efficiency were found to be increased with fortification of mulberry leaves with glycine and SCP (Isaiarasu 1987 and Mathavan *et al* 1984). Mattson (1980) observed that nitrogen content of food influences the assimilation efficiency. Supplementation of animal protein greatly influences the assimilation and larval growth (Velpandi 1993). Nithukumari and J.P.Roy (2011) studied various parameters on feeding, consumption and utilization of mulberry leaves and its effects on growth pattern in mulberry silkworm and tasar silkworm.

Enriching the nutrient quality of mulberry leaves with extraneous nutrient and using them for feeding silkworm larvae is another approach towards attaining maximum larval growth and silk production. (Kumararaj *et al* 1972). Food consumption in the larvae of mulberry silkworm, *Bombyx mori* (L) depends largely upon the nutrient quality of mulberry leaves. It was observed that better growth and development of silkworm larvae as well as good quality of cocoons can be obtained when silkworms fed with leaves of ZincChloride and Zinc Sulphate (Kavitha *et al* 2012). Spirullina and Yeast significantly enhanced their effects on larval growth and the cocoon characters (Masthan *et al* 2011; Kumar & Balasubramaniyam 2013). Efficacy of the fortification of mulberry leaves with amino acid (Radjabi 2010) and folic acid, (Rahmathulla *et al* 2007) has been studied by earlier workers.

This study has been aimed to explore the possibility of recycling the silkworm pupae a bio-waste and employing pupae of insect pest as fortification agents in order to investigate the correlation between the biological value of protein or protein efficiency ratio and the economic character of silkworm. Moreover to find out a solution for the problem of shortage of mulberry leaves in the rainfed areas of the semi-arid zone by evolving a strategy of lowering the feeding rate without sacrificing the growth efficiencies and economic character. The pupae of silkworm, *Bombyx mori* L., are mostly discarded after reeling. This bio-waste is a usable resource since it contains 49% protein, 5% glycogen and 30% fat and many vitamins especially vitamin B_1 & B_2 (Bose & Majumder 1990). The larvae of *Eligmmanaricus*Cram causes damage to the lucifer tree *Ailanthus malabaricus*. DC, araw material for match industry. In this study an attempt has been made to find out the efficacy of pupal solutions as supplementation agent on feeding budget, nitrogen budget and water budget.

Materials and Methods

The bioenergetic components, such as, food utilization and efficiencies were measured using standard gravimetric method of Waldbauer (1968). For the computation of the processes and efficiencies of food utilization, the modified IBP formula of Petrusewicz and Mac Fadyen (1970) was used, using Monopan balance (Dhona, 100Ds, India, Accuracy ± 0.01mg).

The amount of protein present in the normal and fortificated mulberry leaves was estimated following Lowery's method (Lowery *et al* 1951).

From the values of protein, the corresponding nitrogen content was computed using the conversion factor, i.e., the value was divided by 6.25

The biological value of protein and protein efficiency ratio were also calculated (Please vide the feeding budget)

The gain weight of larvae per unit weight of protein consumed was measured and value thus obtained is the protein efficiency ratio (Gopalan *et al* 1980).

The insect selected for the present study is the mulberry silkworm, *Bombyx mori* L. (Hybrid L x NB_4 D_2 local multivoltine race x Nandini bivoltine race).

The egg card containing the eggs of *B. mori* L. hybrid L x NB_4 D_2 at the rate of Rs. 2.10 (|D*f*|D*f* = disease free laying) was collected from the Government Grainage Centre, Sericulture Station, Nannagaram, Tenkasi, Tamilnadu. The number of eggs per laying was approximately 400. Kanva – 2 (M_5) mulberry leaves were procured from the Model Mulberry Plantation Unit and Silkworm Rearing Unit Injar, Sivakasi, Tamil nadu.

The pupae were dried in a hot air oven set at 70°C and were powdered using mortar and pestle. The powdered pupae were stored in a dessicator. This powder dissolved in 100ml of distilled water was treated as 1% pupal solution. This pupal solution was applied evenly on bothsides of the mulberry leaves using synthetic sponges. The coated leaves were then air dried and stored in a cool place and chopped just prior to feeding.

The various feeding parameters like Consumption (C), Assimilation (A) and Production (P) and Metabolism (M) were studied in terms of mg dry wt/larva. Followed by the above said parameter rates of Consumption (Cr), Production (Pr), Defaecation (Fr) and Assimilation (Ar) and Metabolism (Mr) were calculated.

Food utilization efficiencies like Approximate Digestibility (AD) or Assimilation efficiency (Ase), Reference Ratio (RR), Protein extraction efficiency (PEE) were also calculated. Growth efficiency parameter of Efficiency of conversion of Ingested food (ECI), Efficiency of conversion of digested food (ECD) and Assimilation efficiency of N_2 were calculated. The results were analysed statistically by using ANOVA software.

Results

Table: 1 Nutritive analysis of Control and fortificated mulberry leaves.

Parameter	Control	1%BM	1%EN
Protein Content (mg/g)	119	182 (53)	163 (37)
Nitrogen Contents (mg/g)	19.04	29.12 (53)	26.08 (37)
PER	1.9	2.5 (31)	2.2 (16)
BV (%)	63	79 (25)	70 (11)

Table: 2 Effect of pupal solution of *Bombyx mori* and the *Eligmma narcisus* on the feeding budget of the larvae of mulberry silkworm

Parameter and Instar	Control Mean± SD	1% BM Mean ±BD	1% EN Mean ± SD
Consumption			
I	0753 14	0712 4 (-6)	0722 12 (-4)
II	0967 15	0907* 17 (-6)	0919 9 (-5)
III	1060 10	0996* 12 (-6)	1014 29 (-4)
IV	1966 38	1905 53 (-8)	1921 49 (-5)
V	4680 75	4335 51 (-7)	4449 41 (-5)
Defaecation			
I	0456 18	0367* 2 (-19)	0421 17 (-8)
II	0584 26	0486* 19 (-16)	0521* 21 (-11)
III	0612 32	0496* 38 (-19)	0518 53 (-15)
IV	1148 53	1042* 61 (-14)	1080 75 (-6)
V	2763 85	2360* 105 (-14)	2446 112 (-11)
Assimilation			
I	0293 6	0312 9 (6)	0304 9 (4)
II	0386 16	0418 10 (8)	0402 17 (4)
III	0446 21	0505* 13 (13)	0493* 11 (10)
IV	0823 16	0876* 9 (6)	0843 12 (3)
V	1921 85	2029 28 (16)	2001 32 (4)
Production			
I	086 13	106 16 (23)	098 13 (13)
II	111 6	133* 6 (19)	126 4 (-14)
III	127 17	185* 10 (46)	162* 26 (27)
IV	195 6	292 13 (49)	253 16 (29)
V	226 33	408* 57 (80)	383* 56 (09)
Metabolism			
I	0209 15	0204 20 (-2)	0206 24 (-1)
II	0280 22	0283 10 (-1)	0276 34 (-1)
III	0323 49	0320 35 (-1)	0331 42 (2.5)
IV	0620 56	0584 65 (-3)	0590 67 (-5)
V	1695 49	1621* 26 (-4)	1618 34 (-4)

All Values are expressed in mg dry wt/larva.

Each value represents the average of 45 individuals.

Positive/ negative sign within the parentheses.

indicate the percentage of increase / decreases fromthe control.

*Significance at $p < 0.05$ level ('t'-test)

Roman numerals indicate larval instars.

Table: 3 Effect of pupal solution of *Bombyx mori*and *Eligmma narcisus* on the efficiency of food utilization of the larvae of mulberry silkworm

Parameter and Instar	Control Mean ± SD	1% BM Mean ± BD	1% EN Mean ± SD
ASe of AD			
I	38.91 3.4	43.82* 1.2 (3)	42.11 1.0 (8)
II	39.92 1.0	46.09 1.5 (8)	43.74 4.0 (9)
III	42.06 0.7	50.70* 0.7 (20)	48.62 6.4 (16)
IV	41.86 2.8	45.98 3.0 (10)	43.98 2.0 (5)
V	41.05 1.9	46.81 2.1 (14)	44.98 1.9 (9)
RR			
I	1.5 0.03	1.8* 0.02 (20)	1.7 0.02 (13)
II	1.6 0.04	1.8* 0.06 (12)	1.7 0.01 (6)
III	1.7 0.01	1.9* 0.02 (12)	1.8 0.02 (6)
IV	1.7 0.09	1.9* 0.01 (12)	1.8 0.01 (6)
V	1.7 0.04	2.0* 0.04 (18)	1.9 0.02 (12)
PEE & NEE			
I	5 0.62	14* 0.82 (180)	11* 0.82 (120)
II	8 0.92	18* 0.82 (125)	16* 1.3 (100)
III	14 0.48	34* 1.6 (142)	28* 1.5 (100)
IV	35 0.82	43* 2.0 (23)	40* 1.1 (14)
V	64 1.9	82* 1.6 (30)	79 1.9 (11)
EEE V	33	38 (15)	36 (9)
ECI			
I	11.37 1.0	15.48* 1.0 (36)	13.18 4.2 (13)
II	11.5 0.9	15.46* 0.8 (34)	14.25* 3.8 (24)
III	12.0 1.9	18.50* 1.6 (54)	16.95 2.0 (41)
IV	10.7 1.5	15.00* 4.1 (53)	13.00 5.0 (21)
V	5.6 1.9	10.32* 0.8 (84)	9.42 1.6 (68)
ECD			
I	29.0 0.04	35.00* 1.00 (21)	31.05* 6.30 (7)
II	29.53 0.69	33.65 0.38 (14)	31.00 0.48 (5)
III	28.65 1.00	36.63* 2.90 (28)	32.54 5.40 (13)
IV	24.87 0.80	33.88* 0.50 (36)	30.01 1.00 (21)
V	12.5 1.60	20.84 0.80 (66)	19.20 1.90 (54)

Except RR, other values are expressed in %

Each Value represents the average of 45 individuals.

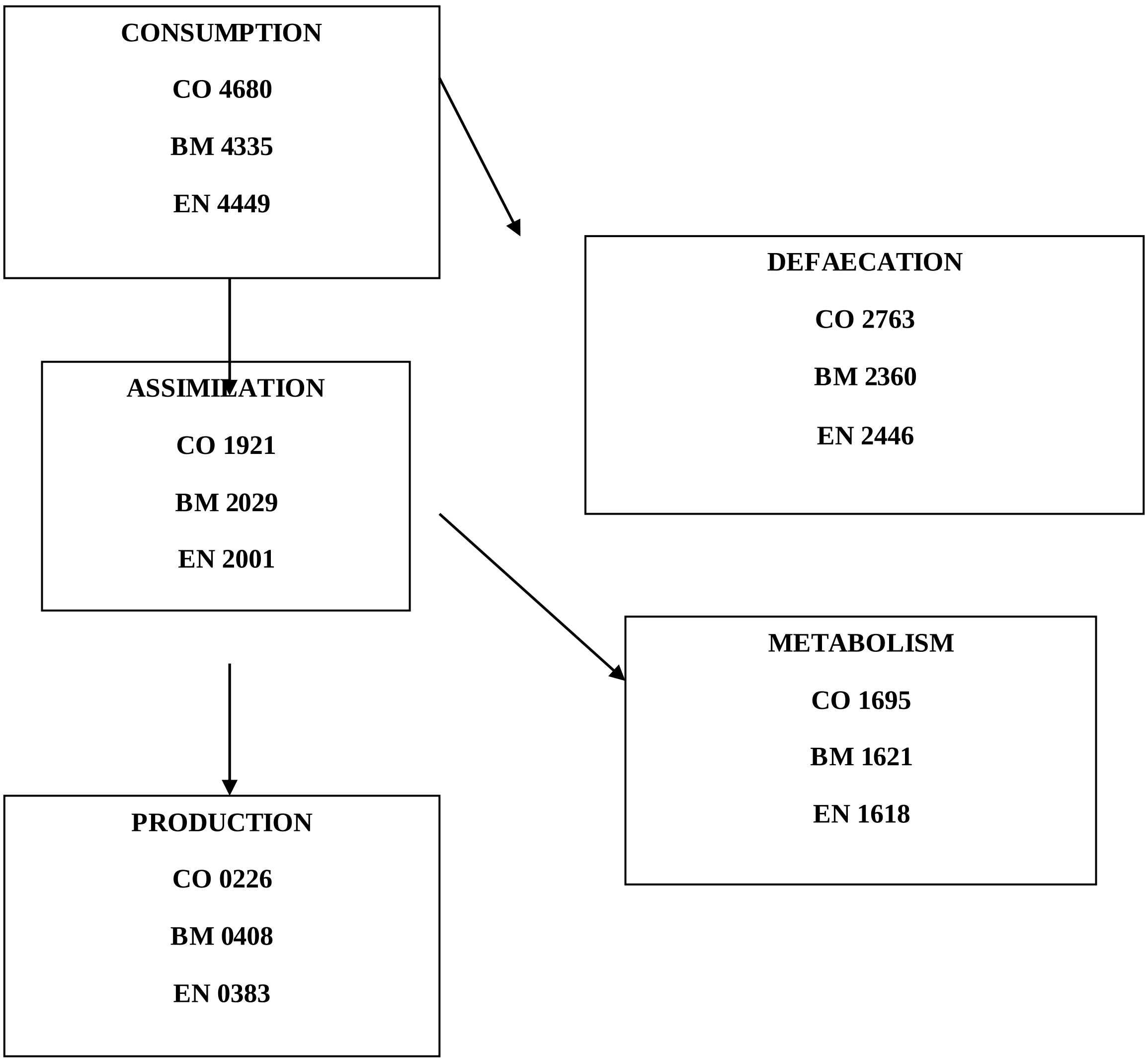
Positive/negative sign within the parentheses

Indicates the percentage increase / decrease from the control

*Significance at $p< 0.05$ level ('t'- test) Roman numerals indicate larval instars.

Values within the parentheses indicate the percentage of increase from the control values.

Fig 1 FEEDING BUDGET



Values Expressed in mg/dry wt/ larva

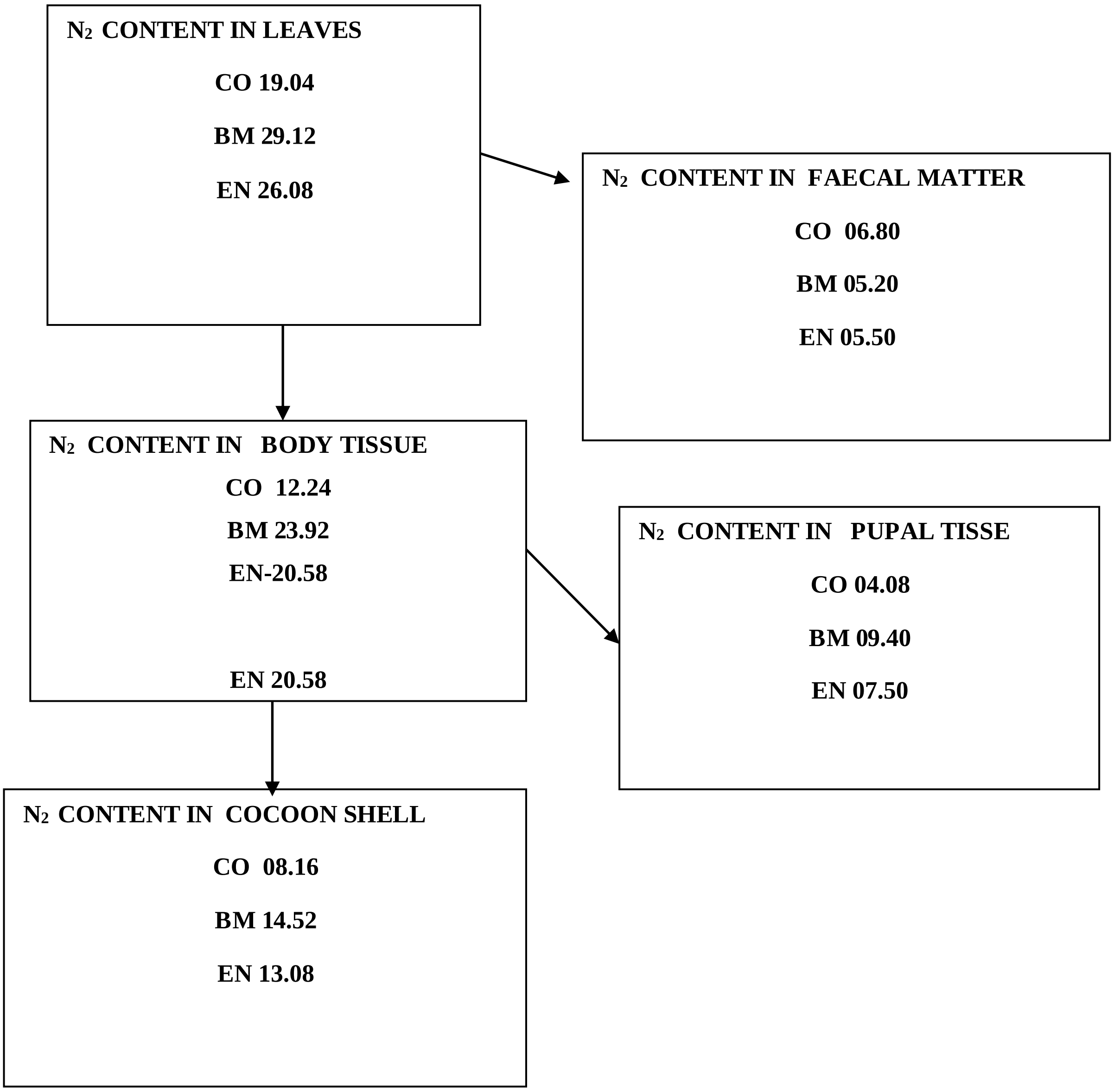
Key

CO – Control

BM- Pupal solution of *Bombyx mori*

EN- Pupal solution *of Eligmmanarcisus*

Fig 2 NITROGEN BUDGET



Values Expressed in mg/g

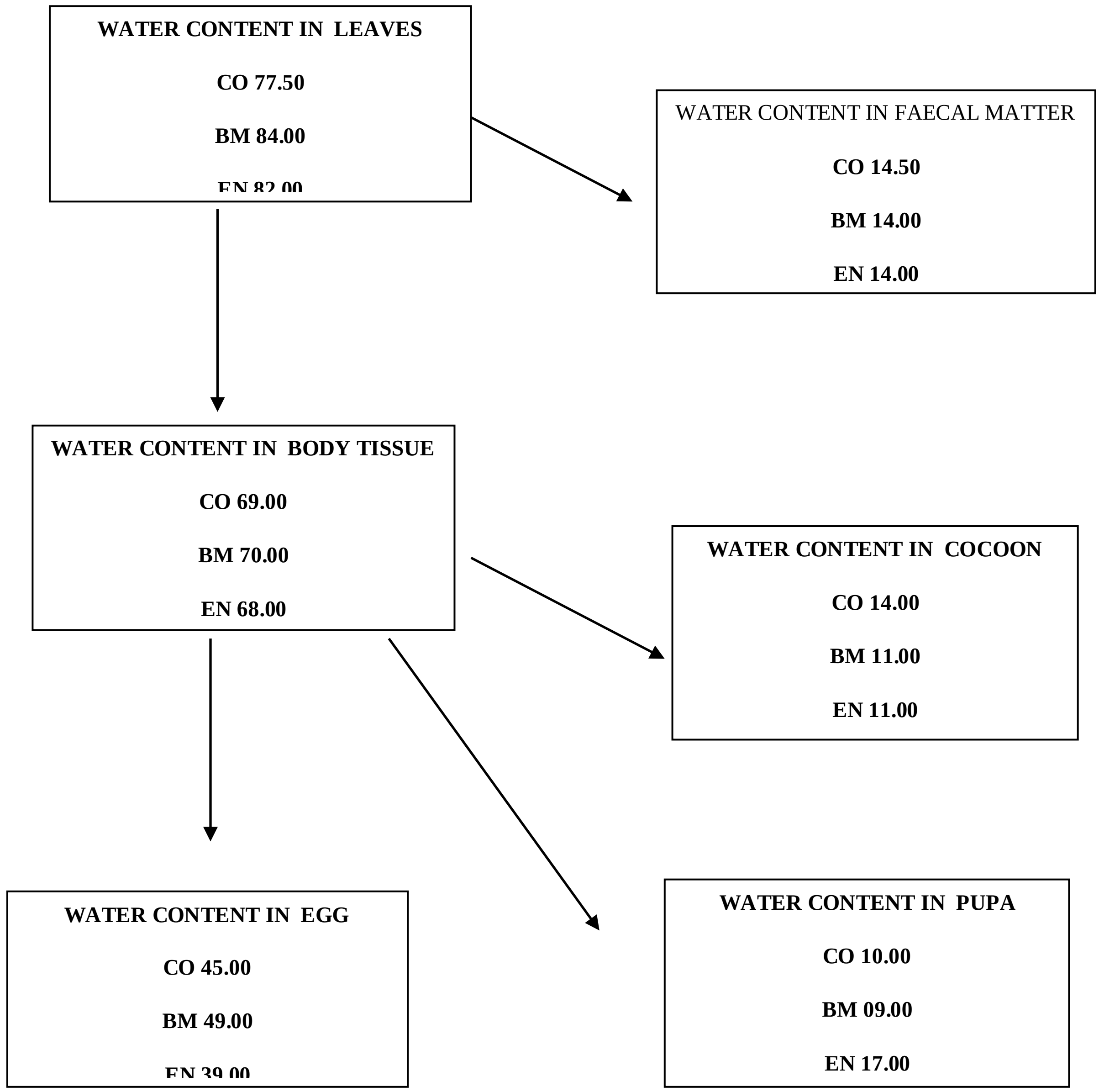
Key : N_2 -Nitrogen,

CO – Control

BM- Pupal solution of *Bombyx mori*

EN- Pupal solution*of Eligmmanarcisus*

Fig 3 WATER BUDGET



Values Expressed in mg/g

Key :

CO – Control

BM- Pupal solution of *Bomby x mori*

EN- Pupal solution of *Eligmmanarcisus*

In the present study the results indicate the impact of pupal solutions on various feeding parameters of silkworm *Bombyx mori* L. The data regarding the use of pupal solution presented in Table 2 and 3.

The protein content was found to be 119 mg/g in normal leaves, 182 mg/g in 1% BM and 161 mg/g in 1% EN fortified leaves. Protein efficiency ratio (PER) was found to be increased namely 2.5% in 1% BM and 2.2% in 1% EN fortification. The biological value (BV) of protein found to be high in all fortified leaves over control (Table 1).

Consumption was found to be decreased in test larvae over control (Table 2). The defaecation showed statistically significant reduction at $p<0.05$ level in 1% BM fortification over control. Statistically significant increase in production or growth by 23%; 19%; 46% and 80% was recorded in respective larval instars in 1% BM solution. There was reduction in metabolism by 4% in 1% BM (Table 2)

Assimilation efficiency (Ase) increases in 1% BM fortification over control (Table 3). There was statistically increase in Reference Ratio (RR) at $p<0.05$ level in 1% BM fortification sets. There was increase in Protein extraction Efficiency (PEE) by 11% in 1% EN fortification. A maximum increase in Efficiency of conversion of ingested food (ECI) was observed by 84% in fifth instarslarvae. Efficiency of conversion of digested food (ECD) or net growth efficiency was observed to be increased in 1% BM and 1% EN fortification. Significant increase in nitrogen content in body tissue by 89% and 68% in 1% BM and 1% EN respectively over control. Water content of control and fortificated mulberry leaves was estimated and illustrated in Fig 3.

Discussion

The results of present study reveal that pupal solution of *Eligmma narcisus* (EN) and *Bombyx mori (*BM) are capable of improving the food utilization. However 1% BM solution is found to have a greater stimulatory influence than that of *E. narcissus* (EN).

Fortification of mulberry leaves by using supplementary nutrients and feeding to the silkworm is a useful modern technique to increase economic value of cocoon character (Etabari 2004). In this study, PER and BV in 1% BM appear to have greatly contributed to the escalation of growth efficiencies. Muthukrishnan and Pandian1987 have reported that extraneous nitrogen in the supplemented leaves can reduce the feeding rate of the insects; this is a desirable feature, especially in the semiarid zone where there is poor yield of mulberry leaves. The increased assimilation may be a tactic of the larvae to compensate the reduction in consumption.

Protein extraction efficiency (PEE) also seems to influence ECI and ECD. This givessupporting evidence to the findings that the larvae of mulberry silkworm are endowed with compensatory tactics or modulatory effects to overcome the environmental stress.

Hori and Watanabe (1986) have reported that silkworm larvae have a efficiency of nitrogen utilization when compared to other species of insects. Particularly during the final instar stage, nitrogen in food ingested is utilized very efficiently for the formation of silk protein. Water content on the food has also a significant influence on the approximate digestibility or assimilation efficiency (Muthukrishnan & Pandian1987) which is a yard stick for food utilization efficiency. The extraneous nitrogen in the form of animal protein has significant effect on the various parameters on feeding and larval growth pattern. Thus budget studies give an overall impact of fortification effect on the mulberry silkworm.

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