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Consumption indices in silkworm as influenced by feeding of transgenic mulberry

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Abstract
Mulberry (*Morus indica* L.) is the sole food plant of the silkworm (*Bombyx mori* L.) and has been exploited commercially in the sericulture industry. Consequently, a major determining factor for enhancing the production of silk is to produce qualitative mulberry foliage that can adapt to different climatic conditions. The present paper aims to know the feeding efficiency of V instar silkworm (PM×CSR2) to find out the quantity of leaf required to produce the silk furthermore, conversion rate of ingested food by the silkworm, so that we can analyse the distribution rate of mulberry intake in silkworm for various factors like growth rate, silk production and fecal matter. The study revealed that Food consumption was significantly different only at the end of fifth instar namely on day 5 to day 7. However, Consumption index was significantly different on day 2, whereas efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were significantly different on day 7 and approximate digestibility (AD) and reference ratio (RR) were significantly different on day 4. Performance of silkworms fed with transgenic mulberry leaves with respect to food consumption and its utilization for growth reveals that these transgenic mulberry lines were effectively utilized by the silkworm as was V1.

Keywords: Mulberry, Vth instar silkworm (PM×CSR2), Food consumption, CI, ECI, ECD, AD, RR

Introduction
Mulberry (*Morus indica* L.) is the sole food plant of the silkworm (*Bombyx mori* L.)and has been exploited commercially in the sericulture industry. Consequently, a major determining factor for enhancing the production of silk is to produce qualitative mulberry foliage that can adapt to different climatic conditions. It has been estimated that various a biotic stress conditions, such as moisture, salinity and alkalinity, can cause yield losses in mulberry of between 50 and 60% (Rao 2002) [7]. In this context, targeted manipulation of a biotic stress tolerance traits was achieved in an earlier study by developing transgenic mulberry plants over expressing stress responsive genes (VR-Rd29A::*HVA1*, ST-CaMV35S::*HVA1*, BT-CaMV35S::*bch1*, *MT*-CaMV35S::*Osmotin*,RD-Rd29A::*Osmotin).*
Nutritional indices helps to find out the quantity of leaf required to produce the silk. It also helps to study conversion rate of ingested food by the silkworm, so that we can analyze the distribution rate of mulberry intake in silkworm for various factors like growth rate, silk production and faecal matter. The consumption indices were estimated daily during V instar. The consumption indices were computed on fresh weight basis by gravimetric method (Waldbauer, 1968) [8]. Maribashetty *et al*. (1999) [1, 3] studied the food utilization in last two instars of newly evolved multi voltine breeds such as MH1and MH2. These two races have higher assimilation and conversion rate to body and shell ratio compare to control, *i.e.*, PM. According to Maribashetty *et al*. (2001) [4], the new bivoltine hybrids of silkworm viz., NTCM×CTM and CTM×NTCM showed decrement of larval duration by two days and increment in food conversion efficiencies (K1 and K2) when compared to control, NB4D2×KA. Rahmathulla *et al*. (2002) [6] studied the nutritional indices and nutritional efficiency parameters of V instar larvae of newly evolved bivoltine hybrids, CSR2×CSR4, CSR2×CSR5 and CSR18×CSR19 under varied environmental conditions. The nutritional indices like ingesta, AD and utilization efficiency of these hybrids were more during un favorable environmental conditions. The present investigation includes to study the feeding efficiency of silkworm PM ×CSR2 on transgenic mulberry during V instar silkworms.

Material and Methods

Location and plant materials used in this study

All experiments were conducted in green house/ containment facility of the Department of Crop Physiology, and Department of Sericulture, University of Agricultural Sciences (UAS) GKVK campus, Bengaluru, Karnataka. The site is situated at 12º 58' North latitude, 77º 35' East longitudes and an altitude of 930M above Mean Sea Level (MSL). The plant species used for the study were transgenic plants of *Morus indica* L. and cultivated mulberry genotype V1. All the transgenic materials (VR series- Rd29A::*HVA;* ST series- CaMV35S::*HVA1;* BT series-CaMV35S::*bch1;* MT series- CaMV35S::*Osmotin* and RD series- Rd29A::*Osmotin*) were developed at the University of Delhi, South Campus, New Delhi and transferred to the Department of Crop Physiology, UAS, Bengaluru as per the Department of Biotechnology, Government of India guidelines.

Treatments detail

Five selected mulberry transgenic lines developed at University of Delhi, South campus, New Delhi and a non-transgenic mulberry, V1 maintained at Department of Crop Physiology, University of Agricultural Sciences, Bengaluru, was used for the study and the details are as follows. Silkworm rearing with Transgenic Mulberry line-1 (T1), Silkworm rearing with Transgenic Mulberry line-2 (T2), Silkworm rearing with Transgenic Mulberry line-3 (T3), Silkworm rearing with Transgenic Mulberry line-4 (T4), Silkworm rearing with Transgenic Mulberry line-5 (T5), Silkworm rearing with Mulberry non-transgenic variety V1/Control (T6) These transgenic lines were maintained under confined conditions. The rearing was conducted during April and May 2017. The cross breed silkworm, PM×CSR2 was utilized for the bioassay. Six treatments comprising of five transgenic mulberry lines and V1 variety (non-transgenic line) were evaluated in four replications with 30 silkworms in each replication. The spacing for worms and other rearing requirements were adopted as recommended by Dandin *et al*. (2003) [2].

Consumption indices comprising food consumption, food digestion, consumption index (CI), growth rate (GR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), approximate digestibility (%) and reference ratio (RR) when determined during the fifth instar by feeding transgenic mulberry leaves were similar to that observed by feeding V1 mulberry variety.

Determination of Food consumption (g)

Weight of fresh food ingested (g) = A - B

Where, A= Weight of fresh leaves offered to larvae (g) and B = Weight of fresh remnants (g)

$$\text{Weight of remnants (g)} = \frac{\text{Fresh weight of aliquot (g)} \times \text{Weight of dry remnants (g)}}{\text{Oven dry weight of aliquot (g)}}$$

Determination of Food digestion (g)

Weight of fresh food digested (g) =Weight of fresh Leaves ingested (g) - Weight of fresh excreta produced (g)

Determination of Consumption index (CI)

Consumption Index explains in a nutshell about the rate at which nutrients enter into digestive system. This is expressed as consumption per mean larval body weight per day.

$$CI = \{(F) / (T*A)\}$$

Where, F- Fresh (or) dry weight of food eaten (g) T- Duration of feeding period (days)

A- Mean fresh (or) dry weight of larvae during feeding period (g)

$$\text{Mean weight} = \frac{\text{Initial larval body weight (g)} + \text{Larval weight after 24 h (g)}}{2}$$

Determination of Growth rate

Growth rate explains how much of fresh/ dry matter increased in the body of the larvae per day per gram of bodyweight.

$$GR = \{(G) / (T*A)\}$$

Where, G - Fresh weight gain of larvae during feeding period (g) T - Duration of feeding period (day)

A – Mean larval weight (g)

Weight gain of larvae (g) = Initial larval weight – Larval weight after 24 h (g)

Determination of Efficiency of conversion of ingested food (%)

The efficiency of conversion of ingested foods (ECI) is an overall measure of an insects ability to utilize for growth the food which it ingests. It was estimated using the formula,

$$ECI (\%) = \{(GR) / (CI)\}$$

Where, GR= Growth rate CI= Consumption indices

Determination of Efficiency of conversion of digested food (%)

The efficiency with which digested food is converted to body substance (ECD) was calculated by the formula,

$$ECD (\%) = \frac{\text{Weight gained (g)}}{\text{Weight of food ingested}} \times 100$$

Determination of Approximate digestibility (%)

The approximate digestibility (AD) was calculated by the formula,

$$AD (\%) = \frac{\text{Weight of food digested}}{\text{Weight of food ingested}} \times 100$$

Determination of Reference ratio

The reference ratio is an indirect expression of absorption and assimilation of food. It is also express ingesta required per unit excreta production.

$$RR = \frac{\text{Ingesta (g)}}{\text{Faecal matter (g)}}$$

Result and Discussion

Food Consumption / Ingestion (g/30worms)

The quantity of leaf consumption on fresh weight basis increased from first day to fifth day and there was a slight decrease in consumption on seventh day among transgenic mulberry lines and V1 variety (Table 1 and Fig. 1). The food consumption showed non- significant differences from day 1 to day 4 and significant differences from day 5 to day 7 among the transgenic lines and control (V1). Significantly highest food consumption on day 5 and day 6 was recorded in VR series (67.94 g/30 worms and 61.61 g/30 worms, respectively) while on day 7 highest food consumption was observed in BT series (49.67g/30 worms).

Food Digestion (g/30worms)

The food digestion was observed to increase from first day to fifth day and thereafter it decreased among transgenic lines and control (V1) on fresh weight basis (Table 2 and Fig. 2). No significant difference was noticed with respect to food digestion on any of the days of fifth instar. However higher food digestion was observed in MT series on day 1, (20.37 g/ 30 worms), in BT series from day 2 to day 3 (22.11, 31.52 and 47.51 g/30 worms, respectively), in VR series on day 5 and day 6 (53.88 and 48.78 g/30 worms, respectively) and in V1 on day 7 (38.20 g/ 30worms).

Consumption Index (CI)

The consumption index in the fifth instar larvae showed non-significant differences on all the days except on second day when fed with leaves of transgenic mulberry and V1 (Table 3 and Fig. 3). Significantly highest food consumption index of 1.07 was observed on second day in the worms fed with leaves of BT series followed by those fed with V1 mulberry leaves (1.01).

Growth Rate (GR)

The growth rate in the fifth instar silkworms showed non-significant differences when fed with mulberry leaves of transgenic lines and V1 variety. However the growth rate decreased gradually from first day to seventh day among the silkworms fed with the leaves of both transgenic lines and the control V1 variety (Table 4 and Fig. 4). The larvae fed on BT series recorded higher growth rate on day 1 (0.35) followed by V1 (0.33) and MT series (0.30), which did not differ significantly from each other and gradually decreased to 0.08 in BT series, 0.07 in V1 and 0.10 in MT and RD series on day 7.

Efficiency of conversion of ingested food (ECI) (%)

The ECI, *i.e.*, the amount of ingested food converted into body substance was found to be highest first day of fifth instar and thereafter gradually decreased as the age of the larvae advanced when fed with all transgenic mulberry lines and control V1 (Table 5 and Fig. 5). No significant difference was observed with respect to ECI from first to fifth day while significant difference was observed on seventh day, where in the worms fed on MT series (15.90) recorded higher ECI followed by RD series (14.54) and BT series (12.53).

Efficiency of conversion of digested food (ECD) (%)

The influence of transgenic mulberry on efficiency of conversion of digested food of silkworm hybrid (PM× CSR2) is presented in table 6 and fig. 6. No significant difference was noticed for ECI from day 1 to day 6 among the worms fed with the leaves of transgenic mulberry and the control V1. However, the significant difference was observed on the seventh day, where in, silkworms fed with MT series leaves showed maximum ECD (21.01%) and least ECD was noticed when fed the leaves of ST series (7.48 %).

Approximate digestibility (%)

Among different days of fifth instar, there was no significant difference for approximate digestibility (AD) (Table 7 and Fig. 7) among the silkworms fed with leaves of transgenic mulberry and the wild type expect on fourth day. However, the AD was significantly maximum on the fourth day when silkworm hybrids were reared on RD series (84.46 %) followed by ST (83.51 %) and BT series (81.94 %).

Reference ratio (RR)

Reference ratio of silkworm hybrid PM×CSR2 reared on transgenic mulberry lines and wild variety during fifth instar showed non-significant differences among the lines tested on all the days except on fourth day (Table 8 and Fig. 8). Significant difference was noticed on the fourth day of fifth instar with highest RR noticed in RD series (6.53) followed by ST series (6.08) and BT series (5.61). The least RR was observed when leaves of VR series (4.62) were used for feeding the silkworms. Consumption indices are known to differ between male and female sexes (Ahmed *et al*., 1999), between multivoltine and bivoltine silkworm races (Maribashetty *et al.*, 1999; Mariba shetty *et al.*, 2001) [4, 1, 3] and with different feeding regimes (Meenal *et al*., 2001) [5]. In the present study consumption indices determined for transgenic mulberry lines along with V1 variety did not show any significant differences thus supporting the view that these transgenic mulberry lines were nutritious to silkworms.

Conclusion

The food consumption showed non-significant differences from day1 to day 4 and significant differences from day 5 to day7 among the transgenic lines and control (V1). Significantly highest food consumption on day 5 and day 6 was recorded in VR series. However, higher food digestion was observed in MT series on day 1, in BT series from day 2 to day 3, in VR series on day 5 and day 6 and in V1 on day. Significantly highest food consumption index of 1.07 was observed on second day in the worms fed with leaves of BT series followed by those fed with V1 mulberry leaves.

Acknowledgment

This work is supported by the Department of Biotechnology, Ministry of Science and Technology, Govt. of India (Ref. No. T/PR/1558/PBD/19/226/22012).

Table 1: Food consumption in V instar larva of silkworm, PM × CSR2 as influenced by transgenic mulberry lines on fresh weight basis (g/30worms)

Transgenic / non- transgenic lines	5th Instar						
	day 1	day 2	day 3	day 4	day 5	day 6	day 7
VR SERIES	22.05	24.66	41.20	49.31	67.94^{c}	61.61^{d}	41.02^{c}
ST SERIES	21.11	22.46	32.89	50.98	56.52^{abc}	55.73^{abcd}	41.51^{cd}
BT SERIES	24.06	28.65	41.21	58.00	61.59^{bc}	49.67^{abc}	49.67^{e}
MT SERIES	25.34	22.84	37.38	48.50	53.67^{ab}	48.70^{ab}	35.11^{a}
RD SERIES	21.40	20.41	32.28	50.41	57.19^{abc}	45.45^{a}	36.99^{ab}
V1	21.18	25.30	35.66	44.57	60.61^{d}	44.36^{a}	46.58^{e}
F TEST	NS	NS	NS	NS	*	*	*
Sem±	3.30	1.85	2.23	3.00	4.36	3.63	3.11
CD at 5%	-	-	-	-	12.95	10.79	9.25

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par

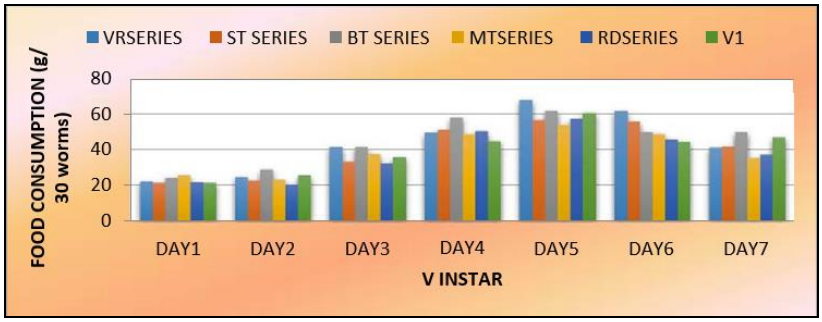

Fig 1: Food consumption in V instar larva of PM × CSR_2 silkworm hybrid as influenced by transgenic mulberry lines on fresh weight basis (g/30worms)

Table 2: Food digestion in V instar larva of silkworm, PM× CSR2 as influenced by transgenic mulberry lines on fresh weight basis (g/30worms)

Transgenic / non-transgenic lines	5th Instar						
	day 1	day 2	day 3	day 4	day 5	day 6	day 7
VR SERIES	16.67	18.24	32.34	38.64	53.88	48.78	30.90
ST SERIES	17.76	17.45	26.91	42.57	45.65	45.19	33.49
BT SERIES	18.73	22.11	31.52	47.51	49.59	39.28	31.62
MT SERIES	20.37	16.87	28.49	38.16	40.84	39.67	26.61
RD SERIES	17.28	15.89	25.67	42.63	46.85	37.89	30.06
V1	16.73	19.50	28.32	35.99	51.89	46.85	38.20
F TEST	NS	NS	NS	NS	NS	NS	NS
Sem±	3.26	1.62	2.09	2.67	3.36	3.58	2.75
CD at 5%	-	-	-	-	-	-	-

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par.

Fig 2: Food digestion in V instar larva of PM× CSR2 silkworm hybrid as influenced by transgenic mulberry lines on fresh weight basis (g/30worms)

Table 3: Consumption Index in V instar larva of silkworm, PM × CSR2 as influenced by transgenic mulberry lines on fresh weight basis

Transgenic / non-transgenic lines	5th Instar						
	day 1	day 2	day 3	day 4	day 5	day 6	day 7
VR SERIES	1.11	0.97^{abc}	1.27	1.19	1.33	1.06	0.67
ST SERIES	1.13	0.92^{ab}	1.09	1.32	1.19	1.05	0.73
BT SERIES	1.19	1.07^{c}	1.23	1.37	1.19	0.85	0.64
MT SERIES	1.31	0.92^{ab}	1.22	1.27	1.15	0.94	0.63
RD SERIES	1.05	0.81^{a}	1.03	1.26	1.21	0.87	0.64
V1	1.12	1.01^{bc}	1.16	1.11	0.91	1.15	0.80
F TEST	NS	*	NS	NS	NS	NS	NS
Sem±	0.17	0.05	0.07	0.07	0.09	0.08	0.05
CD at 5%	-	0.16	-	-	-	-	-

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par

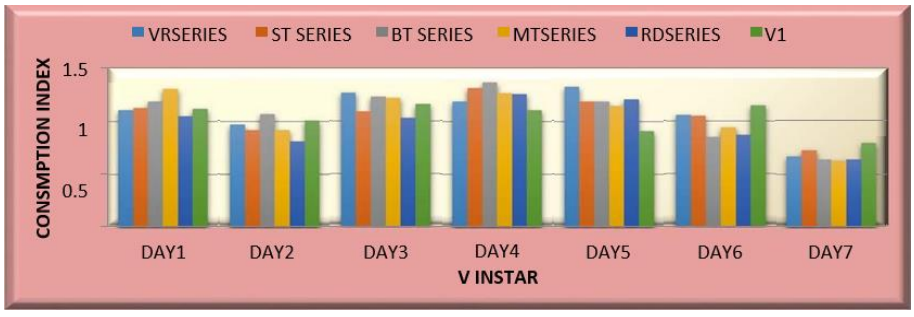

Fig 3: Consumption Index in V instar larva of PM × CSR2 silkworm hybrid as influenced by transgenic mulberry lines on fresh weight basis

Table 4: Growth rate in V instar larva of silkworm, PM × CSR2 as influenced by transgenic mulberry lines on fresh weight basis

Transgenic / non-transgenic lines	5th Instar						
	day 1	day 2	day 3	day 4	day 5	day 6	day 7
VR SERIES	0.30	0.21	0.26	0.24	0.17	0.08	0.04
ST SERIES	0.23	0.26	0.18	0.30	0.14	0.09	0.04
BT SERIES	0.35	0.22	0.23	0.24	0.21	0.10	0.08
MT SERIES	0.30	0.22	0.20	0.25	0.16	0.06	0.10
RD SERIES	0.27	0.19	0.24	0.24	0.12	0.08	0.10
V1	0.33	0.23	0.22	0.26	0.13	0.09	0.07
F TEST	NS	NS	NS	NS	NS	NS	NS
Sem±	0.03	0.03	0.03	0.03	0.03	0.01	0.02
CD at 5%	-	-	-	-	-	-	-

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par

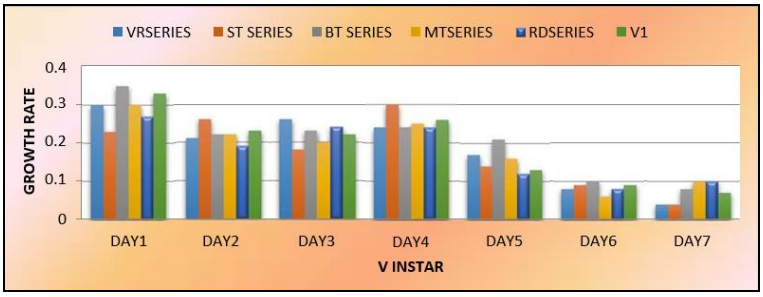

Fig 4: Growth rate in V instar larva of PM × CSR_2 silkworm hybrid as influenced by transgenic mulberry lines on fresh weight basis

Table 5: Efficiency of conversion of ingested food in V instar larva of silkworm, PM×CSR2 as influenced by transgenic mulberry lines on fresh weight basis (%)

Transgenic /non-transgenic lines	5thInstar						
	Day 1	day 2	day 3	day 4	day 5	day 6	day 7
VRSERIES	27.35	21.92	20.76	20.56	13.07	7.74	5.98^{a}
STSERIES	24.06	28.19	16.18	22.99	11.45	9.09	5.99^{ab}
BTSERIES	30.13	20.34	18.41	18.51	17.11	11.56	12.53^{abcd}
MTSERIES	22.87	23.97	16.34	19.90	14.00	6.05	15.90^{d}
RDSERIES	27.80	23.76	23.25	19.17	10.30	9.01	14.54^{cd}
V1	29.33	23.18	20.36	23.30	14.47	8.53	8.67^{abc}
FTEST	NS	NS	NS	NS	NS	NS	*
Sem±	3.53	3.27	2.73	3.05	2.08	1.45	2.33
CD at5 %	-	-	-	-	-	-	6.93

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par

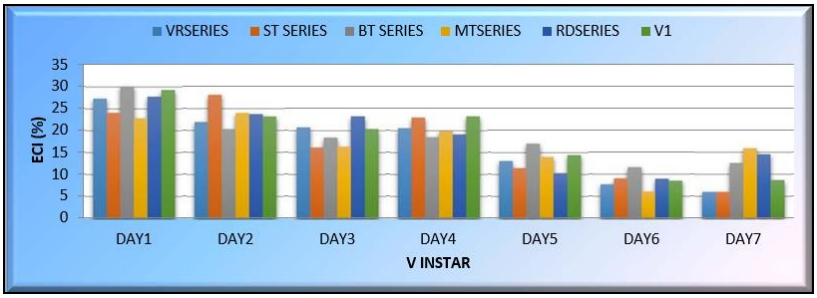

Fig 5: Efficiency of conversion of ingested food in V instar larva of PM×CSR_2 silkworm hybrid as influenced by transgenic mulberry lines on fresh weight basis (%)

Table 6: Efficiency of conversion of digested food in V instar larva of silkworm, PM×CSR2 as influenced by transgenic mulberry lines on fresh weight basis (%)

Transgenic / non-transgenic lines	5th Instar						
	day 1	day 2	day 3	day 4	day 5	day 6	day 7
VR SERIES	36.29	29.64	26.42	26.31	16.47	9.78	8.29^{ab}
ST SERIES	30.53	36.61	20.38	27.56	14.17	11.27	7.48^{a}
BT SERIES	38.57	26.39	24.06	22.30	21.56	14.73	16.09^{abcd}
MT SERIES	29.34	32.79	21.52	25.40	18.52	7.44	21.01^{d}
RD SERIES	36.78	30.68	29.47	22.67	12.66	10.83	17.90^{cd}
V1	37.17	30.14	26.24	28.96	18.90	10.13	10.62^{abc}
F TEST	NS	NS	NS	NS	NS	NS	*
Sem±	5.22	4.47	3.76	3.72	2.83	1.82	2.99
CD at 5%	-	-	-	-	-	-	8.87

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par

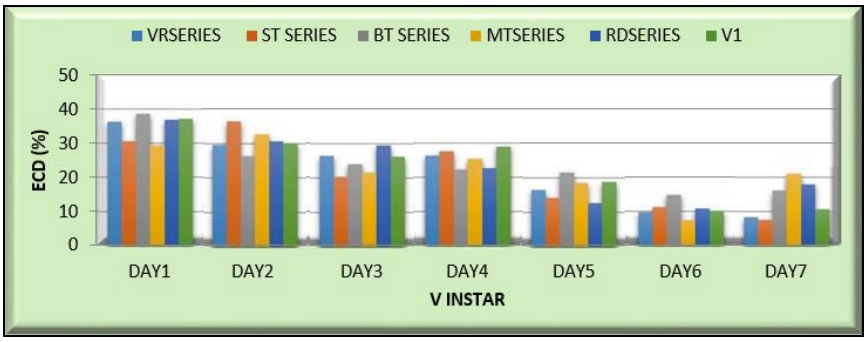

Fig 6: Efficiency of conversion of digested food in V instar larva of PM×CSR$_2$ silkworm hybrid as influenced by transgenic mulberry lines on fresh weight basis (%)

Table 7: Approximate digestibility in V instar larva of silkworm, PM×CSR2 as influenced by transgenic mulberry lines on fresh weight basis (%)

Transgenic / non- transgenic lines	5th Instar						
	day 1	day 2	day 3	day 4	day 5	day 6	day 7
VR SERIES	75.65	74.11	78.54	78.30^{a}	79.34	81.00	75.04
ST SERIES	81.81	77.57	81.88	83.51^{cd}	80.76	81.00	80.60
BT SERIES	77.88	76.99	76.58	81.94^{cd}	80.00	78.64	77.43
MT SERIES	78.64	73.38	75.82	78.43^{ab}	76.01	81.47	75.27
RD SERIES	78.30	77.72	79.18	84.46^{d}	81.82	83.12	80.52
V1	79.00	76.88	79.03	80.63^{abc}	77.36	85.10	81.89
F TEST	NS	NS	NS	*	NS	NS	NS
Sem±	3.29	1.59	1.94	1.02	1.43	1.55	2.48
CD at 5%	-	-	-	3.02	-	-	-

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par.

Table 8: Reference ratio in V instar larva of silkworm, PM×CSR2 as influenced by transgenic mulberry lines on fresh weight basis

Transgenic /non-transgeniclines	5thInstar						
	day 1	day 2	day 3	day4	day 5	day 6	day 7
VRSERIES	4.41	3.94	4.71	4.62^{a}	4.88	4.80	4.24
STSERIES	6.87	4.50	6.18	6.08^{cd}	5.20	5.29	5.31
BTSERIES	4.56	4.37	4.27	5.61^{cd}	5.08	4.81	4.48
MTSERIES	5.09	3.85	4.23	4.69^{ab}	4.18	5.43	4.28
RDSERIES	5.13	4.52	4.88	6.53^{e}	5.53	6.04	5.45
V1	4.78	4.34	4.95	5.21^{abc}	4.60	7.34	5.60
F TEST@5%	NS	NS	NS	*	NS	NS	NS
Sem±	1.06	0.26	0.66	0.30	0.29	0.60	0.54
CD	-	-	-	0.88	-	-	-

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par

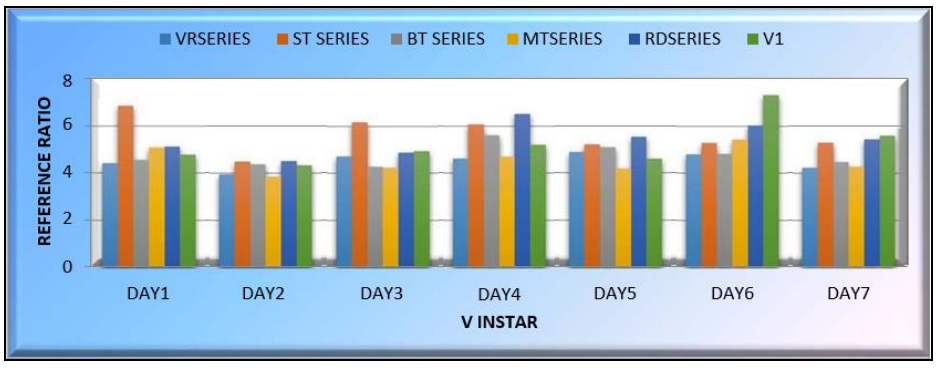

Fig 8: Reference ratio in V instar larva of PM×CSR$_2$ silkworm hybrid as influenced by transgenic mulberry lines on fresh weight basis

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