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EFFECT OF LEAF SUPPLEMENTATION WITH SECONDARY METABOLITES ON NUTRITIONAL PARAMETER OF MULBERRY SILKWORM

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ABSTRACT

Silkworms fed on supplemented leaves dipped in different lower and higher concentrations of three secondary metabolites to all the larval instars four times in a day. Lower concentrations of pectin, amino acid mixture and proline proved to be considerably effective showing higher food consumption, digestibility, reference ratio and growth index. These findings indicated that nutritional indices were found to be highest in Pectin (0.5 %) and Amino acid mixture (0.01 %) when compared with natural diet, which was pointed out the superiority over natural diet, which showed that significantly, improved the silk production and they can recommend as leaf supplementation for increasing the income of silk as well.

Key words: Fortifications, Secondary metabolites, nutritional parameter, silkworm

INTRODUCTION

Nutrition plays an important role in improving the growth and development of *Bombyx mori* L. It is stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves, which plays a very effective role in producing good cocoons (Legay., 1958). Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on factors *viz.*, weather, pests and disease as well as silkworm production (Ito, 1978). Leaf supplementation with secondary metabolites plays one of the important role by which cocoon and silk productivity can be increased and quality can be enhanced and maintained. Sengupta *et al*., (1972) revealed that *Bombyx mori* L. requires specific essential sugars, amino acids, proteins and vitamins for its normal growth of silkworm, survival and also for improvement in the growth of silkgland. Good quality cocoons can be obtained when silkworms fed on nutritionally supplementated leaves their results improved the silk production (Seki and Oshikane, 1959). In silkworms, silk fibroin is derived mainly from 4 amino acids: alanine, serine, glycine and tyrosine (Kirimura, 1962) which come from their dietary source of protein and amino acids (Ito, 1983). Silkworms obtain 72 -86 % of their amino acids from mulberry leaves and more than 60 % of the absorbed amino acids are used for silk production (Lu and Jiang, 1988).

The amino acid plays an important role in glucose, tryptophan and organic acid metabolism. Few studies have been conducted on amino acids supplementation; their results improved the silk production (Etebari and Matindoost, 2005). Thus, in the present study a comprehensive effort was made to determine whether mentioned secondary metabolites like lower concentrations of pectin, proline and amino acid mixture, supplementation influences the growth and development of silkworms along with their nutritional parameters.

MATERIALS AND METHODS

Silkworm Rearing: Eggs of Kolar Gold (PM x CSR_2) were reared in the Sectional Laboratory, Agriculture College, Pune under standard conditions of 29 ^{0}C with a RH of 75±5 % and a photoperiod of 16 L: 8D as described by Harizanis (2004). Fresh mulberry leaves were used for feeding the silkworms.

Treatments: Amino acids was dissolved in distilled water and diluted to lower and higher concentrations of three amino acids *viz.*, pectin (0.5 %) and (1.0 %), proline (1 %) and (2 %) and amino acid mixture (0.01%) and (0.02 %) along with natural diet. Silkworms fed on supplemented leaves to all larval instars. Fresh mulberry leaves were dipped in different lower and higher concentrations of secondary metabolites and then after drying within 15 minutes under fan were fed to the all instars of silkworm larvae were fed five times a day during initial three instars at 6AM, 11AM, 14PM, 18PM and 22PM with succulent leaves whereas later two instars were fed four times a day at 6AM, 13AM, 17PM and 22PM with coarse leaves daily. A batch of 100 larvae was fed with untreated leaves as control. Experiment was accomplished using a completely random design with five replications for each of the treatment by using 10 silkworms per replication for estimating the nutritional parameters was estimated on the basis of gravimetric method described by Waldbauer (1968) and Horie (1976) keeping 10 larvae per replicate. Food consumption, digestibility, reference ratio and growth index were then computed. Quantum of food consumed by a single larva was estimated in the final instar. Food intake material was weighed and fed to larvae; leftover food was collected, weighed and oven dried at 86 ± 5 ^{0}C temperature to a constant weight to estimate amount of dry food ingested. In order to calculate per cent dry matter and to estimate moisture loss during feeding period parallel samples of natural and test diets were weighed and kept under similar environment without larvae. The difference in weights indicated moisture loss from the food. Larval excreta were separated from leftover food, weighed and oven dried at 80 ± 5 ^{0}C temperature. Similarly, dry larval weight was estimated by weighing larva prior to initiation and at the end of instar by freeze-killing and oven drying at 100 ± 5 ^{0}C temperature.

1. Growth index (GI): Calculated by following formula, Growth index = (Final larval weight – Initial larval weight) ÷ Initial larval weight.
2. Ingesta: Ingesta (g) was evaluated by the formula, Weight of food ingested (g) = Weight of food offered – Weight of leftover food.
3. Digesta: Digestibility (digesta in g) was calculated by the formula, Food digestibility (g) = Weight of food ingested – Weight of excreta.
4. Apparent digestibility (AD): Calculated by the formula, Apparent digestibility (%) = (Dry wt. of food ingested - Dry weight of excreta) ÷ Dry weight of food ingested × 100.

5. Reference ratio (RR): It is an expression of absorption of food for a unit excreta production that was computed by the formula, Reference ratio = Dry weight of food ingested ÷ Dry weight of excreta.
6. Consumption index (CI): Calculated by the formula, Consumption index = (Weight of food consumed ÷ Feeding duration) x Mean larval weight during feeding period.

Statistical Analysis: Data were subjected to analysis of variance to determine if the differences found among treatments and the differences between treatments and the untreated control (Natural diet) were significant.

RESULTS

Results of the present investigations on effect of leaf supplementation with secondary metabolites on nutritional parameters of final instar of Kolar Gold (PM x CSR_2) are summarized in Table 1. It seems positive effects of lower concentrations of pectin (0.5%) and amino acid mixture (0.01%) were noticeable as compare with untreated natural diet and other higher concentrations of secondary metabolites. Feeding of larvae with leaf supplementation along with lower and higher concentrations of secondary metabolites to be 1.560. Significantly lowest ingesta were estimated in natural diet (4.430 g). Significantly highest ingesta were recorded in Pectin (0.5 %) (4.595 g) that was at par with Amino acid mixture (0.01 %) (4.576 g). In order of statistical significance, next higher ingesta was estimated as Pectin (1.0 %) (4.480 g), Proline (1 %) (4.469 g), Proline (2 %) (4.431 g) and T6 (4.430 g). Significantly lowest digesta was estimated in natural diet (1.406 g) and significantly highest in Pectin (0.5 %) (1.565 g) that was followed by Amino acid mixture (0.01 %) (1.556 g) and Pectin (1.0 %) (1.553 g) followed by Proline (1 %) and Amino acid mixture (0.02 %) recording the digesta of 1.531 g followed by Proline (2 %) (1.511 g). Significantly lowest apparent digestibility was recorded in natural diet (31.73 %). Significantly highest digestibility was estimated in Pectin (1.0 %) (34.67 %) and was at par with Amino acid mixture (0.02 %) (34.56 %) and Proline (1 %) (34.26 %) followed by Proline (2 %) (34.10%), Pectin (0.5 %) (34.06 %) and Amino acid mixture (0.01 %) (34.00 %), which were statistically in the similar magnitude. Reference ratio in natural diet was estimated to be 1.560. Significantly highest ratio (1.573) was recorded in Pectin (0.5 %) and Proline (2 %) followed by Amino acid mixture (0.01 %) (1.563) and Pectin (1.0 %) (1.533) followed by Proline (1 %) (1.527) and Amino acid mixture (0.02 %) (1.519). Significantly lowest consumption index was computed in natural diet (0.502), significantly highest in Pectin (0.5 %) (0.712) and the intermediate treatments were Pectin (1.0 %) (0.684) followed by Proline (1 %) and Proline (2 %) recording the index to be 0.672 followed by Amino acid mixture (0.01 %) (0.654) and Amino acid mixture (0.02 %) (0.628). Significantly lowest growth index was worked in natural diet (4.287) and significantly highest in Pectin (0.5 %) (4.546) that was followed by Amino acid mixture (0.01 %) (4.533) followed by Pectin (1.0 %) (4.476), Proline (1 %) (4.455) followed by Amino acid mixture (0.02 %) (4.446), which were followed by Proline (2 %) (4.328). These findings indicated that nutritional indices were found to be highest in Pectin (0.5 %) and Amino acid mixture (0.01 %) when compared with natural diet, which was pointed out the superiority over natural diet.

Table. 1 Effect of secondary metabolites on nutritional parameter of final instar

Treatment	Ingesta (g)	Digesta (g)	AD (%)	RR	CI	GI
Pectin (0.5 %)	4.595	1.565	34.06 (35.67)	1.573	0.712	4.546
Pectin (1.0 %)	4.480	1.553	34.67 (36.09)	1.533	0.684	4.476
Proline (1 %)	4.469	1.531	34.26 (35.79)	1.527	0.672	4.455
Proline (2 %)	4.431	1.511	34.10 (35.73)	1.573	0.672	4.328
Amino acid mixture (0.01 %)	4.576	1.556	34.00 (35.67)	1.563	0.654	4.533
Amino acid mixture (0.02 %)	4.430	1.531	34.56 (35.97)	1.519	0.628	4.446
Natural diet	4.430	1.406	31.73 (34.27)	1.560	0.502	4.287
SE ±	0.040	0.013	0.315	0.014	0.006	0.039
C.D. at 5 %	0.115	0.039	0.912	0.040	0.017	0.114

(Figures in parenthesis are mean arcsin transformed values.)

DISCUSSION

The present findings were in conformity with the findings of Chapman, 1998 reported that in order to have best larval growth, insect needs optimum level of amino acids, being used for structural purposes such as enzymes and transport-receptors. Under the studies, lower concentration of each of pectin and amino acid mixture were found promising than their higher concentration. Kabila *et al*. (1994) reported that lower concentrations of aspartic acid increased cocoon characteristics. Tazima (1978) reported that alanine played important role in glucose, tryptophan and organic acid metabolism wherein, aspargine was used as aspartic acids, constituting two groups of essential amino acids and its deficiency leads to growth retardation. Radjabi (2010) observed that lower concentration of aspargine and alanine recorded positive effect of effective rearing rate and had negative effect on shell ratio, therefore not be recommended for commercial silk production. However, Joshi (1985) and Radjabi (2009) found encouraging results with their higher concentration of amino acids. In the present investigations, aspartic acid and aspargine were not incorporated however, pectin and amino acid mixture were found to be promising; at their lower concentration, higher nutritional indices were found to be highest in Pectin (0.5 %) and Amino acid mixture (0.01 %) when compared with natural diet, which was pointed out the superiority over natural diet. Literature in respect of these amino acids being scanty on the whole the results are unable to discuss further.

In conclusion, supplementation of silkworm with selected lower concentrations of amino acids at certain levels may be effective for improved growth, but a higher level of supplementations doesn't have a positive effect on silkworm growth and development. Supplementations of dietary nutrients with the aforesaid promising complementary additives increased content of leaf moisture that might have lead to higher consumption rate. This might have proportionately increased apparent digestibility that in turn resulted into enhanced digestion, absorption, assimilation and utilization of food energy in to larval bio-mass and thereby the cocoon. This might have induced upgrading economic parameters as suggested by Rahamathulla (2007). Further, conversion rate of leaf into silk was also found to be promising depicting higher silk content that represented superiority of silk quality as pointed out earlier by Trivedy (2003).

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