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Influence of Sodium Nitrate ($NaNO_3$) of Different Feeds on Growth and Bioenergetics of Bivoltine NB_4D_2 Race of the Silkworm, *Bombyx mori* L.

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Dietary supplementation of sodium nitrate with different concentrations 50, 100, 200, 500, 700 and 1000 μg / ml of single, two, three and four feeds to fifth instar larvae of biovoltine NB_4D_2 race of the silkworm, *B. mori* resulted in significant increase in the food conversion, conversion rate and conversion efficiency K_1 and K_2. However, there were significant decrease in the food assimilation, assimilation rate and assimilation efficiency in the sodium nitrate treated groups as compared with that of the corresponding parameters of the carrier control. This indicates that the administration of sodium nitrate may stimulate metabolic activities, thereby influencing conversion of food into body weight in the bivoltine silkworm, *B. mori*.

Key words: Sodium nitrate, Growth, Bioenergetics, *Bombyx mori*

Introduction

Nutrition is the most important growth regulating factor in the silkworm, *B. mori,* being a monophagous insect, it derives almost all the nutrients essential for its growth from the mulberry leaf itself. Many scientists have tried to improve the quality and quantity of silk by enriching mulberry leaves with inorganic and organic supplements (Ito and Niminura, 1966; Horie *et al.*, 1967; Vishwanath and Krishnamurthy, 1982; Subburathinam *et al.*, 1990). Minerals are not synthesized within the insects although they are essential elements and affect various metabolic processes. In plants conversion of nitrate to nitrite is a rate-limiting step in the process of nitrate assimilation and is catalysed by an enzyme nitrate reductase (NR) (Schroeder *et al.*, 1968; Beevers and Hageman, 1969). Since the enzyme nitrate reductase is present in mulberry leaves, the supplementation of sodium nitrate may enhance the activity of nitrate reductase and conversion of nitrate to nitrite and ammonia to amino acid and in turn protein synthesis may be possible as many phytohormones have been effective on the silkworm, B. *mori.* Bioenergetics involves the basic thermodynamics laws (Haynie, 2001) *i.e.,* energy can neither be created nor destroyed although it may be interconverted between different forms. In view of the above findings the present investigation was undertaken to study the effect of sodium nitrate with different concentrations and feeds on food utilization budget in the silkworm, *B. mori.*

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Materials and Methods

The rearing room and rearing appliances were disinfected with 2% formalin and the rearing room was closed for a day before the commencement of rearing. The disease free layings (DFLs) of the bivoltine (NB_4D_2) silkworm, *Bombyx mori,* used in the present investigation were obtained from the Government grainage centre, Rayapur, Dharwad, Karnataka and reared in the laboratory by the improved method of rearing techniques (Krishna Swamy, 1978). Immediately after the 4^{th} moult, healthy larvae of uniform sizes were isolated and used for the experiment. Immediately after the 4^{th} moult, the 5^{th} instar larvae were divided into different experimental batches each batch consisting of 50 larvae. Three replications of 50 larvae each were used for the treatment. The chemical sodium nitrate was procured from S. D. Fine Chem Ltd., Mumbai. The chemical sodium nitrate was dissolved completely in distilled water and diluted to form 50, 100, 200, 500, 700 and 1000 μg/ml dilutions. The fresh mulberry leaves were soaked in these concentrations for 15 min and fed to the silkworm *ad libitium*. The feeding of the chemically treated leaves was commenced from the first to fifth day of 5^{th} instar larvae. The silkworms were fed with known weight of the treated leaves.

Experiment I: Single feed was treated with sodium nitrate of 50 to 1,000 μg/ml concentrations and the remaining three feeds were fed with normal leaves.

Experiment II: Two feeds were treated with sodium nitrate of 50 to 1,000 μg/ml concentrations and the remaining two feeds were normal.

Experiment III: Three feeds were treated with sodium nitrate of 50 to 1,000 μg/ml concentrations and the remaining one feed was normal.

Experiment IV: All four feeds were treated with sodium nitrate of 50 to 1,000 μg/ml concentrations.

The carrier control group was fed with leaves dipped in distilled water of single, two three and four feeds. The normal control was fed with untreated leaves.

Food utilization

Nutritional indices were calculated for the total period of 5^{th} instar following the standard gravimetric method described by Waldbauer (1968). The Sacrifice method described by Maynard and Loosli (1962) was adopted to test the growth that was later followed by Tyler (1964), Pandian (1967, 1970), Pandian and Raghuraman (1972) with initiation of each treatment the individuals were weighed and transferred to the experimental terrarium. Parallel batches were weighed and sacrificed for the estimation of growth, unutilized leaves and the faeces were collected daily at 7 am. before giving fresh leaves. The larvae at the beginning and at the termination of the experiment were weighed. The weight of the silkworm, leaves offered, the weight of the left over leaves, the faeces produced, silkglands and control leaves were dried in an oven at 100°C till the constant weight persisted (Waldbaeur, 1968).

Food utilization budget of silkworms was estimated using the IBP terminology (Petrusewicz and Mac Fadyen, 1970). The rates and efficiencies of food was computed according to the method of Delvi (1983). The rates and efficiencies of food utilization were estimated using IBP equation ($I = B + M + F$) where I = Food ingested, B = Biomass gained, M = Food metabolized, $(I - F) - B$, F = Faeces produced, I F = Food digested and assimilated (Waldbauer 1968). The feeding, assimilation, conversion and metabolic rates were calculated for mid body weight (b) and duration of instar (T) for food consumed (I), assimilated $(I - F)$, food converted (B) and food oxidized (M). Food assimilation efficiency was calculated both for ingested K_1 and digested K_2 by taking into account of gained biomass (Delvi, 1983).

Statistical analysis

The experiments were designed by complete randomised block design (CRBD) method. The data collected were fed to the computer for statistical analysis by using the software SPSS version 6, to study the significance of variance among the treatment groups (One way variance test F). To determine the significant difference among the treatment groups, the least significant difference test (Lsd) was carried out.

Results and Discussion

The data on the influence of sodium nitrate on growth and bioenergetics of the silkworm (NB_4D_2) are presented in Table 1 to 4.

Growth

There was significant increase in larval growth in all treated groups. Maximum food consumption/intake was observed in all the groups of various doses and feeds supplemented with sodium nitrate. The food converted into the body matter during the last larval instar not only confirms that mulberry leaves supplemented with sodium nitrate were consumed to the maximum extent but also maximum portion of food were converted into body matter. These findings are in conformity with those of Mahmood (1989) who also recorded better body weight as well as length after feeding minerals, nitrogen, potassium and phosphorus supplemented leaves.

Food intake and feeding rate

In the present study there was a significant increase in food intake/consumption of food in all four treated groups of various doses and feeds. The food consumption plays a very important direct relevance on the weight of the larvae, cocoon, pupae and shell. Similar results have been reported that increase in feeding rate allow the larvae to accumulate sufficient energy required to spin the cocoon, to meet requirement of the non feeding pupae and adult stages (Delvi and Pandian, 1972). However, it has been suggested that the consumption and productivity varies depending on the type of nutrition (Remadevi *et al.*, 1992; Shivakumar *et al.*, 1995)

In the present study there is a significant decrease in feeding rate in single, two, three, lower doses of four feeds and increase in higher doses of four feeds. Feeding rate in the silkworm, *B. mori* decreased with increase in the body weight or age, irrespective of the factors like food quality, scotoperiod or photo-period (Asiya Nuzhat, 1993; Hanifa

Table 1. Food utilisation budget in the silkworm, *B. mori* supplemented with single feed of sodium nitrate

Treatment	Dose µg/ml	Food assimilation	Food conversion	Conversion rate	Assimilation efficiency	Conversion efficiency K_1	Conversion efficiency K_2
Sodium nitrate	50	1229* (96)	698* (126)	0.0422* (120)	24.10* (95)	13.69* (124)	56.79* (131)
Sodium nitrate	100	1257* (98)	653* (118)	0.0386* (110)	24.74* (97)	12.85* (117)	51.99* (120)
Sodium nitrate	200	1212* (95)	721* (131)	0.0418* (119)	23.30* (91)	13.87* (126)	59.48* (137)
Sodium nitrate	500	1259 (99)	783* (142)	0.0476* (136)	24.71* (97)	15.37* (140)	62.19* (143)
Sodium nitrate	700	1257* (98)	710* (129)	0.0443* (126)	24.21* (95)	14.05* (128)	56.48* (130)
Sodium nitrate	1000	1261 (99)	641* (116)	0.0403* (115)	24.85* (95)	12.60* (115)	50.83* (117)
Carrier control	Distilled water	1270 (100)	550 (100)	0.0349 (100)	25.33 (100)	10.96 (100)	43.30 (100)
Normal control	-	1191 (93)	558 (101)	0.0355 (102)	23.79 (93)	11.14 (101)	43.22 (100)
		S	S	S	NS	S	S
S. Em ±		1.704	5.441	0	0.027	0.101	0.442
C.D at 5%		4.723	15.050	0	0.074	0.277	1.225

*, Significant increase / decrease at 5%; S, Significant; NS, Non significant; S. Em ±, Standard error mean; C.D, Critical difference.
Percent increase / decrease over that of the carrier control in parenthesis.
The values are expressed in mg, rates in mg/mg/day.

Table 2. Food utilisation budget in the silkworm, *B. mori* supplemented with two feeds of sodium nitrate

Treatment	Dose µg/ml	Food assimilation	Food conversion	Conversion rate	Assimilation efficiency	Conversion efficiency K_1	Conversion efficiency K_2
Sodium nitrate	50	1271* (104)	686* (118)	0.0420* (109)	24.73 (98)	13.35* (112)	53.97* (113)
Sodium nitrate	100	1268* (104)	682* (117)	0.0418* (109)	25.07 (99)	13.48* (113)	53.78* (113)
Sodium nitrate	200	1281* (105)	730* (125)	0.0427* (111)	25.09 (99)	14.20* (119)	56.98* (119)
Sodium nitrate	500	1244* (102)	662* (114)	0.0414* (108)	25.10 (99)	13.09* (110)	53.21* (112)
Sodium nitrate	700	1276* (104)	752* (129)	0.0475* (124)	25.01 (99)	14.75* (123)	58.93* (123)
Sodium nitrate	1000	1240* (102)	592 (102)	0.0382 (100)	25.55 (101)	12.08 (101)	47.74 (100)
Carrier control	Distilled water	1220 (100)	582 (100)	0.0382 (100)	25.12 (100)	11.91 (100)	47.70 (100)
Normal control	-	1200 (98)	536 (92)	0.0355 (92)	25.71 (102)	11.48 (96)	43.12 (90)
		S	S	S	NS	S	S
S. Em ±		0.002	1.33	5.78	0.04	0.12	0.44
C.D at 5%		0.007	3.68	16.00	0.11	0.33	1.21

*, Significant increase / decrease at 5%; S, Significant; NS, Non significant; S. Em ±, Standard error mean; C.D, Critical difference.
Percent increase / decrease over that of the carrier control in parenthesis.
The values are expressed in mg, rates in mg/mg/day.

Bano, 1997). Feeding rate can be modified by the worm depending on amount of food consumed in *B. mori* (Radhakrishnan, 1987). Muthukrishnan and Pandian, (1983) have suggested that the increasing feeding rates are known to allow the spinning of cocoons in *Acharea janta*. Similarly in the scarabacid beetle *Rhopaea verreuzi*, which feeds on plant roots and organic matter of the soil, increases the feeding rates and allows the insect to grow into a large size even though it excretes organic matter (Cairns, 1982). In the present study increases in the food intake and decrease in feeding rate may be due to increase in body weight.

Faeces defecated

In the present study the faeces defecated significantly increased in various doses and feeds treated with sodium nitrate. The production of excreta depends on quality of food, rate of food intake, absorption rate and also the retention time of food in the gut. Waldbauer (1968) has suggested that higher food intake tends to mobilise the gut contents faster and provides less time for enzyme activity and food absorption, thus making the efficiency poor.

Table 3. Food utilisation budget in the silkworm, *B. mori* supplemented with three feed of sodium nitrate

Treatment	Dose µg/ml	Food assimilation	Food conversion	Conversion rate	Assimilation efficiency	Conversion efficiency K_1	Conversion efficiency K_2
Sodium nitrate	50	1302* (89)	616 (102)	0.0426 (97)	28.30* (88)	13.39 (101)	47.31 (115)
Sodium nitrate	100	1253* (86)	614 (102)	0.0448 (101)	27.14* (84)	13.38 (101)	49.00* (118)
Sodium nitrate	200	1310* (90)	654* (108)	0.0456 (104)	28.15* (88)	14.05 (106)	49.92* (120)
Sodium nitrate	500	1340* (92)	670* (111)	0.0453 (103)	28.03* (87)	14.01 (106)	50.00* (121)
Sodium nitrate	700	1271* (87)	650* (108)	0.0448 (101)	27.11* (84)	13.87 (105)	51.14* (124)
Sodium nitrate	1000	1375* (94)	612 (102)	0.0446 (101)	29.48* (91)	13.30 (100)	44.50 (108)
Carrier control	Distilled water	1462 (100)	604 (100)	0.0440 (100)	32.14 (100)	13.28 (100)	41.31 (100)
Normal control	-	1377 (94)	614 (102)	0.0468 (106)	31.30 (97)	13.95 (105)	44.58 (108)
		S	S	NS	S	NS	S
S. Em ±		2.05	12.12	0	0.01	0.25	0.88
C.D at 5%		5.68	33.50	0	0.02	0.69	0.63

*, Significant increase / decrease at 5%; S, Significant; NS, Non significant; S. Em ±, Standard error mean; C.D, Critical difference.
Percent increase / decrease over that of the carrier control in parenthesis.
The values are expressed in mg, rates in mg/mg/day.

Table 4. Food utilisation budget in the silkworm, *B. mori* supplemented with four feed of sodium nitrate

Treatment	Dose µg/ml	Food assimilation	Food conversion	Conversion rate	Assimilation efficiency	Conversion efficiency K_1	Conversion efficiency K_2
Sodium nitrate	50	1469* (112)	682* (116)	0.0493* (114)	30.02 (98)	13.93 (101)	46.42* (103)
Sodium nitrate	100	1428* (109)	654* (111)	0.0456 (105)	29.83* (97)	13.66 (99)	45.79 (102)
Sodium nitrate	200	1344 (102)	591 (100)	0.0362* (83)	29.89* (97)	13.14* (95)	43.97* (98)
Sodium nitrate	500	1412* (107)	616 (104)	0.0370* (85)	24.18* (79)	13.40 (97)	43.63* (97)
Sodium nitrate	700	1384* (105)	504* (85)	0.0320* (74)	31.66* (103)	11.53* (83)	36.41* (81)
Sodium nitrate	1000	1255* (96)	354* (60)	0.0279* (64)	32.06* (104)	9.04* (65)	28.20* (63)
Carrier control	Distilled water	1313 (100)	590 (100)	0.0434 (100)	30.76 (100)	13.82 (100)	44.93 (100)
Normal control	-	1262 (96)	572 (97)	0.0391 (64)	28.43 (92)	13.33 (96)	45.32 (101)
		S	S	S	S	S	S
S. Em ±	1.71	1.41	11.4	0.16	0.23	0.85	
C.D at 5%	2.35	3.29	31.50	0.44	0.63	2.35	

*, Significant increase / decrease at 5%; S, Significant; NS, Non significant; S. Em ±, Standard error mean; C.D, Critical difference.
Percent increase / decrease over that of the carrier control in parenthesis.
The values are expressed in mg, rates in mg/mg/day.

Food assimilation, assimilation rate and assimilation efficiency

In the present investigation there was a decrease in food assimilation, assimilation rate and assimilation efficiency in sodium nitrate treated groups of single feed. There was an increase in food assimilation, and assimilation efficiency in two feeds. In three feeds increase in food assimilation, assimilation rate in lower doses and decrease in assimilation efficiency in higher doses. There was an increase in food assimilation in all the doses and assimilation rate in lower doses, decrease in assimilation rate in higher doses, and increase in assimilation efficiency in four feeds except in 50 µg and 100 µg doses of sodium nitrate treated groups. In the present study there is decrease in assimilation rate and assimilation efficiency that may be due to the decrease in feeding rate, which in turn may be due to the maximum increase in body weight.

Food conversion, conversion rate and conversion efficiency

The present study showed an increase in food conversion, conversion rate and conversion efficiency in all doses of single, two, lower doses of four feeds and decrease in higher doses of four feeds of sodium nitrate treated groups. However, there was a significant increase in food conversion and conversion efficiency K_2, but there was no significant change in the conversion rate and conversion efficiency K_1 in three feeds of sodium nitrate treated groups. In the present study conversion efficiency increased in one and two feeds. However, there was no significant change in conversion efficiency in three feeds but there was decrease in four feeds of sodium nitrate treated groups may be due to toxic effect. It indicates that lower feeds stimulated the effective conversion where as more intake of sodium nitrate inhibited the conversion. Gross conversion efficiency K_2 was found to be inversely proportional to assimilation efficiency. The efficiency of silkworm to convert ingested (ECI) and/or the efficiency of digested food into body substance (ECD) indicator for the insects ability to utilize ingested or digested food for growth (Assal *et al.,* 1994). This probably explains that more feeds with sodium nitrate may have affected conversion rate and conversion efficiency. High rate of metabolism indicates that energy is utilized for physiological processes.

Food oxidation and metabolic rate

The present observation reveals that the decrease in oxidation and metabolic rate were due to increased conversion efficiency in single and two feeds sodium nitrate treated

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