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Effect of Natural and Artificial Diets on Growth Parameters of Kolar Gold, Silkworm

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A B S T R A C T

Three types of artificial diets *viz.*, Artificial diet$_1$, Artificial diet$_2$ and Artificial diet$_3$ with different compositions were used for rearing Kolar Gold race of mulberry silkworm, *Bombyx mori* L. where the diets were fed up to initial three instars and the later two instars were reared on natural diet (untreated leaves). Formulated diets were compared with natural diet and the observations on growth parameters were recorded. The study revealed that amongst the three formulated artificial diets, artificial diet$_1$ (mulberry leaf powder - 30 g, soybean powder - 28 g, cellulose powder - 15 g, corn starch -6 g, citrate - 3.5 g, salt mixture - 4 g, sucrose - 4 g, agar agar - 7 g, along with vitamin B mixture - 0.40 g, soybean oil - 1.30 g and antibiotic chloramphenicol - 1.00 g and water - 302.0 ml) was found to be promising in almost all the growth parameters of the silkworm under study and selected for further studies. Growth parameters like larval weight and larval length were significantly maximum in silkworms which were fed with artificial diet$_1$, followed by artificial diet$_3$. Larval weight and larval length was maximum in artificial diet$_1$ with 4.338 g and 7.25 cm respectively, followed by artificial diet$_3$ with 4.213 g and 7.00 cm respectively in final instar. In artificial diet$_1$, larval duration was extended by 83 hr followed by artificial diet$_3$ in which larval duration was prolonged by 94 hr in comparison with natural diet,. In artificial diet$_1$, moulting period was curtailed by 3 hr. However, in artificial diet$_3$ it was extended by 20 hr in comparison with natural diet. In all the instars flacherie disease occurrence was meager in artificial diet$_1$ with 0.75 per cent, followed by artificial diet$_3$ with 1.00 per cent in initial instar and in later instars, disease occurrence was minimum in artificial diet$_1$ with 2.53 per cent followed by artificial diet$_3$ with 2.55 per cent.

Introduction

India is the only country that produces all four kinds of silk *viz.*, mulberry, tasar, eri and muga (Anitha, 2011). All these four varieties of silk are produced through commercial rearing of *Bombyx mori*, *Antheraea mylitta*, *Philosamia ricinii* and *Antheraea assama*, respectively. However the best quality silk is obtained from the cocoons of the larvae of mulberry silkworm and is produced mostly in many states of India accounting for 70.21 per cent (25,348 MT) followed by Eri 19.84 per cent (7,175 MT), Tasar 9.32 per cent (3,370

MT) and Muga 0.66 per cent (240 MT) of the provisional total raw silk production of 36,152 MT (CSB, 2020).

Mulberry leaves are complete diet for silkworm sometimes it is possible that some deficiencies occur due to different reasons. The supplementation of extra nutrients along with mulberry leaves results higher yield because the production of superior quality and quantity of silk depends mainly on nutritional status and healthiness of the larvae (Rahmathulla *et al.,* 2007). Studies on the nutrition experienced real progress due to the introduction and development of artificial diets that enhances nutritional status to obtain good cocoon yield and shell ratio (Zah Cristina and Marghitas, 2011).

Materials and Methods

Artificial diets

Three diets were formulated and were fed to initial three instars and later two instars were fed with natural diet. Ingredients and the quantity of ingredients used to formulate the diets are given below

Procedure for formulating test diet

The procedure used for formulating the test diets of the silkworm consists of a series of steps including measuring the requisite amounts of the feed ingredients, mixing the feed ingredients (namely, dried diet), mixing water with the dried diets and steaming them, pouring them in trays and cooling them in deep freezer and transferring the prepared diet to the rearing rooms whenever required. Young age mulberry leaves of var. Victory 1 were used in powder form as a constituent of the diet. Required amount of ingredients were measured by making dry to green factor. Volume of distilled water added to the dried diet was adjusted by multiplying the weighed diet's ingredients with 2.8 factor. At 80^0 C temperature distilled water was heated and required amount of agar agar was added to bring gel formation and all the ingredients were thoroughly mixed and steamed in autoclave for 30 minutes at 110^0 C. Soon after the process of steaming before its temperature lowers down to 80^0 C, the material was poured in pre-sterilized plastic trays that was latter covered with aluminum foil; these test diet trays were placed in deep freezer for about 1 hr and then maintained in normal refrigerated temperature until its utilization. 1 hr. prior to the feeding, test diets were taken out of the refrigerator. Temperature and relative humidity for first three and later two instars were maintained at 30 ($\pm$1)^0 C and 85 ($\pm$5) per cent and 25 ($\pm$1)^0 C and 75 ($\pm$5) per cent, respectively. Last two larval instars were then fed with natural diet (Bhojne, 2013).

Method of recording observation

Larval weight (g)

$$\text{Weight of single larvae} = \frac{\text{Weight of 10 larvae (g)}}{\text{Total number of larvae (10)}}$$

Larval length (cm)

Length of the larvae was measured in mm with the help of the vernier caliper at initiation and at the end of each instar when the larvae were in relaxed state taking utmost care not to disturb the larvae.

Disease incidence (%)

$$\text{Disease incidence} = \frac{\text{Total number of diseased larvae}}{\text{Total number of larvae}} \times 100$$

Moulting duration (hours)

Moulting duration was recorded from the time worms started settling for moult up to 50 per cent worms came out of moult.

Instar larval duration (hours)

Larval duration was recorded from the time worms came out of the moult up to settling for next moult, for each instar up to spinning.

Results and Discussion

In T_4 (natural diet), mean larval weight recorded for first, second, third, fourth and fifth larval instars were as 0.006, 0.058, 0.382, 1.140 and 4.033 g, respectively. In first instar all the remaining three treatments T_1, T_2 and T_3 (0.005 g) recorded same larval weight. In second instar, weight was significantly highest in T_4 (0.058 g) which was at par with T_1 (0.057 g), followed by T_3 (0.055 g). In third instar, highest weight was recorded in T_1 (0.383 g) that was found at par with T_4 (0.382 g), followed by T_3 (0.379 g). In fourth instar, significantly lowest weight was recorded in T_2 (1.045 g), while the highest weight was recorded in T_1 (1.288 g), followed by T_3 (1.205 g). In fifth instar T_1 (4.338 g) was found to be superior among all the treatments, next highest weight in order of statistical significance was exhibited in T_3 (4.213 g) and T_4 (4.033 g) and the lowest weight was recorded in T_2 (3.996 g). In initial two instars, larval weight of diets was observed to be lesser in comparison with T_4 (natural diet), in third instar it was noticed almost similar in range with T_4 (natural diet). However in later two instars fed with leaves, the weight was remarkably higher in T_1 in comparison with T_4 and other formulated diets. These findings are in harmony with the results of Roychoudhury *et al.,* (1994) found that the larvae reared on artificial diet has recorded accelerated larval growth and weight of the larvae was higher, Rajaram *et al.,* (2012) reported that the larval growth was better in silkworms reared on artificial diet in early instars than those reared on mulberry leaves, Jatuporn and Nijura (2017) reported that silkworms raised on artificial diets had higher larval weights than those raised on natural diet.

Mean larval length for first, second, third, fourth and fifth instars were recorded in T_4 (natural diet) as 0.500, 1.352, 1.550, 4.85 and 7.00 cm, respectively. In first instar, larval length was observed to be same in T_1, T_3 and T_4 (0.50 cm). In second instar significantly longer length was recorded in T_4 (1.352 cm) which was at par with T_1 (1.334 cm), followed by T_3 (1.313 cm) and the shorter length was recorded in T_2 (0.950 cm). In third instar, significantly more length was observed in T_4 (1.550 cm), followed by T_1 (1.400 cm) that was found at par with T_3 (1.390 cm) and less length was recorded in T_2 (1.200 cm). In fourth instar significantly longer length was recorded in T_1 (5.00 cm) which was at par with T_3 (4.90 cm), followed by T_4 (4.85 cm). In fifth instar longer length was recorded in T_1 (7.25 cm), followed by T_3 and T_4 both with same length (7.00 cm) while the shorter length was recorded in T_2 (6.50 cm). In all the races of silkworm, larval length in initial two instars was observed to be lesser, in intermediate instar in similar range with natural diet and in later two instars remarkably higher than T_4 (natural diet). These findings are in harmony with the results of Roychoudhury *et al.,* (1994) found that the larvae reared on artificial diet has recorded accelerated larval growth, Rajaram *et al.,* (2012) reported that the larval growth was better in case of silkworms reared on artificial diet in early instars than those reared on mulberry leaves, Moise *et al.,* (2020) reported that the length of the larvae grown on artificial diet was remarkably higher than those larvae which were reared on natural diet.

Mean larval duration for first, second, third, fourth and fifth instars were found significantly lowest in T_4 (natural diet) amongst the treatments recording 72, 84, 96,

114 and 140 hr, respectively. In first instar, duration was significantly prolonged in T_2 (131 hr) extended by 59 hr, followed by T_3 (124 hr) extended by 52 hr. In second instar, duration was significantly extended by 10 hr in T_2 (94 hr) that was at par with T_1 (92 hr) extended by 8 hr, and at par with T_3 (91 hr) extended by 7 hr. In third instar significantly enhanced larval duration was observed in T_2 (120 hr) extended by 24 hr, followed by T_3 (114 hr) extended by 18 hr, followed by T_1 (112 hr) that was extended by 16 hr. In fourth instar, duration was significantly extended by 16 hr in T_2 (130 hr), followed by T_3 (124 hr) extended by 10 hr, followed by T_1 (120 hr) extended by 6 hr. In fifth instar, duration was significantly extended by 8 hr in T_2 (148 hr) that was at par with T_3 (147 hr) extended by 7 hr, followed by T_1 (144 hr) extended by 4 hr. In initial two instars, larval duration of the treatments was found to be extended as compared to T_4 (natural diet) similar trend was observed in third instar. However in later two instars, duration was not much extended or prominently extended in T_1 when compared to T_4 (natural diet). These findings are in accordance with Mottaghitlab and Pourali (2004) found that larval duration was recorded to be highest in all the artificial diets formulated, Bhojne (2013) found that the larval duration was extended for the larvae reared on semi- synthetic diets in comparison to natural diet, Jatuporn and Nijura (2017) reported that larval periods were extended on artificial diets as compared to those reared on natural diet (Table 1–8).

Table.1 Treatment details

Treatment	Details
T_1	Artificial $diet_1$
T_2	Artificial $diet_2$
T_3	Artificial $diet_3$
T_4	Natural diet

Table.2 Composition of the artificial diet

Sr.No	Ingredients	Artificial diets		
		$Diet_1$	$Diet_2$	$Diet_3$
1	Mulberry leaf powder (g)	30.00	30.00	45.00
2	Soybean powder (g)	28.00	20.00	20.00
3	Cellulose powder (g)	15.00	25.00	10.00
4	Corn starch (g)	06.00	06.00	06.00
5	Citrate (g)	03.50	03.50	03.50
6	Salt mixture (g)	04.00	04.00	04.00
7	Sucrose (g)	04.00	02.00	02.00
8	Agar agar (g)	07.00	07.00	07.00
9	Ascorbic acid (g)	00.50	00.50	00.50
10	Vitamin B mixture (g)	00.40	00.40	00.40
11	Soybean oil	01.30	01.30	01.30
12	Chloramphenicol (ug)	01.00	01.00	01.00
13	Water (ml)	302.0	302.0	302.0

Table.3 Effect of natural and artificial diets on mean larval weight of Kolar Gold

Treatment	Mean larval weight(g)				
	Initial instars			Later instars	
	First	Second	Third	Fourth	Fifth
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
T_1	0.005±0.00032	0.057±0.0017	0.383±0.0024	1.288±0.010	4.338±0.025
T_2	0.005±0.00022	0.050±0.0027	0.359±0.0085	1.045±0.064	3.996±0.101
T_3	0.005±0.00021	0.055±0.0005	0.379±0.0021	1.205±0.021	4.213±0.115
T_4	0.006±0.00010	0.058±0.0017	0.382±0.0008	1.140±0.018	4.033±0.052
SE ±	0.00011	0.0009	0.0022	0.017	0.040
C.D at 5%	0.00035	0.0029	0.0070	0.054	0.125
C.V. %	4.13	3.38	1.21	3.01	1.97

Table.4 Effect of natural and artificial diets on mean larval length of Kolar Gold

Treatment	Mean larval length (cm)				
	Initial instars			Later instars	
	First	Second	Third	Fourth	Fifth
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
T_1	0.500 ± 0.008	1.334 ± 0.011	1.400 ± 0.041	5.00 ± 0.183	7.25 ± 0.041
T_2	0.452 ± 0.005	0.950 ± 0.035	1.200 ± 0.058	4.50 ± 0.041	6.50 ± 0.041
T_3	0.500 ± 0.014	1.313 ± 0.003	1.390 ± 0.013	4.90 ± 0.082	7.00 ± 0.183
T_4	0.500 ± 0.008	1.352 ± 0.003	1.550 ± 0.030	4.85 ± 0.041	7.00 ± 0.183
SE ±	0.004	0.009	0.019	0.052	0.066
C.D at 5%	0.014	0.02	0.059	0.160	0.203
C.V. %	1.93	1.50	2.80	2.16	1.90

Table.5 Effect of natural and artificial diets on mean larval duration of Kolar Gold

Treatment	Mean larval duration (hr)				
	Initial instars			Later instars	
	First	Second	Third	Fourth	Fifth
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
T_1	121 ± 0.82	92 ± 1.63	112 ± 2.45	120 ± 1.63	144 ± 1.83
T_2	131 ± 1.15	94 ± 0.82	120 ± 1.63	130 ± 1.41	148 ± 1.63
T_3	124 ± 2.31	91 ± 2.58	114 ± 0.82	124 ± 2.16	147 ± 0.82
T_4	72 ± 1.63	84 ± 1.63	96 ± 1.63	114 ± 2.45	140 ± 1.63
SE ±	0.790	0.889	0.866	0.978	0.763
C.D at 5%	2.44	2.74	2.67	3.01	2.35
C.V. %	1.41	1.97	1.56	1.60	1.05

Table.6 Effect of natural and artificial diets on mean moulting period of Kolar Gold

Treatment	Mean moulting period (hr)			
	First	Second	Third	Fourth
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
T_1	25 ± 1.41	26 ± 0.81	27 ± 1.08	27 ± 0.81
T_2	28 ± 0.81	30 ± 1.25	34 ± 1.63	36 ± 1.63
T_3	27 ± 1.08	28 ± 1.15	28 ± 0.50	28 ± 1.15
T_4	24 ± 1.63	26 ± 0.81	28 ± 1.15	30 ± 1.41
SE ±	0.637	0.515	0.581	0.645
C.D at 5%	1.96	1.59	1.79	1.99
C.V. %	4.90	3.73	3.96	4.26

Table.7 Effect of natural and artificial diets on total larval phase of Kolar Gold

Treatment	Duration of test treatments in comparison with natural diet (hr)					
	Feeding phase			Non- feeding phase		
	Larval	Extended	Curtailed	Moulting	Extended	Curtailed
T_1	589.00	83	-	105	-	3
T_2	623.00	117	-	128	20	-
T_3	600.00	94		111	3	-
T_4	506.00			108		

Table.8 Effect of natural and artificial diets on disease occurrence of Kolar Gold

Treatment	Disease occurrence (%)	
	Initial three instars	Later two instars
T_1	0.75 (4.96)	2.53 (09.15)
T_2	1.75 (7.60)	4.60 (12.39)
T_3	1.00 (5.73)	2.55 (09.19)
T_4	1.50 (7.00)	3.01 (10.00)
SE ±	0.007	0.016
C.D at 5%	0.02	0.05
C.V. %	1.18	1.01

(Figures in parenthesis are mean arcsine transformed values)

In T_4 (natural diet), moulting period required for first, second, third and fourth moults was recorded as 24, 26, 28 and 30 hr, respectively. In first moult, the period required for moulting was significantly extended by 4 hr in T_2 (28 hr) that was found to be at par with T_3 (27 hr) extended by 3 hr wherein T_1 (25 hr) was observed to be extended by only 1 hr when compared to natural diet. In second moult, requisite time was extended by 4 hr in T_2 (30 hr) followed by T_3 (28 hr) extended by 2 hr and T_1 reported similar time as that of natural diet (26 hr). In third moult, the requisite time was extended by 6 hr in T_2 (34 hr) while T_3 (28 hr) recorded similar period as that of natural diet. Wherein, the time period required for moulting in T_1 (27 hr) was curtailed by 1 hr. In fourth moult, the

requisite time was extended by 6 hr in T_2 (36 hr). It was curtailed by 2 and 3 hr in T_3 (28 hr) and T_1 (27 hr), respectively. Moulting period was more or less extended in almost all the treatments except in T_1 wherein, it was prominently curtailed in last two moults than T_4 (natural diet). The requisite period for moulting was observed to be more or less extended in all the treatments when compared to the natural diet except for T_1. These findings are in accordance with Bhojne *et al.,* (2013) reported that larval and moulting period was prolonged in the artificial diet in comparison with natural diet, however the results do not correspond the findings of Roychoudhury *et al.,* (1994) who observed it to be reduced.

Larval feeding duration was extended by 117 hr in T_2 (623 hr), followed by T_3 (600 hr) extended by 94 hr, followed by T_1 (589 hr) extended by 83 hr. Moulting period was extended by 20 and 3 hr in T_2 and T_3, respectively, whereas curtailed by 3 hr in T_1 during non-feeding phase.

In initial instars, flacherie disease occurrence was significantly lowest in T_1 (0.75 %) that was followed by T_3 (1.00 %), followed by T_4 (1.50 %), however the highest disease occurrence in initial instars was reported in T_2 (1.75 %). In later instars, significantly highest disease occurrence was reported in T_2 (4.60 %), followed by T_4 (3.01 %) that was followed by T_3 (2.55 %) and minimum disease occurrence was reported in T_1 (2.53 %). The results are in accordance with Matsubara *et al.,* (1989) reported that aseptically prepared diet in bioclean room led to meager disease occurrence, Gomma *et al.,* (1976) reported feeding of the larvae on artificial diet from the beginning of second of second instar had least percentage of mortality, Sannamvong and Quiniones (1990) reported that formulated diet recorded lesser larval mortality in comparison with natural diet.

In conclusion, amongst all the three formulated diets of all the races *viz*., Kolar Gold, FC_1 x FC_2 and FC_2 x FC_1; T_1 (artificial $diet_1$) exhibited better performance. In the entire instars disease incidence was meager. When compared to the natural diet both larval weight and larval length was found to be lower in initial instars and remarkably higher in later instars. Larval duration was prolonged in silkworms fed on artificial diet.

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