EFFECT OF CHLOROMYCETIN AND GLYCINE ON THE GROWTH AND PRODUCTION OF SILK BY *BOMBYX MORI* L.

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Summary

Two batches of silkworms, one in the IV instar and the other in the V, have been fed on alternate days with various concentrations of chloromycetin and the minimum quantity of the antibiotic required to produce the maximum beneficial effect has been determined to be of the order of 50–60 mg./kg. body weight of the larvæ.

Simultaneous supplementation of chloromycetin and glycine has been shown to possess the distinct advantage of reducing the quantities of the two substances to half the concentrations previously employed to produce the desired effects. It has also been indicated that the administration of chloromycetin to the larvæ twice on alternate days is not as effective as supplementing the antibiotic once daily along with glycine.

Introduction

The growth-promoting effect of antibiotics is more or less a recognised fact. Likewise, amino acids are also known to play an important role in the nutrition and metabolism of man and domestic animals. Luecke *et al.* (1950) and other workers (Lawrence and McGinnis, 1952; Vijayaraghavan *et al.*, 1952) have reported on the beneficial influence of streptomycin on the growth of chicks and that of aureomycin and terramycin on the development of rabbits and rats. Since then Murthy and Sreenivasaya (1953, 1954) have demonstrated the beneficial effect of chloromycetin on the growth of silkworm and have pointed out the increase in the yield of silk as due to the simultaneous supplementation of the antibiotic and glycine. However, the concentration of chloromycetin employed by the above workers was of so high an order (50 mg./kg. body weight of larvæ) as to render the application of this finding impractical on an industrial scale. It was therefore considered worthwhile to investigate further into this problem and find out if the dosages fixed by them for the antibiotic as well as the amino acid could further be reduced without adversely affecting either the growth and health of the organism or the production of silk therefrom and the results obtained during the investigation are presented in this paper.

Materials, Methods and Results

Disease-free Mysore XC *Nichi* 1 cross-breed layings were obtained from Channapatna Silk Farms and allowed to hatch at the room temperature (22–24° C.). When the larvæ just completed their third or fourth moult as the experiment demanded, they were reared in batches of 30 each in one experiment and individually in the rest. Batch rearing was done in ply-wood trays and wide-mouthed bottles were employed for individual rearings. Three series of experiments were carried out as outlined below.

In the first experiment, the larvæ were reared in batches of 30, both in the IV and the V instars. Chloromycetin solution was prepared in distilled water in concentrations of 20, 30, 40, 50, 60 and 70 mg./100 ml. For each feed, 0·05 ml. per g. of larvæ was administered by smearing the solution on fresh mulberry leaves. The antibiotic was given only on alternate days, the first and the last feeds being with chloromycetin. This constituted the concentrations 20, 30, 40, 50, 60 and 70 mg./kg. body weight of silkworms, the total number of chloromycetin feeds not exceeding 6 for the entire instar. As control batches, worms were reared on (1) leaves supplemented with an equal volume of water, (2) with 50 mg. per kg. of chloromycetin daily during the V instar, and (3) unsupplemented fresh leaves. Duplicates were run for all experiments.

When the silkworms were administered chloromycetin during the IV instar, they were dosed heavily on the first day with about three times the concentrations specified above and kept on the proposed dosages during the subsequent feeds. In the V instar, they were allowed to feed on untreated leaves.

All the worms were transferred on to mountages at the time of spinning (for results, see Tables I, II, III and IV).

In the second experiment, the larvæ were reared individually with ten worms reserved for each treatment. Chloromycetin was employed in concentrations 12·5 mg., 25 mg. and 50 mg./kg. body weight of larvæ. Glycine solution was prepared in water separately and that volume of the solution was used which would give a concentration of 2 mg. per larva per day. On the days when chloromycetin was not given (since chloromycetin was supplemented only on alternate days) only glycine solution was smeared on the leaves. The control batches consisted of (1) worms fed with untreated fresh leaves, and (2) worms fed with leaf *plus* water.

In the third experiment also the worms were reared individually. Chloromycetin concentration was kept at 25 mg. and 50 mg. levels. Glycine supplied was in the same concentration as specified in the previous experiment. In addition to the two controls already mentioned two other sets were reared amongst which one received only glycine in the above concentration and the other, 25 mg./kg. of chloromycetin together with half the quantity of glycine. The total number of

TABLE I

Growth response of silkworms when chloromycetin is fed during the IV instar

(Weights of 10 larvæ in g.)

No. of days after III moult	Leaf	Leaf + Water	Chloromycetin					
			20 mg. kg.	30 mg. kg.	40 mg. kg.	50 mg. kg.	60 mg. kg.	70 mg. kg.
1	0·8829	0·8804	0·8830	0·8918	0·8813	0·9016	0·9036	0·9101
2	1·3077	1·5089	1·4824	1·4951	1·4903	1·5159	1·4915	1·5243
3	2·1015	2·3190	2·2452	2·3425	2·2452	2·2811	2·3138	2·3347
4	3·2902	3·5989	3·5648	3·5652	3·5695	3·6155	3·6743	3·6994
5 } 6 }		In moult	Weights not taken		In moult			
7	4·1860	4·1335	4·1781	4·1852	4·2025	4·2643	4·2770	4·4070
8	7·1040	7·4252	7·0345	6·8225	6·8860	7·4280	7·3235	7·6577
9	10·8415	11·2645	10·7982	10·1445	10·2438	11·0372	10·9367	11·4472
10	13·6589	15·2494	14·1309	14·0051	14·0904	14·9182	14·9909	15·7121
11	18·9481	19·0365	19·3583	18·6068	18·5700	19·7810	19·4691	20·3054
12	20·3170	19·8030	19·6432	19·3656	19·1646	19·6880	19·9878	19·8054
13		Spinning		Weights not taken		Spinning		

TABLE II

Weights of cocoons, pupæ and shells corresponding to the larval weights given in Table 1

Description	Leaf	Leaf + Water	Chloromycetin					
			20 mg./kg.	30 mg./kg.	40 mg./kg.	50 mg./kg.	60 mg./kg.	70 mg./kg.
Cocoons ..	10·4010	10·1275	10·2433	10·1192	10·1394	10·8956	10·3423	10·3395
Pupæ ..	9·0101	8·7473	8·8544	8·7517	8·7662	9·4486	8·9341	8·9252
Shells (wet wt.) ..	1·3909	1·3802	1·3889	1·3675	1·3732	1·4470	1·4082	1·4143
Shells (dry wt.) ..	1·3389	1·2735	1·2943	1·3004	1·2869	1·3563	1·3283	1·3466
Yield of silk (%) ..	100	95·1	96·68	97·14	96·02	101·4	99·19	100·6

TABLE III

Growth response of silkworms to chloromycetin during the V instar

(Weights of 10 larvæ in g.)

No. of days after IV moult	Leaf	Leaf — Water	Chloromycetin						
			20 mg. kg.	30 mg. kg.	40 mg. kg.	50 mg. kg.	60 mg. kg.	70 mg. kg.	50 mg. kg. daily
1	3·5242	3·5858	3·5943	3·5242	3·5137	3·5035	3·6496	3·6099	3·5579
2	6·0790	6·4277	6·4765	6·3191	6·3367	6·4845	6·7460	6·5742	6·4515
3	9·1830	10·0008	9·8846	9·7031	9·9516	10·0907	10·3597	10·0232	10·0655
4	13·4095	13·8292	13·6865	13·8933	14·3392	14·0160	14·6205	14·3267	14·2475
5	17·4462	18·5099	18·1817	18·1037	18·4102	18·4429	19·0417	18·7209	18·5822
6	20·3622	21·7851	21·3210	20·9386	21·4775	21·6336	22·6466	22·7490	22·7203
7	Spinning		Weight not taken				Spinning		

TABLE IV

Weights of cocoons, pupæ and shells corresponding to the larval weights given in Table III

Description	Leaf	Leaf + Water	Chloromycetin						
			20 mg./kg.	30 mg./kg.	40 mg./kg.	50 mg./kg.	60 mg./kg.	70 mg./kg.	50 mg./kg. daily
Cocoons ..	10·3281	10·4827	10·4994	10·5756	10·5423	10·9987	10·9383	11·1307	10·6711
Pupæ ..	8·9195	9·0510	9·0754	9·1295	9·0917	9·4990	9·4547	9·6195	9·2429
Shells (wet wt.) ..	1·4086	1·4317	1·4240	1·4461	1·4506	1·4997	1·4836	1·5112	1·4282
Shells (dry wt.) ..	1·3173	1·3367	1·3243	1·3509	1·3612	1·4035	1·3849	1·4191	1·3550
Silk yield (%) ..	100	101·4	100·6	102·5	103·3	106·5	105·1	107·6	102·8

feeds with the antibiotic was kept constant at six and distributed over the entire instar at the rate of one feed a day. The remaining feeds consisted of glycine alone.

In all the three series of experiments, the cocoons were harvested on the fifth day of mounting. Shells and pupæ were separated and weighed and the yield of silk was computed taking the value for leaf control as 100 on the basis of dry weight of the shells (for results of experiments 2 and 3, see Tables V, VI, VII and VIII).

DISCUSSION

The beneficial effects conferred on the animal by an antibiotic have been attributed to an alteration in the animal's intestinal microflora (Sieburth *et al.*, 1951; Anderson *et al.*, 1952) which is probably brought about in anyone of the following ways, *viz.*, (1) by bringing about inhibition or death of the bacteria which produce toxins, (2) by affecting a reduction in the microbial population and thereby diminishing the competition for available nutrients between the micro-organisms and the host and (3) by selective inhibition of certain types of micro-organisms and thereby bringing about conditions favourable for the synthesis of nutrients valuable for the growth of the animal. Chloromycetin does not appear to cause any of the above changes in silkworms during the IV instar as no alteration has been observed either in growth or in the yield of silk (Tables I and II). On the other hand, supplementation of chloromycetin during the V age promoted remarkable increase in growth although the production of silk was not influenced appreciably (Tables III and IV). During this advanced stage the antibiotic has probably acted as a growth-promoting factor at the cost of the precursors of silk (Murthy and Sreenivasaya, 1953), and/or by way of enhancing the transaminase activity (Shyamala and Bhat, 1955).

It was also observed that chloromycetin in concentrations as high as 850 mg./kg. body weight and above causes the death of the silkworms, and this appears to be due to the direct toxic effects exerted by the antibiotic on the animal itself rather than due to any substantial alteration of the type of flora of the intestines of the silkworms. Aureomycin, for example, has been reported to have caused the death of guinea-pigs on account of its toxicity (Roine and Ettala, 1952).

From Table V, it is clear that the incorporation of chloromycetin and glycine does not affect the larval weights to any considerable extent. Nevertheless, the results from Table VI indicate that a similar treatment in the proportion of 25 mg./kg. body weight of chloromycetin and 2 mg. glycine per larva per day gives increased cocoon and pupa weights as well as a 10% increase in the yield of silk. A further increase in the concentration of chloromycetin as for instance, 50 mg./kg. with the same amount of glycine has an adverse effect on the weight of the larvæ without any improvement being registered in the production of silk. It is also noticed that the quantity of glycine can be profitably halved to obtain the most beneficial effects.

TABLE V

Effect of different concentrations of chloromycetin on the larval growth when glycine is incorporated

(Weights of 10 larvæ in g.)

No. of days after IV moult	Leaf	Leaf + Water	Glycine 2 mg. + Chloromycetin 12·5 mg.	Glycine 2 mg. + Chloromycetin 25 mg.	Glycine 2 mg. + Chloromycetin 50 mg.
1 ..	4·0670	3·9680	3·9880	4·0770	4·1340
2 ..	6·3020	6·7160	6·4630	6·9560	7·6180
3 ..	10·2740	10·3910	10·0320	10·5780	11·6790
4 ..	13·9770	15·2750	14·6810	14·8170	15·6160
5 ..	19·0380	20·0360	18·9460	19·2850	18·4050
6 ..	22·6880	23·0460	21·2650	23·0250	21·8660
7 ..	Spinning		Weights not taken		Spinning

TABLE VI

Weights of cocoons, pupæ and shells corresponding to the larval weights in Table V

Treatment	Cocoons	Pupæ	Shells wet wt.	Shells dry wt.	Yield of silk
Leaf	13·6320	12·3250	1·3070	1·2000	100
Leaf + Water	13·1140	11·8600	1·2540	1·1620	97
Glycine 2 mg. + Chloromycetin 12·5 mg.	12·7620	11·4070	1·3550	1·2605	105
Glycine 2 mg. + Chloromycetin 25 mg. ..	14·0970	12·6844	1·4126	1·3204	110
Glycine 2 mg. + Chloromycetin 50 mg. ..	12·4500	11·0165	1·4335	1·3320	111

TABLE VII

Results of feeding chloromycetin and glycine together only once a day for six days

(Weights of 10 larvæ in g.)

No. of days after IV moult	Leaf	Leaf + Water	Glycine 2 mg.	Glycine 2 mg. + Chloro. 25 mg.	Glycine 2 mg. + Chloro. 50 mg.	Glycine 1 mg. + Chloro. 25 mg.
1	4·4800	4·4000	4·5470	4·4340	4·4070	4·5330
2	5·7210	6·1050	6·1640	5·9270	5·8130	6·0650
3	8·4360	9·6970	9·7720	9·5740	9·3040	9·8080
4	12·8830	14·9040	14·9330	14·8380	14·4560	15·4300
5	19·5730	21·1110	20·6490	20·6970	19·5220	20·9300
6	23·1170	25·6240	25·6490	25·7020	23·9840	26·0570
7	Spinning		Weights not taken		Spinning	

Table VIII

Weights of cocoons, pupæ and shells corresponding to the larval weights in Table VII

Treatment	Cocoons	Pupæ	Shells wet wt.	Shells dry wt.	Yield of silk
Leaf	14·3072	12·8842	1·4230	1·3160	100
Leaf + Water	14·7178	13·2308	1·4870	1·3755	104·5
Glycine	15·1868	13·6678	1·5190	1·4580	110·7
Glycine 2 mg. + Chloro. 25 mg. ..	14·9320	13·3850	1·5470	1·4920	113·0
Glycine 2 mg. + Chloro. 50 mg. ..	14·0028	12·4318	1·5710	1·5090	114·6
Glycine 1 mg. + Chloro. 25 mg. ..	15·4934	13·8984	1·5950	1·5380	116·8

Although Table VII does not indicate any marked difference in larval weights, it is interesting to note from the results presented in Table VIII that glycine has its beneficial influence on the yield of silk when the proportions of chloromycetin and glycine are 25 mg. and 1 mg. respectively. This is significant from the practical standpoint inasmuch as it affords a reduction of the expensive antibiotic to half the original concentration fixed as optimal for this supplement. Moreover, the minimum quantity of glycine needed is also reduced to half the amount thereby lowering the cost of feeding and minimising at the same time the chances of glycine toxicity (Grosehke *et al.*, 1946; Murthy, 1955).

Another significant observation is that chloromycetin is effective to its maximum extent when supplemented along with glycine on all the six days of the V instar instead of only two times on alternate days. The optimum concentrations are of the order of 25 mg./kg. for chloromycetin and 1 mg. per larve per day for glycine. It is also evidenced from these experiments as well as various other protocols in our possession that the beneficial influence of chloromycetin or glycine is of a lower order of magnitude individually as compared to that of their combination. This combination, it may be emphasised, brings about a 16 per cent increase in the yield of the valuable product, silk.

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