EFFECT OF CHLOROMYCETIN ON FEED UTILISATION BY THE SILKWORM *BOMBYX MORI* L.

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Received April 18, 1956

SUMMARY

Data are presented for the leaf consumption, digestion and increase in weight of larvæ reared on untreated mulberry leaves and leaves supplemented with chloromycetin. It is found that chloromycetin increases the feed utilisation of the silkworm. The possible role of chloromycetin in the feed efficiency of the silkworm is discussed in the light of the above findings.

The growth-promoting effect of antibiotics has been demonstrated in farm animals and birds (Carpenter, 1951; Stokstad and Jukes, 1951; Elam and Couch, 1951). However, the mechanism of action of antibiotics is still incompletely understood. There is controversy as to whether the antibiotic acts entirely through its antibacterial property or by affecting favourably the physiology and metabolism of the host organism itself. In the latter event, it may be expected to manifest by an increase in the feed efficiency or by the activation of enzymes or through hormones which control and regulate growth (Braude *et al.*, 1953; Stokstad, 1954).

Previous reports from this laboratory have shown the growth-promoting activity exerted by chloromycetin and aureomycin in the larvæ of the silkworm. It has also been shown that an increased yield of silk protein occurs when an extra nitrogen source is provided along with the antibiotic to the silkworm diet (Murthy and Sreenivasaya, 1953 and 1954; Sharada and Bhat, 1956). Furthermore, evidence has been provided in a recent report (Shyamala and Bhat, 1955) on the activation of the glutamic-aspartic acid transaminase in the tissues of the silkworm on chloromycetin supplementation. Preliminary studies on amylase activity of the silkworm intestines have also indicated an increase in amylase activity as a result of chloromycetin supplementation. The present paper describes experiments conducted in order to determine the influence of chloromycetin on the feed efficiency and digestibility of the silkworm.

MATERIALS AND METHODS

Two series of experiments were conducted. In the first series, an aqueous solution of chloromycetin was administered and hence a control batch of worms reared on water alone as the supplement to the mulberry leaves was maintained. The results given below show that water itself has a profound effect on the consumption of leaf and digestion. Hence, another experiment was designed in which

chloromycetin was dissolved in acetone and mulberry leaves were dipped in the solution and the acetone allowed to evaporate from the leaf surface leaving a uniform distribution of chloromycetin, so that there was as little interfering substance as possible. This procedure was adopted after ascertaining that the leaves so treated did not affect the worms to any appreciable extent.

A. Aqueous solution of chloromycetin

(i) *Set-up.*—150 Cross-breed (Mysore × C. Nichi) silkworms just out of the III moult were selected (equal size and weight) from a batch of worms reared in the laboratory from disease-free eggs. They were divided into 3 groups each containing 5 replicates of 10 larvæ. The larvæ were reared and maintained in clean plywood trays in a place of uniform lighting and aeration. The first group of 5 batches received fresh mulberry leaves whereas the second and the third groups were fed on supplements of water and chloromycetin respectively along with the mulberry leaf.

(ii) *Feeding.*—Fresh mulberry leaves were brought from the garden just before feeding and the larvæ were fed four times a day. The amount of leaf supplied to each batch was carefully weighed before feeding.

(iii) *Collection of material for estimation.*—It was considered useful to record all results collected in the course of the experiment on the moisture-free basis so that the variation in the amount of water in the different materials under consideration may not interfere in the correct interpretation of the data.

At exactly the same time every morning, just before the first feeding, the litter in the trays was separated carefully into the excretory portion and the unconsumed leaf portion. The materials were dried in an electric oven at 110° C. and weighed. These gave values for unconsumed leaf and excreta. Three separate samples of fresh mulberry leaves were collected at random from the same supply of leaves and the dry weights were determined. This value was adopted for calculating the dry weights of leaves fed to the experimental larvæ.

In order to obtain the moisture-free weights of larvæ for each day of experiment, the following procedure was adopted.

Six hundred healthy silkworms were collected from the same batch from which the experimental larvæ were taken and were divided into three groups of 200 each (general batches). Rearing was conducted in suitable trays so as to secure as much uniformity in spacing as was obtaining in trays in which rearing of 10 worms (experimental batches) was carried out. One group was fed on fresh unsupplemented mulberry leaves and the other two with supplements of water and chloromycetin respectively. The chloromycetin was administered both for the general and experimental batches by dipping the leaves in an aqueous solution containing 50 μg./ml. of the antibiotic during the IV instar and 200 μg./ml. during the V instar. The supplements were administered only on alternate days as it has been our experience that silkworms dosed continuously with water for a long time tend to suffer from intestinal disorders towards the time of spinning.

From each of these general batches, 10 larvæ were selected every morning in such a way that they had approximately the same size and body weight as the corresponding experimental larvæ. Their dry weights were determined and these were used as the basis for calculating the dry weights of the experimental larvæ. It was found, however, that the rate of larval development in the general batches did not always keep pace with that of the experimental batches, particularly during the V instar, the former taking a much longer time to attain maximum growth. It was, therefore, not feasible to make daily computations of dry weight of experimental larvæ on the basis of values obtained for the general batches. The average dry content of the larvæ for the total number of days in the V instar was therefore calculated and this value employed for obtaining the gain in dry weight of the experimental larvæ during this physiological period.

B. Acetone as solvent for chloromycetin

The layout of the experiment was the same as in the first series with water as the solvent. Feed utilisation studies were conducted with Mysore × C. Nichi worms from the IV moult onwards since in the previous experiment it was observed that the effect of chloromycetin decreased on the continued administration of the drug. Three hundred worms were taken, divided into 3 groups, each group containing 5 replicates of 20 larvæ each.

The concentration of chloromycetin employed was 200 μg./ml. The leaves were dipped in the acetone solution, the acetone evaporated by keeping the leaves exposed to the atmosphere for a few minutes and the treated leaves were then given to the worms.

To overcome the discrepancy observed in the growth of the worms in the experimental batches maintained for studying the feed utilisation and the general batches maintained for the daily determination of the dry weights of larvæ, group rearing (20 worms in each tray) was adopted in general batches also instead of mass rearing of 200 worms. The experimental period was 5 days and 15 trays containing 20 worms each were taken of which 5 trays were meant for each treatment, *viz.*, untreated leaves, acetone-treated leaves, leaves treated with a solution of chloromycetin in acetone. One tray from each treatment was used up daily to find out the dry weights. The dry weights of the experimental batches were computed on the basis of the dry weights obtained from the general batches.

Results and Discussion

It will be seen from Table I that during the IV instar, the total amount of leaf consumed or digested do not show any significant differences in differently treated feeds. But during the same period, the larvæ dosed with water and chloromycetin reveal 15 per cent and 17 per cent more of dry weight as compared with the unsupplemented series. During the V instar supplementation of water results in a consumption increase of 15 per cent over the control whereas the antibiotic supplementation registers an increase of 8 per cent only. In spite of the increased consumption the total amount of leaf digested is actually 22 per cent less in the

TABLE I

Leaf consumption, digestion, increase in larval weight

(expressed in gms./10 larvæ)

Water solvent	Fourth instar			Fifth instar		
	Leaf only	Leaf + Water	Leaf + Chloro.	Leaf only	Leaf + Water	Leaf + Chloro.
Dry weight of leaf consumed ..	2·337	2·261	2·233	14·03	16·425	15·761
Dry weight of leaf digested ..	0·891	0·944	0·883	5·531	4·318	3·392
Increase in larval dry weight ..	0·269	0·309	0·316	0·999	1·495	1·483
% Utilisation (consumption)* ..	11·5	13·67	14·15	7·12	9·10	9·41
% Utilisation (digestion)† ..	30·18	32·73	35·78	18·10	34·60	43·70
Acetone solvent	Experiment Started after IV moult in view of the			Leaf only	Leaf + Acetone	Leaf + Chloro.
Dry weight of leaf consumed ..	results obtained in the			16·896	17·198	17·236
Dry weight of leaf digested ..	previous experiment			6·005	6·254	5·961
Increase in larval dry weight ..				3·183	3·225	3·272
% Utilisation (consumption) ..				18·83	18·75	18·98
% Utilisation (digestion) ..				52·99	51·57	54·89

* Percentage utilisation on the basis of consumption $= \frac{\text{Inc. in dry weight of larvæ during a given interval of time, } \times 100}{\text{The dry weight of leaf consumed during the same period}}$

† Percentage utilisation on the basis of digestion $= \frac{\text{Inc. in dry weight of larvæ during a given interval of time } \times 100}{\text{The dry weight of leaf digested during the same period}}$

case of water and 38 per cent less in the case of chloromycetin. Crampton and Lloyd (1954) observed that food intake and utilisation are considerably reduced in rats whose water-supply was restricted, thus stressing the importance of adequate amount of water with the diet. The results obtained with water only as the supplement give ample evidence to the essentiality of adequate amounts of water for the silkworm.

The significant response obtained with the aqueous solution of chloromycetin is not observed with acetone solvent even though the decreased digestion of leaf and increased body weight are observed in both cases. However, the discrepancies observed in growth of silkworm between the experimental batch and the batches from which larvæ were selected for the determination of dry weight make the values for the dry weight of experimental larvæ less accurate. But with acetone solvent the growth of the experimental batches and batches maintained for dry weight determination synchronised very well and hence give a correct estimate of the dry weights of the experimental larvæ.

A comparison of the efficiency of feed utilisation on the basis of the digested leaf, between the controls and chloromycetin treated larvæ, shows that there is about 25 per cent increase in the feed utilisation when an aqueous solution of chloromycetin is given and about 6 per cent increase when given in acetone solvent. It has already been shown that chloromycetin supplementation increases the transaminase activity of the intestines and the hæmolymph of the silkworm (Shyamala and Bhat, 1955). Preliminary studies also indicate an increase in the amylase activity of the intestines of chloromycetin fed worms. The increase in feed efficiency observed above may be due to a better utilisation of the food by increased enzyme activity. However, a comprehensive study of other enzyme systems participating in metabolism may throw more light on the mode of action of the antibiotic.

Figure 1 depicts the daily change in the digestibility (amount of leaf-digested/100 gm. dry weight of leaf consumed). A steep rise is observed just after IV moult, a decrease in the middle of the instar and again a slight increase just before the end of the instar. Administration of chloromycetin appears to result in an increased digestibility compared to the acetone control. This advantage, however, is lost after a continued administration of this drug to the larvæ. This observation is probably suggestive of the role of chloromycetin as an antibacterial agent in nutrition. It is possible that initial increases in digestibility quotients are due to the action of chloromycetin in rearranging and conditioning intestinal flora of the silkworm in a way beneficial to the larva from the point of view of its nutrition. This gain is lost towards the end, probably because a continued administration of chloromycetin results in the elimination of more of those microbial types which are necessary for a normal nutritional balance in the silkworm. This view is expressed as a tentative suggestion and requires further investigation.

The feed utilisation on the basis of consumption shows (Fig. 2) alternatively high and low values and chloromycetin supplementation increases the feed utilisation except on one day as compared with the acetone control.

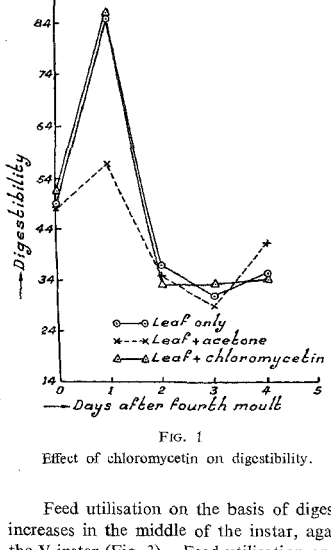

FIG. 1
Effect of chloromycetin on digestibility.

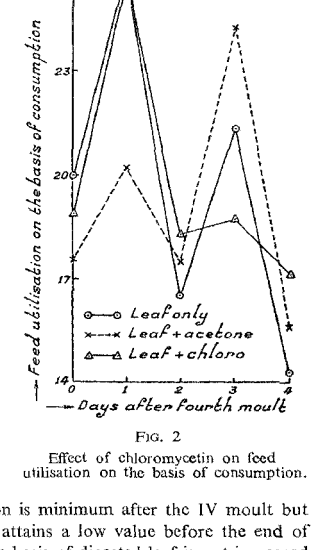

FIG. 2
Effect of chloromycetin on feed utilisation on the basis of consumption.

Feed utilisation on the basis of digestion is minimum after the IV moult but increases in the middle of the instar, again attains a low value before the end of the V instar (Fig. 3). Feed utilisation on the basis of digested leaf is not increased as a result of chloromycetin supplementation except before the end of the instar when chloromycetin favourably affects the feed utilisation.

The data presented in Table II reveal that during the IV and the V instars, supplementation of water to the larval diet results in a decrease in the dry content of the larval body from 17·56 per cent to 14·58 per cent. at the time of maturity. In other words, the moisture content of the tissues increases. This property is not consistently displayed for all the individual days of the instar, but the total effect of an increased uptake of water appears to favour its increased deposition in the tissues. A simultaneous administration of chloromycetin is able to increase the dry content although the percentage dry weight still remains significantly lower than the control batches for the first few days. This effect of chloromycetin is particularly evident during the second half of the V instar when the supplementation

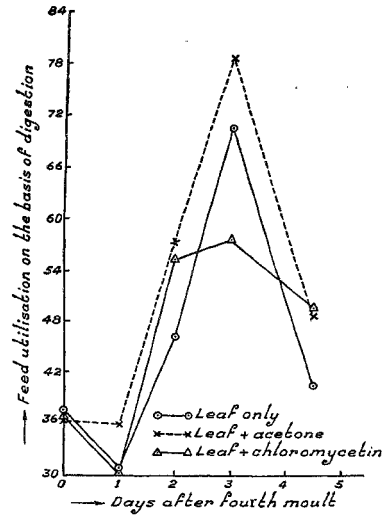

FIG. 3. Effect of chloromycetin on feed utilisation on the basis of digestion.

of this antibiotic is able to bring back the dry weight content of the larval body to the normal level of the control. This might mean that chloromycetin either helps in a better utilisation of water for building up of the tissues and/or that there is an elimination of the excess water as a result of the administration of chloromycetin. Data presented in Table III do not show any significant increase in the dry weight of chloromycetin administered larvæ as compared with the acetone treated control batches, but the increase in larval weight (Table I) show that even when there is a reduction in the water intake in the acetone control batches as a result of partial dehydration of the treated leaves, chloromycetin is able to assist in the growth of silkworms. Slinger and Pepper (1955) observed that the response of chicks to penicillin was as great when water was given *ad libitum* as when water intake was restricted and indicated that penicillin might have a sparing effect on the water requirement of chicks and poults. However, the elimination of excess of water might also have been effected by chloromycetin, when water was used as the solvent. Braude and Johnson (1953) found that aureomycin supplementation to young pigs increased the rate of growth and feed utilisation and also brought about higher urinary excretions. Hence, chloromycetin when given as an aqueou-

solution to the silkworms might help in a better utilisation of water for growth as well as in the elimination of excess water.

TABLE II

Percentage dry weight of larvæ with water solvent for chloromycetin

Days after III moult	Leaf only	Leaf + Water	Leaf + Chloro.	Days after III moult	Leaf only	Leaf + Water	Leaf + Chloro.
1	8·83	9·43	9·34	9	10·92	10·08	10·19
2	10·36	8·83	9·39	10	11·64	..	9·71
3	9·14	8·30	8·12	11	11·86	10·57	10·52
4	10·29	9·22	9·84	12	12·66	10·78	12·00
5		In IV moult		13	12·62	11·31	12·09
6	11·96	10·52	10·81	14	12·89	12·67	13·89
7	10·45	9·67	9·98	15	15·63	14·59	15·19
8	..	10·22	9·90	16	17·56	14·58	17·71

TABLE III

Percentage dry weight of larvæ with acetone as solvent for chloromycetin

Days after IV moult	Leaf only	Leaf + Acetone	Leaf + Chloro.
Just after IV moult	11·08	11·08	11·08
1	12·26	11·55	11·69
2	13·75	12·44	13·75
3	14·62	14·16	14·92
4	17·16	17·80	16·75
5	19·87	19·88	19·91

When acetone is used as the solvent, the leaves dipped in either acetone alone or a solution of chloromycetin in acetone, lose part of the moisture content. The slight increase in the body weight of chloromycetin fed larvæ might thus be due to a better utilisation of water or due to other changes which might have been brought about by acetone treatment.

ACKNOWLEDGEMENTS

Our thanks are due to the Central Silk Board for the financial aid and to the Director, Indian Institute of Science, for his keen interest.

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