

AN APPROACH TO NUTRITIONAL CONTROL MECHANISMS
IN THE SILKWORM, *BOMBYX MORI*

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ABSTRACT

The effects of the various diet components on the body composition and metabolism of the silkworm, *Bombyx mori*, are reviewed in order to formulate an understanding of the nutritional control mechanisms in this insect.

INTRODUCTION

The growth rate of silkworm larvae and the amount of silk which they produce are influenced by the quality of their food, i.e., mulberry leaves. The weight increment of this insect is very large, as a newly-hatched larva of the silkworm increases its weight within three weeks (the period required to become a fully-grown caterpillar) by a factor of over 10,000. The nutrients derived from mulberry leaves are metabolized and used for making up the composition of the silkworm body, which is characteristic for each of the tissues, organs, and haemolymph. There are many reports on the chemical composition of the whole body, tissues, organs, and haemolymph of the silkworm, showing that the values, especially that of haemolymph, change with growth and development. The physiological meaning of these changes is not, however, sufficiently understood.

As the nutritional requirements of the silkworm come to light (Ito, 1967), the effects of variations in the dietary composition on larval growth and cocoon production are gradually becoming clear, and some of the mechanisms involved have been elucidated. In addition, it has recently been proved that dietary conditions affect various functions in the body.

The effects of dietary conditions on the chemical composition and metabolic function of the silkworm body may be of two types. One of them includes cases where a specific compound in the body is directly derived from the diet, its amount in the larval body thus being influenced directly by the diet. The diet can be said, in this case, to have a *direct effect*. It is considered that the ability of the larva to synthesize the compound is lacking or relatively scanty. The diet not only has a direct effect but also exerts various secondary effects derived from the direct effect on the body.

In other cases a deficiency of a certain nutrient or an imbalance of nutrients in a diet causes some changes in the composition of larval body or in metabolic activity. It may be said that in these cases the diet has an *indirect effect*. Some abnormal metabolism may take place or some change may appear to suppress an abnormal strain produced in the larval body under the abnormal dietary condition. This change is a kind of adaptation, but it does not necessarily follow that such an adaptation occurs in all the metabolic mechanisms in the body.

Vitamins

One of the typical examples of the direct effect can be demonstrated with water-soluble vitamins. The levels of riboflavin (Horie *et al.*, 1966) and ascorbic acid (Ito and Arai, 1965) in the larval body are entirely dependent on the amounts of these vitamins which were added to the diet. Similar dietary effects will be seen with other vitamins required by the silkworm. In larvae fed on a biotin-deficient diet the secondary effect has been reported; fatty acid content of these larvae decreases considerably and fatty acid composition differs largely from the control (Horie and Nakasone, 1968). The system synthesizing fatty acids in the silkworm seems to involve acyl CoA carboxylation.

Proteins and Amino Acids

With an increase in the level of dietary soybean meal, xanthine oxidase activity of the fat body, midgut, and Malpighian tubes of silkworm larvae increases, and the excretion of uric acid is accelerated (Ito and Mukaiyama, 1964). It may be interpreted that the larva is forced to catabolize excess amino acids rather quickly when kept on a high-protein diet.

The composition of free amino acids in the haemolymph is influenced directly or indirectly by the amino acid composition of the defined diet. When a larva is allowed to feed for thirty hours on a diet from which one of the essential amino acids was omitted, the same amino acid in a free state completely disappears from the haemolymph (Inokuchi, 1970). At the same time the protein level in the haemolymph decreases and ninhydrin-positive substances accumulate in the haemolymph as secondary effects. Furthermore, in such cases there is a tendency for most of the other free amino acids to increase to varying levels. When *D*-methionine is added in place of the *L*-isomer, for instance, a large amount of free methionine is recovered from the haemolymph, but the amino acid pattern undergoes a considerable change because *D*-methionine has very small nutritive activity (Ito and Inokuchi, in preparation). The amino acid pattern obtained in the presence of dietary *D*-amino acid in place of its *L*-isomer is more or less similar to that in the absence of dietary *L*-isomer, except that the free methionine disappears completely from the haemolymph in the latter case. Even when the diet lacks either alanine, glycine, or serine which are classed as non-essential amino acids, some changes occur in the amino acid pattern, which are smaller than those caused by deleting an essential amino acid (Inokuchi, 1970). Comparisons of haemolymph amino acid patterns contribute to evaluations of the dietary efficiency.

The rate of conversion of amino acids by larvae is also influenced by dietary conditions. The conversion from citrulline to arginine is accelerated on the arginine-deficient diet, and those from arginine to proline and ornithine to proline are accelerated on the proline-deficient diet (Inokuchi, 1969b; Inokuchi *et al.*, 1969). A higher recovery of cystine of the tissue protein as well as that of the free cystathionine in the haemolymph from labelled methionine is obtained on the cystine-deficient diet than on the control diet (Inokuchi, 1969a). Furthermore, the levels of free cystathionine, free lanthionine, free methionine, and free cystine in the haemolymph vary according to the dietary levels of both methionine and cystine (Inokuchi, 1972).

These results are clear indications of the occurrence of several metabolic pathways in the silkworm, and radiochemical methods have simultaneously been employed to demonstrate these pathways.

An unusual reddish coloration of the larval body, considered to be due to the accumulation of epidermal pigments, can be obtained either by the excess addition of dietary tryptophan or by the deletion of dietary proline (Arai and Ito, 1967).

Sterols

The sterol composition of the larva is dependent on the dietary sterol (Ito *et al.*, 1970). When fed on a defined diet containing only cholesterol as a sterol source, the larva possesses cholesterol exclusively. When the diet contains either β -sitosterol, stigmasterol, or campesterol, the major larval sterol is cholesterol, but the sterol which was derived directly from the diet also occurs at a substantial level. This may be related to the ability of larvae to convert C_{29} - or C_{28} -sterol to cholesterol. The conversion of β -sitosterol to cholesterol was demonstrated in the silkworm (Ikekawa *et al.*, 1966). Furthermore, the cholesterol content of the whole larva is dependent on its level in the diet.

Utilization of dietary sterol by the silkworm is greatly accelerated in the presence of dietary fatty acid or oil (Ito and Nakasone, 1967). The rate of esterification of ingested cholesterol by the larva is higher in the presence of fatty acid than in its absence (Ito *et al.*, 1970).

Fatty Acids

The silkworm requires several fatty acids for normal growth and development. These include linoleic and linolenic acids which are of high nutritive value, and oleic acid which is of some value. Myristic, palmitic, and stearic acids possess small activity (Ito and Nakasone, 1966). On the other hand, the silkworm synthesizes fatty acids from glucose (Horie *et al.*, 1968). The ^{14}C of labelled acetate (Sridhara and Bhat, 1964) and labelled glucose and pyruvate (Horie *et al.*, 1968) is incorporated into several fatty acids, but neither into linoleic nor into linolenic acid.

Fatty acid composition of the silkworm is dependent on the fatty acids in the diet (Ito and Nakasone, 1969). It is considered that larval linoleic and linolenic acids are derived directly from dietary sources. In the absence of myristic, palmitic, stearic, or oleic acid in the diet, there is almost no effect in the content of each of these acids in the larva. Without any dietary polyunsaturated fatty acid, secondary effects are brought about in the composition of larval fatty acids; namely, the level of oleic acid in the larva increases and comprises over 50% of the total fatty acids, and the level of palmitoleic acid exceeds over 10% of the totals. Larvae which barely reached the third instar in the absence of any dietary fatty acid or oil possess a pattern of fatty acids rather similar to that obtained from larvae reared in the absence of polyunsaturated fatty acids. Thus, in larvae growing on mulberry leaves linolenic acid predominates, making up approximately one-third of the total larval fatty acids, because this fatty acid predominates in mulberry leaves (Ito and Nakasone, 1966; Nakasone and Ito, 1967). The fact that both oleic and palmitoleic acids in the larva increase in the absence of the dietary polyunsaturated fatty acids suggests the presence of a mechanism which keeps the proportions of total unsaturated and saturated fatty acids almost constant.

The rate of fatty acid synthesis in silkworm larvae is regulated by the dietary levels of fatty acids and carbohydrates (Horie and Nakasone, 1971). The synthesis from glucose is depressed by dietary fatty acids, and enhanced by increased dietary sucrose levels.

Carbohydrates

The levels of haemolymph trehalose and fat body glycogen decrease and reach a minimum when the larva is starved for a certain period. The original levels are resumed when the larva is offered some sugars, such as sucrose, glucose, and fructose, but their levels are not or only partly maintained with some other sugars including pentoses (Horie, 1961). The rates of carbohydrate utilization by the silkworm are correlated to a high degree with their nutritive value for this insect (Ito and Tanaka, 1961).

In the silkworm there are several genetic mutant strains which lack amylase in the digestive juice or possess weak activity of it. No synthesis or only a slight synthesis of haemolymph trehalose and fat body glycogen from the dietary starch or dextrin is obtained in such larvae (Mukaiyama *et al.*, 1964).

When the dietary level of sucrose is elevated, the rate of fatty acid synthesis by the larva is accelerated (Horie and Nakasone, 1971). Furthermore, it was recently shown that the contents of haemolymph trehalose, fat body glycogen, and total lipids in the larva can be increased following increases in the dietary glucose, accompanied by a drop in the quantity of free amino acids in the haemolymph (Yanagawa, 1973). The dietary level of glucose also influenced the turnover rate of haemolymph trehalose and the rate of relative participation of the pentose phosphate pathway in glucose metabolism.

To summarize: In the present paper the author tried to describe several examples of nutritional control in the silkworm, *Bombyx mori*. It is clear that dietary conditions affect larval composition and metabolic function, in addition to their obvious effects on growth and development and on cocoon production of the silkworm. Direct dietary effects are cases where the lack of a certain nutrient in a diet causes a deficiency of this nutrient to occur in the body of a larva and a supply of the nutrient results in its accumulation in the body. However, there remain some problems about the mechanical distinction between the direct and indirect effects of a diet.

There have been many discussions of the control mechanisms of growth and development in insects. Needless to say, nutrition plays one of the fundamental roles in these processes. Data on the nutritional control have been accumulating recently in regard to several insects (e.g., Ito and Fraenkel, 1966). Nutritional studies are thus expected to contribute more and more to the solution of metabolic problems in insects.

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