Quantitative Requirement of Nutrients for Growth of the Silkworm, *Bombyx mori* L.

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Basic studies on the utilization of food stuff by the silkworm, *Bombyx mori*, have been carried out since sixty years ago. Hiratsuka (1917)[2] reported the detailed information of ingestion and utilization of food stuff as well as consumption of nutrients for both the growth and the maintenance of life of the silkworm.

Recently, much information has been accumulating on nutritional significance of individual substances in the silkworm by using the chemically defined diet which has been established in the last ten years.[1,23] Namely, the requirements of nutrients for growth and survival of silkworm have been demonstrated qualitatively and quantitatively, and several reviews on this subject are also available.[16,17,18]

In the present paper, emphasis is placed on quantitative requirement of each nutrient for growth and survival of the silkworm, *Bombyx mori*, with reference to metabolism.

Dry matter[7]

The amounts of dry matter ingested and digested by a single larva and those of the whole body of the silkworm, and cocoon layer are illustrated in Fig. 1. About 54 and 59% of the digested dry matter were stored in the mature larval body of male and female, while 47 and 42% were consumed by male and female larvae, respectively. Approximately one fourth of digested dry matter was distributed into cocoon layer, and 11% into eggs. Both 4.6 and 4.4 g of dry matter ingestion were required for g of body dry weight gain in male and female larvae, respectively.

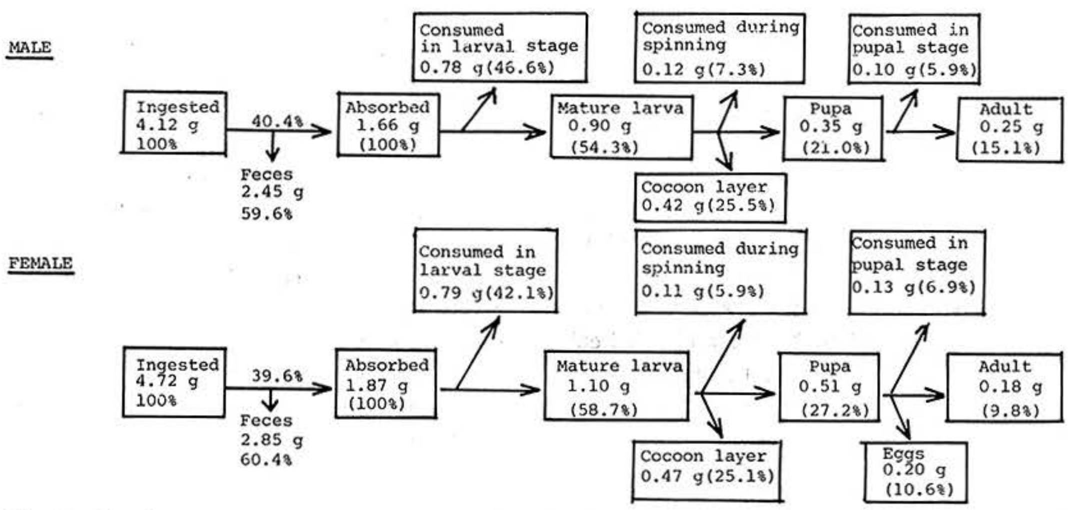

Fig. 1. The dry matter economy of male and female silkworm, *Bombyx mori*, through it's life cycle[7]

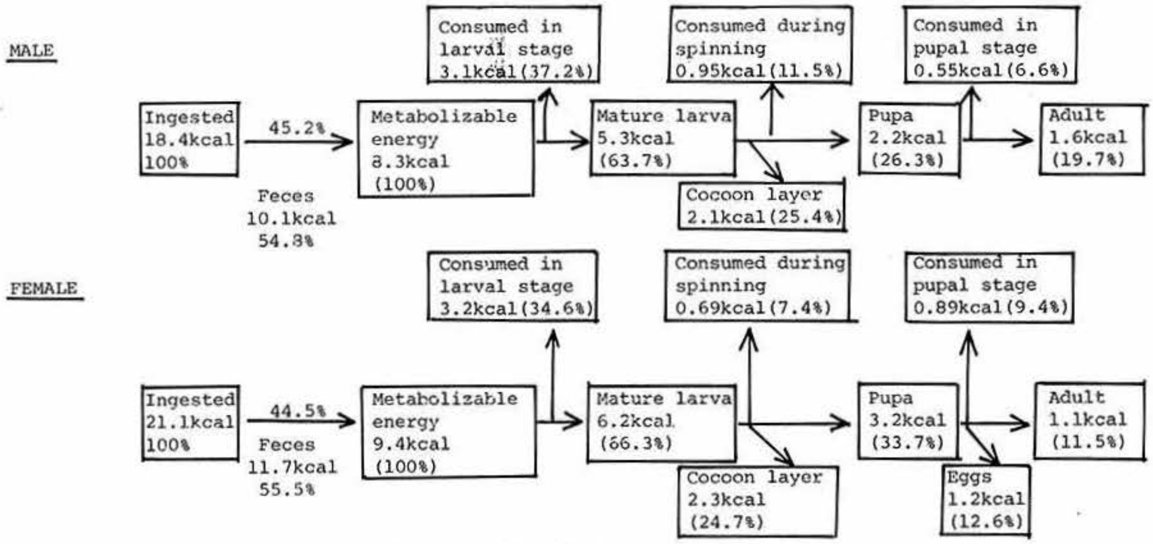

Fig. 2. The energy economy of male and female silkworm, *Bombyx mori*, through it's life cycle[7]

Energy[7]

The gross energy ingested by individual in both 4th and 5th instars was 18.4 and 21.1 kcal, and that metabolized was 8.3 and 9.4 kcal in male and female larvae, respectively (Fig. 2). The ratio of the metabolizable energy to the ingested gross energy was approximately 45% in both sexes. The gross energy stored in the body were found to be 5.2 and 6.1 kcal for male and female and these caloric values were corresponding to 28 and 29% of the gross energy ingested and to 64 and 66% of the gross metabolizable energy. Approximate 26 and 34% of the metabolizable energy were stored in the pupal bodies of male and female, and over 12% went into the eggs. The gross energy consumed in 4th and 5th instars was 3.1 and 3.2 kcal for male and female larvae, respectively, and these values correspond to 37 and 35% of the metabolizable energy. The daily consumption of energy per kg of body weight was calculated to be 130 and 115 kcal in larval stage for male and female, and these values reduced to one third in pupal stage.

Carbon[7]

The carbon of food ingested in 4th and 5th instars by the individual silkworm larva was 145 and 166 mmoles for male and female, respectively, and that metabolized was 56 and 64 mmoles (Fig. 3). The ratio of metabolizable carbon was approximately 39% in both sexes. Of the metabolizable carbon, 40 and 49 mmoles were stored in male and female mature larvae and the recoveries were 73 and 77%. Approximate 27 and 24% of metabolizable carbon were consumed by male and female larvae and 25 and 24% were consumed during a period from spinning initiation to just before pupal-adult ecdysis. The efficiencies of storage of ingested carbon in cocoon layer were 10.9 and 10.5% in male and female larvae, respectively, and those of metabolizable carbon were 28.2 and 27.2%. It was found that the cumulative output of carbon dioxide expired by a single silkworm in larval and pupal stages were 24 and 28 mmoles in male and female, respectively. These amounts of carbon dioxide correspond to more than 40% of metabolizable carbon and these amounts were exactly in agreement with the amounts of carbon consumption in the same period (Fig. 3).

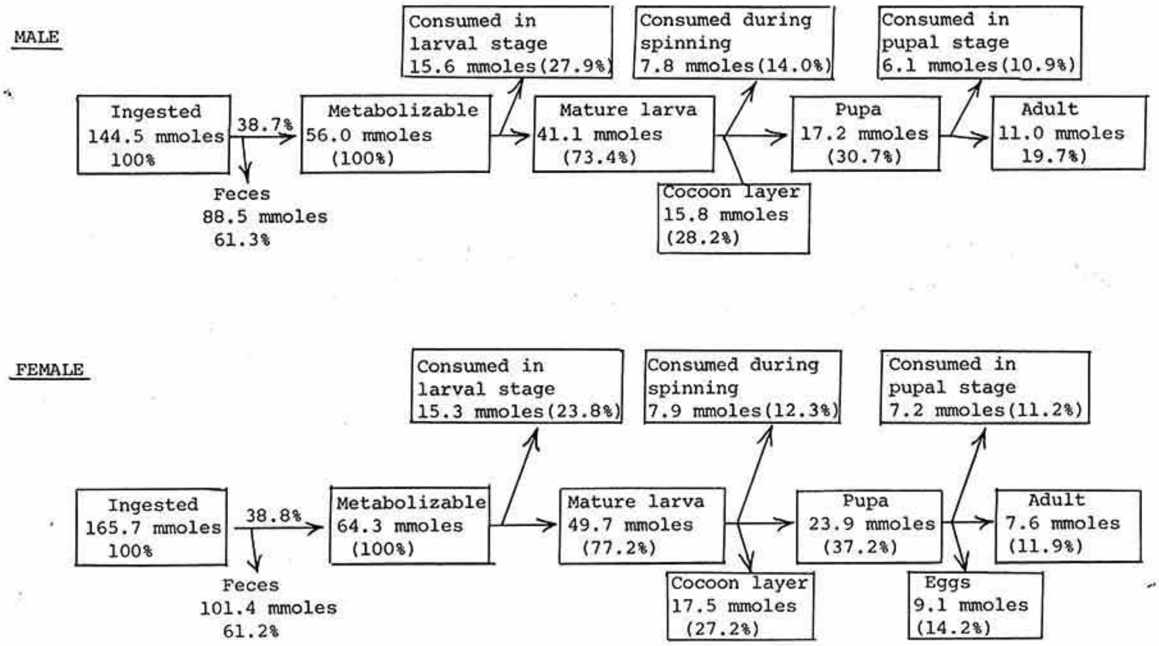

Fig. 3. The carbon economy of male and female silkworm, *Bombyx mori*, through it's life cycle[7]

Carbohydrate

Carbohydrates are utilized by the silkworm for energy source and for synthesis of both lipid and amino acids. Of course, digestibility and nutritive effect of various kinds of carbohydrate are largely dependent on the activity of hydrolyzing enzyme in their digestive system. Amylase is the only carbohydrase found in the digestive fluid of the silkworm and other glycisidases occur only in the epithelial tissue. With these glycosidases in the epithelial tissue hydrolyzing activity for sucrose is the strongest, being nicely corresponding with adequate content of sucrose in mulberry leaves.[3] From these distribution of digestive enzymes, it is probable that oligosaccharides are generally absorbable into the epithelial tissue in the silkworm without any degradation in the digestive fluid and, in turn, are hydrolyzed by the enzyme present in the epithelial tissue. On the contrary, polysaccharide, starch, seems to be hydrolyzed by the amylase in digestive fluid, being followed by further cleavage in the epithelial tissue. During starvation, the silkworm larvae decreased their levels of fat body glycogen much more quickly than that of their hemolymph trehalose, suggesting the evidence of some physiological mechanism maintaining a relatively constant level of hemolymph trehalose. The administration of certain amount of each sugar into larvae just after starvation causes the lowered levels of fat body glycogen and hemolymph trehalose to rise to the original level. The carbohydrates good for increasing hemolymph trehalose are generally most effective in increasing fat body glycogen. A number of carbohydrates including glucose, fructose, sucrose, maltose and raffinose were more effective than others for the increase of both levels.[4] The degree of increase of fat body glycogen and hemolymph trehalose are also dependent on the content of carbohydrate in the diet.[27] The amount of carbohydrates ingested by the silkworm larva through the life cycle is approximately 3—4 mmoles and about 70% of this amount is absorbed. In the mature larva 0.6—0.7 mmole of carbohydrate is accumulated in the form of glycogen and chitin. The rates of conversion of glucose were measured by using radiochemical method, suggesting the occurrence of an active pentose phosphate cycle and glycolytic pathways in the silkworm. The pathway participation of pentose phosphate cycle to glucose catabolism is calculated to be approximately 35%.[11]

Vitamins

The silkworm larvae require several vitamins for their growth and survival. The essential vitamins are choline-Cl, inositol, nicotinic acid, pantothenate pyridoxine, riboflavin, thiamine, biotin and folic acid. The minimal optimal levels of these vitamins in the diet were determined as shown in Table 1, in which the values are compared with those from other species of insect. It is interesting that there is a much similarity in dose requirements by each vitamin among insect species.[8)12)] It is generally accepted that vitamins are required at a catalyzer's level because of their function. Choline and inositol are, however, required by the silkworm at a far higher level than other vitamins. Thus, choline and inositol are not useful for catalytic function, but for lipogenic precursors. The minimal optimal levels of nicotinic acid and pantothenate are much lower than those of choline and inositol, suggesting the function of these vitamins as precursor of coenzymes. It is almost certain that dose differences among various vitamins reflect their specificity of metabolic function. Concerning with function of biotin, the retardation of growth on lack of biotin has a bearing on the alteration of fatty acid composition which is caused by disturbance of fatty acid synthesis.[9]

Lipids

The silkworm larvae require polyunsaturated fatty acids such as linoleic and linolenic acids for their normal growth and development.[17] The visible abnormalities occurred in adult wing on lack of polyunsaturated fatty acids in the diet.[25] The nutritive efficiency was much higher with the addition of fatty acids mixture to the diet than that of single one. The minimal optimal level in the diet for growth was about 8 μmoles/g of dry diet. The nutritive value of vegetable oils are dependent on the composition of fatty acids, and oils from soybean, corn, linseed and safflower are useful for promotion of growth. The fatty acids of mulberry leaves are consisted of palmitic, linolenic and linoleic acids and there are enough amounts to maintain good growth of the silkworm in mulberry leaves. The composition of fatty acids in the silkworm tissues altered according to the com-

Table 1. A comparison of the minimal optimal level of vitamins for growth and survival of insect in the diet[12)]

	Biotin	Choline-Cl	Inositol	Nicotinic acid	Pantothenate-Ca	Pyridoxine HCl	Riboflavin	Thiamine HCl
	(nmoles per g of dry diet)							
Bombyx mori	4	5,370	5,550	160	80	20	15	1
Tenebrio molitor[a)]	0.7	2,150	—	130	30	10	13	
Drosophila melano.[b)]	2	3,220	—	110	90	15	24	6
Phomia regina[c)]	—	570	—	20	13	20	16	10
Musca domestica[d)]	1.6	—	—	40	40	24	13	6
Anthonomus grandis[e)]	—	2,360	2,780	140	70	10	10	3
Schistocerca gregaria[f)]	—	6,270	1,730	40	60	150	10	11
Aedes aegypti[g)]	6	5,000	—	1,140	1,200	34	190	40

a) Fraenkel et al. (1950); b) Sang (1956); c) Brust and Frankel (1955); d) Brookes and Fraenkel (1958); e) Vanderzant (1959, '63); f) Dadd (1961); g) Akov (1962)

These values are obtained by calculation from the original data on the basis of dry weight.

Table 2. Effects of dietary levels of sucrose and oleic acid on the synthesis of fatty acid in larvae of the silkworm[10]

	Sucrose content in the diet (mg/g)	Oleic acid content in the diet (mg/g)	Fatty acid synthesis in the larva (cpm/mg/dose)
Exp. 1	120	0	1,310
	120	3	1,120
	120	6	816
	120	12	407
	120	24	240
Exp. 2	40	0	1,018
	80	0	1,495
	120	0	2,384
	160	0	2,261

position of dietary fatty acids.[17]

It is evident that palmitic, stearic and oleic acids are synthesized from glucose in the silkworm[11] and that the rate of this synthesis is influenced by the levels of fatty acids and carbohydrates in the diet (Table 2). The addition of fatty acids to the diet inhibited fatty acid synthesis, whereas, it was accelerated by an increased sucrose level in the diet. It is interesting that similar control mechanism of lipogenesis to that found in mammalian tissues[10] probably exists in insect.

The silkworm larvae require sterols essential for growth and survival, indicating inability for *de novo* synthesis of sterol in the silkworm.[15][20] β-Sitosterol was found to be main sterol in mulberry leaves and it was of high nutritive value, whereas, a great portion of sterol of the silkworm tissues was comprised by cholesterol. Using ^{3}H-β-sitosterol, cholesterol formation was demonstrated to occur in the silkworm.[11] It is quite certain that the conversion of phytosterols to cholesterol by removing the C-24 alkyl group is a prerequisite to the utilization of these sterols. The possible intermediates of β-sitosterol dealkylation such as fucosterol and desmosterol were very effective for maintenance of good growth.[21] The sparing effect of fatty acids and vegetable oils to sterol requirement of the silkworm was confirmed and the minimal optimal level of sterol for normal growth of the silkworm was about 5 μmoles/g of dry diet in the presence of fatty acids, being of similar order to those of choline, inositol and fatty acids.[21]

Proteins and amino acids

Nutrition of proteins and amino acids is particularly of importance for the silkworm larvae because of their active utilization of nitrogen substances involving the synthesis of silk protein. The optimal level of dietary protein for growth of the silkworm is in the range of 22 to 26% and nutritive values of various kinds of protein are greatly dependent on their composition of amino acids. The supplementation with limiting amino acids markedly improve the nutritive value of gluten and zein.[19] The amino acids essential for growth and survival of the silkworm larvae are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The minimal optimal levels of these essential amino acids have been determined, and the content of essential amino acid in mulberry leaves, not to mention, satisfy the requirements of the silkworm.[19] The requirement of either phenylalanine or methionine are partly replaced with tyrosine or cystine, respectively, indicating occurrence of synthesis of tyrosine and cystine in the silkworm.[20]

The ratio of essential amino acids over total amino acids in the diet (E/TA) optimal for growth is in the range of 0.43 to 0.54. It is interesting that this upper limit of E/TA ratio is corresponding to that calculated from mulberry leaves. The addition of glutamate and aspartate partly replaced the requirement of non-essential amino acids. There is optimal combination of the amounts of acidic and non-essential amino acids for growth acceleration in the silkworm and this quantitative ratio alters according to larval development. Furthermore, the female larvae are more susceptible to amino acid imbalances in the diet than male.[5]

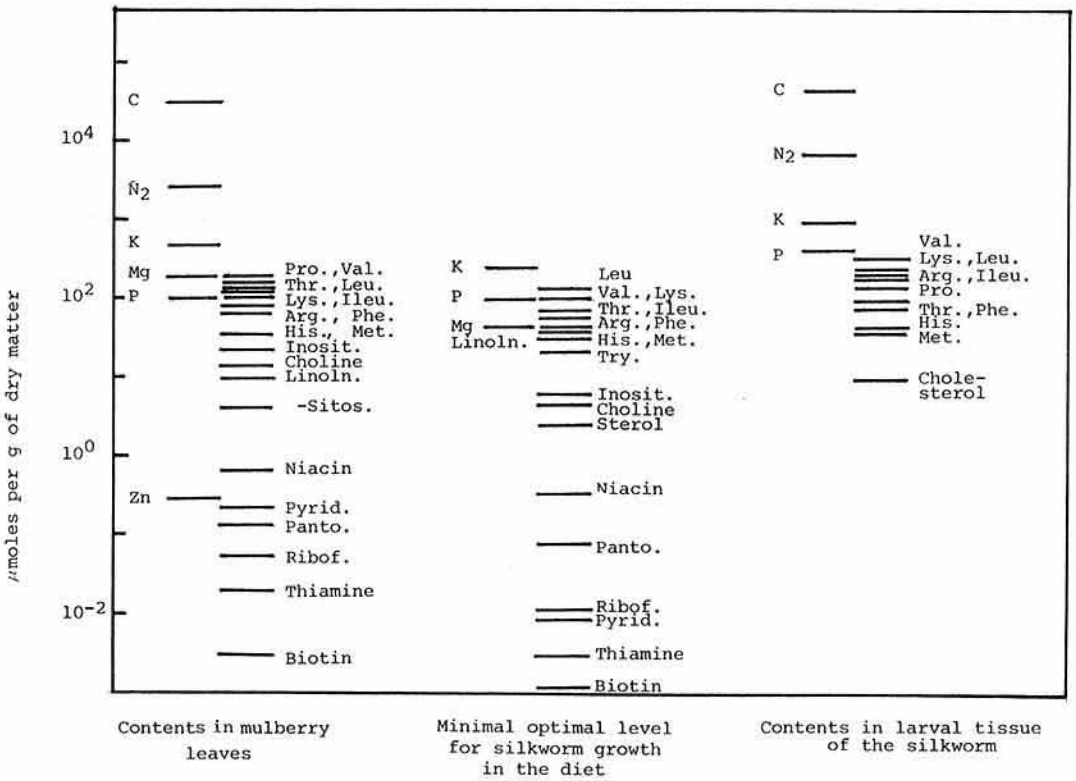

Fig. 4. Minimal optimal levels of nutrients for growth and survival of the silkworm in the diet and compositions of mulberry leaves and the silkworm larvae

The total amount of nitrogen ingested by the silkworm larva through it's life cycle is 12—14 mmoles and approximate 85% of this amount consists of amino nitrogen. Digestibility of nitrogen is approximately 60% and a large part of digested nitrogen is stored in the silkworm as source of silk protein. Producibility of silk protein is lower in female than male because of the transfer of nitrogen into eggs.[9]

Minerals

The silkworm larvae require essentially potassium, phosphorus, magnesium and zinc for their growth and survival. It is interesting that there are similarities in dose requirements by each element among insect species, and that mulberry leaves contain the adequate amounts of minerals to maintain good growth.[13,22]

These quantitative requirements of nutrients by the silkworm larvae are summarized in Fig. 4 in which dose requirement by the silkworm and compositions of mulberry leaves and tissues of the silkworm are indicated on the logarithmic scale. It is noteworthy that the order of dose requirement of nutrient is almost closely correlated with content of respective nutrient in mulberry leaves, and each order is specific for each nutrient.

These accumulated informations are useful for further improvement of the artificial diet of the silkworm.

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