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THE EFFECTS OF THREE DIFFERENT DIETS ON HAEMOLYMPH PROTEIN CONCENTRATION AND XANTHINE DEHYDROGENASE ACTIVITY OF TOBACCO CUTWORM, *SPODOPTERA LITURA*

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Gwo Jen Liaw and Chun Fu Peng (1982) The Effects of Three Different Diets on Haemolymph Protein Concentration and Xanthine Dehydrogenase Activity of Tobacco Cutworm, *Spodoptera litura. Bull. Inst. Zool., Academia Sinica*, 21(1): 67-74. Three diets, namely, (A), taro plant leaf, (B), casein plus wheat germ, and (C), soybean plus brewer's yeast were used in our laboratory to rear tobacco cutworm, *Spodoptera litura.* Diet B provided the best conditions for insects to grow, develop and emerge. Haemolymph protein concentration and xanthine dehydrogenase activity of insects were markedly increased. Diet C produced slightly better conditions than diet A for insect growth. In addition, haemolymph protein concentration and xanthine dehydrogenase were elevated when tobacco cutworms were reared with diet C. These result suggest that positive correlation exist between elevated haemolymph protein concentration, increased xanthine dehydrogenase activity and growth rate of the insect larva. It is concluded from this study that a casein plus wheat germ diet is a perfect nitrogen source for the tobacco cutworm growth. Haemolymph protein concentration and xanthine dehydrogenase activity instead of dietary protein concentration may served as a growth index of the insects.

Xanthine dehydrogenase (Molybdoflavoprotein xanthine: oxygen oxidoreducase, EC 1.2.3.2) is involved in purine metabolism and catalyzes the biosynthesis of uric acid, the major end product of nitrogen catabolism in insects[3]. Two of many factors which can alter xanthine dehydrogenase activity are the stage of metamorphosis and the rearing conditions of the insect. In addition, Hayden and Duke[6] demonstrated that, in Locust, allopurinol, sodium molybdate and xanthine activated xanthine dehydrogenase, while copper sulphate inhibited the activity of this enzyme. In chicks, purine was reported to regulate xanthine dehydrogenase activity[8]. Purine was also found to activate the enzyme in the fruit fly, *Drosophila melanogaster*, and the blow fly, *Aldrichina grahami*[7]. Furthermore, feeding on a high protein diet increased the level of xanthine dehydrogenase activity in *Bombyx mori*[3], *Tenebrio molitor*[19], and *Drosophila melanogaster*[4]. Bhattcherarya and Waldbauer[1] reported that feeding a high protein diet to *Tribolium confusum* resnlted in a stimulation of uric acid production and a reduction of the level of xanthine and hypoxanthine. A positive correlation between xanthine dehydrogenase activity and dietary protein content in the blow fly, *Aldrichina grahami*, was also reported by Huynh *et al.*[7].

The tobacco cutworm, *Spodoptera litura*, is a polyphagous pest and is known to feed on about 112 host plants[12]. This insect has been experimentally reared on artificial diet[2,9,14,15,16,17,18,20]. The nitrogen compounds in these artificial diets are provided come by one of the following three sources; 1) the natural host plants[15,16]; 2) soy-, pinto-, or green-bean with brewer's yeast[9,14,20]; 3) casein and wheat germ[18]. The quality of these nitrogen sources can lead to a fluctuation in haemolymph protein constituents, and can therefore alter the growth rate of insects. Thus, a positive correlation between dietary protein content and the growth rate of insects is held in general belief[5,11,13]. Three artificial diets, namely, taro plant leaf[18], soybean with yeast[20] and casein with wheat germ[18] were used by our laboratory to ascertain the suitability for mass rearing. However, little knowledge is known about the correlation between these dietary protein sources, xanthine dehydrogenase activity and the growth rate of tobacco cutworm. The present study was thus undertaken to investigate the effects of the above three type of protein sources on xanthine dehydrogenase activity and the growth rate of the larva of the tobacco cutworm and probed the relationship between the xanthine dehydrogenase activity, haemolymph protein concentration and the growth rate of the larva.

MATERIALS AND METHODS

Insects

The larvae of tobacco cutworms (*Spodoptera litura*) were obtained from the field and reared in the laboratory at 25±1°C with taro plant leaves as a food source. These larvae served as the stock. Egg masses from the stock adult female were collected and placed in bottles covered with fine nylon cloth. Three different diets were prepared and placed in corresponding bottles. Diet A contained fresh taro-plant leaves, a natural host plant of tobacco cutworm. Diet B contained casein 5 gm, wheat germ 4 gm, sucrose 5 gm, Chinese kale powder 2 gm, Wesson's salt 1.2 gm, L-ascobic acid 0.6 gm, aureomycin 0.016 gm, 4N KOH 0.6 ml, 15% Methyl-*p*-hydroxybenzoate (in 95% EtOH) 1.2 ml, 10% choline chloride 1.2 ml, Linseed oil 0.5 ml, vitamin mixture 0.2 ml, agar 3 gm, and water 100 ml[18]. The composition of the Wesson's salt and the vitamin mixture are shown as previously described[18]. Diet C, it modified the Okada's composition[14], contained soybean powder 10 gm, rice bran 6 gm, brewer's yeast 4 gm, L-ascobic acid 0.4 gm, 15% Methyl-*p*-hydroxybenzoate (in 95% EtOH) 1.2 ml, aureomycin 0.016 gm, agar 3 gm, and water 100 ml. After hatching, the larvae were reared in the bottles with diet A, B, and C, respectively until the third instar and were then transferred to plastic boxes (24.5×15.5×8 cm) containing diets corresponding to those in the bottles. The growth and development of the larvae under these dietary conditions were recorded, i.e., the length of the larval period, weight of larva and percentage of pupation and emergence.

Preparation of Crude Enzyme Solution

Crude enzyme solution was prepared according to the method of Huynh *et al.*[7]. The food of the alimentary canal and haemolymph was removed from the 6th instar larvae. The whole body was washed then diced in a cold Tris-HCl (50 mM) buffer solution, pH 9. The body tissues were homogenized in a Potter-Elvehjem glass homogenizer with 5 volumes of cold solution containing 50 mM Tris-HCl buffer, pH 9, 0.25 M sucrose and 1 mM $MgCl_2$. The homogenates were centrifuged for 30 minutes at 20,000 g, and activated carbon was then added to the supernatant in a concentration of 100 mg/ml. The mixture was allowed to stand for one hour with occassional stirring and was then centrifuged again at 20,000 g for 10 minutes. The pellets were discarded and the supernatants used for enzymatic assay. The protein concentration of the crude enzyme solution was determined as described by Lowery *et al.*[10].

Enzymatic Assay

Xanthine dehydrogenase activity was measured spectrophotometrically following the NADH formation as previous described[7].

Haemolymph and Dietary Protein Determination

(1) The haemolymph of larvae from each dietary group was collected and diluted by adding an equal volume of 1N NaOH. The aliquot volume of alkaline haemolymph solution was then used to determine proteins.

(2) A total of 2 gram of each diet was placed in test tubes which contained 4 ml of 1N NaOH. The alkaline dietary extracts were then boiled for 4 hours until the solids were completely dissolved. The aliquot volume of alkaline solution in eath dietary group was taken for the determination of protein concentration by the method of Lowery *et al.*[10].

RESULTS

Table 1 shows that the rate of growth and development of the tobacco cutworm was influenced by dietary conditions. Diet B containing casein and wheat germ provided the best conditions for the tobacco cutworm to grow, develop and emerge. Diet B also produced the biggest larvae and pupae among the three groups. Although the larvae which were reared with diet C (containing soybean and yeast) had shorter larval periods than those reared with diet A (containing fresh taro-plant leaves), the weight of the larvae and percentage of pupation and emergence in both groups were quite similar.

Table 1 shows that the weights of 12-day old larvae reared under three different dietary conditions varied. The comparison arbitarily made on 12-day old larvae were intended merely to demonstrate the net weight gain of the larvae in each dietary group. As noted in the table 1, the length of larval period was not identical among three groups. Nevertheless, the data shown in this table indicate that casein plus wheat germ is the best diet for tobacco cutworm growth.

Fig. 1 demonstrates the xanthine dehydrogenase activity of the 6th instar larvae reared under the three different dietary conditions. Xanthine dehydrogenase activity of the insects reared with diet C was greater than with diet A, yet less than with diet B. A plot of the enzymatic activity of the insects forms a hyperbolic curve in diet B and C group, whereas the activity of the diet A group is represented as a straight line. This result suggests that xanthine dehydrogenase activity is stimulated when insects are reared with artificial diets.

Fig. 2 shows that haemolymph protein concentration of the 6th instar larvae reared with diet B and C was significantly higher than the protein concentration of those reared with diet A. To evaluate the relationship

TABLE 1

The comparison of the rate of growth and development, the percentage of pupation and emergence of tobacco cutworm, *Spodoptera litura*, which were reared under the three different dietary conditions.

Diet	Length of the larval period (day)	Weight of* larva (mg)	Weight of** pupa (mg)	Pupation (%)	Emergence (%)
A	20-23	150±56	394±16	72.00	81.36
B	14-17	243±67	473± 9	80.36	86.36
C	16-18	161±43	417±19	72.76	83.33

A: diet contained fresh taro-plant leaves.
B: diet contained casein and wheat germ.
C: diet contained soybean and yeast.
* Twenty five of 12th day old larvae were used to determine the weight of larva in each group. Data expressed as mean±SE of mean. $p<0.1$ when A vs B. $p<0.1$ when B vs C. $p<0.5$ when A vs C.
** Twenty five pupae were used to determine the weight of pupa in each group. Data expressed as mean±SE of mean. $p<0.005$ when A vs B. $p<0.005$ when B vs C. $p<0.1$ when A vs C.

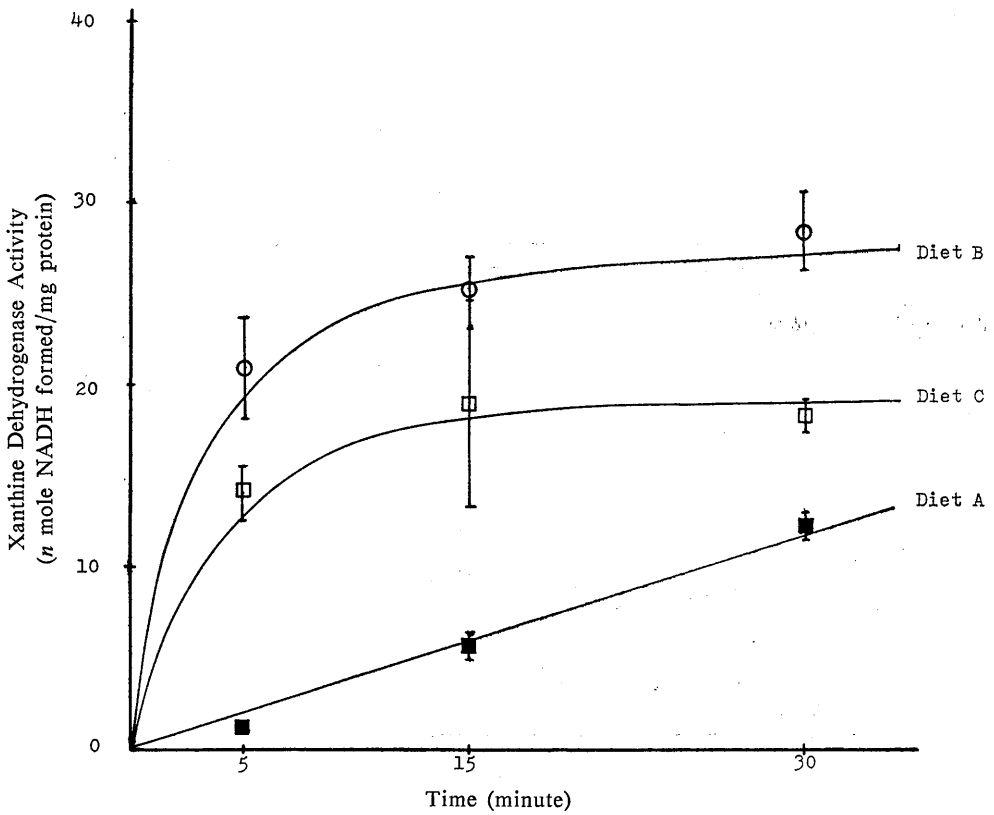

Fig. 1. The xanthine dehydrogenase activity of 6th instar tobacco cutworms which were reared under the three different dietary conditions. Each point represents the mean and SE of mean of six experimental determinations.

between the haemolymph protein concentration of the larvae and the amounts of nitrogen sources in each dietary group, the protein concentration of each diet was determined. Fig. 3 shows that diet C contained the highest amount of protein per gram of food diet A, the next highest amount; and diet B, the least amount. These results indicate that haemolymph protein concentration in 6 th instar larvae is not necessarily correlated with the concent ration of dietary protein.

DISCUSSION

This study demonstrates that a casein plus wheat germ diet is a better nitrogen source for tobacco cutworm growth, development, and emergence than diets of soybeans plus brewer's yeast or taro-plant leaves. In addition, the artificial diets (casein plus wheat germ and soybean plus brewer's yeast) appear to stimulate the xanthine dehydrogenase activity of insects when compared to natural host plant (taro leaf) diet (Fig. 1). The two artificial diets also result in the elevation of haemolymph protein concentration (Fig. 2) in the 6th instar larvae even though these two dietary protein concentrations are not necessarily higher than the natural host plant (Fig. 3). Although a positive correlation between xanthine dehydrogenase activity and dietary protein content was previously reported by a number of authors[1,3,4,7,19], the data demonstrated in this study show that haemolymph protein concentration—not dietary protein content—has a positive

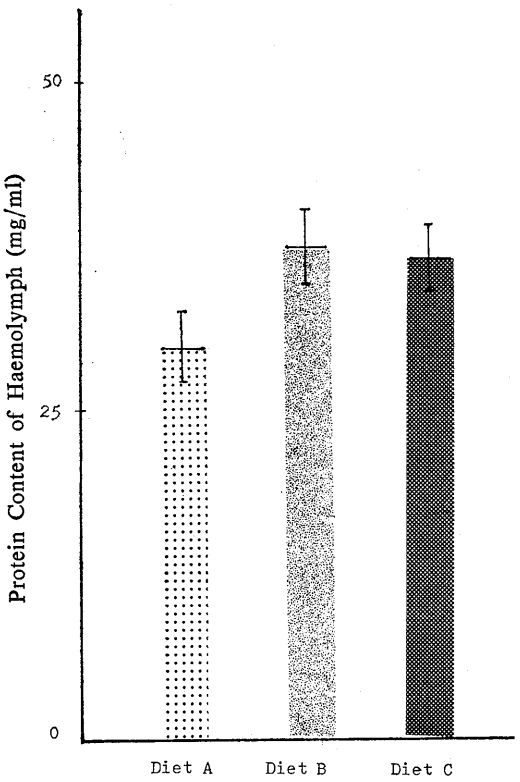

Fig. 2. Haemolymph protein concentration of the 6th instar larvae which were reared with diets A, B, and C. Each bar represents the mean and SE of mean of six experimental determinations. $p<0.1$ when diet A vs diet B. $p<0.5$ when diet B vs diet C. $p<0.1$ when diet A vs diet C.

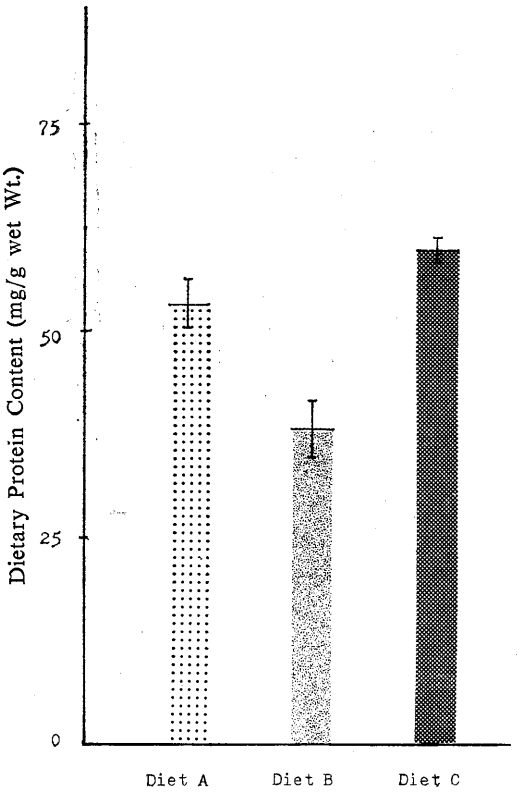

Fig. 3. Protein content in each diet. Each bar represents the mean and SE of mean of six experimental determinations. $p<0.005$ when diet A vs diet B. $p<0.005$ when diet B vs diet C. $p<0.1$ when diet A vs diet C.

correlation to the rate of growth and xanthine dehydrogenase activity. This positive relationship between the protin concentration of haemolymph and the growth rate of larvae of many insect has been previously reported[5,11,13]. It is likely that dietary protein concentration may not be as good an index as haemolymph protein concentration to show the rate of growth and development of insects because not all of the protein is completely hydrolyzed during food digestion, and not all of the amino acids are released at the same time. Therefore, certain concentrations of specific amino acids might not be satisfactory for the excellent growth of insects. It is conceivable that, although taro-plant leaves contain no less protein than the artificial diet C (Fig. 3), the complete hydrolysis of proteins and the absorption of all available amino acids from the natural host plants may take longer to achieve than with artificial diets. In addition, other factors such as carbohydrate, lipid, vitamin and trace element contents of each diet may also control the growth rate of insects. The difference in these nutritional contents among three dietry groups is currently under investigation. In this communication, however, we focused merely on the effect of dietary protein sources on both the xanthine dehydrogenase activity and haemolymph concentration

of the insects.

The close relationship observed between the haemolymph protein concentration and xanthine dehydrogenase is unique. Free amino acids from dietary proteins are absorbed and transferred into haemolymph. The insect utilizes the absorbed amino acids to constitute the structure of its body through anabolic processes. The result of anabolism represents the rate of growth and development. However, xanthine dehydrogenase activity generally represents a catabolic process; therefore, high activity of this enzyme reflects a high rate of catabolism. It is conceivable that when the haemolymph protein concentration exceeds the capacity of the anabolic pathways, the biosynthesis of uric acid from amino acids such as aspartate, glutamine and glycine is most likely elevated when haemolymph protein concentration increases. The activation of xanthine dehydrogenase which responds to high haemolymph protein concentration thus not only serves as an important index for the growth rate of insects, but also accelerates catabolic pathways for the excretion of nitrogen compounds. It is thus concluded from this study that xanthine dehydrogenase activity and haemolymph protein concentration instead of dietary protein concentration may served as a growth index of the insects. This index is particularly essential for the preparation or modification of protein in artificial diets.

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三種不同的飼料對斜紋夜盜蛾的血淋巴蛋白質濃度和黃嘌呤去氫酶活性之影響

廖 國 楨　　彭 俊 輔

在實驗室內飼養斜紋夜盜蛾的飼料主要的有(A)芋頭葉(B)酪蛋白加麥胚和(C)黃豆加酵母粉。斜紋夜盜蛾在飼料B的生長、發育和羽化爲最好，而且蟲體內的血淋巴蛋白質濃度和黃嘌呤去氫酶活性的顯著的增高。飼料C比飼料A提供稍好的生長條件，亦可提高血淋巴蛋白質的濃度和黃嘌呤去氫酶的活性，這個結果暗示提高血淋巴蛋白質濃度，增高黃嘌呤去氫酶活性和昆蟲幼蟲的生長速率之間有正相關的關係。如此可以得到結論：酪蛋白加麥胚的飼料是一個對斜紋夜盜蛾生長所需的良好氮源；血淋巴蛋白質的濃度和黃嘌呤去氫酶的活性或許可以取代飼料蛋白質的濃度作爲昆蟲生長的指標。