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Quantitative Changes of Carbohydrate, Lipid and Protein during the Post-embryonic Development and Metamorphosis of *Tribolium castaneum* and *T. confusum* (Coleoptera; Tenebrionidae: Insecta)

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Quantitative variation of total carbohydrate, protein and lipid, were studied during the post-embryonic development and metamorphosis of *Tribolium castaneum* and *T. confusum*. Of the nutrients, total carbohydrate presents more or less uniform concentration throughout the grub stages and attains maximum level in the pupa. Protein shows gradual increase in concentration to its peak in the last grub instar while the lipid presents a fluctuating concentration, with maximum level in the fourth grub instar. A major part of the total carbohydrate remains stored as glycogen (17.8%–70.68%). Of the total lipid 36.9%–64.1% accounts for cholesterol. Variations of the contents are seen in pre-, inter- and postmoult periods of each instar. Pupa presents notable sexual dimorphism in the contents. Concentration of almost all components is higher in *T. castaneum* than in *T. confusum*.

Key Words: **Nutrients, Ontogeny, Metamorphosis,** *Tribolium castaneum, T. confusum*

Introduction

Quantitative variation of different nutrients during the post-embryonic development and metamorphosis of insects have received some attention in the past few decades. Such variations in relation to different insects have been interpreted from different viewpoints by Gilbert and Schneiderman (1961), Bade and Wyatt (1962), Agrell (1964), Janda et al. (1966), and Bhattacharya and Pant (1968), as reflecting the balance of synthesis, storage and degradation of the nutrients in response to the developmental needs. Since only a few of the previous investigations have dealt with the biochemical changes during the ontogeny of stored grain pests (Pant & Oberoi 1958, Bernard et al. 1963, Pant & Dang 1965, Bhattacharya et al. 1968 and Singh & Sinha 1976), the present investigation was

undertaken to provide informations on the quantitative variation of carbohydrate, protein and lipid during the post-embryonic development and metamorphosis of *T. castaneum* and *T. confusum.*

Material and Methods

Adults were collected from infested rice and from the collected pool of specimens, *Tribolium castaneum* and *T. confusum* were separated on the basis of antennal characters. Both the species were cultured separately in a jar containing sterilized whole wheat flour (94.5%), dried yeast (5%) and 'Rovimix'—a vitamin mixture containing A, B_2 and D_3 (0.5%) at 30±2°C and 70% RH maintained in an incubator, keeping a water tray inside. Grubs of different age groups were classified into six instars on the basis of head capsule/body length ratio. Sex of the pupa was identified on the basis of morphological characters on the genitalia—female pupa shows two raised projections while male pupae are with two depressions on the genitalia. Pre-, inter-, and postmoult periods of each instar were identified on the basis of chronological age of the developing grubs and colour of the integument.

For quantitative analysis of nutrients grubs of different instars, pupae of both sexes and emerging adults of both the species were taken in test tubes, anesthesized with mild chloroform vapour, weighed and finally homogenized with specific extraction medium for respective nutrient estimation. Total carbohydrate was estimated by the method of Umbreit et al. (1957). Glycogen estimation was done on the basis of the method of Carrol et al. (1956). Protein extraction was made by 0.1N NaoH and estimated by folinphenol method (Lowry et al 1951). Lipid was estimated by the method of Folch (1957) and cholesterol estimation was done on the basis of a modified method of Roy et al. (1955). All the experiments were replicated ten times to minimize errors and the mean values are incorporated in the tables and figures. The contents in each case were expressed as μg/100 mg wet body wt.

Results

The results clearly present a basic feature of the fluctuations in the concentration of carbohydrate, protein and lipid during the post-embryonic development. Though the trend of fluctuation is characteristic to each nutrient, yet it presents some common features of the whole developmental period. The developmental profiles of each nutrient in different physiological state of each instar are also variable. The levels of almost all components are high in premoult period and are followed by sharp decline in the postmoult period and show a slight increase in the intermoult period (figure 1). But the picture in cholesterol variation is different; it shows considerable decrease in the premoult period (figure 2). Of all the components, protein shows highest level (1.33%–2.91% of the body weight) followed by lipid (1.38%–2.18% of the body weight). Total carbohydrate concentration accounts for 1.07% to 1.55% of the body weight (tables 1 and 2). Total carbohydrate level shows more or less uniform concentration upto fourth grub instar, after which it presents moderate increase to its peak in pupa (male). Of the total carbohydrate a major part remains stored as glycogen, ranging from 17.8–70.66% in *T. castaneum* and 19.09–70.58% in *T. confusum.* Unlike total carbohydrate level, glycogen level presents a gradual substantial rise in the concentration upto the sixth grub instar, with temporary decline in the levels in

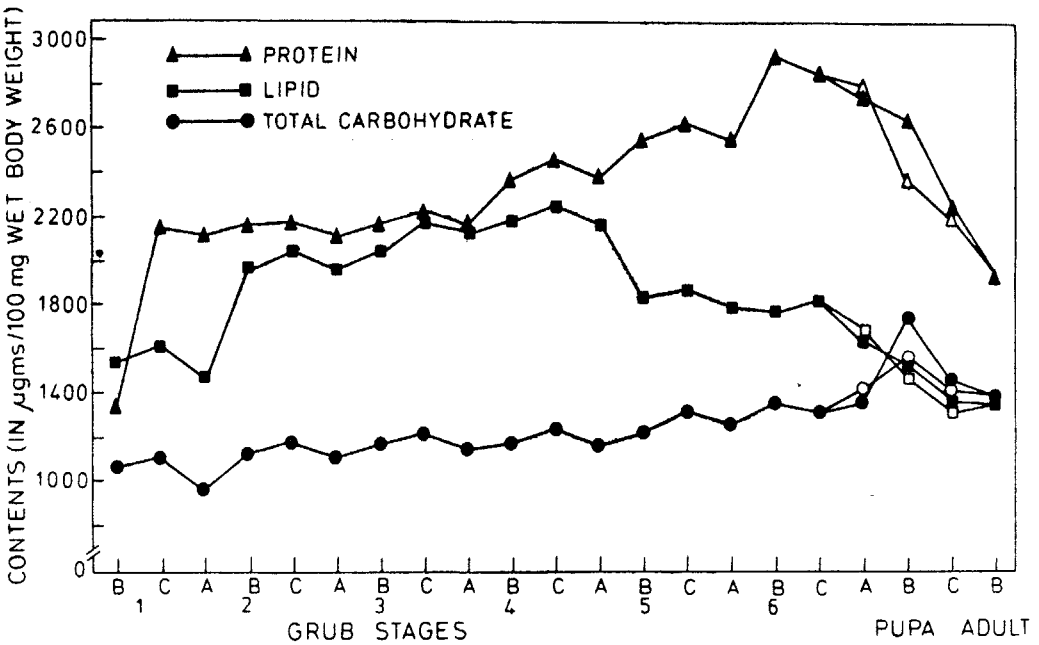

Figure 1 Concentration of protein, lipid and total carbohydrate in different morphogenetic periods of the post-embryonic development of *T. castaneum*. *A*, *B* and *C* denote post-, inter- and premoult periods. Vacant symbols in pupa represent female sex

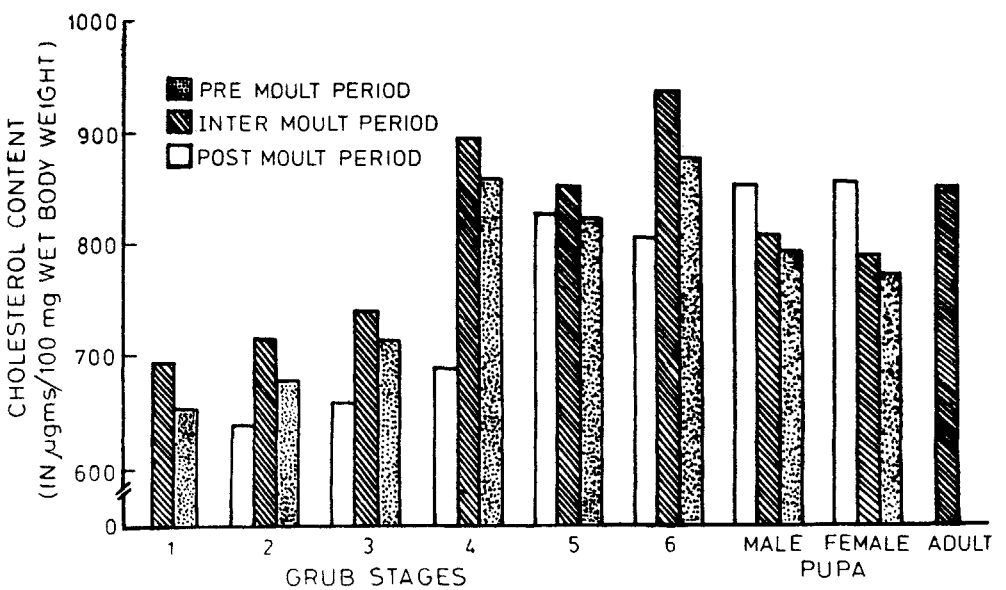

Figure 2 Cholesterol content in different morphogenetic periods of the post-embryonic development of *T. castaneum*

Table 1 *Concentration of various nutrients (μg/100 mg body weight) during the intermoult periods of the post-embryonic development of* Tribolium castaneum

Nutrients	Grub stages						Pupa		Adult
	1	2	3	4	5	6	Male	Female	
Total carbohydrate	1071 (±2.38)	1117 (±3.35)	1102 (±1.37)	1172 (±2.25)	1205 (±2.25)	1372 (±1.29)	1730 (±4.39)	1550 (±1.35)	1386 (±2.07
Glycogen	500 (±1.87)	600 (±1.35)	590 (±2.35)	495 (±1.39)	525 (±0.89)	690 (±1.27)	405 (±3.39)	350 (±1.29)	379 (±1.49)
Protein	1333 (±1.25)	2144 (±1.19)	2160 (±2.96)	2375 (±1.25)	2561 (±1.26)	2917 (±2.79)	2631 (±3.95)	2380 (±1.29)	1997 (±1.29)
Lipid	1535 (±3.25)	1976 (±2.28)	2016 (±2.19)	2185 (±1.19)	1805 (±2.35)	1780 (±1.09)	1520 (±1.49)	1480 (±3.25)	1385 (±1.25)
Cholesterol	721 (±2.01)	717 (±1.25)	743 (±3.79)	895 (±1.35)	721 (±2.39)	738 (±1.39)	851 (±2.35)	795 (±1.35)	890 (±2.39)

Note: Data within the parentheses represent standard errors (n=10)

Table 2 *Concentration of various nutrients (μg/100 mg body weight) during the intermoult period of the post-embryonic development of* Tribolium confusum

Nutrients	Grub stages						Pupa		Adult
	1	2	3	4	5	6	Male	Female	
Total carbohydrate	1021 (±1.98)	1098 (±2.29)	1092 (±1.95)	1195 (±2.25)	1105 (±1.25)	1225 (±1.09)	1705 (±2.85)	1510 (±3.25)	1315 (±2.29)
Glycogen	459 (±2.1)	575 (±1.25)	529 (±2.19)	499 (±1.26)	509 (±1.95)	625 (±1.95)	405 (±3.39)	348 (±1.36)	365 (±1.02)
Protein	1297 (±1.35)	2038 (±2.09)	2097 (±3.19)	2290 (±1.89)	2519 (±1.26)	2859 (±2.15)	2457 (±1.95)	2310 (±3.19)	1985 (±1.38)
Lipid	1505 (±2.89)	1949 (±1.39)	1989 (±2.05)	2125 (±1.89)	1985 (±0.89)	1820 (±3.35)	1500 (±2.29)	1425 (±1.25)	1310 (±1.75)
Cholesterol	695 (±1.45)	690 (±1.96)	760 (±4.56)	781 (±2.09)	697 (±1.75)	757 (±1.59)	805 (±2.05)	790 (±4.39)	850 (±1.19)

Note: Data within the parentheses represent standard errors (n=10)

different morphogenetic periods (figure 3). Lipid presents a gradual increasing level upto the fourth grub instar, after which it gradually declines upto the adult. Of the total lipid a major part accounts for total cholesterol 36.9%–64.1%) which shows statistically insignificant variations throughout the post-embryonic development (figure 2). Another notable feature is that the levels of almost all contents are higher in *T. castaneum* than in *T. confusum.*

Discussion

The mode of fluctuations of different nutrients during the post-embryonic development and metamorphosis of two species of *Tribolium*, distinctly corroborates the heterogenic morphogenetic

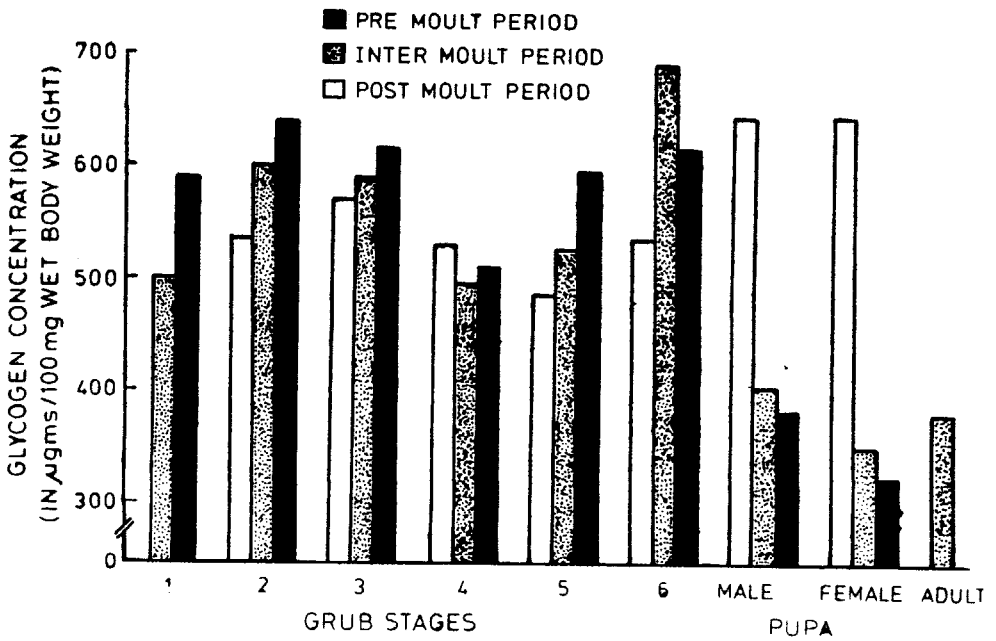

Figure 3 Glycogen content in different morphogenetic periods of the post-embryonic development of *T. castaneum*

events occurring in these periods. The trend of fluctuation of the nutrients concerned, which are highly essential for morphogenetic changes, not only reflects the metabolic pattern of the nutrients concerned and the nutritional requirement of the developmental stages but also exhibits the presence of a metabolic rhythm caused by morphological and physiological changes during ontogeny.

The level of total carbohydrate was more or less uniform throughout the grub stages, but the glycogen level exhibited fluctuating changes in different instars reaching its peak in sixth instar which is in consistence with the findings of Rousell (1955), Shigematsu (1956), Pant and Oberoi (1958), Zaluska (1959), Ludwig et al. (1964), Pant and Dang (1965) and Srivastava (1965). The level of carbohydrate in different instars as found here suggests that the grubs feed voraciously to provide substrate for glycogen synthesis leading ultimately to gluconeogenesis. Glycogen synthesized during the developmental stages is deemed to be a 'mobile reservoir' whose concentration depends upon the period of development, mode of nutrition and the energy requirement for development (Shigematsu 1956). The relative decline in the glycogen level at the fourth grub instar might be either due to less synthesis of glycogen or its utilization for trehalose synthesis. High glycogen concentration in the grub stages is also known to be directly related with the development of nervous system. Substantial rise of lipid level upto fourth instar grub stage and gradual decline in later stages, seem to be due to its active participation to meet the energy

requirements in the later phases of grub development (Wightman 1978). Moderate level of cholesterol throughout the development clearly proves its indispensibleness for growth and development. Sharp rise of cholesterol level at the fourth grub instar might be due to decreased mobilization of cholesterol for unknown reasons. Gradual increasing level of protein corresponds to the high rate of its synthesis by the fat body (Birt et al. 1969). Instar specific content of protein reflects both qualitative and quantitative variation since appearance of new proteins and disappearance of others are specific to each instar (Firling 1977).

Relatively high level of almost all components (except lipid) in the last grub instar, which represents a transitional phase between grub and pupal stage, may be due to the storage of nutrients for the nonfeeding pupal stage and for the provision of energy required for reconstruction of the important imagine organs in the pupal stage and is surely indicative of the synthesis of these components in this stage.

The morphogenetic changes associated with the moulting at each larval instar is reflected in the level of various nutrients, Usually in premoulting period almost all components show higher level from the respective preceding stage as evidence of enhanced rate of synthesis of these nutrients prior to moulting. Increased concentration of carbohydrate and lipid in this stage is related with increased rate of oxygen consumption in premoulting period (Gilbert & Schneiderman 1961). In premoulting period increase of protein level may be due to the appearance of new proteins associated with moulting processes only. (Firling 1977). Declining level of all components in the postmoult period clearly indicates their utilization in the complex biochemical processes associated with moulting. Contrary to this basic feature, due to active participation of cholesterol in the synthesis of moulting hormone, it shows considerable decline in premoulting period (Robbins et al. 1061). Moreover cell multiplication prior to moulting also accounts for its diminishing level since, a considerable part of it is utilized for the construction of subcellular membrane structures (Clayton & Lasser 1964).

The morphogenetic and biochemical changes associated with metamorphosis begin in pharate pupal (late last grub) stage in holometabolous insects in which larval and adult organisations are so divergent that only a few larval organs retain the functions in the adult life. A radical transformation accompanied by a varied degree of histolysis of the larval tissues may account for the diminishing level of almost all components in this stage. The notable decline in the content of cholesterol in this stage is surely due to its conversion for the synthesis of ecdysone prior to grub-pupal moult.

In pupa the main energy-providing substance seems to be lipid supplemented by a small amount of carbohydrate which explains the declining level of lipid (Ludwig et al. 1964). It is also conjectured that a conversion often occurs from lipid to carbohydrate which positively correlates high level of carbohydrate. Declining level of glycogen may be due to glycolysis in the fat body producing active free sugar for the differentiating cells. Protein level in the pupa not only shows quantitative variation from the preceding grub stage but it also reflects synthesis of new proteins associated with pupal-adult moult (Firling 1977). Marked sexual dimorphism in

the level of almost all components, i.e. less concentration in female pupae is due to greater utilization of the components by the female pupae than male and this increased rate of utilization is not only associated with egg development since certain non-ovarian tissues also utilize far more nutrients than corresponding male tissues (Domroese & Gilbert 1964, Singh & Sinha 1976).

From theoretical knowledge it is established that prior to pupal-adult moult the pharate adult (pre-adult) utilizes the nutrients for the reconstruction of a few imagine structures which accounts for the declining level of almost all components in emerging adults.

It may thus be assumed that the variation of these nutrients during the post-embryonic development reflects a balance of their anabolism and catabolism; the mechanisms controlling these variations are not properly understood though it is probable that hormonal agents play important role in such morphogeentic and biochemical changes associated with the phenomenon of metamorphosis.

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References

Agrell I 1964 Physiological and biochemical changes during insect development; in *The Physiology of Insecta* Vol I. pp 91–148 ed M Rockstein (New York : Academic Press)

Bade N L and Wyatt G R 1962 Metabolic conversion during pupation of the *Cecropia* Silkworm: Deposition and utilization of nutrient reserves; *Biochem. J.* **83** 470–478

Bernard R and Lemonde A 1963 Sugar requirement of *Tribolium* larva; *Rev. Can. Biol.* **8** 498–503

Bhattacharya A K and Pant N C 1968 Growth and development of Khapra beetle *Trogoderma granarium* Everts (Coleoptera; Dermestidae) on pulses; *Bull. ent. Res.* **59** 383–387

Birt L M and Christian B 1969 Changes in the nitrogenous compounds during the metamorphosis of the Blowfly *Lucilia cuprina; J. Insect. Physiol* **15** 709–711

Carrol N V, Longley R W and Roe J H 1956 The determination of glycogen in liver and muscle by use of anthrone reagent; *J. biol. Chem.* **220** 583–593

Clayton R B and Lasser N L 1964 The intracellular distribution of sterols in the tissues of the cockroach, *Eurycotis floridana; Fedn. Proc. Fedn. Am. Soc. exp. Biol.* **23** 275

Domroese K A and Gilbert L I 1964 The role of lipid in adult development and flight muscle metabolism in *H. cecropia*; *J. exp. Biol.* **41** 573–590

Firling C E 1977 Amino acid and protein changes in the haemolymph of developing fourth instar *Chironomus tentans*; *J. Insect. Physiol.* **23** 17–22

Folch J M S, Lees M and Stanley G H S 1957 A simple method for the isolation and purification of total lipids from animal tissues; *J. biol. chem.* **226** 497–509

Gilbert L I and Schneiderman H A 1961 Some biochemical aspects of insect metamorphosis; *Am. Zool.* **1** 11–51

Janda Jr V, Sehnal F and Simek V 1966 Changes in some chemical constituents of *Galleria mellonela* larvae in relation to growth and morphogenesis; *Acta Biochem. Polon.* **13** 329–335

Lowry O H, Rosenbrough N J, Farr A L and R J Randall 1951 Protein measurement with Folin-Phenol reagent; *J. biol Chem.* **193** 265–275

Ludwig D, Crowe P A and Hassemer M H 1964 Free fat and glycogen during metamorphosis of *Musca domestica* L.; *J.N.Y. ent. Soc.* **72** 23–28

Pant N C and Oberoi N K 1958 On the carbohydrate utilization by the larvae of *Trogoderma granarium* Everts; *Experientia* **14** 71

—— and Dang K 1965 Effect of different carbohydrates on the growth and development of *Tribolium castaneum* Hebrst; *Indian J. Ent.* **27** 432–441

Robbins W E, Kaplanis, J N, Monroe R E and Tabor L A 1961 The utilization of dietary cholesterol by German cockroaches; *Ann. ent. Soc. Am.* **54** 165–168

Rousell P G 1955 Determination of glycogen content during the metamorphosis of the meal worm (*Tenebrio molitor* L); *J.N.Y. ent. Soc.* **63** 107–110

Roy A C, Dutta S N and Sur R N 1955 A rapid method for estimation of total adrenal cholesterol; *J. Sci. indus. Res.* **140** 124–125

Shigematsu H 1956 Study on the glycogen in the mulbery silkworm with special reference to fat body; *J. Sericul. Sci. Japan* **25** 122–127

Singh N B and Sinha R N 1976 Carbohydrate, lipid and protein in the developmental stages of *Sitophilus oryzae* and *S. granarius* (Coleoptera, Curculionidae); *Ann. ent. soc. Am* **70** 107–111

Srivastava P H 1965 Total glycogen in the pea aphid *Acyrthosiphonpisum; Can. J. Zool.* **43** 225–227

Umbreit W W, Burris R H and Stauffer J F 1957 in *Manometric Techniques* pp 272–279 ed Rev (Burgess: Minneapolis)

Wightman J A 1978 Ecology of *Callosobruchus analis* (Coleoptera: Bruchidae); Energetics and energy reserves of the adults; *J. anim. Ecol.* **47** 131–142

Zaluska A H 1959 Glycogen and chitin metabolism during development of the silkworm (*Bombyx mori* L.); *Acta Biol. Exp. Lodz.* **19** 339–351