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EFFECT OF FEEDING DIFFERENT DIETS ON THE CRUDE PROTEIN CONTENT AND FREE AMINO ACIDS IN THE HAEMOLYMPH OF LUCILIA SERICATA (MEIGEN) AND *CHRYSOMYA ALBICEPS* (WIED) PREPUPAE (DIPTERA-CALLIPHORIDAE)

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SUMMARY

Larvae of both blowflies *Lucilia sericata* and *Chrysomya albiceps* were fed bovine meat, bovine liver, fish and chicken. The content of crude protein in the haemolymph of *L. sericata* prepupae when its larvae were fed on the four different diets, showed higher concentration when fed on bovine liver than when fed on chicken and bovine meat. The least protein content recorded was when larvae were offered fish as diet. Meanwhile the protein content in the haemolymph of *C. albiceps* prepupae showed slightly higher concentration when larvae were fed on bovine liver than when fed on bovine meat, chicken and fish. Using (HPLC), the concentration of histidine was the most predominant amino acid in haemolymph of *L. sericata* prepupae when its larvae were fed on bovine liver followed by threonine when its larvae were fed on fish. While the amino acid leucine and serine were significantly low when larvae were fed bovine liver. The amino acid threonine in haemolymph of *C. albiceps* prepupae was high when its larvae were fed bovine meat and chicken and cysteine was significantly high in case of larvae fed on bovine liver and fish. The concentration of the amino acid glutamic acid was the least when fish was offered as diet and the amino acid isoleucine and leucine were significantly low when larvae were fed bovine liver.

INTRODUCTION

Insects like animals appear to have a general pattern of nutritional needs i.e they require diets containing a nitrogen source, vitamins, an energy source (most often a carbohydrate) and some mineral salts (Karowa and Martin, 1989; Horie and Watnabe, 1983; Zucoloto, 1987). Although many studies have been devoted to the determination of essential nutrients, many investigations are concerned with studying the feeding habits of food preferences of insects (Busse and Barth, 1985).

The larvae of *L. sericata* and *C. albiceps* feed predominantly on meat or a rich protein diet. The nutritional quality of protein is a function of its amino acid composition which is important in growth and reproduction in both types of flies.

The present work was undertaken to determine the effects of different kinds of protein diets on the content of crude protein and free amino acids in the haemolymph of both *L.sericata* and *C. albiceps* prepupae.

MATERIALS AND METHODS

Colonies of both *L.sericata* and *C.albiceps* were established in the laboratory from flies initially collected from Abou-Rawash Farm, 30km far from Cairo City, Giza Governorate, Egypt, during June 1995. In order to attract naturally gravid females, fly traps as that described by Roy and Dasgupta (1975) were used. Flies caught in the traps were removed daily, and transported to the laboratory, sorted, sexed and identified according to Zumpt (1965) and Tantawi and El-Kady (1997). Stock colonies of both adults *L. sericata* and *C. albiceps* flies were reared in the laboratory of Parasitology Department, National Research Center, Dokki, Cairo, Egypt, according to the method of Omar et al. (1992). The ambient mean temperature ranged between 14.6 and 32.1°C and mean relative humidity between 32.5 and 53.5%. Laboratory illumination of the mass colony was controlled during both summer and winter seasons by using Philips fluorescent tubes (24 W.) (Saunders et al., 1986). Newly hatching larvae were reared on different test diets (bovine meat, chicken, fish or bovine liver) till reaching the prepupal stage, then sacrificed. Haemolymph samples from prepupae of *L.sericata* and *C.albiceps* were performed according to El-Gindi and Abdel Meguid (1994). Estimation of protien was determined according to the methods of Bradford (1976). Amino acid determination was performed using High Pressure Liquid Chromatography (HPLC) according to the method of Steven et al. (1989).

RESULTS

In *L.sericata*, the total crude protein content in the haemolymph of the prepupae obtained from larvae fed on bovine liver was much higher than when fed on chicken and bovine meat, 9.4 ±0.57; 8.21±1.24 & 8.15 ± 0.94g/dl, respectively. However in *C. albiceps*, the total crude protein content in the haemolymph of the prepupae obtained from larvae fed on bovine liver, bovine meat and chicken was more or less similar being 8.71 ±1.02; 8.56 ± 1.02 and 8.18 ±1.06 g/dl, respectively. A lowest value was recorded in both *L. sericata*

(7.30 ± 0.53 g/dl) and *C. albiceps* (7.33 ±1.13 g/dl) when larvae were fed on fish diet. Statistical analysis showed no significant difference (P>0.05). Table (1) and Fig. (1).

Using HPLC, 17 amino acids were separated from the haemolymph of both *L.sericata* and *C.albiceps* prepupae (Tables and Figs. 2 & 3). In the haemolymph of *L.sericata* prepupae the most predominant amino acid was histidine (284.76 ±16.77 mg/100ml) and significantly the least were leucine (0.23 ± 0.001 mg/100ml) and serine (3.89 ± 0.04 mg/100ml) when its larvae were fed on bovine liver. Statistical analysis showed a highly significant variations in the values of the free amino acid between the four diets (P<0.01). However the level of threonine in the haemolymph of *L. sericata* did not significantly vary between the four test diets (P>0.05). Also in the haemolymph of *C. albiceps* the level of the separated amino acids differed according to the test diets. Significant high values of cysteine were obtained with bovine liver (256.2 ± 5.11 mg/100ml) and fish (198.47± 4.32 mg/100ml). Arginine, threonine, proline and tyrosine showed highly significant variations (P<0.01). Meanwhile glycine, histidine, valine and lysine showed significant differences (P<0.05).

DISCUSSION AND CONCLUSION

Proteins are the principal components of all animal tissues. From a nutritional standpoint the requirement for protein is a requirement for the individual amino acids that comprise them, because proteins are complex nitrogen compounds composed of chains of amino acids. The amino acids play an important role in insect metabolic activities during metamorphosis. Results obtained during this study revealed that, the effect of various animal protein diets on the crude protein content and free amino acids in the haemolymph of both *L.sericata* and *C.albiceps* prepupae showed different results according to the type of diet.

When *L.sericata* larvae were fed on bovine liver, the protein content in the haemolymph of the prepupae was much higher than in those fed on chicken and bovine meat. The least protein content recorded when larvae were offered fish as diet. In case of *C. albiceps*, the protein content in the haemolymph of the prepupae showed slightly higher concentration when larvae were fed on bovine liver than in those fed on bovine meat, chicken and fish. Similar results were reported by El-Gindi and Abdel Meguid (1994), in case of *Parasarcophaga argyrostoma*. The authors reported that the crude protein in the haemolymph of larvae fed on bovine liver showed higher concentration than in those fed on bovine meat and fish.

Table (1): Crude protein content (g/dl) in the haemolymph of *Lucilia sericata* and *Chrysomya albiceps* prepupae.

Type of diet	Crude protein (g/dl)	
	Lucilia sericata (Mean ± SE)	Chrysomys aalbiceps (Mean ± SE)
Fish	7.30 ± 0.53	7.33 ± 1.13
Chicken	8.21 ± 1.24	8.18 ± 1.06
Bovine meat	8.15 ± 0.94	8.56 ± 1.02
Bovine liver	9.4 ± 0.57	8.71 ± 1.02
F valaue	0.98	0.45
LSD		

Results aare mean (± SE) of five replicate experiments
LSD: Least significant difference

Table (2): Free amino acid content in the haemolymph of *Lucilia sericata* prepupae obtained from larvae fed on differet diets.

Amino acid	Amino acid in haemolymph of larvae fed on different diets (mg/100 ml)				F-value	LSD
	Bovine meat (mean ± SE)	Bovine liver (mean ± SE)	Fish (mean ± SE)	Chicken (mean ±SE)		
Aspartic acid	14.91± 1.76^{c}	22.47± 0.97^{b}	38.32± 3.10^{a}	10.87± 0.65^{c}	41.93***	6.13
Glutamic acid	32.97± 2.30^{b}	35.4± 4.8^{b}	70.89± 2.78^{a}	28.27± 0.44^{b}	42.19***	9.82
Serine	4.09± 0.25^{b}	3.89± 0.04^{b}	34.51± 3.15^{a}	4.03± 0.22^{b}	92.77***	5.16
Glycine	15.71± 0.36^{c}	35.14± 2.89^{a}	19.81± 0.87^{cb}	22.39±1.81^{b}	22.39***	5.78
Histifine	170.72± 2.55^{b}	284.76±16.77^{a}	63.06± 2.74^{c}	72.2± 2.18^{c}	142.96***	28.25
Arginine	60.53± 3.49^{b}	75.8± 4.07^{ab}	82.74± 6.86^{a}	44.2± 3.52^{c}	13.34**	15.31
Threonine	140.11± 7.51^{a}	162.32± 3.13^{a}	175.78± 3.95^{a}	160.7± 28.74^{a}	0.96	---
Alanine	28.49± 0.43^{ab}	30.62± 0.89^{b}	50.53± 3.07^{a}	20.93± 4.68^{c}	19.70***	9.28
Proline	41.79± 2.26^{b}	47.99± 3.96^{b}	74.21± 3.38^{a}	21.57± 4.18^{c}	37.93***	11.5
Tyrosine	20.49± 0.75^{b}	20.68± 1.74^{b}	52.75± 5.88^{a}	16.07± 0.92^{b}	29.53***	10.18
Valine	43.69± 0.11^{b}	40.36± 0.63^{b}	50.73± 0.13^{a}	29.82± 2.22^{c}	56.65***	3.77
Methionine	11.89± 0.83^{b}	8.77± 0.37^{c}	36.15± 0.62^{a}	7.02± 0.08^{c}	604.07***	1.81
Cysteine	25.67± 2.04^{cb}	34.87± 2.91^{b}	62.6± 8.31^{a}	17.12± 1.32^{c}	18.68***	14.89
Isoleucine	16.19± 1.01^{a}	4.13± 0.27^{b}	18.11± 1.99^{a}	6.13± 1.54^{b}	26.59***	4.45
Leucine	1.9± 0.40^{b}	0.23± 0.001^{b}	29.5± 2.36^{a}	0.89± 0.12^{b}	141.30***	3.91
Phenyl alanine	4.48± 0.76^{b}	6.34± 0.43^{b}	35.04± 2.49^{a}	3.98± 0.01^{b}	130.30***	4.3
Lysine	38.36± 5.76^{ab}	44.27± 5.08^{a}	36.73± 5.93^{ab}	30.13± 0.74^{b}	2.12	---
Total	671.99±32.57	858.04±48.95	9.31.46±57.61	496.32± 53.37	---	

Results are mean (±SE) of three replicate experiments ** P<0.01 ***P<0.001
Same letter do not differ significantly.
LSD: Least significant difference F-value: ANOVA test

Table (3): Free amino acid content in the haemolymph of *Chrysomya albiceps* prepupae obtained from larvae fed on different diets.

Amino acid	Amino acid in haemolymph of larvae fed on different diets (mg/100 ml)				F-value	LSD
	Bovine meat (mean ± SE)	Bovine liver (mean ± SE)	Fish (mean ± SE)	Chicken (mean ±SE)		
Aspartic acid	59.96 ± 3.91[b]	20.28± 1.99[c]	25.98± 0.73[c]	95.67± 4.09[a]	133.06***	9.85
Glutamic acid	49.94 ± 2.78[a]	36.41± 4.47[b]	0.96± 0.08[d]	25.65± 3.65[c]	41.87***	10.45
Serine	23.12 ± 1.41[a]	4.86± 0.04[c]	10.64± 0.77[b]	5.66± 0.93[c]	78.92***	3.09
Glycine	14.26 ± 0.62[b]	12.65± 1.92[b]	18.66± 1.12[a]	14.93±0.16[ab]	4.81*	3.78
Histifine	46.98 ± 2.89[a]	47.32± 7.26[a]	32.07± 1.72[b]	34.57± 0.74[ab]	4.02*	13.09
Arginine	52.43 ± 2.28[a]	46.22± 4.74[ab]	27.56± 0.89[c]	36.4± 5.5[cb]	8.15**	12.5
Threonine	146.75± 11.18[a]	97.18 ± 7.52[b]	109.8± 4.85[b]	151.22±11.48[a]	8.54**	29.94
Alanine	9.39± 0.32[c]	7.32 ± 0.99[c]	18.94± 0.81[b]	30.38± 2.73[a]	48.30***	4.95
Proline	31.27± 2.87[b]	36.91± 1.60[b]	44.72± 2.76[b]	61.22± 8.36[a]	7.69**	15.32
Tyrosine	13.33± 0.37[b]	35.0 ± 1.35[a]	51.04± 1.3[a]	37.78± 9.66[a]	10.06**	16.07
Valine	43.15± 1.93[b]	57.48± 5.38[a]	37.61± 1.16[b]	49.05± 4.57[ab]	5.27*	12.08
Methionine	14.05± 1.45[c]	30.53± 4.10[b]	49.27± 1.36[a]	9.39± 0.76[c]	61.24***	7.53
Cysteine	91.36± 3.41[c]	256.2± 5.11[a]	198.47± 4.32[b]	62.41± 3.21[d]	309.1***	13.32
Isoleucine	37.7± 2.99[a]	1.09± 0.19[c]	22.06± 1.48[b]	3.88± 0.19[c]	104.2***	5.46
Leucine	10.2± 0.87[b]	1.17± 0.27[c]	38.61± 2.74[a]	6.97± 0.35[b]	131.23***	4.75
Phenyl alanine	8.84 ± 1.2[c]	25.83± 1.23[b]	28.92± 4.35[a]	16.05± 1.74[c]	48.85***	8.14
Lysine	66.55 ± 1.59[a]	41.52± 2.68[b]	43.29±5.3[b]	52.17± 0.95[ab]	5.41*	16.03
Total	719.28± 42.07	757.96± 51.2	758.6±35.74	693.4± 59.07		

Results are mean of three replicate experiments
Same letter do not differ significantly.
LSD: Least significant difference ** P<0.01 ***P<0.001
F-value : ANOVA test

Also Araujo, et al. (1995) found a significant difference between the protein content of the 3rd larval instar of *Chrysomya megacephala* reared on ground beef than on bovine blood.

Results obtained in the present work also showed that, the total free amino acids in haemolymph of both blowflies prepupae showed its highest value when *L.sericata* larvae were fed on fish, and when *C.albiceps* larvae were fed on fish as well as on bovine liver. The free amino acids present in the haemolymph are mostly derived from : a) the protein in the food; this depend to a great extent on the presence of digestive proteolytic enzymes found in the insect midgut., b) represent storage of nitrogenous materials that can be drawn on, according to the needs of the tissues, c) stored in the blood until they are eliminated by normal excretion, (Pattan, 1963), d) due to histolysis of the larval tissues (protein) into amino acids by amino acid dehydrogenase as the larvae are at the beginning of the pupal period (Agrell, 1949).

In case of the blowfly *L.sericata* the amino acid histidine, which is an essential amino acid linked to the pathway of purine synthesis, was significantly high after feeding bovine liver. The amino acid threonine, considered as one of the end products of succinyl coenzyme A catabolism, was significantly high after feeding larvae on fish. The amino acids leucine and serine were significantly low in the haemolymph of larvae fed bovine liver. Low value of these amino acids could be due to deficiency of their metabolism from the amino acids that they arise from. Arginine was moderately high in larvae fed bovine meat, bovine liver and fish. Glutamic acid was more or less similar in case of bovine meat and bovine liver diet. Glycine and lysine were more or less similar in case of all diets. The amino acid proline was higher in case of fish diet. Since the amino acids arginine and proline, are synthesized from glutamate, this might explain its lower value. Lysine is synthesized from aspartate and glycine from glycerate -3- phosphate.

In case of the blowfly *C.albiceps* the amino acid threonine was high in larvae fed bovine meat and chicken, but cysteine was significantly high in case of larvae fed on bovine liver and fish. As methionine is known to supply sulphur to cysteine, the low value of methionine could be attributed to cysteine synthesis. Glutamic acid was low after a fish diet , also the amino acids isoleucine and leucine were significantly low after feeding bovine liver. The low value of these amino acids could be due to their depletion or degradation in metabolic pathways.

The amino acids threonine and histidine were more abundant in *L.sericata* while threonine and cystine were the most abundant in *C.albiceps*.

Similar results were reported by Yousef (1988), who stated that threonine, lysine, serine, histidine and arginine are also the most abundant amino acids in the haemolymph of the 6th larval instar of *Spodoptera littoralis*. The amino acid tyrosine showed highest value after fish diet in both blowflies *L.sericata* and *C.albiceps*, and lowest value in case of both bovine liver and bovine meat diet. During the few days preceding each moult tyrosine accumulates in order to play a role in cuticle formation by transforming to quinones (tyrosinase enzyme) which gives the darkening of the cuticle. (Duchateau-Bosson et al., 1962 and Yousef 1988). Proline showed highest value after a fish diet in *L.sericata,* while in *C.albiceps* after fish and chicken diet. Proline is considered as an important amino acid in relation to some of the biological oxidation pathways in cells. Proline was found to be predominant in the haemolymph of fifth instar larvae and adult of Rhodnius prolixus (Barrett and Friend, 1975); and also present in high concentration in the thoracic muscles of some Trichopteran hydropsychidae (Haag and Sullivan, 1984); as well as in *Aedes aegypti* (Thayer and Terzian, 1970); *Glossina moristans* (Bursell, 1963); *Leptinotarsa decemlineata* (Dekort et al., 1973); and *Apis mellifera* (Barker and Lehner, 1972). Yousef (1988), reported that proline amino acid contributes to about 30% of the total amino acids in the larval stage of *Spodoptera littoralis.*

Evans and Crossley (1974), determined the concentration of 18 amino acids in the cells and plasma of the haemolymph of third-instar larvae of *Calliphora vicina* R.-D 72 hr before puparium formation. All but two of the amino acids occurred mainly in the plasma, which was found to contain 86.7-99.5% of the total concentrations present. The two exceptions were aspartate (69.2%) and glutamate (61.7%) in the cells of larvae 24 and 96 hr before puparium formation. The choice of feeding depends on the physiological status of the species. Teran (1977 and 1978), Inokuchi (1971) and Galun et al. (1981) in their studies indicated that certain food contain highly phagostimulating amino acids, whereas, other foods may have an inhibiting action. Collett (1976) reported that the amounts of nearly every amino acid in *Calliphora* remained unchanged even under adverse conditions. Also the author found that the free amino acid pool was always regulated intracellularly because the presence of greater concentrations of amino acid within cells than in the haemolymph indicates that a great deal of the regulation took place intracellularly. With a dietary source of amino acid, the rates of incorporation into protein account for only a part of the total turnover of each amino acid. With a sufficiency of amino acid, substantial amounts are probably used as an energy source. Anderson (1984) stated that individual amino acid concentrations in *Calliphora vicina* R.-D. (erythrocephala Mg.) showed complex patterns of change with development. However El-Gindi and Abdel-Meguid (1994), stated that, most of the essential amino acids of *Parasarcophaga argyrostoma* larvae arginine, lysine, leucine, isoleucine, histidine, phenyl alanine, valine and threonine were present in high concentration in the haemolymph of the last larval instar fed on bovine meat diet. Arginine was absent in larvae fed on fish and bovine liver diet, isoleucine was absent in larvae fed on bovine liver diet only, but methionine was not present in case of all three different diets.

According to the present results it appeared that, feeding larvae of both *L.sericata* and *C.albiceps* on different protein diets showed variation in total protein of haemolymph as well as variation in values of different amino acids with slight higher values in case of bovine liver diet which can be used as diet for laboratory colonization of these two blowflies.

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