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CULTURE OF CHIRONOMID LARVAE (INSECTA- DIPTERA-CHIRONOMIDAE)UNDER DIFFERENT FEEDING SYSTEMS

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ABSTRACT

Rearing of chironomid larvae was conducted under laboratory conditions, to study the growth rate (weight & length) of chironomid larvae under different feeding systems. Three types of food were used , the first was Tetramin Flaked commercially available fish food (D1) , the 2nd food was algae (Scenedesmus sp., D2) and the 3rd was baker yeast (D3). Larvae have mean initial length 1.5mm. and mean weight 0.99 µg. Three replicates were performed for each treatment. The three diets have significantly ($P<0.05$) effect on both weight and length of chironomid larvae, but larvae fed on D1 showed the highest weight and length which represented by 36.68 µg and 9.29 mm, respectively. Followed by those fed on D3 was represented by 7.03mm for length and 31.03 µg for weight, while D2 showed the lowest values for both length (6.28mm.) and weight (27.08 µg.). The highest percentage of protein (51.15%) was for larvae fed on D1, followed by those fed D3 (49.45%), while the larvae fed D2 showed the lowest value of protein which was represented by 26.45%. The lowest lipid content (12.04%) for larvae fed D1, while it represented by 13.25 and 14.22% for larvae fed on both D2 and D3, respectively.

Most essential amino acids such as arginine, histidine methionine, phenylanine isoleucine, leucine and lysine were significantly ($P<0.05$) higher in chironomid larvae fed onTetramin, than those fed the other two diets.

The present work revealed the good nutritional value and growth rate for chironomid larvae fed on tetramin.

INTRODUCTION

Insects play an important role in food chain of aquatic system, and among Diptera the chironomid larvae (Midge larvae) are recognized as an important food item for many fishes and cultured invertebrate (Shaw and Mark, 1980; Habib *et al.,*1992; Yusoff *et al.,*1996; Fernando, 1994; Branco *et al.,* 1997; Tidwell *et al*., 1997 and Wais *et al*.,1999). Also they can be used as frozen food when added to shrimp meal for the freshwater prawn, *Macrobrachium rosenbergii*, can promote and improve its growth rate and survival, under laboratory conditions (Abdel-Razek *et al.,* 1998).

The chironomid larvae known as blood worms due to the presence of hemoglobin in their bodies. They represent an abundant group of benthos insects in freshwater ecosystem. They have high reproductive capacity, each female lay about 2300 eggs in one batch which hatch in about three days at temperature(18-22^{o}C). The larvae attain a size suitable for feeding purposes in 16-20 days (Guerrero,1982). Its growth and development can be influenced by numerous environmental factors including temperature and photoperiod (Maier *et al*.,1990), food availability and food quality and quantity (Mackey 1977 a ;Vos *et al.,* 2000).

Chironomid larvae are excellent source of protein (De La Noue and Choubert, 1985),

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lipid, vitamins and minerals (Mclarney *et al*., 1974). Habib *et al*.(1997) stated that for aquaculture practices minerals may be supplemented to fish and shrimps by feeding them with chironomid larvae.

Due to this increasing demand for chironomid larvae in aquaculture researches and scientists try to study its production, behavior, developmental, growth, feeding, and nutritive value (Armitage, 1995).

The food value of chironomids was studied by Sugden (1973) who reported a value of 56% protein for chironomid larvae. The relatively high protein content, the high digestibility represented by 73.6% as mentioned by De La Noue and Choubert (1985), and the apparent function in small quantities as a growth promoter in fish and crustacean diets (Tidwell *et al.,* 1997) make chironomid larvae a rich food for many organisms. It was suggested by Armitage (1995) that the preference of bottom feeder organisms for chironomid larvae and pupae as food source is related to their high energy content (% mean values: moisture content 86, protein 48 to 55, lipid 14, carbohydrate 23, chitin 4, ash 9, with an utilization energy of 4.1 to 6.1K Cal g^{-1}).

Rasmussen (1985) studied the effect of density and micro-detritus enrichment on the growth of two species of chironomid larvae (*Chironomus riparius* and *C. paripes*) in small ponds over the 25- days experimental period and found that density effect significantly on their growth both with and without organic enrichment. Vos *et al*.(2000) reported that the nutritional requirement of sediment -feeding invertebrates are poorly understood, they performed a growth experiment with larvae of chironomids to study the interaction between food availability and food quality during the growth of early instar.

Generally ,few studies were performed on growth and nutritive values of chironomid larvae, so the objective of this study was to determine the growth and nutritive value of these larvae when fed on three types of food (Tetramin, algae and yeast) under laboratory conditions.

MATERIAL AND METHODS

Larvae collection and culture method

Larvae of chironomid were collected from shallow ponds at Abo-Rawash region (Giza Governorate) and reared under laboratory conditions as described by Maier *et al.* (1990), using the breeding aquarium (Fig.1) which was filled to a depth of approximately 15 cm with artificial pond water (APW) used by Aly (1993) (Table 1).

Table 1. Artificial pond water (APW)

Chemicals	Weight in grams
$Ca\ Cl_2.2H_2O$	11.76
$Mg\ SO_4.\ 7H_2O$	4.93
$NaHCO_3$	2.59
KCl	0.23

Each one of the above chemicals is dissolved in and made up to one litre with distilled water to make stock solution.
The water is made up by mixing 25 ml. of each stock solution and making up to one litre.

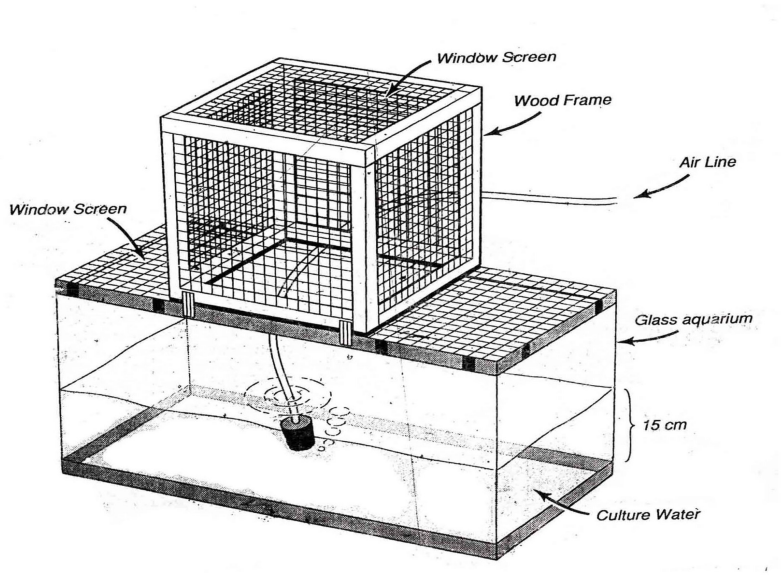

Fig . (1) Breeding aquarium of chironomid larvae

Experimental design and food items

Completely randomized design in factorial arrangement (3x4) was employed. The experimental treatments were done in three replicates.

Food items

Three different food items were tested; the first ,fish food which is used in laboratory experiments, Tetramin Flaked stable food are commercially available fish food (D1). The 2nd was algae, *Scenedesmus* sp. (D2) which purchased from algal laboratory in National Research Center Cairo. Its culture was performed according to Hussien (1988) . The last food item was yeast which was bought at a bakery (D3). Villegas (1982) reported that yeast has been used successfully as feed in aquaculture. All food items were dried, ground, sieved through a 106 µm mesh, and stored at –18Cº before conducting chemical analysis and growth experiment.

Growth experiment

Growth experiments were conducted in glass containers (20x20x10 cm.) containing 50 g of pre washed and combusted sand (<500 µm) heated at 550 ºC for 6 h.) . Five hundred ml. of APW (pH 8.2) were added per container, and the water was constantly aerated. Hundred larvae (first instars) were placed in each container by using a small eye dropper. (Three container for each treatment). The rate of feeding was 0.15 mg / container twice a week . Samples of 20 larvae were taken and stored in 70% ethanol for initial growth determination. Body length was measured from the anterior end of the head to the anus by using a compound microscope fitted with a calibrated eye piece micrometer. Total 20 larvae were measured and the mean body length was estimated. To measure live wet weight, groups of 20 individuals of approximately equal size were placed in small culture dishes of water. Most of water was removed, leaving the larvae

accumulating on the bottom. Later, larvae were transferred to a piece of filter paper with forceps. After about 20 seconds these larvae were transferred to a piece of tared filter paper and weighed to the nearest 0.01 mg. Larval mean weight was estimated. Both length and weight were measured at the beginning of experiment (T1) and at 7 (T2), 14 (T3) and 21 days (T4) intervals. The duration of the growth experiment was restricted to three weeks (from 1 to 21 June ,2005) to ensure that larvae did not reach the fourth instar .This instar was avoided because that stage both growth and development towards the pupal and adult stage occur, and the larvae become sexually diamorphic (Gilbert , 1967 and Beenakkers *et al.,*1981).

Chemical analysis of food items

By the termination of the tests, the diets used and all chironomid larvae were stored frozen for proximate analysis and amino acids analysis for larvae (Tables 2, 3, 9).

The organic matter ,carbohydrate, proteins and lipid contents of food sample were determined according to Luczak *et al.*(1997) , Du Bois *et al.*(1956), Lowry *et al.*(1951) and (De-Boer 1988) respectively. Energy content was calculated by assuming that 1g. of fat releases 38.9 KJ, 1g. of protein releases 17.3 KJ, and 1 g. of carbohydrate yields 16.9 KJ. Ash content was estimated by ignition of a weighed dried samples in a muffle furnace at 600 ºC over night (17 h.)

Amino acids analysis

The extraction of amino acids content of larvae from each treatment at the end of experiment were performed according to the method of the AOAC (1990) and the amino acids were analyzed by using LC3000, Amino Acid Analyzer, Flow rate: 0.2ml/min. Pressure of buffer 0 to 50 bar, Pressure of reagent 0 to 150 bar and reaction temperature 123ºC.

Statistical analysis

Statistical analysis was made by Factorial design (3x4), Means were separated using LSD (Least Significant Difference test) according to the procedure by Steel and Torrie (1980).

Table 2. Food composition expressed in percentages of organic matter of the test diets.

Parameters	Types of food		
	Tetramin (D1)	Algae (D2)	Yeast (D3)
Organic matter	87.6	95.8	93.7
Protein	37.6	36.8	42.3
Lipid	6.3	9.6	2.2
Carbohydrate	30.1	42.6	41.1
Energy content (KJ/g)	14.4	17.7	15.5

Table 3. Proximate composition (%) of chironomid larvae fed on different test diets.

Proximate composition(%)	Chironomid larvae fed on D1	Chironomid larvae fed on D2	Chironomid larvae fed on D3
Crude protein (CP)	51.15 [a]	26.45 [b]	49.45 [a]
Crude lipid	12.04 [a]	13.25 [b]	14.22 [b]
Ash	13.25 [a]	18.62 [b]	14.24 [a]
Nitrogen free extract (NFE)	16.75 [a]	10.15 [b]	16.08 [a]

Means of each raw with different letters indicate significant differences (P<0.05)

Table 4. Effect of different diets and time (days) on growth parameters (weight-Length) of chironomid larvae (ANOVA –Factorial design).

Treatments	Length(mm.)	Weight (µg.)
Diets		
Tetramin (D1)	4.89	18.88
Algae (D2)	3.69	13.91
Yeast (D3)	3.99	14.35
L.S.D.	0.156	0.393
Time (days)		
0 (T1)	1.50	0.99
After 7 days (T2)	3.00	7.21
After 14 days (T3)	4.74	23.05
After 21 days (T4)	7.53	31.60
L.S.D.	0.181	0.454

T1= Time at the beginning of experiment.

Table 5. Correlation values between lengths of chironomid larvae and time in different feeding systems.

	D1	D2	D3
D1	1		
D2	0.991**	1	
D3	0.992**	0.998**	1
Time	0.982**	0.991**	0.989**

** Correlation values were significant at the 0.01 level.

Table 6. Correlation values between weight of chironomid larvae and time in different feeding system.

	D1	D2	D3
D1	1		
D2	0.995**	1	
D3	0.960**	0.977**	1
Time	0.968**	0.985**	0.988**

** Correlation values were significant at the 0.01 level

Table 7. Effect of interaction between different diets and time(days) on growth parameters (length and weight) of chironomid larvae (ANOVA)

Interaction	Length (mm.)	Weight (µg.)
D1 X T1	1.50	0.99
D1 X T2	3.29	7.34
D1 X T3	5.50	30.52
D1 X T4	9.29	36.68
D2 X T1	1.50	0.99
D2 X T2	2.81	6.83
D2 X T3	4.19	20.73
D2 X T4	6.28	27.08
D3 X T1	1.50	0.99
D3 X T2	2.90	7.47
D3 X T3	4.53	17.90
D3 X T4	7.03	31.03
L.S.D.	0.313	0.786

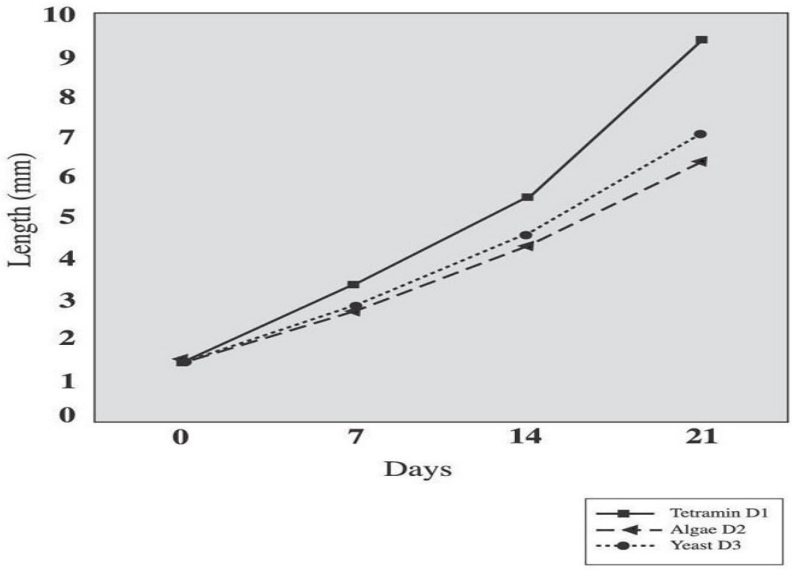

Fig.(2): Growth in length(mm) of chironomid larvae fed different test diets (D1, D2,D3) during the experimental period.

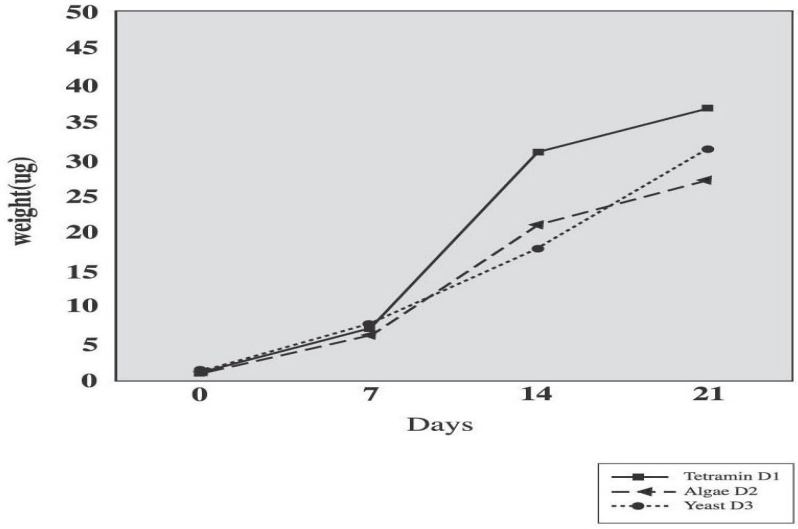

Fig.(3): Growth in weight(ug) of chironomid larvae fed different test diets (D1, D2,D3) during the experimental period.

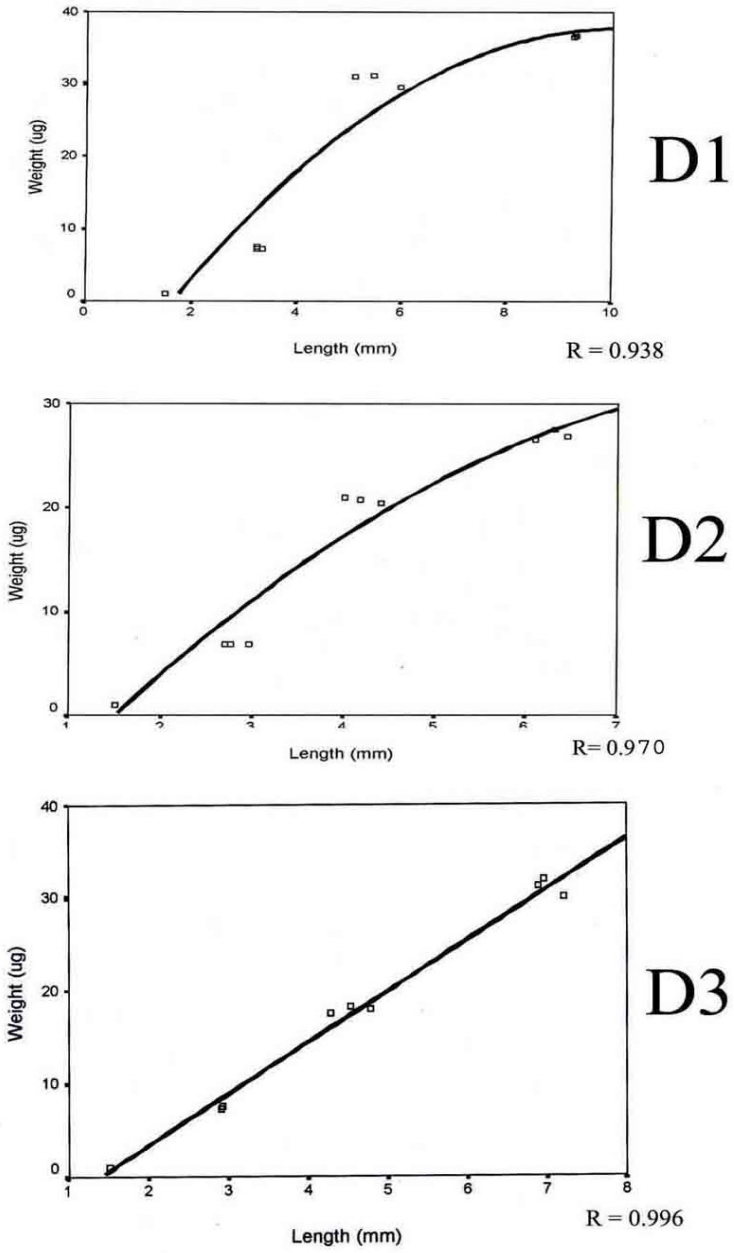

Fig (4) : Relations between length (mm) and weight (ug) of chironomid larvae fed on different test diets.
(D1 , D2 , D3)

Table 8. Regression equations developed for length and weight of chironomid larvae over time in the three different diets and their correlation (R).

Different diets	Length		Weight	
	Regression equations Y = a + b X	R	Regression equations Y = a + b X	R
D1	Y = 1.058 + 0.365 X	0.982	Y = -0.657 + 1.861 X	0.968
D2	Y = 1.337 + 0.225 X	0.991	Y = 0.106 + 1.312 X	0.985
D3	Y = 1.259 + 0.260 X	0.989	Y = -0.736 + 1.437 X	0.988

Table 9. Amino acids of chironomid larvae fed on different test diets(g/100 g protein).

Amino acids	Chironomid larvae fed on D1	Chironomid larvae fed on D2	Chironomid larvae fed on D3
Essential amino acids			
Arginine	5.66 [a]	3.12 [b]	3.88 [b]
Histidine	3.96 [a]	1.05 [b]	2.12 [c]
Threonine	2.77 [a]	5.44 [b]	4.33 [c]
Phenylalanine	4.76 [a]	2.10 [b]	2.88 [b]
Valine	3.56 [a]	5.84 [b]	5.68 [b]
Methionine	10.92 [a]	2.03 [b]	5.45 [c]
Isoleucine	3.43 [a]	2.17 [b]	2.66 [b]
Leucine	5.18 [a]	4.98 [b]	4.45 [a]
Lysine	3.77 [a]	2.50 [b]	2.20 [b]
Total	44.01	29.23	33.65
Non essential amino acids			
Aspartic acid	7.18	8.83	7.96
Clutamic acid	8.61	9.55	9.44
Serine	4.09	6.28	5.92
Glycine	6.96	7.11	8.25
Alanine	4.67	10.64	8.92
Proline	4.49	6.51	4.12
Tyrosine	3.56	3.48	3.92
Cystine	4.66	5.28	6.68
Total	44.22	57.68	55.21

Means of each row of essential amino acids with different letters indicate significant differences (P<0.05).

RSULTS AND DISCUSSION

Food composition

The food items had comparable organic matter contents ranging between 87.6% (Tetramin, D1) and 95.8% (algae, D2) while yeast represented 93.7% (Table 2), crude protein (CP) was higher (42.3%) in D3 and the two other diets showed about similar values of CP, represented by 37.6 , 36.8% for both D1 and D2, respectively. The lowest lipid contents was found in D3 (2.2%), whereas the highest value was observed in D2 represented 9.6% and its value was 6.3% for D1 . Carbohydrate content was lowest (30.1%) for D1, and its values for D2 and D3 were almost similar and represented by 42.6, 41.1% , respectively. Energy contents for different food items were represented by 14.4%(lowest value) for D1 followed by D3 (15.5%), whereas the highest energy content was observed in D2 (17.7%).

Proximate composition of chironomid larvae fed different test diets

Table (3) shows that CP, ash and nitrogen free extract (NFE) are insignificantly different (P>0.05) for larvae fed on both D1 and D3 ,whereas larvae fed on D2 shows a lowest significant difference (P<0.05) in CP and NFE, which represented by 26.45 and 10.15%, respectively. At the same time the ash content is significantly higher (P<0.05) for larvae fed on D2 than those fed on the other two diets(D1 and D3). While the lowest value of crude lipid (12.04%) for larvae fed on D1 which significantly difference (P<0.05) than those fed on D2 and D3.

Growth experiment

The effect of different diets and time on growth parameters of chironomid larvae (ANOVA) is shown in table (4). It is found that the three diets have significant effect (P<0.05) on both weight and length. However, D1 give the highest mean length and weight for all intervals, represented by 4.89mm. and 18.88 µg., respectively. It is followed by D3 (3.99 mm. for length and 14.35 µg. for weight) while D2 represented the lowest values for both length (3.69mm.) and weight (13.91µg.) and this may be due to the best utilization of protein found in fish food (Tetramin, D1) than protein found in the two other diets(D2 & D3). The gut content of those larvae fed on algae looked green, whilst it was brown in those fed on Tetramin. Berg(1995) reported that the color of chironomid larvae guts depend on food type. Table (4) shows also significant differences (P<0.05) between zero time (beginning of experiment) and different intervals (7 ,14 , 21 days) for both length and weight of larvae, but the higher significant differences were found between 14 and 21 days ,mean length for all diets was 4.74 mm. at 14 days, and 7.53 mm. at 21 days. For weight it represented by 23.05 and 31.60 µg for both 14 and 21 days, respectively.

There were highly significant correlations (P<0.01) between length and time and between weight and time for all diets used, this means that both length and weight of larvae increase with time (Tables 5 & 6) . This finding is in line with that obtained by Vos *et al.* (2000) who reared larvae of midge *Chironomus riparius* on different food items of different composition and found that both quality and biochemical composition of food influenced growth of midge larvae and maximum length attained by larvae reared on fish food was higher than maximum length reached on food items of plant origin (algae, living leaves of aquatic plants) after one week rearing period. Habib *et al*. (1997) reported that growth of chironomid larvae in algal culture was significantly (P<0.01) lower than in artificial diets.

Other studies further support the observation that some chironomid diets generally consist of animal matter which may be important for larval growth (Berg, 1995). Vodopich and Cowell (1984) reported that *Procladius culiciformis* (Linnaeus)

(chironomidae) did not moult beyond the third instar when fed only on algae and dertritus. Similarly, growth rate of *Alabesmyia monilis*(Linnaeus) (chironomidae) decreased when denied animal food (Mackey 1977 b). Also Biever (1971) and Credland (1973) who showed that Tetramin is an excellent food source for many species of chironomid larvae and enhance the growth rate in laboratory culture. Rasmussen (1985) recorded that the growth rate of *Chironomus riparius*_when fed on organic enrichment with microdetritus (1g. of Tetramin to 100 ml. of fine mud) significantly enhanced its growth, for densities <2.8 cm^2 and in contrast, the enrichment had no effect on another species of chironomid known as *Glyptotendipes paripes.*

The present study revealed that larvae fed D2 (algae, *Scenedesmus* sp.) showed the lowest growth rate probably due to the indigestibility of this diet as mentioned by (Johnson *et al.,* 1989). These authors stated that some algae especially blue green algae have been reported to be unimportant as food source for chironomids because they are either relatively undigestible or not ingested at all. These results are in contrast with these obtained by Rasmussen (1984 a&b) who stated that higher growth rate of chironomid larvae often coincide with increased availability and ignition of planktonic diatoms. Also suspended blue green algae are also reported to be a major portion (68% of gut contents) of the diet of filter feeding *Chironomus crassicaudatus* (Ali, 1990). However, benthic green algae have been reported to be a major food source as stated by Brook (1954) who observed that chironomid larvae on a sand- filter bed ingested only filamentous blue green algae and filamentous diatoms.

The effect of interaction between each diet with different intervals (time) is illustrated in table (7). Comparing the differences between different means in both length and weight of larvae fed on different diets with the values of LSD, it was found that highly significant differences in the case of larvae fed on D1 with different intervals especially between the last intervals T3 ,T4. The same trend was found for those fed on D2 & D3. It was noted that the highest significance was observed between T3 & T4 for all diets. While, at day 21, the larvae fed on D1 showed the best and the highest length rate 9.29 mm. and for weight was 36.68 µg. followed by those fed on D3 at the same time. Lastly, larvae fed on D2 showed the lowest growth rate represented by 6.28 mm. for length and 27.08 µg. for weight, this is may be because the larvae began to enter the fourth instar and at this stage the largest growth rate in both absolute and relative terms occurs. Tokeshi (1995) reported that the fourth instar of chironomid larvae are on average 5-8 times larger in mass than third instar, which in turn are 5-8 times larger than second instar. Field observation with some chironomid larvae showed that environmental factors such as temperature, pH, toxic substances, photoperiod, oxygen content, and biotic interaction may influence growth in the chironomidae (Tokeshi 1995). Thus, where overwintering occurs as second or third instar, growth curves tend to show a sharp upturn towards the end of larval life. In this respect, Ladle *et al*.(1984) demonstrated that growth in *C. zealandicus* was positively related to temperature, similarly the length of life cycle was reduced at higher temperature in laboratory experiment.

The regression equations of body length (mm.) and weight (µg.) of larvae on culture time (days) for different treatments were significant (Table 8), indicating that the rate of growth in length and weight increase as time increase and from day 14 to 21 a sharp increase in both length and weight occurs (Figs.2&3) .The value of b was found to depend on the size and weight of the larvae, the highest value of b was observed for larvae fed on D1 which have highest growth rate, this result is in agreement with that obtained by Mackey (1977 b) who stated that the larger the larvae at maturation, greater the value of b for 12 species of chironomid

larvae from River Thames,UK. Also table (8) shows highly significant correlations (R) between length and time , weight and time for all diets. This means that the time affects positively on both length and weight.

Again there were highly positive correlations between length and weight in each diet represented by 0.938 for larvae fed on D1, 0.970 for those fed D2 and the highest correlation (0.996) was found for those fed on D3.This means that D3 (yeast) has a similar effect on both length and weight. This effect is described by a straight line for larvae fed D3, while those fed on D1 & D2 slightly differ as shown in figure(4).

Amino acids profile (essential and non essential) of chironomid larvae fed on different diets are shown in table (9).

Essential amino acids (EAA)

All the EAA of chironomid larvae as arginine, histidine, phenylanine, methionine, isoleucine, leucine and lysine except threonine and valine were significantly higher ($P<0.05$) in larvae fed on D1 than those fed the other two diets (D2 & D3). It was cleared that EAA showed maximum quantity 44.01 for larvae fed D1 followed by those fed on D3 (33.65) and the lowest value (29.23) was observed for those fed on D2 (Table 9) :

Non essential amino acids (NEAA)

Results of the eight NEAA of chironomid larvae fed on D1 showed the highest values of aspartic acid, clutamic acid, glycine, alanine and cystine while serine, proline and tyrosine represented the lowest values, whereas larvae fed on D2 showed the highest values for all NEAA , except tyrosine and cystine. On the other hand, larvae fed on yeast (D3) showed the highest values of clutamic acid,aspartic acid, glycine, alanine and cystine, while proline and tyrosine represented the lowest values. The results showed maximal total NEAA for larvae fed on D2 (57.68) which slightly higher than those fed on D3 (55.21) and the lowest value represented by 44.22, was reported for larvae fed D1 (Table 9). In this respect, Watanabe *et al.* (1978) recorded that the nutritional quality of living organisms as protein source was investigated by determining their amino acid composition, digestibility, protein efficiency ratio and net protein utilization. As shown in table (9) most EAA significantly higher for larvae fed Tetramin (D1) this may be due to the best and highest digestibility of Tetramin protein than the other two diets. So the food value of chironomid larvae was improved by allowing them to fed on Tetramin. This finding is in line with that obtained by Habib *et al*.(1997) who reared chironomid larvae on algae and artificial diet and found that most of EAA were significantly ($P<0.05$) higher in larvae fed on artificial diet than those reared on algal culture.

Wilson and Poe (1985) found a significant correlation ($r^2= 0.96$) between dietary amino acid requirements and whole body amino acid composition of channel catfish, *Ictolurus punctatus*. However, Das *et al*. (1996) stated that the content of EAA in the feed cannot form the sole measure of evaluation of the feed quality.

Blood worms have been previously reported in many literatures to be very adequate for growth in fish (e.g. Ling, 1966 ;Yashouv, 1970; Koh and Shim, 1980) and invertebrate such as freshwater prawn (e.g. Tidwell *et al*., 1997; Abdel-Razek *et al.*,1998). These authors concluded that the positive effect on the addition of blood worms was due to the supply of substances essential in minimal quantities to the growth of the body. The relatively high digestibility (73.6%) and the apparent function as a growth promoter in fish diets make chironomid larvae a rich energy source for many fish (De La Noue and Choubert 1985). Ali (1995) and Lobon-Cervia and Bennemann (2000) showed that midge larvae and pupae comprised 40-70% by volume and 40-80% by weight, of the total food contents of benthophagous fishes. Callisto *et al*.(2002) recorded that chironomid larvae are considered an important food resource for both *Leporinus amblyrhynchus* (Anostomidae, Characiformes) and *Pimelodus maculates* (Pimelodidae, Siluriformes) and found a trophic relation

between fish and chironomids assemblages in Araguari River (Brazil).

So attention must be given to insects in general especially Chironomid group and more future studies should include their biology, behavior, feeding, growth, production and culture.

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