Comparative Investigations on Some Biological and Biochemical Aspects in Freshwater Crayfish (Procambarus clarkii) Fed on Eichhornia crassipes, Echinochloa stagnina L. and Polygonum tomentosum L.

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Abstract: The present work was designed to evaluate the nutritional potential as well as the effect of 6 weeks feeding with three aquatic macrophytes namely Eichhornia crassipes (Mart) Solms. (Ward en-nil), Echinochloa stagnina L. (Niseela) and Polygonum tomentosum L. (Qordaab) collected from Damatta branch of the River Nile on the growth parameters, muscles biochemical composition and two digestive enzyme activity in the haemolymph and hepatopancreas of crayfish Procambarus clarkii collected from water stream at El-Bagour, El-Menofia governorate, Egypt. Results showed that the three studied plants were characterized by their high protein content, low lipids, high cellulose and crude fiber contents with a maximum, 14.25±0.35, 3.2±0.28, 28.85±0.92 and 24.2±0.85 % of DW respectively. The elemental analysis revealed that, the three studied plants meet the P, Cu, Pb and Zn dietary requirements of aquatic animals but, exceed the K, Fe and Mn requirements. In addition, the phytochemical screening revealed the presence of most biologically active substances in the investigated plants but with different concentrations. Further, obtained data indicated a significant increase in final body weight, gain body weight, specific growth rate and condition factor of crayfish fed on Polygonum tomentosum compared with the other investigated plants. Statistical analysis revealed that significant increase in muscles biochemical composition of crayfish fed on three investigated plant at 6 weeks compared with those animals at 3 weeks. At the same time the activity of the digestive enzymes amylase and lipase in the haemolymph and the hepatopancreas of crayfish were affected by plant species. It is tempting to conclude that, the crayfish consumed all three investigated plants preferring Polygonum tomentosum which give the highest specific growth in those animals fed on it, thus, it is recommended to use P. tomentosum in feeding crayfish.

Key words: Feeding • Nutritional potential • Macrophytes • Crayfish • Growth parameters • Muscles biochemical Composition

INTRODUCTION

Crayfish are the dominant macro crustacean in many freshwater ecosystems, omnivorous and are capable of feeding on a variety of plants, animals and microorganisms, including bacteria [1, 2]. Cultivated crayfish are of great economic value in some countries, mainly in Europe, Chile, the southern United States and Australia however, in Egypt, freshwater crayfish are not usually consumed until now.

Aquaculture feeds are amongst the most expensive animal feeds and typically account for half of the total cost of aquaculture production, with protein being the most expensive component [3-5]. Due to their high nutritional content, marine protein meals such as fish meal, squid meal and shrimp meal have long been the main protein sources used in feed for most aquaculture species. Plant meals, which are considerably cheaper than marine meals are being studied as partial replacement for marine meals in aquaculture feed [5, 6]. However, with the increasing cost and periodic shortages of marine meals on the global markets, the aquaculture industry is interested in reducing its dependence on fish meal through the development of alternative protein sources preferably

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those unsuitable for human consumption. *Eichhornia crassipes* (C Mart.) Solms., *Echinochloa stagnina* L. and *Polygonum tomentosum* L. are warm water aquatic plants which widespread in many countries, particularly during summer months with their highest growth in July. They are very widely distributed weeds in the River Nile. There is some work concerning the use of extracted leaves protein in fish meals [7], substitution of soybean meal by different levels of water hyacinth leaf protein (WLP) in Nile tilapia diets [8], macrophytes suitability as fish feed [9] and nutrition value of *E. crassipes* and *P. tomentosum* [10].

Studies that have investigated digestibility of various feed stuffs in freshwater crayfish are scarce however, despite the ability of crayfish to consume a wide range of food types. In general, natural food material consumed by freshwater crayfish consists of both animal and plant matter [11]. Digestive enzyme activities are an effective tool for identifying particular components of an animal diets [12-14]. Several enzymes have been identified in crustacean digestive systems which appear to play major roles in liberating essential nutrients from materials ingested during feeding [15].

There is a growing awareness of the value of biochemical estimation of aquatic organisms for two purposes; the first is for understanding the process of its metabolism and physiological adaptations, while the second is for determining the possibility of using it as an alternative source of food. The chemical composition of crayfish *P. clarkii* was investigated by many authors [16-17]. Some studies were focused on the feeding impacts of different items on biochemical composition of crayfish [18-20].

Recently, considerable attention has been given to harvesting aquatic plants for practical uses to partially defray the cost of removing plants from waterway and use as economical source of nutrients in many parts of the world. Hence, the present work was designed to compare, the nutritional value besides, phytochemical screening of some active substances of aquatic plants *Eichhornia crassipes* (C Mart.) Solms, *Echinochloa stagnina* L. and *Polygonum tomentosum* L. In addition, emphasis was given to compare the effect of feeding with investigated plants on growth performance, muscles biochemical composition and the activity of digestive enzymes amylase and lipase in haemolymph and hepatopancreas of crayfish *Procambarus clarkii* to investigate their ability to utilize these plants.

**MATERIALS AND METHODS**

**Biochemical and Elemental Analysis of Investigated Macrophytes**: Macrophytes were hand picked from the freshwater habitat (Damiette branch of the River Nile near to El-Serw Village South of Manzalah Lake at the eastern north part of Egypt) during January, 2008. In the laboratory, they were identified into three species (Fig. 1) including, the free floating *Eichhornia crassipes* and the emergent species *Echinochloa stagnina* L. and *Polygonum tomentosum* L. according to the standard taxonomic identification [21, 22].

**Sample Processing**: The roots of the selected plants were removed and the rest of plants were cleaned and rinsed in distilled water. Then, the fresh plants were divided into two parts one of them was used for a purpose of animal feeding, while the other part was dried at 60°C to constant weight, ground to fine powder and preserved in well stopper sample vessels for chemical analysis. The water contents were considered as the losses in mass from wet sample after drying at 60°C to constant weight while, humidity was considered as the losses in mass from a shade dry sample after drying at 100°C.

**Biochemical Analysis**: A mixture of chloroform, methanol and water was used for lipid separation [23] however, lipids determination takes place following March and Weinstein [24]. The protein fraction (% of DW) was calculated from the elemental N determination using the nitrogen-protein conversion factor of 6.25. While carbohydrate contents were assayed by the phenol-sulfuric acid method [25]. The amount of starch was calculated by multiplying its glucose content by 0.9 [26] and Cellulose contents was determined following Harborn [27].

**The Gross Energy**: were determined on the basis of the biochemical composition (lipid, protein and carbohydrate contents) using the standard conversion factor [28]. The mean gross energy value for carbohydrates, lipids and proteins has been estimated to be 17.2, 39.8 and 23.4 k J g⁻¹, respectively. The results were expressed in K J g⁻¹ of DW, where, J= Joule, DW= dry weight.
Fig. 1: Photograph showing: A) Eichhornia crassipes, B) Echinochloa stagnina and C & D) Polygonum tomentosum

The nitrogen free extracts (NFE) were calculated according to Pachas et al. [29]. NFE = 100 – (humidity + CP + EE + CF + Ash).

Where CP = Crude Protein, EE = Ether Extract (total lipids) and CF = Crude Fiber.

Metabolizable Energy (ME) was calculated using the value of 14.59, 33.86 and 17.56 KJ g⁻¹ for carbohydrate, fat and protein, respectively [30].

Protein to energy ratio (P/E) = mg crude protein / KJGE.

Elemental Analysis: Two types of plants extracts were prepared, the first extract was prepared according to the method described by Burkai [31] using (sulphuric acid and perchloric acid) and used for determination of N, P and K. Total N concentration was determined by standard micro-kjeldahl method [32]. Then phosphorus was assayed spectrophotometrically [33], potassium by flame emission spectrometry [34]. The second extract was prepared according to Allen [35] and used for determination of micro-elements using (Perkin Elmer 2100 Flame Atomic Absorption Spectrophotometer with an Autosampler).

The contents of ash, water soluble ash and crude fibers were estimated following the Egyptian Pharmacopia [36] while acid insoluble ash was estimation as described by Humphries [37].

The Preliminary Phytochemical Screening: The preliminary phytochemical screening was assessed following the methodology of Harborn [38] and Kokate [39].

Comparative Investigation on Freshwater Crayfish Fed on the Investigated Plants: Adult specimens of 120 crayfish Procambarus clarkii were collected from water stream at El-Bagour, El-Menofia governarate,
Egypt. Collected crayfish with total length 6.5 to 11 cm were brought to the laboratory and maintained in plastic containers. These containers were exposed to atmospheric air, natural light and at the room temperature and fed on lettuce leaves. Water was maintained at a depth of 12-14 cm. After the acclimation period the crayfish were divided to three groups with triplicate subgroups for each one. Each group was fed on 3 macrophytes for six weeks as follow Eichhornia crassipes (Mart.) Solms, Echinochloa stagnina L. and Polygonum tomentosum L.

**Performance Parameters:** Mean Final body weights (FW), Average weight gain (AWG), daily growth rate (DGR), Specific growth rate (SGR), condition factor (K) and feed conversion ratio (FCR) were calculated according to the following equations [8]:

\[
\text{FW (g)} = \frac{\text{Total weight of crayfish}}{\text{Number of crayfish}}.
\]

\[
\text{AWG (g/crayfish/day)} = \frac{\text{Average final weight - Average initial weight}}{\text{Experimental period (d)}}.
\]

\[
\text{ADG (g/crayfish/day)} = \frac{\text{Average final weight}}{\text{Experimental period (d)}}.
\]

\[
\text{SGR (% day)} = \frac{\text{Ln (final weight)} - \text{Ln (initial weight)}}{\text{Experimental period (d)}} \times 100.
\]

\[
\text{K} = \frac{\text{Mean final weight} \times \text{FW}'' (g)}{(\text{Mean final length})^2 \times 100}.
\]

\[
\text{FCR} = \frac{\text{diet fed (g)/net weight gain (g)}}{\text{Experimental period (d)}}.
\]

**Chemical Composition and Nutritive Value of Crayfish:** Moisture content was determined as described by A.O.A.C. [40]. The total hydrolysable carbohydrates were determined spectrophotometrically using Beckman DU-7400 spectrophotometer at 490 nm using phenol-sulfuric method as described by Dubois et al. [25].

Lipid was extracted from dried samples (0.5g) in Soxhlet apparatus for 6 hours by using chloroform: methanol at ratio of 2:1, then the solvent was evaporated using the rotary evaporator under vacuum, then residues was dried at 95°C to remove all solvent residues and have a constant weight, then total lipid percentage was calculated, according to the A.O.A.C [40].

**Determination of Total Soluble Protein:** Samples were defatted with n-hexane (1:5, w/v) for three times according to A.O.A.C.[40], then 0.5g defatted sample was extracted with 0.03M Tris-HCL buffer at PH 8.0 at the ratio of 1:20, w/v, at room temperature, according to Iwabuchi and Yamauchi [41]. Soluble protein was estimated spectrophotometrically at 595 nm in the previous extract using the coomassie brilliant blue G-250 as described by Bradford [42] in triplicates using bovine serum albumin (BSA) as a standard.

**Variation of the Digestive Enzymes Amylase and Lipase in Crayfish**

**Preparation of Enzyme Extracts:** Crayfish were anesthetized for several minutes in ice water, blotted dry, weight and then dissected. The hepatopancreas was removed from each crayfish, weighed, mixed with equal amounts of homogenate buffer solution (PH 7.4). The hepatopancreas was homogenized using hand-held Tissue Tearor (Biospec Products Inc.) and centrifuged (Beckman model TJ-6) at 3000 x g for 20 min at room temperature, after which the supernatant was stored at -20°C until required.

**Enzyme Assay:** Amylase activity was measured according to Gella et al. [43], using α-Amylase- Direct kit Bio systems. Lipase was assayed with colorimetric test, kinetic using lipase-F colorimetric (DGMR kit). The enzymes activities were assayed with spectrophotometer 4040 at 405 and 580 nm, respectively.

**Statistical Analysis:** One-way analysis of variance (ANOVA) was used to determine significant changes among groups.

**RESULTS**

**Biochemical and Elemental Analysis of Investigated Macrophytes**

**Biochemical Analysis:** The main nutritional components of the three selected macrophytes species were determined and the values were recorded in Table 1. The water contents of the studied plants was found to range from 91.05±0.01% of W.W. in E. crassipes leaves to 81.80±0.71% of W.W. in Echinochloa stagnina while, the highest moisture content was recorded for E. crassipes 10.44±0.23 % of DW and the lowest 8.81±0.27% of DW for P. tomentosum. The ash content of the studied plants fluctuated in a very wide range from 9.15±0.49% of DW in Ech. stagnina leaves to 20.60±0.57% of DW in P. tomentosum. Different values of water soluble and
Table 1: Values of some estimated biochemical components of the studied plants (Mean±SD)

<table>
<thead>
<tr>
<th>Plant components</th>
<th>Plants species</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%) of DW</td>
<td>E. crassipes</td>
</tr>
<tr>
<td>Water contents % of W. W.</td>
<td>91.0±0.01</td>
</tr>
<tr>
<td>Moisture</td>
<td>10.44±0.23</td>
</tr>
<tr>
<td>Ash</td>
<td>15.55±1.34</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>9.3±1.48</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>3.82±0.45</td>
</tr>
<tr>
<td>Organic matter</td>
<td>84.45±1.34</td>
</tr>
<tr>
<td>Total lipids</td>
<td>2.95±0.64</td>
</tr>
<tr>
<td>Crude protein nitrogen</td>
<td>14.25±0.35</td>
</tr>
<tr>
<td>Total available carbohydrates</td>
<td>3±0.28</td>
</tr>
<tr>
<td>Starch</td>
<td>2.73±0.25</td>
</tr>
<tr>
<td>Cellulose</td>
<td>28.8±0.92</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>17.9±0.47</td>
</tr>
</tbody>
</table>

Where: W. W. – Wet Weight. And DW – Dry Weight.

Table 2: Some calculated parameters for the three studied plants (Mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>E. crassipes</th>
<th>Ech. stagnina</th>
<th>P. tomentosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFE</td>
<td>38.86±0.59</td>
<td>45.65±0.46</td>
<td>32.5±0.62</td>
</tr>
<tr>
<td>P/L</td>
<td>4.93±0.94</td>
<td>3.22±0.34</td>
<td>5.41±0.58</td>
</tr>
<tr>
<td>P/S</td>
<td>5.3±0.68</td>
<td>3.00±0.16</td>
<td>2.58±0.09</td>
</tr>
<tr>
<td>C/L</td>
<td>1.05±0.32</td>
<td>1.19±0.06</td>
<td>2.33±0.17</td>
</tr>
<tr>
<td>P/E (mg P / kg ME)</td>
<td>12.76±0.18</td>
<td>9.39±0.27</td>
<td>12.88±0.11</td>
</tr>
<tr>
<td>GE (kJ/g of DW)</td>
<td>11.17±0.43</td>
<td>11.49±0.15</td>
<td>9.19±0.27</td>
</tr>
<tr>
<td>ME (kJ/g of DW)</td>
<td>8.26±0.28</td>
<td>9.65±0.2</td>
<td>7.17±0.25</td>
</tr>
</tbody>
</table>

Where, NFE = Nitrogen Free Extracts, P/L = Protein / Lipid, P/S = Protein/ Starch, C/L = Carbohydrates/ Lipids, GE = Growth Energy, ME = Metabolizable Energy and P/E = Protein to Energy Ratio.

Table 3: Values of macro(% of DW)- and micro(ppm)-elements of the studied plants (Mean±SD)

<table>
<thead>
<tr>
<th>Plants Parameters</th>
<th>E. crassipes</th>
<th>Ech. stagnina</th>
<th>P. tomentosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>2.25±0.01</td>
<td>1.66±0.03</td>
<td>1.87±0.04</td>
</tr>
<tr>
<td>P</td>
<td>0.28±0.01</td>
<td>0.29±0.0</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>K</td>
<td>3.48±0.03</td>
<td>2.18±0.03</td>
<td>2.2±0.01</td>
</tr>
<tr>
<td>Cu</td>
<td>44.4±0.14</td>
<td>34.05±0.07</td>
<td>40.25±0.35</td>
</tr>
<tr>
<td>Zn</td>
<td>56.15±0.21</td>
<td>37.05±0.07</td>
<td>43.5±0.42</td>
</tr>
<tr>
<td>Fe</td>
<td>1152.5±3.53</td>
<td>1171.5±2.12</td>
<td>1092.5±3.53</td>
</tr>
<tr>
<td>Mn</td>
<td>524±1.41</td>
<td>552.5±3.53</td>
<td>541±1.41</td>
</tr>
<tr>
<td>Pb</td>
<td>45.06±0.08</td>
<td>24.9±0.09</td>
<td>35.05±0.07</td>
</tr>
</tbody>
</table>

Table 4: A P. phytochemical screening of the studied plants extracts

<table>
<thead>
<tr>
<th>Tested substances</th>
<th>E. crassipes</th>
<th>Ech. stagnina</th>
<th>P. tomentosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosides</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenes</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Small amount ++ Moderate amount +++ High amount - Not detected

As shown in Table 2, the nitrogen free extracts (NFE), metabolizable energy (ME) and growth energy (GE) attended the highest values in Ech. stagnina compared with other studied plants while, the highest P/L (5.41±0.58) and C/L (2.33±0.17) ratio were recorded for P. tomentosum at the same time the highest P/S ratio was found for E. crassipes 2.58±0.09.

Elemental Analysis: The results in Table 3 show the variations in concentrations of all studied elements with plant species. The highest levels of N and K' were recorded for dry tissue leaves of the floating species E. Crassipes (2.25±0.01 and 3.48±0.03 % of DW, respectively) followed by the emergent species Polygonum tomentosum 1.87±0.064 and 2.24±0.01% of DW for the two elements, respectively, while the highest value of P was recorded for Echinocloa stagnina 0.29±0.0 % of DW. The results also showed the variations in micro-elements concentrations with the highest Cu”, Zn” and Pb” values in samples of E. Crassipes while, the highest values of Fe” and Mn” were recorded in samples of Echinocloa stagnina.

The Preliminary Phytochemical Screening: The results obtained in the present investigation (Table 4), of the
dried plants leaves extracts revealed the presence of some active substances (glycosides, tannins, flavonoids, sterols, terpenes and alkaloids) in all plants extracts, but with different concentrations depending on the plant species while, saponins was recorded only in very low concentration in extract of P. tomentosum.

Comparative Investigation on Freshwater Crayfish Fed on the Investigated Plants

Growth Performance: Table 5 shows that values of FW, AWG, ADG, SGR and K did not differ significantly (P> 0.5) among the fed crayfish with Eichhornia crassipes. While the results clearly showed that crayfish fed on Echinocloa stagnina and Polygonum tomentosum were significantly (P<0.05)increase in ADG and specific growth rate and condition factor. The result obtained showed that P. clarkii fed on Echinocloa stagnina and Polygonum tomentosum recorded the highest increase in FW since they recorded 15.73±3.95 and 18.56±4.47 g, after 6 week (Table 6), respectively. The average weight gain was 2.76±0.017 and 5.59±0.031 g, respectively. As for crayfish fed on Eichhornia crassipes, they came in the second order in increase of mean final body weight, since they reached 15.22±3.83 and this increase is not significant. Crayfish fed with Polygonum tomentosum exhibited the highest specific growth rate. SGR of P. clarkii fed on investigated plant was increased in the following order Polygonum tomentosum, Echinocloa stagnina and Eichhornia crassipes.

Feed Utilization: Expressed as feed conversion ratio (FCR) was given in Table 5. FCR showed significant decrease in crayfish fed on Polygonum tomentosum compared with the other two investigated food items.

Table 6: The effect of the food items on the total body weight and length of the crayfish Procambarus clarkii (Mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Eichhornia crassipes</th>
<th>Echinocloa stagnina</th>
<th>Polygonum tomentosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure period</td>
<td>Length(cm)</td>
<td>Weight (g)</td>
<td>Length(cm)</td>
</tr>
<tr>
<td>0</td>
<td>8.1±0.78</td>
<td>13.83±2.09</td>
<td>8.59±0.75</td>
</tr>
<tr>
<td>3 weeks</td>
<td>8.1±0.78</td>
<td>13.83±2.09</td>
<td>8.59±0.75</td>
</tr>
<tr>
<td>6 weeks</td>
<td>8.1±0.78</td>
<td>13.83±2.09</td>
<td>8.59±0.75</td>
</tr>
</tbody>
</table>

Chemical Composition: The estimated percentages of moisture in muscles of P. clarkii fed on Eichhornia crassipes, Echinocloa stagnina and Polygonum tomentosum for 3 weeks were 79.12, 80.19 and 83.13, respectively. The values of moisture (%) in muscles of crayfish fed on Eichhornia crassipes and Echinocloa stagnina at 6 weeks were slightly higher than the same specimens fed at 3 weeks. Changes in the macronutrients like proteins, lipids and carbohydrates in the abdominal muscles are seen in Figure 2, 3. In crayfish fed on three investigated plants, the estimated values of muscles proteins were 14.44±0.04, 14±0.36 and 14.22±0.017 after 3 weeks, respectively, while these were 19.13±0.03, 18.49±0.018 and 20.19±0.02, respectively in the crayfish muscles after feeding for 6 weeks. The rates of increase were 32.48, 32.07 and 41.98%, respectively.

It was obvious from Figure 2 that the estimated percentage of lipids in muscles of fed crayfish on Eichhornia crassipes, Echinocloa stagnina and Polygonum tomentosum for 6 weeks was higher than that muscle after 3 weeks with percentage changes 28.87, 47.16 and 85.08%, respectively.

The level of carbohydrates in muscles of fed crayfish on Echinocloa stagnina and Polygonum tomentosum was higher than that muscle fed P. clarkii on Eichhornia crassipes at 6 weeks (Fig. 3). After 3 weeks, the carbohydrate levels in abdominal muscles of crayfish fed on Echinocloa stagnina and Polygonum tomentosum were 0.05±0.02 and 0.04±0.02, which were increased to 0.05±0.003 and 0.06±0.027 at 6 weeks with percentage changes 18 and 50%.

Enzyme Activities: The effects of feeding with Eichhornia crassipes, Echinocloa stagnina and Polygonum tomentosum on amylase and lipase activities in haemolymph hepatopancreatic extracts of crayfish at 3 and 6 weeks are shown in Table 7.
Table 7: The effect of the food items on the digestive enzyme activities amylase and lipase of the crayfish Procambarus clarkii (Mean±SD)

<table>
<thead>
<tr>
<th>Parameters/Macrophytes</th>
<th>Echinochloa crusipes</th>
<th>Echinochloa stagnina</th>
<th>Polygonum tomentosum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amylase activity</strong></td>
<td>1000±1.27</td>
<td>858±0.63</td>
<td>1793±0.89</td>
</tr>
<tr>
<td><strong>Lipase activity</strong></td>
<td>18±0.84</td>
<td>18±0.18</td>
<td>17±0.45</td>
</tr>
<tr>
<td><strong>Hepatopancreas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amylase activity</strong></td>
<td>2590±1.78</td>
<td>1697±1.78</td>
<td>2748±2.68</td>
</tr>
<tr>
<td><strong>Lipase activity</strong></td>
<td>22±2.36</td>
<td>20±2.6</td>
<td>20±1.8</td>
</tr>
</tbody>
</table>

* = not significant  " < 0.05  "" < 0.01

Fig. 2: Values of biochemical analysis of Crayfish feed on different plants for three weeks (A) and six weeks (B)

Fig. 3: Values of carbohydrates contents in Crayfish muscles feeding on different plant items
The crayfish fed on *Eichhornia crassipes* and *Polygonum tomentosum* at 6 weeks had increased (P<0.01) in haemolymph amylase activities and highly significant decreased (P<0.01) in haepatopancreatic amylase activities compared with at 3 weeks. High amylase activity was demonstrated in the haemolymph of crayfish fed on *Eichhornia crassipes* and *Polygonum tomentosum* with maximal activity for this enzyme observed in the crayfish fed on *Polygonum tomentosum*. While, at 6 weeks, amylase activity levels highly significant decreased with different percentage reaching -49.4, -15.7 and -85% in the hepatopancreas of crayfish fed on *Eichhornia crassipes*, *Echinocloa stagnina* and *Polygonum tomentosum* after 6 weeks compared to 3 weeks.

The activity level of lipase was much lower than those observed for amylase. Relatively, low level of lipase activity was observed in hepatopancreas of crayfish fed on *Eichhornia crassipes* and haemolymph of crayfish fed on *Echinocloa stagnina* and *Polygonum tomentosum*. The lipase activity highly significant increased (P<0.01) in the hepatopancreas of fed crayfish on *Echinocloa stagnina*.

**DISCUSSION**

In general, crayfish are omnivorous and consume a variety of aquatic plants [2, 44]. Chambers *et al.* [45] suggested that the feeding preferences of crayfish, *Orconectes virilis* were not determined by plant chemistry, but are rather related to the ease of handling small and short, bottom-dwelling plants, such as *Chara* and *Lemna*, as compared with Large, erect rosette- or floating- leaved forms. During this investigation the feeding preferences of crayfish was being affected to some extent by the morphological feature of the macrophytes leaves where, *Polygonum tomentosum* with leaves like preceding but covered with short oppressed hairs and acute apex appeared to be the most preference compared with *E. crassipes* which have a thick, waxy leaves and *Echinocloa stagnina* the plant with flat leaves, 0.3-2cm wide and 5-50 cm long, ligules a fringe of hairs, only in upper leaves and sometimes absent.

Regarding the plant components six major components were considered when analyzing the selected plants as potential crayfish feed: energy, carbohydrates, crude protein, crude fat, macro-elements and micro-elements. Energy is essential for the maintenance of life processes including, cellular metabolism, growth, reproduction and physical activity. So, the ability of a food to supply energy is of a great importance in determining its nutritional value to animals. At the same time, excess dietary energy may result in high fat deposition in the animal, decreased feed intake and reduced weight gain [46]. These findings were coming in agreement with our results where feeding with *P. tomentosum* the plant with low energy values resulting in low lipids content in crayfish muscles compared with the two other plants at the same time the results of energy values are coming in agreement with those reported by Haroon [10] and Lopes *et al.* [47] for *E. crassipes*. Proteins are high molecular weight organic compounds essential to the structure and function of all living cells. The protein content in the plants under experiment ranged from 14.25±0.35 to 10.26±0.18 % of DW and these values were being enough to meet the dietary requirements of the animal as the changes in animal growth rate can be observed. However, it is clearly apparent during the present investigation that a noticeable increase in growth rate of the crayfish *P. clarkii* were significant in specimens fed on macrophyte plant *Polygonum tomentosum* and *Echinocloa stagnina*, while a non significant increase in growth rate was detected in those eating *Eichhornia crassipes* although the highest protein contents were recorded for *E. crassipes* and this can be explained by the presence of tannins at higher concentrations in this plant compared by the another two plants. In the plant cell, the tannins are located separately from the proteins and enzymes of the cytoplasm but when tissue is damaged, e.g. when animals feed, the tanning reaction may occur, making the protein less accessible to the digestive juices of the animal. [27, 48] resulting in decreasing its digest ability and absorbance by animal cell. Coming in agreement with our suggestion the finding of El-Serafy *et al.* [49] who recorded that the extraction of water hyacinth showed satisfactory results for both extracted protein and fibrinous residues these processes may eliminate some anti-nutrients, such as tannins, nitrates and oxalates. At the same time, *P. tomentosum* was characterized by its higher carbohydrates contents compared by the rest of plants and this also increase the absorbance of protein resulting in increasing the weight and length of animal. However, lipids represent a major nutrient source for crustaceans [50, 51] the highest value of lipids contents in the investigated plants is 3.2 ±0.28% of DW in *Echinocloa stagnina* which mean that, these plants are poor in their lipids contents and do not meet the dietary fat requirement of the animal. Changes in carbohydrate contents in muscle of fed crayfish on the *Polygonum tomentosum* investigated plants were
relatively limited and do not appear to represent significant energetic reserves.

The preliminary phytochemical screening of the three investigated plants extracted suggested the presence of some biologically active substances which have been recorded for their therapeutic effects. At the same time, Haroon [48] recorded the antimicrobial activity of E. crassipes and P. tomentosum besides the later was characterized by the highest flavonoid contents compared by other plants which were recorded as antioxidant substance.

Investigating the activity of digestive enzymes under different dietary treatments will be essential to provide information the nutritional requirements for crayfish and to determine which macrophyte components are most likely being assimilated. Evidence exists to show that growth of aquatic animals can be limited by the capacity of their digestive system to break down and assimilate specific nutrients [12, 52]. Typically, a strong correlation was observed between the activity levels of specific digestive enzymes and animals’ dietary preference [53]. There was also evidence to suggest that digestive enzymes may set physiological limits on growth rates in fish [54]. The current study examined the activity of crayfish digestive enzyme amylase and lipase. The sharp increase in amylase specific activity noticed in crayfish with a large increase in size. Amylase activity in hepatopancreas was higher in crayfish fed on Polygonum tomentosum after 3 weeks than the specimen fed on Eichhornia crassipes and Echinochloa stagnina, reflecting the highest starch and cellulose content of Polygonum tomentosum compared by the two other species. Similar finding were given by Figueiredo et al. [55] and Johnston [56].

After 6 weeks, amylase activity in hepatopancreas was lower in crayfish fed on Eichhornia crassipes and Polygonum tomentosum in contrast, it was higher in the haemolymph. An interpretation of this finding is that the ability of crayfish to alter amylase activity in the hepatopancreas is not substrate specific, but instead represents a more general adaptive response to changes in the level of dietary carbohydrate. Otherwise, lipase activity was lower than amylase activity in the hepatopancreas and haemolymph of crayfish fed on the investigated plants. It was clear from the present result that, crayfish retain a suite of digestive enzymes (including lipase and amylase) that enable them to utilize many different dietary components as a source of energy and materials for growth. They also appear to be capable of adjusting their digestive enzymes to suit the available herbivorous diet.

The finding of this study suggested that, the highest FW, AWG, SGR and K were achieved by feeding with fresh leaves of P. tomentosum with low (energy value, P/S) ratio, high (P/E, P/L and C/I) and the suitable morphological features was the best feeding item for crayfish.

REFERENCES


54. Lemieux, H., P. Blier and J.D. Dutil, 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (Gadus morhua); Fish Physiology and Biochemistry, 20: 293-303.
