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Seasonal Variation of Vitamin and Sterol Content of Chironomidae Larvae

Tuba Kara
Department of Biology, Faculty of Science, Firat University, Elazig, Turkey

Abstract: In the present study, seasonal variation of vitamin and sterol content of Chironomidae larvae were determined by using HPLC. As the result of vitamin analysis, we found α-tocopherol, retinol, K_1, K_2, D_2 and D_3. When the seasonal variation of vitamin groups were compared, a significant increase was observed in vitamin K_1, K_2, D_2 and α-tocopherol in all seasons. A significant increase was observed in vitamin D_3 in spring. And also vitamin A level high in autumn and winter. α-tocopherol level was significantly high among vitamins. When vitamin groups were compared statistically, differences were detected between seasons ($p<0.001$). Analyzing the content of sterol, we found ergosterol, cholesterol, stigmasterol and β-sitosterol in all seasons. Cholesterol level was found to be significantly high in sterols. When sterol contents were compared statistically, differences were detected between seasons ($p<0.001$). In conclusion, the reasons for these differences are larval development feature and the variety of food in different seasons.

Key words: Chironomidae, seasonal variation, sterol, vitamin, diptera

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*., 1997; Yusoff *et al*., 1996; Fernando, 1994; Tidwell *et al*., 1997; Wolfram-Wais *et al*., 1999). Chironomid larve are excellent source of lipid, vitamins and minerals (McLarney *et al*., 1974).

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). Benthic communities of the profundal regions of freshwater ecosystems are dependent on sedimentation processes for their food supply (Brinkhurst, 1974). Food availability and feeding biology are two important factors determining seasonal changes in food ingestion, growth and reproduction of Chironomid larvae (Jonasson, 1972). Several studies have shown that Chironomus growth is closely correlated with the availability of algae or detritus of algal origin in their food (Kajak and Warda, 1968; Jonasson, 1972; Lindegaard and Jonasson, 1979; Johnson and Pejler, 1987).

Insects, protein, mineral, vitamin and carbohydrates need (Hagen *et al*., 1974). Sterols are required for cell membrane and are produced by plants and animals. B-sitosterol, stigmasterol and ergosterol are abundant in plants. Cholesterol, kaprosterol and allosterol are sterols in the oils of animal origin (Liu, 2003). ADEK vitamins are fat-soluble vitamins. Vitamin E functions as an antioxidant in cell membrane and lipoproteins (Keskin, 1987; Belitz *et al*., 2005).

The aim of this study is to analyze of vitamin and sterol content of Chironomidae larvae and then statistically compare the variation between seasons.

MATERIALS AND METHODS

Chironomid larvae were collected on spring, summer, autumn and winter 2010 in Büyük stream (Pelte/Elazig). Then samples were stored at deep-freeze until analysis. The weights of samples were weighed and then were put into tubes. Vitamins and sterols were extracted from lipid extract by the method of Sanchez-Machado *et al*. (2004) and Lopez-Cervantes *et al*. (2006) with minor modifications. Five mL n-hexane/isopropyl alcohol mixture was treated 5 mL KOH solution (0.5 M in methanol) were added and immediately vortexed for 20 sec. The tubes were placed in a water bath at 80°C for 15 min. Then after cooling in iced water, 1 mL of distilled water and 5 mL of hexane was added and the mixture was rapidly vortexed for 1 min, then centrifuged for 5 min at 5000 rpm. The supernatant phase were transferred to another test tube and dried under nitrogen. The residue was redissolved in 1 mL of the HPLC mobile phase (68:24:4 (v/v/v) methanol: acetonitrile: water). Finally, an aliquot of 20 μL was injected into the HPLV column. Before injection, the extracts were maintained at -20°C away from light.

Chromatographic analysis was performed using an analytical scale (15×0.45 cm I.D) Supelco LC 18™ column with a particle size 5 μm (sigma, USA). HPLC conditions were as follows: mobile phase 60:38:2 (v/v/v): acetonitrile/methanol/water; a flow rate of 1 mL min^{-1}; column temperature 30°C. The detection was operated using two channels of a diode-array spectrophotometer, 326 nm for retinol, 265 nm for vitamin D and vitamin K, 202 nm for alpha-tocopherol and phytosterols (Lopez-Cervantes *et al*., 2006).

Statistical analysis was performed using SPSS software (ver. 10.0). The experimental results were reported as Mean±SEM (standard error of means). Analysis of variance (ANOVA) and an LSD (least significant difference) test were used to compare the experimental groups.

RESULTS

When the vitamin groups were compared, α-tocopherol level was found to be significantly high among vitamins. When α-tocopherol was compared between seasons, a significant increase was observed on summer ($p<0.001$) (Table 1).

Partial increases were observed in vitamin K_1 and vitamin D_3 in spring ($p<0.001$). And also partial increases was observed in K_2 in summer ($p<0.05$). When the seasonal variation of vitamin content were compared, a significant increase were observed in vitamin retinol and vitamin D_2 in summer ($p<0.01$, $p<0.05$). On the other hand, vitamin content was decreased in autumn when vitamin groups compared statistically between seasons ($p<0.001$, $p<0.01$, $p<0.05$) (Fig. 1).

When compared of sterol contents of Chironomidae larvae, were found a significant increase in cholesterol and stigmasterol level ($p<0.001$). Cholesterol, stigmasterol and β-sitosterol increased significantly in summer according to other seasons. Cholesterol level was found to be significantly high among sterols. Unlike partially increase was observed in ergosterol in winter ($p<0.05$).

DISCUSSION

When the vitamin groups were compared, α-tocopherol level was found to be significantly high among vitamins and in summer ($p<0.001$). And when sterol groups were compared statistically, cholesterol level was found to be significantly high in all seasons and especially in summer ($p<0.001$) (Fig. 2). We think feeding activity and development properties of Chironomidae larvae as the cause of this situation. Especially during summer months, larvae feed on plant origin food.

Table 1: Seasonal variations of vitamin and sterol content of chironomidae larvae (mg g^{-1})

Lipophilic vitamins and sterols	Spring	Summer	Autumn	Winter
α-tocopherol	28.40±1.100	109.70±2.910^{d}	16.00±0.760	33.50±0.760
Retinol	0.10±0.007	0.20±0.007	0.34±0.008	0.58±0.008^{c}
Vitamin K_1	31.17±0.730^{c}	0.84±0.340	0.40±0.007	0.75±0.140
Vitamin K_2	1.25±0.380	2.17±0.440^{b}	1.75±0.380	1.58±0.300
Vitamin D_2	19.34±5.100	14.50±1.150	10.84±0.850	21.00±0.860^{b}
Vitamin D_3	0.50±0.140^{c}	0.20±0.050	0.10±0.007	0.10±0.008
Ergosterol	3.34±0.440	2.67±0.670	3.50±0.570	6.42±0.870^{b}
Kolesterol	228.75±2.160	538.00±19.43^{d}	420.42±2.920	313.67±2.030
Stigmasterol	100.50±2.140	125.607±2.34^{d}	75.67±1.100	35.00±3.820
Betasitosterol	0.50±0.140	6.67±0.730^{d}	0.92±0.008	0.34±0.008

^{a}: $p>0.05$, ^{b}: $p<0.05$, ^{c}: $p<0.01$, ^{d}: $p<0.001$

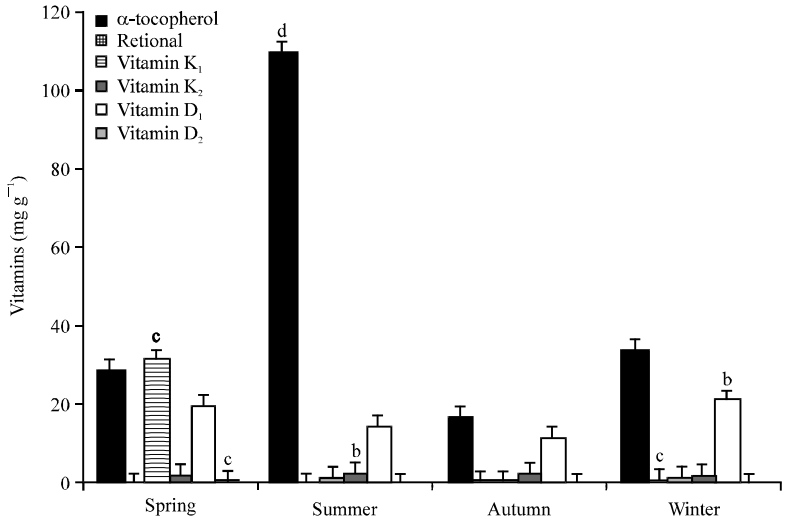

Fig. 1: Levels of vitamins in chironomidae larvae (mg g^{-1}), a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

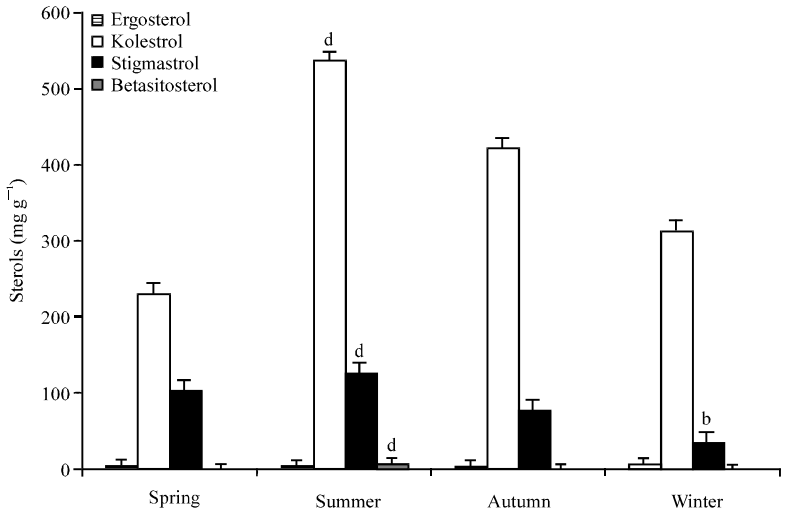

Fig. 2: Levels of sterols in chironomidae larvae (mg g^{-1}), a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001

It is known that insects contain almost entirely cholesterol, even if their food contains significant amounts of phytosterols. This is due to dealkylation of dietary phytosterols to cholesterol, a process, characteristic for Arthropoda (Behmer and Elias, 2000). Stefanov *et al*. (2002) analyzed lipid and sterols of *Musca domestica* L. larvae. They obtained the opposite results cholesterol was comparatively low for insects and phytosterols appeared in significant concentrations, suggesting that phytosterols are dealkylated slowly in *Musca domestica* larvae. In contrast to we determined cholesterol level was high Chironomidae larvae (Table 1).

Also Stefanov *et al*. (2002) checked the possibility that phytosterols are derived from the diet, so they investigated the sterol composition of dry milk dissolved in water which is the food of larvae. They found very low sterol concentrations, with cholesterol accounting for more than 90% of the total sterol mixture. Less than 3-4 % sitosterol and campesterol were present. Under these conditions the only alternative source for phytosterols is the wheat bran. Observations of the midgut contents showed the presence of chewings, originating from the wheat bran. It is possible that the larvae are able to scrape the substrate with their mouthparts (Sareen *et al*., 1990). These findings, difference feeding Chironomidae larvae, is evidence that showing how it affect of vitamin and sterol content of larvae.

Vitamin E was included in the diet of some insects for its antioxidant activities to protect the integrity of fatty acids and perhaps of other substances (Fraenkel and Blewett, 1946; Beck *et al*., 1949; Vanderzant, 1957). Fraenkel and Blewett (1946) found that vitamin E improved the growth of *Anagasta* (*Ephestia*) *kühniella* (Zeller), but they reasonably favoured the supposition that its role was principally as a protective antioxidant on unsaturated fatty acids rather than as a nutritional requirement of the insect.

Both vitamin A and vitamin E increased the rate of larval growth and improved the rate of development of pupae so as to increase the number of adult emergent (House, 1951; Coppel *et al*., 1959). House (1966) studied effects of vitamin E and vitamin A on growth and development *Agria affinis* larvae (Diptera) and determined vitamin E is requirement for reproduction and vitamin A increased growth. Vitamin E (α-tocopherol) level was found significantly high in our study (Fig. 1). As the cause of this situation, is shown Chironomidae larvae stored vitamin E for later stages of development.

Several studies have demonstrated the importance of several pulses of pelagic algal detritus to Chironomus growth (Hilsenhoff, 1966; Jonasson and Kristiansen, 1967; Jonasson, 1972; Kajak, 1977; Lindegaard and Jonasson, 1979; Johannsson, 1980; Johnson and Pejler, 1987). Furthermore, invertebrate growth has been shown to be slower when detritus is the dominating dietary constituent, whereas the ingestion of algae has been positively correlated with increased growth (Cummins, 1973). One study was showed that blue-green algae are a major portion (68% of gut contents) of the diet of filter feeding *Chironomus crassicaudatus* (Ali, 1990). Benthic green algae have been reported to be a major food source as stated by Brook (1954) who observed that Chironomidae larvae on a sand-filter bed ingested only filamentous blue green algae and filamentous diatoms.

In conclusion, we determined seasonal variation of vitamin and sterol content of Chironomidae larvae. We have detected difference in vitamin and sterol content between seasons. As the cause of this situation, we think the reasons for these differences are larval development feature and the variety of food in different seasons. Our findings and our research is new for our country. Therefore, these findings are important for biochemical analysis of future.

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