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Estimate The Amount of Amino and Fatty Acids in The Liver Tissue of Uromastyx Aegyptius Microlepis During the Periods of Hibernation (December) And Activity (May)

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Abstract
Amino and fatty acids in the liver tissue of Uromastyx aegyptius microlepis during the periods of hibernation (December) and activity (May) were estimate by a high performance liquid chromatography, liver of a lizard during the activity and hibernation seasons, contained 18 amino acids, which include, 10 essential amino acids and 8 non-essential amino acids, and the liver in the male lizard contained five fatty acids during each season, the concentration rates of all the amino acids during the activity season were higher than their counterparts during the hibernation season, the total concentrations of essential and non-essential amino acids during the activity season were 19434.8 µg/ml, which was greater than the total concentrations levels during the hibernation season (7941.5) µg/ml, the total concentrations of saturated fatty acids (SFA) (70.5) µg/ml and a percentage of (75.9%) were higher than the total levels of concentrations of unsaturated fatty acids MUFA and PUFA, which were (22.3) µg/ml and a percentage of (24%) during hibernation, while during the activity season, the total concentrations of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) amounted to (125.6) µg/ml and a percentage (59.1%) is higher than the total average concentrations of saturated fatty acids SFA (86.8) µg/ml and in percentage (40.7%).

Keywords: Amino acids, Fatty acids, Liver tissue, Hibernation, Activity.

Introduction

Uromastyx aegyptius microlepis belongs to Class: Reptile, Order: Squamata, Family: Agamidae, Genus: Uromastyx (Afrasiab *et al*., 2018). Herdt and Sayegh (2013) and Aspinall *et al*. (2020) Noting that the liver in different animals, including reptiles, has several important roles in protein metabolism, including the regulation of amino acids: there are 20 amino acids necessary for the formation of body protein and liver cells use these amino acids to make new proteins, and they are the building blocks of other organic compounds and can be converted Non-essential amino acids are converted to more useful acids through a process known as transamination. Amino acids in excess of the body's need cannot be stored, but are converted by the liver into ammonia and then urea. This process is known as deamination and urea is excreted with urine. As well as fat metabolism: fat is a source of energy for the body, where the liver converts fatty acids and glycerol into phospholipids to form cell membranes and converts cholesterol to bile salts, and excess fat is stored in the form of deposits in certain places of the body. An adaptive change in the concentration of amino acids in response to a decrease in temperature is one of the essential features of thermophilic animals that live in regions with large temperature differences (Karanova, 2013). linoleic acid (C18:2) and linolenic acid (C18:3) are fatty acids that are required as precursors for the production of most long-chain polyunsaturated fatty acids, which are of particular interest in animals with an external heat source (Hazel, 1988). Also, several studies have shown that both oleic acid and arachidonic acid biosynthesized from linoleic acid play an important role in thermoregulation (Larsson *et al*. 2004 and Ben-Hamo *et al*. 2011).

Materials and Methods

Ten adult male animals of *U. aegyptius microlepis* were collected to hibernation season (December 2019), and ten animals to activity season (May 2020), and the animals were placed in an animal cage during hibernation and activity seasons. These animals were collected from Al-Najaf province and were classified by the Natural History Museum. Organ have been identified as described Kotpal (2010), the liver was extracted, and a piece of the peripheral part of the left lobe of the liver was taken from each animal and frozen at a temperature of -18 C° inside plastic bottles shown on it the number and date of each animal until the use of High Performance Liquid Chromatography (HPLC) to analyze the amino and fatty acids. Amino acids found in the liver are diagnosed according to the general method of derivation (Fierabracci *et al*., 1991; Fürst *et al*., 1990). Depending on the concentration of amino acids using the following equation:

Concentration of amino acid (mM/L)= $\frac{Beam\ area\ of\ acid\ in\ the\ sample}{Beam\ area\ of\ satandard\ acid}$ × Standard concentration of acid × Number of times dilution

The analysis of fatty acids was carried out according to Cortinas *et al*. (2004) and Chouinard *et al*. (1999). The fat separation process was completed using a rapid method proposed by Feng *et al.* (2004). Fatty acids were measured directly on the medium-inverse liquid chromatographic device and it was used the following equation to calculate the concentration of fatty acids:

Concentration of fatty acid (mM/L)= $\frac{Beam\ area\ in\ the\ sample}{Satandard\ beam\ area}$ × Standard concentration of acid × Number of times dilution

Results

Amino acids concentrations during active and hibernation seasons

The result of Table (1) and Figure (1) showed that the liver of a lizard, during the activity season, contained 18 amino acids, which include, 10 essential amino acids (EAA) and 8 non-essential amino acids (NEAA) which are the same as those found in the winter hibernation season, but their concentration rates It was different from what it is in the hibernation season, the concentration rates of all the essential amino acids EAA during the activity season were higher than their counterparts during the hibernation season, and it was noted that there were high concentrations of the following acids: leucine (Leu), lysine (Lys), phenylalanine (Phe). The essential amino acids are listed in decreasing rates of concentration, which are threonine (Thr), tryptophan (Try), arginine (Arg), and methionine (Met), while the lowest concentrations of the following essential amino acids are isoleucine (Ile) and histidine (His), and valine (Val). While the concentrations of the non-essential amino acids NEAA were also higher during the activity season than their counterparts during the hibernation season, and the acids had the highest concentrations as follows: Proline acid (Pro), glycine (Gly), and glutamic acid (Glu), and tyrosine acid (Tyr) and aspartic acid (Asp), while the nonessential amino acids with the lowest concentration rate included the following: alanine acid (Ala), glutamine acid (Gln), and cysteine acid (Cys). High significant differences appeared at the probability level $P < 0.001$ between the activity (May) and hibernation (December) seasons in the average concentrations of the following amino acids: glutamic acid (Glu), glycine (Gly), threonine (Thr), and alanine (Ala), and proline (Pro), tyrosine (Tyr), tryptophan (Try), and isoleucine (Ile), as well as significant differences at the probability level $P < 0.05$ in the average concentrations of the following amino acids: aspartic (Asp), histidine (His) and leucine (Leu) And phenylalanine (Phe) and lysine (Lys), while there was no significant difference in the results of the test-T test at the level of probability $P < 0.05$ between glutamine (Gln), arginine (Arg), valine (Val), methionine (Met) and cysteine (Cys), the total concentrations of essential and non-essential amino acids during the activity season were 19434.8 µg/ml, which was greater than the total concentrations levels during the hibernation season (7941.5) µg/ml.

Table 1. Changes in the levels of average concentrations (µg/ml) for each amino acid in the liver of the male *U. aegyptius microlepis* lizard during the active season and the hibernation season.

Amino acids (µg/ml)	Active season (n=4) Average ± SD	Hibernation season (n=4) Average ± SD	Difference of means	*P*-value
Asp	821.5 ± 195.9	441.6 ± 154.8	379.9	0.023
Glu	1323.5 ± 192.5	400.6 ± 99.6	922.9	< 0.001
Gln	747.1 ± 249.7	740.3 ± 148.1	6.8	0.967
Gly	1461.6 ± 228.0	373.9 ± 129.2	1087.7	< 0.001
Ala	790.9 ± 74.7	373.6 ± 78.0	417.3	< 0.001
Pro	1648.0 ± 160.4	530.0 ± 474.5	1118.2	< 0.001
Tyr	1140.2 ± 114.7	356.3 ± 112.8	783.9	< 0.001
Cys	731.8 ± 176.3	617.8 ± 59.3	113.9	0.266
His	570.7 ± 97.34	437.9 ± 41.1	132.8	0.046
Arg	944.9 ± 478.6	477.7 ± 54.0	467.2	0.145
Thr	1161.1 ± 135.3	233.1 ± 43.6	927.9	< 0.001
Val	340.9 ± 225.7	247.4 ± 26.4	93.6	0.469
Met	921.7 ± 427.1	659.7 ± 61.3	262.0	0.308
Try	987.6 ± 110.0	461.2 ± 133.3	526.5	< 0.001
Ile	613.4 ± 662.2	287.4 ± 96.4	325.9	< 0.001
Leu	2181.0 ± 507.6	258.0 ± 31.6	1923.1	0.005
Phe	1441.9 ± 367.5	472.0 ± 102.6	969.8	0.002
Lys	1607.0 ± 488.3	573.0 ± 145.6	1033.6	0.007

Significant differences are high at the probability level P<0.001 and significant differences at the probability level P<0.05.

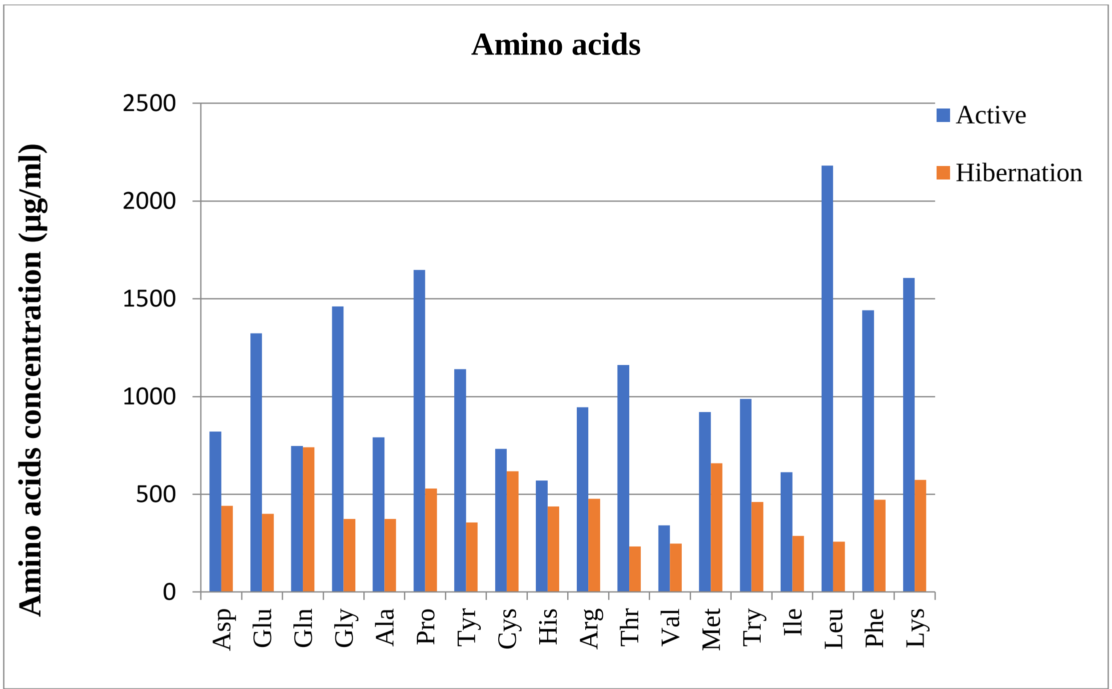

Figure 1. Changes in the levels of average concentrations (µg/ml) for each amino acid in the liver of the male U. aegyptius microlepis lizard during the active season and the hibernation season.

Fatty acids concentrations during active and hibernation seasons

The result of Table (2) and Figure (2) showed that the liver in the male lizard contained five fatty acids during the activity season (May). It included three types of saturated fatty acids (SFA), which are from the highest concentration rate to the lowest concentration rate, including: Stearic acid (C18:0), which has the highest concentration rate among the saturated fatty acids in this season, arachidic acid (C20:0), and palmitic acid (C16:0). There are two types of unsaturated fatty acids, including a type of monounsaturated fatty acids (MUFA) that appeared during the activity and hibernation seasons. It is oleic acid (C18:1) or the so-called omega-9, which is the highest concentration rate of the rest of the saturated and unsaturated fatty acids the other type is polyunsaturated fatty acids (PUFA), which is α-linolenic acid (C18:3) or the so-called omega-3, compared to the hibernation season in which this acid was absent, and there is no presence of linoleic acid C18:2) or the so-called omega-6, which is one of the types of polyunsaturated fatty acids (PUFA) in the activity season. It is clear from the result of table (2) that the total concentrations of saturated fatty acids (SFA) (70.5) µg/ml and a percentage of (75.9%) were higher than the total levels of concentrations of unsaturated fatty acids MUFA and PUFA, which were (22.3) µg/ml and a percentage of percentage (24%) during hibernation, while during the activity season, the total concentrations of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) amounted to (125.6 µg/ml) and a percentage (59.1%) is higher than the total average concentrations of saturated fatty acids SFA (86.8) µg/ml and in percentage (40.7%), also there are highly significant differences at the probability level $P < 0.001$ between the activity and hibernation seasons in the levels of fatty acids concentrations, namely oleic and alpha-linolenic acid, and there is a significant difference at the probability level $P < 0.05$ in the average concentration of linoleic acid between the two seasons, but the results of the T-test did not show any significant differences at the probability level $P < 0.05$ in the mean concentrations of palmitic, stearic and arachidic acid.

Table 2. Changes in the levels of average concentrations (µg/ml) of each fatty acid in the liver of a male lizard *U. aegyptius microlepis* during the active season and the hibernation season.

Fatty acids g/ml)µ)	**Active season (n=4) Average ± SD**	**Hibernation season (n=4) Average ± SD**	**Difference of means**	***P*-value**
Palmitic acid C16:0	26.1 ± 8.7	22.4 ± 2.6	3.7	0.444
Stearic acid C18:0	31.5 ± 11.0	15.1 ± 1.3	16.3	0.058
Arachidic acid C20:0	29.2 ± 4.7	33.0 ± 2.7	-3.76	0.214
Oleic acid C18:1	79.6 ± 8.3	11.5 ± 2.1	68.1	< 0.001
Linoleic acid C18:2	0.0 ± 0.0	10.8 ± 4.3	-10.86	0.002
α-Linolenic acid C18:3	46.0 ± 3.0	0.0 ± 0.0	45.98	< 0.001

Significant differences are high at the probability level P<0.001 and significant differences at the probability level P<0.05.

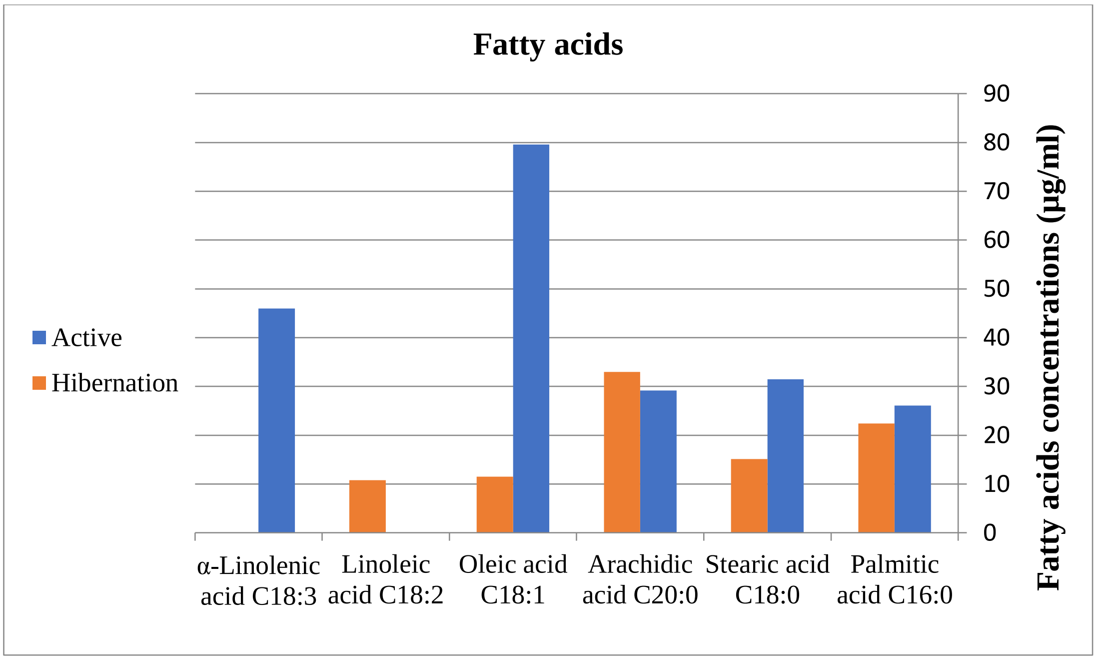

Figure 2. Changes in the level of concentrations (µg/ml) of each fatty acid in the liver of a male lizard *U. aegyptius microlepis* during the active season and the hibernation season.

Discussion

The lizard *U. aegyptius microlepis* feeds mainly on herbs and fresh leaves of vegetables, and the animal enters winter hibernation, which is usually from the beginning of December until the end of March, when it stops feeding and depends on the food stored inside its body, and when the spring season begins, the season of activity and reproduction Begins with feeding and partner search (Kotpal, 2010), Depending on the dietary requirements for nitrogen balance or growth, amino acids are traditionally classified as nutritionally essential EAA (indispensable) or NEAA non-essential (dispensable) for humans and animals (Wu, 2009), The current study includes that the liver contains 18 amino acids, 10 EAA and 8 NEAA during the activity and hibernation seasons. It was noted that there were large concentrations of the following acids: Leu, Lys, and Phe, while lower concentrations appeared for the following acids: Ile, His, and Val, which had the lowest concentration among them. While an increase in the concentration of Arg was found in the cerebral cortex, blood serum and liver during the hibernation season in the desert lizard *Varanus griseus* by Abdel-Raheem and Mosallamy (1979), and in the study of Abu-Tarboush *et al*. (1996a) on the nutritional quality and protein properties of Egyptian lizard meat *Uromastys aegyptius*, some essential amino acids (Phe, Tyr, Thr, His, Try) were lower while Ile was higher in lizard meat protein. As for the average concentrations of NEAA, which was found in this study to be large during the active season compared to the hibernation season, it includes the acids with the highest concentration, which are Pro, Gly, and Glu, and the least concentrated acids are Ala, Gln, and Cys. This result is consistent with the study of El-Deib (1990), which included measuring the concentration of amino acids in the brain, liver and kidneys in a type of snake, which is *Naja haje*, and the study of Abdel-Raheem and Mosallamy (1979) found that during the hibernation period of the desert lizard *Varanus griseus* increased In the concentration of Asp in the cerebral cortex, blood serum and liver compared to the activity period, as for Glu and Gln, its concentration was greater in the liver tissue during the activity.

The increase in the rate of concentration of essential and non-essential amino acids in the current study during the activity season compared to the hibernation season may be due to the fact that amino acids play a key role in the adaptation of animals (temperature change) to the winter season at temperatures close to zero. In the essential and non-essential amino acids during the activity season to increase the activity of the liver in metabolism in order to maintain the nutritional balance of the body, and unlike glucose, which inhibits the secretion of glucagon and stimulates the secretion of insulin, amino acids stimulate the release of both hormones, but their stimulating effect on the secretion of glucagon is More effective at lower glucose concentrations (Islam, 2014). It was found in the current study of the male lizard that the liver contained five fatty acids during the activity season (May) as well as during the hibernation season (December), while Mohamed (2013a) found twenty-three fatty acids in the body fat and three Ten fatty acids in liver oil in two types of lizards are *U. dispar* and *U. ocellat*, and the total concentrations of monounsaturated fatty acids, both MUFA and PUFA, in the liver of male lizards in the current study were about (125.6) µg/ml with a percentage (59.1%), which is greater than the total concentrations of saturated fatty acids SFA, which reached (86.8) µg/ml with a percentage of (40.7%) in the activity season, while in the hibernation season, the total concentrations of saturated fatty acids SFA (70.5) µg/ml at a percentage (75.9%), which is greater than the total concentrations of unsaturated fatty acids with its two types of monounsaturated fatty acids MUFA and multiple PUFA (22.3) µg/ml as a percentage (24%), while Mohamed (2013a) study found that the total SFA was (49.8) mg/g and in percentage (49.6%) in body fat and its total was about (24.9) mg /g and in percentage (24.5%) in liver oil, while the total concentrations of USFA of both types, MUFA and PUFA in body fat (50.5) mg/g at percentage (50.4%), and in liver oil (76.7) mg/g by percentage (75.5%), while in the study of Abu-Tarboush et al. (1996b) the percentage of SFA within a ranged between (33% - 37.7%) and the range of USFA ranged between (62.3-67%) in the meat of *Uromastyx* lizards, while it was found in the study of Mohamed and Al-Sabahi (2013) that the percentage of SFA in the oil of liver of freshwater fish ranged between (57.5% - 65.5%) and the percentage of USFA ranged between (34.5% - 42.5%) in those fish, and in the result of the current study three types of SFA were found, which are from the largest to the lowest concentration and dominance that include stearic acid And arachidic acid and palmitic acid in the activity season, while in the dormant season, Arachidic acid was dominant, which increased by a difference of (3.76) from its previous concentration in the activity season, while it was found in Mohamed (2013a) study that palmitic acid was the most abundant in both body fat and liver oil in lizards are *U. dispar* and *U. ocellat*, while found in the study of

Abu-Tarboush et al. (1996b) Dominance of palmitic and stearic acid in meat of *Uromastyx* lizards. In the study of Mohamed and Al-Sabahi (2013) and Mohamed (2013b), palmitic acid was the most abundant in liver oil, muscle and adipose tissue in freshwater fish *Labeo niloticus* and *Clarias lazera*. The current study showed that the only type of MUFA, which is oleic acid, had a greater concentration than the rest of the saturated and unsaturated fatty acids during the activity season, but in the hibernation season it decreased by a large difference (-68.1) than its concentration in the activity season. While it was found in the study of Mohamed (2013a) that the types of MUFA that were more abundant in body fat and liver oil were Oleic, Palmitoleic and Ecosenoic, while in the study of Abu-Tarboush et al. (1996b) showed the presence of a high percentage of oleic acid in meat of *Uromastyx* lizards, but found a predominance of oleic and palmitoleic fatty acids in muscle tissue, and a predominance of elaidic acid was found in fatty tissues and a predominance of oleic acid in liver oil of freshwater fish by Mohamed and Al-Sabahi (2013) and Mohamed (2013b), as observed in the study McCue (2008) fasting changes in the formation of fatty acids within body fat in six species of reptiles subjected to semi-lethal fasting periods lasting (0, 56, 112, and 168) days, and all of these species showed An overall decrease in their relative content of SFA, while the relative levels of MUFA in snakes were not affected in general during fasting, but they decreased by more than (17%) in lizards and the relative levels of USFA increased during starvation in all species, the relative levels of USFA arachidonic acid were less than (1%) of fatty acids in snakes at all times, but increased from (0.7%) to (3.5%) in hungry lizards, This study also showed the presence of one type of PUFA, α-linolenic acid and the absence of linoleic acid in the activity season. Also, one type of PUFA was found in the hibernation season, which is linoleic acid, while there was no α-linolenic, and Mohamed (2013a) study showed the presence of two types of PUFA, namely eicosatrienoic acid and linolenic acid, in body fat, but in liver oil, it was observed that two types of acids linolenic acid and linoleic acid were dominant in two types of lizards which is the lizard *U. dispar* and *U. ocellat*, also recorded in the study of Abu-Tarboush et al. (1996b) Predominance of linolenic acid in meat of *Uromastyx* lizards, and it was also observed in Mohamed and Al-Sabahi (2013) study on liver oil in freshwater fish that two types of PUFA were dominant: linoleic acid in *Polypterus senegalus* and docosahexaenoic acid in *Clarias larias* and *Lates niloticus* and this study suggested that the diversity in the composition and quantity of fatty acids present in the two studied species may be due to differences in species, diet, spawning cycle, season and environment. Smaller reptiles metabolize faster than larger reptiles (Espinoza and Tracy, 1997) and maintaining cell membrane fluidity in lizards may be related to a change in membrane lipids as an adaptive strategy when ambient temperature changes (Huey and Stevenson, 1979; Bennett, 1980; Hertz et al. 1993).

Conclusions

The general importance of this study was the detection of amino and fatty acids in the male *U. aegyptius microlepis*, that the liver has a characteristic chemical structure where it contains a lot of good food sources and how can that organism organize its biological activities during the active and hibernation seasons.

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