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SEASONAL VARIATION ON BIOCHEMICAL AND HORMONAL PROFILE OF UROMASTYX FAT OIL USING GC-MS TECHNIQUE

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ABSTRACT

Uromastyx is genus of agamid lizard and known as spiny tailed lizard. By nature, Uromastyx are herbivorous but in younger age love to eat insects and small animals. Uromastyx borrows in desert, rocky and hilly areas. Size of Uromastyx is up to 91cm in adults and 10cm in neonates. Oil of Uromastyx is used for treatment of different disease. Aim of current study was to investigate fatty acid composition and seasonal variations in biochemical and hormonal profile. In two different seasons five samples of Uromastyx were collected and then fat extraction process done in laboratory, from fats of Uromastyx pure fats oil was extracted with Folch method by using chloroform and methanol in 2/1 ratio. GC-MS technique was used for analysis. From five samples, twenty-three fatty acids were identified and percentage of saturated fatty acids was 47.8% while unsaturated fatty acids were 30.4%. Benzenedicarboxlic, an aromatic fatty acid was present in all five samples of two seasons. Metabolites of vitamin B-12 were also identified in it. Hormonal studies of all three parameters luteinizing hormone, follicle stimulating hormone and cortisol were same except testosterone which was high in samples of December month. Study of fatty acids and hormonal profile of different seasons conclude that saturated fatty acids were higher as compared to unsaturated fatty acids. Sample of month December

values of saturated fatty acids were lower as compared to October and similarly testosterone level were higher in December with all other parameters remained same.

Keywords: Gas chromatography Mass spectrometry (GC-MS), Uromastyx, Vitamin B-12, Luteinizing Hormone (LH)

INTRODUCTION

Uromastyx is Asian and African genus of agamid lizard, collectively they are known as spiny tailed lizards. Uromastyx, mastigures or dab lizards belong to the kingdom Animalia and class Reptilian. Uromastyx are primarily herbivorous while they eat small animal and insects mostly the young lizards. Beingterrestrial species inhibiting unproductive habitat and relatively harsh as well [1]. The population of Uromastyx may be found exaggerated over the different areas where it inhabits itself according to the suitable environment. Whereas, its population may be found affected in some of the areas where it does not inhabit due to the suitable environment. Areas occupying the larger densities of the discussed species include spiny and rocky areas, deserts, and they also inhabit in the areas with some rocky surfaces as well. Their population is scarce in the areas where the desert area is open and sandy [2]. They are found to occur near or under the rocky surfaces where they dig for themselves and the discussed areas are mostly beneath the cliffs or bushes where they can hide themselves from the predators and the areas made easier for their prey to be captured in such areas. Whereas, they may live over the wider ranges of such rocky lands with greater variations in the external environment. Environment may range from -7° to 60°C. Uromastyx is coped up to maintain its body temperature due to its cold bloodiness. Optimum temperature that is required by specie to live a healthy life is around 20° therefore; in up and down temperatures it maintains its body temperature to certain limit such as around 20°C [3].

For the purpose it burrows land to a deeper side and hid itself there where there is a great variation in the temperature as compared to the surface of land. It relishes the hard environments such as humid environment it easily stays in the areas where the humidity is quite enough. And mostly the rocky areas including in the habitat of Uromastyx have very rare amounts of rainfall therefore, availability of water and humidity varies a lot. Apart from the discussed situation Uromastyx can tolerate wider ranges of humidity and can even tolerate the scarcity of available water [4]. Their size ranges from 25 cm to 91cm and neonates are

more than 7-10 cm in length. Like reptiles, these lizard colors change according to the temperature during cool weather they appear dull and dark but the color become lighter in warm weather, especially when basking Uromastyx body have dark pigmentation that allow them to absorb sunlight more effectively. Tail of the Uromastyx is muscular, heavy can swung and attack with great velocity. Uromastyx generally sleep in their burrows in position that their tails closest to the opening, in order to thwart intruders [5]. Many Uromastyx species can be very easily confused. All the Uromastyx species have thought to have identical spherical head circumference which is usually larger in its size and have spiny tails which are quite long. Uromastyx have different intricate designs when it comes to their outer skin usually the patterns are of similar type. Whereas, the most known practice to differentiate among its species is to study their specific patterns as they differ in the case of one species to another.

The specie is adopted to have brighter colors in their background such as orange, yellow, green, red which are spreaded over with spots of brown or grey color. The spots mentioned are mostly denser over the region of its neck and shoulders [6]. As discussed most of the species are darker in the color while they differ in species on the basis of its habitat and eating behavior. In most of the cases Uromastyx is believed to be herbivorous but in very rare cases it may also eat insects usually in the earlier age of their life. Most of the time in their life span they spend their time walking around in the warm surfaces under the brighter sunlight whereas, whenever they find some danger they get hideout in the underground chambers of the ground mostly rocky and spiny surfaces. Uromastyx strives to find out its friendly habitat in the spiny, hilly or rocky areas and these are areas where it finds out its safer shelter at the time it encounters any danger and discovers its accessible vegetation [7]. Uromastyx inhabit a range stretching through most of North Africa, the Middle East, ranging as far east as Iran and southern Iraq. Revision of the genus *U. ocellata* was found to be wide spread and regionally abundant. Two species of Uromastyx are known from Sudan: Uromastyx dispar Heyden, 1827 and Lichtenstein, 1823. These agamid lizards are regularly eaten and sold in produce markets by Local peoples, especially Bedouins of central and northern Arabia [8]. Hunting of dab lizard still exists in Arabia, because it is considered a delicious meat that provides strength. In a study conducted on the meat of *Uromastyx aegypitus*, the meat was found to

contain high protein (82.64%) and cholesterol but low levels of fat and saturated fatty acids. The nutritional quality and protein characteristics were also studies by Abu-Tarboush, where low levels of some amino acids were recorded. However, since this lizard is still traditionally eaten in some African and Arab countries, more studies are needed to verify the nutritional quality of its different edible parts [9]. There is much information available on the fatty acid composition of the lipids present in meat oil of many edible animals while very little is published on fatty acid contents of these lizards. Analysis of fatty acids profile provides information about the essential fatty acids contents of Uromastyx lizards which are willingly consumed.

The liver and fat oils may provide a rich and underexploited source of polyunsaturated fatty acids, including essential omega-3s and Omega-6s, thus increasing the commercial value of the lizard [10]. The consumption of the highly unsaturated fatty acids: eicosapentaenoic (C20:5n-3) and docosahexaenoic (C22:6n-3) is linked to the development of the brain and nervous tissue in infants and visual function, and reduces the incidence of coronary heart disease [11]. In the state of Arabia *Uromastyx aegypitus* is widely spread which is largest agamid lizard. In warm part of the day it becomes active due to which it known as diurnal animal. In numbers of terrestrial ectoderms considerable capacity for physiological thermoregulation and behavioral like in *Uromastyx aegypitus* [12]. At low ambient temperature large numbers of ectoderms remain survive because of physiological adaptation. On the other hand freezing conditions are susceptible for terrestrial ectoderms and different strategies are present to that enable terrestrial ectoderm against the freezing condition.

MATERIALS AND METHODS

For dissection process injection ketasol (ketamine) 1ml was injected intraperitoneal to make them unconscious. After the dissection the fat and adipose tissues was collected from the parts around the start of tail. The fat was collected in falcon tubes and store in the laboratory for further process. The initial average weight of collected fat was 12g and the average final purified weight of fat was 3.15g.

The most popular Folch method was for extraction;

- One gram of fat tissue is homogenized with chloroform/ methanol (2/1), after dispersion at room temperature the whole mixture

is agitated during 15-20 minutes in orbital shaker.

- The mixture/homogenate is centrifuge to recover the liquid phase.
- The solvent is washed with 0.9% of Nacl.
- For the separation of mixture into two phases it was centrifuged at low speed 2000rpm
- The upper layer was removed by evaporation and the pure sample was collected from the bottom chloroformic portion.

After the extraction of the purefat sample by Folch method, Gas chromatography-Mass spectrometry is analytical technique used for the detection of simple molecules like fatty acids, steroid and hormones. The main advantages of GC/MS for compounds analysis, including quantifying analytes, ability to separate complex mixture and determine trace levels of contamination. Sample is volatized in the chromatogram which is the start of this technique. A capillary column packed with solid phase used to separate the sample in various components and vaporize too. In GC/MS helium gas is used to propel the compounds. After the separation of components they elutes from the column at different times, this is generally known as their retention times. When the components left the GC, column electrons are used to ionize them in mass spectrometer; mass analyzer is an instrument through which ionized molecules are accelerated. The separation of ions based upon their mass to charge ratio. The peaks of the compounds appear as a function of their m/z ratio. There will be several different peaks for complex sample; mass spectrum will be the final readout. The compounds will be known be using the database of the computer and quantity as well.

RESULTS

Results of the following study execute levels of fatty acid in the samples remained as following i.e., in sample one the identified fatty acids were Undecane, 2-methyl for the compound retention time was about 7.726 mins. Likewise, hexadecane represented retention time of 12.973 mins, retention time for other compounds such as, heptdecenal, 9-Octadecenoic acid(Z)-, methyl ester, 1,2 Benzenedicarboxlic acid, mono (2-ethyhlexyl) ester, Lathosterol remained 13.910, 18.648, 23.310 and 28.981 respectively. In sample one of Uromastyx fat oil retention time for Undecane, 2-methyl and Hexadecane, 2-methyl was recorded 7.726 and 12.973 minutes. For others compounds like E-15-Heptadecenal, 9-Octadecenoic acid (Z)-, methyl ester, 1, 2

Benzenedicarboxlic acid, mono (2-ethyhlexyl) ester, Lathosterol retention time was recorded as 13.910, 18.648, 24.310 and 28.981 minutes. Lathosterol was compound with most percentage (8.807 %) detected in sample one. In sample two the retention time recoded for Methoxyacetic acid, 3-tridecyl ester 6.680 minutes, for Tetradecane and Dodecane, 5, 8-diethyl retention time was 11.045 and 12.987 minutes. On the other hand for the remaining compounds like Tetradecane, 2,6,10-trimethyl, 1-Docosene, Pentadecanoicacid, 14-methyl-, methyl ester, Octadecenoic acid, methyl ester, Nonadecanoic acid retention time noticed was 12.987, 13.917, 15.539, 18.655 and 19.137 minutes respectively. The maximum time retention was for compound 3-Eicosene, Benzenedicarboxlic acid which was 20.176 and 24.330 minutes monounsaturated fatty acid Octadecenoic acid, methyl ester was detected with high percentage (6.730%). In sample three of Uromastyx fat oil the retention time for the compounds Dodecane, Undecane, Undecane, 2-methyl was 4.766, 5.350 and 7.745 minutes. Similarly, for the other compound identified in this sample which were Dodecane, 2, 6, 11-trimethyl, Tetradecane, Hexadecane, 2, 6, 11, 15-trimethyl retention time was increased gradually from 8.357, 9.362, and 11.059 minutes respectively. For Benzoic acid which is unsaturated fatty acid retention time was 12.390 and for Benzenedicarboxlic acid member of aromatic fatty acid retention time was maximum with 24.351 minutes. In sample four of fat oil retention time recoded for Methoxyacetic acid, 2-pentadecyle ester, 2, 4-Dodecadienal, 2-Undecenal, 1-docosene was 7.726, 8.567, 9,063 and 13.903 minutes. For Oleic acid and 10-Octadecenoic acid, ethyl ester both are unsaturated fatty acids in nature and their retention time was 18.567 and 18.723 minutes. 8-Androsten-3-ol, 17-(2-methylallyl)-4, 4, 14-trimrthyl is metabolites of vitamin B12 was detected with retention time 25.261 minutes. Time retention for 10, 12, 14-Nonacosatriynoic acid was 25.831 minutes. In fifth sample of Uromastyx fat oil retention time for saturated fatty acids like Undecane, 2-methyl, Octadecane, 6-methyl, Octadecane, 2-methyl, Dodecane, 2, 6, 10-trimethyl, was 7.726, 9.334, 10.495 and 12.980 minutes. For aromatic fatty acid Benzenedicarboxlic acid retention time as 24.324 minutes. The unsaturated fatty acids 9-Octadecenoic acid (Z)-, methyl ester, 3-Eicosene were detected and their retention time was recorded 18.648 and 20.162 minutes. From detected compounds of this sample 9-Octadecenoic acid (Z)-, methyl ester was highest in percentage with 7.210%.

Table 01 shows the compound identification in 1g of fat sample of Uromastyx, molecular weight, chemical formula, retention time, percentage of identified compound in 1g of the fat sample and the nature of the fatty acid detected in the sample.

Table 02 shows the compound identification in 1g of fat sample of Uromastyx, molecular weight, chemical formula, retention time, percentage of identified compound in 1g of the fat sample and the nature of the fatty acid detected in the sample.

Table 03 shows the compound identification in 1g of fat sample of Uromastyx, molecular weight, chemical formula, retention time, percentage of identified compound in 1g of the fat sample and the nature of the fatty acid detected in the sample.

Table 04 shows the compound identification in 1g of fat sample of Uromastyx, molecular weight, chemical formula, retention time, percentage of identified compound in 1g of the fat sample and the nature of the fatty acid detected in the sample.

Table 05 shows the compound identification in 1g of fat sample of Uromastyx, molecular weight, chemical formula, retention time, percentage of identified compound in 1g of the fat sample and the nature of the fatty acid detected in the sample.

Harmonal Study Results Of Uromastyx Serum

Hormonal studies of the samples according to their seasonal changes have shown quite similar results whereas, some results of the hormones such as., Testosterone have shown significant difference in the different time frames.

The table 6 shows the hormonal values analyzed by the serum of Uromastyx blood with their units.

Table 1: Identification of the fatty acids in the fat of Uromastyx through GC-MS

PEAK #	RETENTION TIME (MIN)	IDENTIFIED COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	PERCENTAGE OF TOTAL IN 1G OF FAT SAMPLE	TYPE OF FATTY ACIDS
1	7.726	Undecane, 2-methyl	$C_{12}H_{26}$	170	2.233%	Saturated Fatty Acid
2	12.973	Hexadecane, 2-methyl	$C_{17}H_{36}$	240	3.077%	Saturated Fatty Acid
3	13.910	E-15-Heptadecenal	$C_{17}H_{32}O$	252	2.508%	Poly Unsaturated Fatty AcidOmega 6
4	18.648	9-Octadecenoic acid(Z)-, methyl ester	$C_{19}H_{36}O_2$	296	3.507%	Poly Unsaturated Fatty Acid Omega 3
5	24.310	1,2 Benzenedicarboxlic acid, mono (2-ethyhlexyl) ester	$C_{16}H_{22}O_4$	278	4.463%	Aromatic
6	28.981	Lathosterol	$C_{27}H_{46}O$	386	8.807%	Saturated Fatty Acids
7	32.613	Unknown	$C_{33}H_{46}CINO$	603	4.580%	Unknown

Table 02: Identification Of The Fatty Acids in the fat of Uromastyx through GC-MS

PEAK #	RETENTION TIME (MIN)	IDENTIFIED COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	PERCENTAGE OF TOTAL IN 1G OF SAMPLE	TYPE OF FATTY ACIDS
1	6.680	Methoxyacetic acid, 3-tridecyl ester	$C_{16}H_{32}O_3$	272	2.134%	Saturated Fatty Acid
2	9.328	Tetradecane	$C_{14}H_{30}$	198	1.942%	Saturated Fatty Acid
3	11.045	Dodecane, 5,8-diethyl	$C_{16}H_{34}$	226	3.043%	Saturated Fatty Acid
4	12.987	Tetradecane, 2,6,10-trimethyl	$C_{17}H_{36}$	40	2.453%	Saturated Fatty Acid
5	13.917	1-Docosene	$C_{22}H_{44}$	308	2.143%	Unsaturated Fatty acid
6	15.539	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	3.966%	Saturated Fatty Acid
7	18.655	Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	6.730%	Monounsaturated Fatty Acid
8	19.137	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	2.054%	Saturated Fatty Acid
9	20.176	3-Eicosene	$C_{20}H_{40}$	280	2.481%	Unsaturated fatty acids
10	24.330	Benzenedicarboxlic acid,	$C_{16}H_{22}O_4$	278	5.475%	Aromatic Fatty Acids

Table 03: Identification Of The Fatty Acids in the Fat Of Uromastyx Through GC-MS

PEAK #	RETENTION TIME (MIN)	IDENTIFIED COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	PERCENTAGE IN 1G SAMPLE	TYPE OF FATTY ACID
1	4.766	Dodecane	$C_{12}H_{26}$	170	1.039%	Saturated Fatty Acid
2	5.350	Undecane	$C_{11}H_{24}$	156	0.831%	Saturated Fatty Acid
3	7.746	Undecane, 2-methyl	$C_{12}H_{26}$	170	1.487%	Saturated Fatty Acid
4	8.357	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212	0.728%	Saturated Fatty acid
5	9.362	Tetradecane	$C_{14}H_{40}$	198	2.652%	Saturated Fatty Acid
6	11.059	Hexadecane, 2,6,11,15-trimethyl	$C_{20}H_{42}$	282	1.045%	Saturated
7	12.390	Benzoic acid, 2,4-bis (trimethylsilyl)oxy-trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	0.911%	Unsaturated Fatty Acid
8	12.994	Heptadecane, 2,6,10,15-tetramethyl	$C_{21}H_{44}$	296	1.099%	Saturated Fatty acid
9	24.351	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	1.712%	Aromatic Fatty Acids

Table 04: Uromastyx Fat Analysis BY Gas Chromatography Mass Spectrometer

PEAK #	RETENTION TIME (MIN)	IDENTIFIED COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	TOTAL PERCENTAGE IN 1G OF FAT	TYPE OF FATTY ACID
1	7.726	Methoxyacetic acid, 2-pentadecyle ester	$C_{18}H_{36}O_3$	300	0.820%	Saturated Fatty Acid
2	8.567	2,4-Dodecadienal	$C_{12}H_{20}O$	180	0.704	Unknown
3	9.063	2-Undecenal	$C_{11}H_{20}O$	168	0.651%	Unsaturated fatty Acid
4	13.903	1-docosene	$C_{22}H_{44}$	308	0.573%USFA	Unsaturated Fatty Acid
5	15.532	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.703%	Unsaturated fatty Acid Omega 12
6	17.101	n-Hexadecanosic acid	$C_{16}H_{32}O_2$	256	3.120%	Saturated Fatty Acid
7	18.567	Oleic acid	$C_{18}H_{34}O_2$	282	0.976%	Unsaturated Fatty acid Omega 9
8	18.723	10-Octadecenoic acid, ethyl ester	$C_{19}H_{36}O_2$	296	19.188%	Unsaturated Fatty Acid
9	24.351	1,2-Benzenedicarboxylic acid	$C_{16}H_{22}O_4$	274	1.381%	Aromatic Fatty Acids
10	25.261	8-Androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimrthyl	$C_{28}H_{44}O_2$	412	2.235%	Metabolites of B12
11	25.831	10,12,14-Nonacosatriynoic acid	$C_{29}H_{46}O_2$	426	3.563%	Unknown
12	28.994	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	2.130%	

Table 05: Identification of Fatty Acids in Uromastyx through GC-MS

PEAK #	RETENTION TIME (MIN)	IDENTIFIED COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	PERCENTAGE IN 1G SAMPLE	TYPE OF FATTY ACID
1	7.468	Benzene, 1,3bis(1,1-dimethyetyl)	$C_{14}H_{22}$	190	8.491%	Unknown
2	7.726	Undecane, 2-methyl	$C_{12}H_{22}$	170	1.981%	Saturated Fatty Acid
3	9.334	Octadecane, 6-methyl	$C_{19}H_{40}$	268	1.901%	Saturated Fatty Acid
4	10.495	Octadecane, 2-methyl	$C_{19}H_{40}$	268	1.747%	Saturated Fatty Acid
5	12.980	Dodecane, 2,6,10-trimethyl	$C_{15}H_{32}$	212	2.420%	Saturated fatty Acid
6	13.920	10-Heneicosene	$C_{21}H_{42}$	294	2.016%	Saturated
7	18.648	9-Octadecenoic acid(Z)-, methyl ester	$C_{19}H_{36}O_2$	296	7.210%	Unsaturated fatty Acid
8	19.124	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	2.336%	Saturated Fatty Acid
9	20.162	3-Eicosene	$C_{20}H_{40}$	280	2.486%	Unsaturated Fatty acid
10	24.324	1,2-benzenedicarboxylic acid	$C_{16}H_{22}O_4$	278	5.007%	Aromatic
11	29.008	Cholesterol	$C_{27}H_{46}O_4$	386	2.209%	Saturated

Table 06: Sample # 01 Results

Sr. No	Hormones names	Value
1	Luteinizing Hormone	<0.12mIU/ml
2	Follicle Stimulating hormone	<0.11mIU/ml
3	Cortisol	<0.1µg/dl
4	Total Testosterone	189.12ng/dl

Table 07: Sample # 2 Results

Sr. No	Hormones names	Value
1	Luteinizing Hormone	<0.12mIU/ml
2	Follicle Stimulating hormone	<0.11mIU/ml
3	Cortisol	<0.1µg/dl
4	Total Testosterone	24.25ng/dl

Hormonal values of the Uromastyx serum are shown in table with their units.

TABLE 08: Sample # 3 Results

Sr. No	Hormones names	Value
1	Luteinizing Hormone	<0.12mIU/ml
2	Follicle Stimulating hormone	<0.11mIU/ml
3	Cortisol	<0.1µg/dl
4	Total Testosterone	35.05ng/dl

Hormonal values of the Uromastyx serum are shown in table with their units.

DISCUSSION

Five samples of Uromastyx of two different seasons were analyzed by Gas Chromatography Mass spectrometer for the identification of the fatty acids in the oil prepared by the fat of Uromastyx. During this study 23 fatty acids were detected from the samples and numbers of fatty acids were same in all the samples. The percentage of saturated fatty acids was 47.8% in five samples analysis. On the other hand unsaturated fatty acids were 30.4% in which are lesser than percentage of the saturated fatty acids. Only one aromatic fatty acids Benzenedicarboxlic was present in all the five samples of Uromastyx fats oil. Metabolites of vitamin B 12 also identified in one sample. Few compounds were also detected which was not present in the database. In case of unsaturated fatty acids omega-3, omega 6 and omega 9 were detected in reasonable amount. The most important thing was that in December samples the percentages of saturated fatty acids were lesser than the samples of October. In first sample of fat oil the compound Lathosterol (C_{27}) was predominant with presence in the sample 8.807%. Saturated fatty acids (SFA) Hexadecane and Undecane were identified in sample by percentage 3.077% and 2.233 % respectively. Poly unsaturated fatty acids (PUSFA) E-15-Heptadecenal (C_{17}) which is included in omega 6 fatty acid class was identified with 2.508% and poly unsaturated fatty acid omega 3 (9-Octadecenoic acid) was identified with high quantity 3.507% as compare to omega 6 fatty acid. Aromatic

fatty acid also shown in graph Benzenedicarboxlic acid (C_{16}) was with prevalence 4.463% and present with high quantity as compare to saturated fatty acids and unsaturated fatty acid in 1g of the fat sample. An unknown compound with molecular mass 603 and chemical formula $C_{33}H_{46}CINO$ was identified with quantity 4.580%. In second sample of same season the numbers of saturated fatty acids were large in numbers as compare to other fatty acids. In saturated fatty acids (SFA) Pentadecanoic acid was identified with higher amount of 3.966% and followed by identifications of Dodecane (C_{16}) 3.043%, Tetradecane (C_{17}) 2.453%, Methoxyacetic acid was identified in sample by percentage of 2.134% and Nonadecanoic acid (C19) which is also fatty acid by 2.054% was present in fat oil sample of Uromastyx. On the other hand in this sample monounsaturated fatty acid (MUSFA) Octadecenoic acid (C_{19}) contents were higher than the individual saturated fatty acid with quantity 6.730%. Appreciable level of unsaturated fatty acid docosene and Eicosene with percentage (2.143% and 2.481%) respectively. Like the first sample of the Uromastyx fat oil aromatic fatty acid Benzenedicarboxlic acid with reasonable content 5.475% was detected.

Last three samples were collected in the month of December and in the third sample of fat oil saturated fatty acids were dominants in the numbers as compare to other fatty acids. Dodecane, Hexadecane, Undecane, Tetradecane, Undecane, 2-methyl, Dodecane, 2, 6, 11-trimethyl were detected in low contents (1.039%, 1.045%, 0.831%, 2.652%, 1.487%, 0.728%) respectively. Among all the saturated fatty acids in sample this sample Tetradecane was the highest in percentage. Benzoic acid was the only unsaturated fatty acids with C_{16}that was found in this sample in low contents 0.911%. Like the first two samples of October month Aromatic fatty acid was detected in sample of December as well with low quantity of 1.712%. In another sample of December moths showed the large number of unsaturated fatty acids and couple of saturated fatty acids. Methoxyacetic acid (C18) which is saturated fatty acid was present in low content about 0.820% in fat oil sample containing 1g of fat while n-hexadecanosic acid (C16) was the leading saturated fatty acid with contents 3.120%. In case of unsaturated fatty acids 10-octadecenoic unsaturated fatty acid was the most leading with high contents 19.188% and member of omega 12 Hexadecanoic acid (C17) was second dominant with 6.703%.

Oleic acid was found in very low amount 0.976% it is member of omega 9 fatty acids and other USFA was detected 2- Undecenal (C11) 0.651%, 1- Docosene (C22) 0.573%. Like all the samples Benzenediccarboxylic acid which is aromatic fatty acid is present in amount 1.381%. In opposite to the other entire sample a metabolites of vitamin b12 was detected named 8-Androsten-3-ol, (C28) in 2.25% and molecular weight 214. Couple of unknown compounds were detected and not identified by the database including Dodecadienal (C12) and low contents were present 0.704% and Ethyl iso-allocholate (C26) was also identified with quantity 2.130%. An acid was detected whose nature unknown is named Nonacosatriynoic (C29) was present in quantity 3.563%. In fifth sample of only two saturated acid was present the dominant unsaturated fatty acid was 9-octadecenoic acid (C19) was found with 7.210% and Eicosene (C20) was detected in quantity 2.486%. Aromatic fatty acid Benzenedicarboxlic acid who was detected in all the samples was present in high amount of 5.007%. In case of saturated fatty acid Undecane, 2- methyl (C12), Octadecabe, 6- methyl(C19), Octadecane,2-methyl(C19), Dodecane(C15), heneicosene (C21) and Nonadecanoic acid were detected in appericial level percentage were (1.981%, 1.901%, 1.747%, 2.420%, 2.016% and 2.336% respectively). Cholesterol (C27) was present in 2.209%. Like other samples Bezene, 1,3 bis (1,1 dimethyetyl) was detected as unknown compound with molecular mass 190 and quantity in sample was 8.491%.

Number of studies conducted by [13] twenty-three fatty acids was identified in the fat oil. In fat oil palmitic acid which is saturated fatty acid was dominant, on second and third numbers tricosanoic (C23) and Myristic (C14) were dominant. The most prevailing monounsaturated fatty acids were Ecosenoic (C20), palmitic (C16) and oleic acid (C18). For polyunsaturated fatty acids percentage for fat oil was 22.7%. Lenolenic and eicosatrienoic were the dominant polyunsaturated fatty acids. In that study total fatty acids contents were more or less same in oils of fat and liver but the main difference was between the percentages of saturated fatty acids which was 49.6% and 24.5%. In the meat of Uromastyx saturated fatty acids range between 33% - 37.5%. In the fat oil the saturated fatty acids was in the range (47.9%-55.1%) reported in the muscles of beef and cow but too much lower than the fat oil of fresh water fish. In that study of Uromastyx fat and liver oil, as compare to the liver oil of fresh water fish saturated fatty acids were too

much low in liver oil of Uromastyx [14]. In that study the percentage of unsaturated fatty acids in fat oil was 50.4%, on the other hand oleic, Ecosenoic and Palmitoleic were most dominant monounsaturated fatty acids and in polyunsaturated unsaturated fatty acids group lenolenic was most prevailing. High percentage of unsaturated fatty acids in the meat of Uromastyx ranging from 62.3- 67%. In fat oil low contents of lenolenic was reported by and same results were finalized by study in meat of beef and cow. Similar to this study polyunsaturated fatty acids were varying in fat oil and there is similarity in level of polyunsaturated fatty acids like omega 6. In human diet these components are consider to be essential and play role against disease and in health promotion [15]. A diet with balanced amount of omega 3 and omega 6 can prevent human from the treatment of coronary artery disease, diabetes, hypertension and cancer [16]. Hormonal study of Uromastyx serum was performed for the comparative study of hormones values. In hormonal profile luteinizing hormone (LH), follicle stimulating hormone (FSH), cortisol and total testosterone of three samples were performed. One sample was from the month of October while two were from the month of December. The levels of LH, FSH and cortisol were very same in the entire three sample which were <0.12mIU/ml, <0.11mIU/ml and <0.1µg/dl respectively. On the other hand, total testosterone level in October sample was 35.05ng/dl while the level was 24.25ng/dl and 189.12ng/dl in December samples of Uromastyx serum. In overall hormonal study the level of total testosterone was high in December Uromastyx sample as compare to sample of October.

Comparative study shows in month of February testosterone level in plasma start to increase and reached its maximum limit in month of spring months (March and April). All other factors like active spermatogenesis; in testis mature sperm appearance and increase in weight of testicular are linked with increase in plasma testosterone concentration [17]. While decrease in testosterone concentration was low in tissues of testis in same season when level of total testosterone level was at maximum in per testis. Spermatogenic compartments enlargement and in blood testosterone release in large quantity linked with low level of testicular concentration of androgen in summer season [18]. There was difference of findings in other like *picta*, *Chrysemys* and *turtle* as compare to Uromastyx. In the month of May sharp fall start in both level of

testosterone and weight of testicular level of androgens and it reached to minimal values in second week of May. The testicular testosterone starts to increase in month of summers and testicular weight remained low while semniferous epithelium based on stem spermatogonia and sertoli cells mainly [19]. When this morphological profile was low, in early September and in late august in 100 mg of tissues peak of testicular testosterone was observed. In June month level of testosterone start to increase. In Uromastyx tailed lizard radiogenic activity increase and suppress occurs in spermatogenic activity by hyperthermia [20] Due to high temperature there is enhancement in androgensis in testis.

CONCLUSION

The results of the following study help to elucidate that study conducted on the animals i.e., Uromastyx collected from the region south Punjab Pakistan in two different seasons. Twenty-three fatty acids were detected from the five samples of fat oil that was extract by the dissection and collection of fat form the pelvic region. Then Folch methods was used to extract pure fat oil. As chromatography mass spectrometry was used to identify the fatty acids present in that sample in high or low contents. From twenty-three fatty acids 47.8 % were saturated fatty acids and 30.4 % unsaturated fatty acids were detected. In case of unsaturated fatty acids polyunsaturated omega 3, omega 6 and omega 9 were also present in reasonable amount. The aromatic acid percentage was just 4.34. In all the five samples aromatic fatty acid was present and couple of unknown compound were also detected in sample. The main point was the low value of saturated fatty acids in the sample of December as compare to October. In the hormonal study of Uromastyx serum the levels of luteinizing hormone, follicle stimulating hormone and cortisol were similar in samples of both the seasons of October and December while in one sample of December month the value of total testosterone was 189.12ng/dl which was very high as compare to sample value of 35.05ng/dl.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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