

Comparative Studies of Amino Acid, Fatty Acids and Proximal Chemical Composition Content of Donkey, Dog, Camel, Beef and Goat Liver

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THE OBJECTIVE of the present study was to compare the nutritional value of liver samples of donkey, dog, camel, beef and goat by using standard methods. All samples showed a good content of amino acids, fatty acids, total protein content and moisture, data illustrated that the differences among all samples under study were significant ($p < 0.05$). The high level of protein (67.47% dw) and the low level of crude fat (6.49% dw) were determined in donkey liver, while the highest level of fat (11.69%) was determined in goat liver ($P < 0.05$). Leucine was present in the highest percent among essential amino acids followed by lysine. Camel liver has the highest value of myristic acid (3.11%) and palmitic acid (33.29%), beef liver has the highest value of margaric acids (3.26%) and stearic acid (42.93%). γ -Linolenic acid was absent only in dog's and donkey's livers.

Keyword: Liver, Chemical composition, Amino acids, Fatty acids.

Introduction

The Liver is a valuable source of nutrients especially various kinds of fatty acids, amino acids, minerals and vitamins (Kinsella and Butler, 1970). The liver contains all types of vitamins especially Vitamin A. The liver is a good source of amino acids and fatty acids. Amino acids, the building blocks of protein are very important in almost biological processes. They also affect the function of organs, hormones..., etc. They are also important for tissue repairing in muscles, skin and bones. Liver is a useful source of amino acids as well especially the essential amino acids. Amino acids are very important for growth (Smith et al., 1992). Essential amino acids are mostly responsible for the amino acid stimulation of muscle protein synthesis (Smith et al., 1998). Protein from meat especially liver has almost all the essential and non-essential amino acids (Lemon et al., 1992). Many polyunsaturated fatty acids (PUFA) are also present in the liver which is good for health as they reduce the cholesterol (Katan et al., 1994). Lipid has a very important effect on human health, but high consumption may cause high plasma and heart disease (Chizzolini et al., 1999). Saturated fatty acids are an important source of energy and the unsaturated fatty acids especially, polyunsaturated fatty acids play vital

role in reducing serum cholesterol concentration, low-density lipoprotein-C, they have some important role in modulation of immune function and inflammatory system (Rudel et al., 1995 and Stables & Gilroy, 2011).

In Egypt, dog and donkey meat and its cuts are inedible owing to religious and cultural reasons. However, some food producers tend to market donkeys and dogs as edible animals for low prices. The present investigation was conducted to compare the percentage of amino acids, fatty acids and proximate chemical composition in the liver of donkey, dog, camel, beef and goat.

Material and Method

Liver samples

Fresh donkey and dog livers were collected from Alexandria zoo, Alexandria, Egypt. Lamb, Camel and beef livers were obtained from local market. All the samples (mince) were kept frozen (-20 °C) at Kilner jar until analyzed.

Methods

The minced liver samples were analysed for moisture, total protein, saturated and unsaturated fatty acids, essential and non-essential amino acids and ash contents in accordance with standard AOAC methods (AOAC, 1995). Determination of

essential and non-essential amino acids

Amino acids were analysed by using AOAC method (AOAC 1995, method no 985.28). The minced liver samples were hydrolysed with 6 N HCl for 24 hr and amino acids were quantified using the Beckman Amino Acid Analyser (model 6300; Beckman Coulter Inc., Fullerton, Calif., U.S.A.) employing sodium citrate buffers as step gradients with the cation exchange post-column ninhydrin derivatization method. The data was reported as mg of amino acid per 1 g of sample.

Tryptophan content

The colorimetric method of (Miller, 1967) was followed to determine tryptophan in the alkaline hydrolysate using dimethyl amino benzaldehyde (DMAB). The alkaline hydrolysate was prepared by refluxing 1 g. sample with 10 ml. of barium hydroxide for 20 hrs. The hydrolysate was neutralized to pH 7 using 0.5 M Sulphuric acid and then filtered to remove the precipitate. The blue colour was developed with 0.2% sodium nitrate solution. Tryptophan was determined by using a standard curve of tryptophan prepared under the same conditions.

Determination of saturated and unsaturated fatty acid

Saturated and unsaturated fatty acids were analysed by method described by Folch et al., (1957) and AOAC (1995: method no. 996.06). 10 gram of sample (each) was taken for fatty acid analysis. Briefly described as 5ml chloroform/methanol (2: 1, v/v) solution was added to each sample and shook thoroughly for 3 min. 1ml of 0.9% NaCl was added to the mixture and vortexed again. The chloroform phase containing lipids were collected. Lipid extracts were converted to fatty acid methyl esters as described by AOAC (1995). It was prepared after alkaline hydrolysis following procedures. Briefly, lipid extract was mixed with 0.5N 2ml methanolic NaOH and held at 100°C for 10 min. This solution was cooled to room temperature and mixed with 2ml of (14% Borontrifluoride, 86% Methanol). After that, solution was incubated at 100°C for 2 min and cooled down to room temperature. 1ml n-heptane was added to final solution and thoroughly mixed by vortex for 3 min and reheated to 100°C for 1min. Final solution was cooled down to room temperature and centrifuged at 300 rpm for 5 min. The upper layer (heptane phase) was transferred to glass tube for the GC analysis. The fatty acid analysis was done by gas

chromatography (Perkin Elmer Auto system XL) equipped with flame ionization detector a DB5 silica capillary column (60m x 0.32 mm i.d.). the oven temperature was maintained initially at 45 °C and programmed to 60°C. at a rate 1°C/ min, then it programmed from 60°C to 240°C at a rate of 3°C/ min. Helium was used as the carrier gas at flow rate 1ml/min. The injector and detector temperatures were set at 230°C and 250°C, respectively. The final concentration of the fatty acid methyl esters analyses was approximately 7mg/ml in heptane. Determination of proximate chemical composition

Proximate compositions of the various liver were determined using AOAC methods (1995, method no 934.06). All analysis was done in triplicate. The moisture content was analyzed by the differences in weight before and after drying in oven at 100°C, the ash content was determined by igniting the sample at 550°C for 56- hr until the sample was completely free from carbon particles in a carbolite muffle furnace while the total nitrogen was determined by Kjeldahl method as described by AOAC (1995, method no. 981.10) and a factor of 6.25 was used for converting the total nitrogen to crude protein.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out on the data obtained to determine any significant difference in the various types of liver. Significance was assumed at ($P < 0.05$) (SAS, 1996).

Results and Discussion

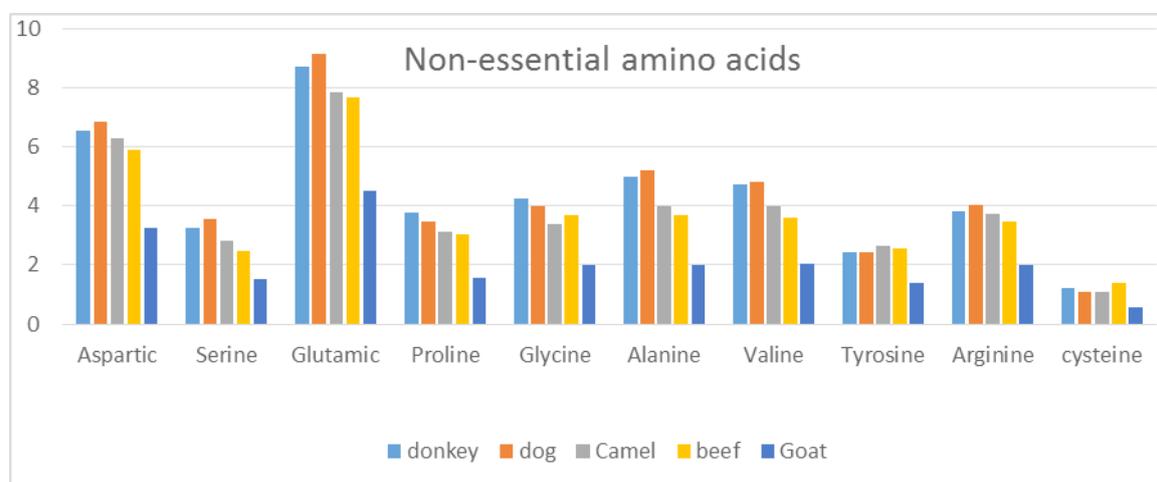
Essential and non-essential amino acids (in percentage)

Liver is a very good source of essential and non-essential amino acids (Rose, 1949). Liver contains almost all the essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) and has no limiting amino acids (Lemon et al., 1992). Results in Table 1 showed that the differences in non-essential amino acids contents between all samples were significant ($p > 0.005$). Glutamic acid is present in liver with the highest amounts (9.16%), followed by aspartic acid, alanine and valine. Cysteine was present in the lowest amount in all types of livers as shown in Fig 1. The dog liver had the highest significant level of aspartic acid, serine, glutamic acid, alanine,

TABLE 1. Non-essential amino acids (in percentage).

Non-essential Amino acid	Donkey	Dog	Camel	Beef	Goat
Aspartic acid	6.54±0.08 ^{ab}	6.84±0.16 ^a	6.30±0.05 ^b	5.89±0.31 ^c	3.26±0.04 ^d
Serine	3.26±0.17 ^b	3.55±0.07 ^a	2.84±0.07 ^c	2.47±0.03 ^d	1.52±0.02 ^e
Glutamic acid	8.70±0.06 ^b	9.16±0.11 ^a	7.87±0.06 ^c	7.67±0.08 ^d	4.51±0.03 ^e
Proline	3.76±0.03 ^a	3.45±0.02 ^b	3.12±0.03 ^c	3.02±0.04 ^c	1.58±0.04 ^d
Glycine	4.27±0.21 ^a	4.00±0.05 ^b	3.40±0.03 ^d	3.67±0.06 ^c	1.99±0.07 ^c
Alanine	5.01±0.06 ^a	5.21±0.04 ^a	3.98±0.07 ^b	3.70±0.04 ^c	2.01±0.06 ^d
Valine	4.74±0.14 ^a	4.83±0.05 ^a	3.98±0.08 ^b	3.62±0.06 ^c	2.05±0.05 ^d
Tyrosine	2.42±0.06 ^b	2.42±0.02 ^b	2.65±0.02 ^a	2.55±0.11 ^a	1.40±0.03 ^c
Arginine	3.82±0.04 ^b	4.03±0.06 ^a	3.73±0.04 ^c	3.49±0.01 ^d	2.00±0.02 ^e
Cysteine	1.21±0.05 ^{ab}	1.10±0.05 ^b	1.07±0.06 ^b	1.37±0.02 ^a	0.55±0.07 ^c

*Means ± SD in a row not sharing the superscript are significantly different at p<0.05.

**Fig. 1. Non-essential amino acids (in percentage).****TABLE 2. Essential amino acids (in percentage).**

Essential Amino acid	Donkey	Dog	Camel	Beef	Goat
Methionine	1.87±0.05 ^a	1.92±0.04 ^a	1.5±0.04 ^b	1.32±0.03 ^b	0.68±0.03 ^c
Isoleucine	3.48±0.17 ^a	3.66±0.06 ^a	3.03±0.05 ^b	2.79±0.05 ^c	1.58±0.02 ^d
Leucine	6.76±0.21 ^b	6.99±0.17 ^a	6.04±0.10 ^c	5.46±0.06 ^d	3.12±0.05 ^e
Phenylalanine	3.23±0.08 ^b	3.23±0.20 ^b	3.69±0.07 ^a	3.33±0.02 ^b	1.95±0.01 ^c
Histidine	1.89±0.03 ^b	1.94±0.02 ^b	2.23±0.02 ^a	1.85±0.03 ^b	1.18±0.02 ^c
Lysine	5.7±0.05 ^b	6.01±0.13 ^a	4.2±0.02 ^c	3.45±0.05 ^d	1.87±0.07 ^e
Tryptophan	0.34±0.03 ^a	0.27±0.04 ^{ab}	0.21±0.01 ^b	0.26±0.02 ^{ab}	0.22±0.04 ^b
Threonine	3.38±0.12 ^a	3.56±0.13 ^a	2.94±0.03 ^b	2.53±0.06 ^c	1.53±0.06 ^d

*Means ± SD in a row not sharing the superscript are significantly different at p<0.05.

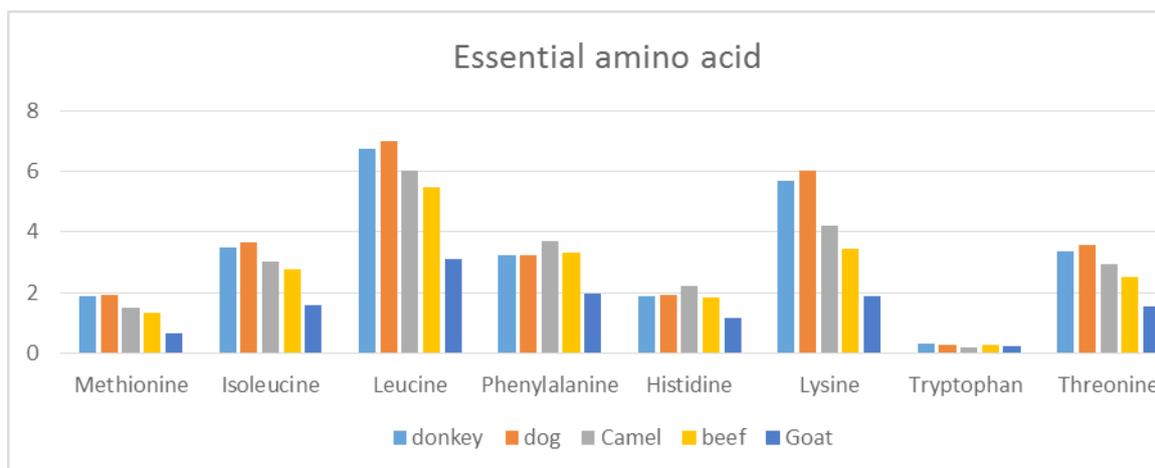


Fig. 2. Essential amino acids (in percentage).

valine and arginine compared to other species, while the donkey liver had a significant highest level of glutamic acid, proline, glycine, alanine and valine.

As shown in Table 2 and Fig. 2, Data revealed that the differences in essential amino acids were significant ($p < 0.05$) in all types of liver under study. Leucine was present in the highest percent followed by lysine in all the samples.

Amino acids balance the pH of the body, help in cell repairing and provide energy and repair bones and cells (Li et al., 2007). Glutamic acids act as neurotransmitters and helps in facilitating the communication of the brain and nerve cells. Arginine plays very important role in the development of the immune system. Cysteine play role in shape maintenance of cells. The presence of amino acids also enables vitamins and minerals to perform all their essential functions. Essential amino acids have many other functions. Without these essential amino acids, the human body is

unable to function normally and in some extreme cases, their lack causes death (Wu et al., 2001).

Saturated and unsaturated fatty acid

Liver is the thorough source of both saturated and unsaturated fatty acids (Katan et al., 2005). We found that all the five kinds of liver used in this study contain saturated and unsaturated fatty acids. For saturated fatty, Data illustrated that the differences among all samples understudy were significant ($p < 0.05$) as shown in Table 3. Camel liver has the highest value of myristic acid (3.11%) and palmitic acid (33.29%), whereas the lowest value for these acids was found in donkey (0.40%) and beef (15.02%), respectively. Beef liver has the highest value of margaric acids (3.26%) and stearic acid (42.93%), the lowest value of margaric acid (0.38%) and stearic acid (42.93%) were found in dog liver and camel liver, respectively. Saturated fatty acids are important in different biological functions as being a source of metabolic energy and in cell membrane structure formation (Chizzolini et al., 1999).

TABLE 3. Saturated Fatty acid (in %).

Fatty acid	Donkey	dog	Camel	Beef	Goat
C14:0 Myristic acid	0.40±0.02 ^b	0.57±0.02 ^b	3.11±0.03 ^a	0.49±0.02 ^b	2.96±0.05 ^a
C16:0 Palmitic acid	16.18±0.36 ^d	27.39±0.32 ^b	33.29±0.27 ^a	15.02±0.21 ^d	22.48±0.26 ^c
C17:0 Margaric acid	0.57±0.04 ^d	0.38±0.09 ^d	2.12±0.04 ^b	3.26±0.06 ^a	1.36±0.04 ^c
C18:0 Stearic acid	16.28±0.09 ^b	11.33±0.05 ^d	7.80±0.16 ^c	42.93±0.37 ^a	12.87±0.09 ^c

*Means ± SD in a row not sharing the superscript are significantly different at $p < 0.05$.

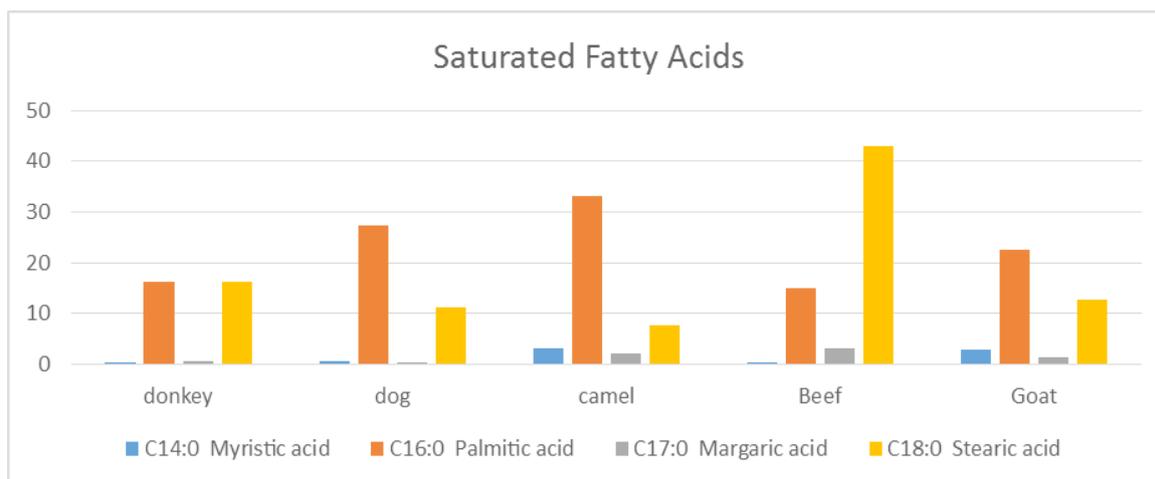


Fig.3. Saturated fatty acids (in percentage).

TABLE 4. Unsaturated Fatty acids (in %).

Fatty acid	Donkey	Dog	Camel	Beef	Goat
Oleic acid	17.72±0.19 ^c	14.60±0.14 ^d	25.33±0.29 ^b	17.90±0.25 ^c	48.12±0.19 ^a
Linoleic acid	48.59±0.31 ^a	22.40±0.31 ^b	—	10.16±0.07 ^d	—
γ-Linolenic acid	—	—	25.94±0.63 ^a	4.68±0.19 ^b	10.76±0.11 ^a
Eicosapentaenoic acid	0.26±0.04 ^d	19.26±0.15 ^a	2.41±0.06 ^c	5.56±0.03 ^b	0.74±0.02 ^d
Docosapentaenoic acid	—	4.08±0.07 ^a	—	—	0.73±0.01 ^d

*Means ± SD in a row not sharing the superscript are significantly different at p<0.05.

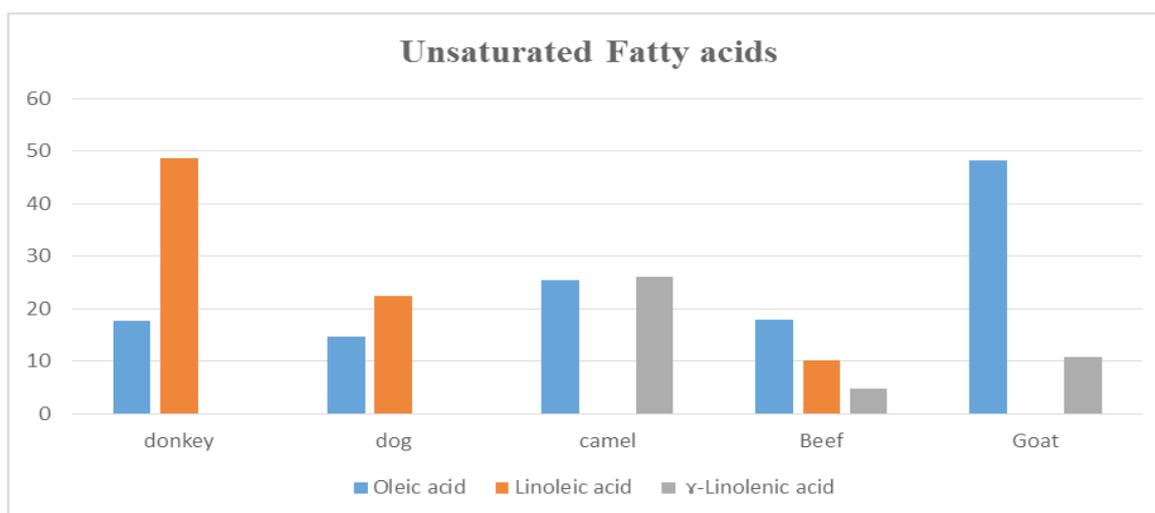


Fig. 4. Unsaturated fatty acids (in percentage).

Although fatty acids have an important impact on human health, the high consumption of saturated fatty acids is associated with CVDs, and obesity (Lichtenstein et al., 2006). Our study indicates that the liver is a good source of unsaturated fatty acids as well. Oleic acid was present in all the livers we studied and was in the highest percentage (48.12%) in goat

liver, while the lowest percentage (14.60%) in dog liver. Linoleic acid was absent in camel and goat as shown in Fig. 4. Interestingly γ -Linolenic acid was present only in camel, beef and goat liver which are commonly consumed as a food source. This suggests that the detection of γ -Linolenic acid can be used as a base of differentiating between edible and non-edible livers.

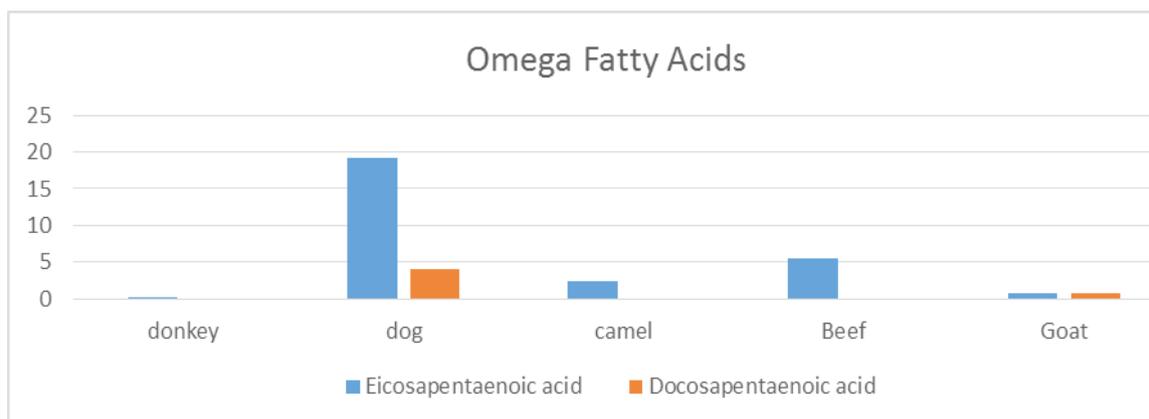


Fig. 5. Omega fatty acids (in percentage).

TABLE 5. Proximate chemical composition.

Sample	% Moisture	Dry matter	Organic matter	Ash	% in dry matter	
					Ether Extract	Total protein
Donkey	70.12±0.44 ^b	29.88±0.21 ^b	95.67±0.63 ^b	4.33±0.07 ^a	6.49±0.16 ^d	67.47±1.86 ^a
Dog	71.97±0.42 ^a	28.03±0.18 ^c	95.16±0.48 ^b	4.84±0.04 ^a	10.12±0.32 ^c	65.67±0.46 ^b
Camel	70.69±0.51 ^b	29.31±0.13 ^b	95.46±0.30 ^b	4.54±0.13 ^a	11.42±0.27 ^a	63.99±1.29 ^c
Beef	70.68±0.38 ^b	29.32±0.23 ^b	95.78±0.27 ^{ab}	4.22±0.08 ^a	10.89±0.19 ^b	61.04±0.49 ^d
Goat	67.70±0.47 ^c	32.30±0.20 ^a	96.77±0.41 ^a	3.23±0.08 ^b	11.69±0.06 ^a	40.95±0.37 ^c

*Means ± SD in a column not sharing the superscript are significantly different at $p < 0.05$.

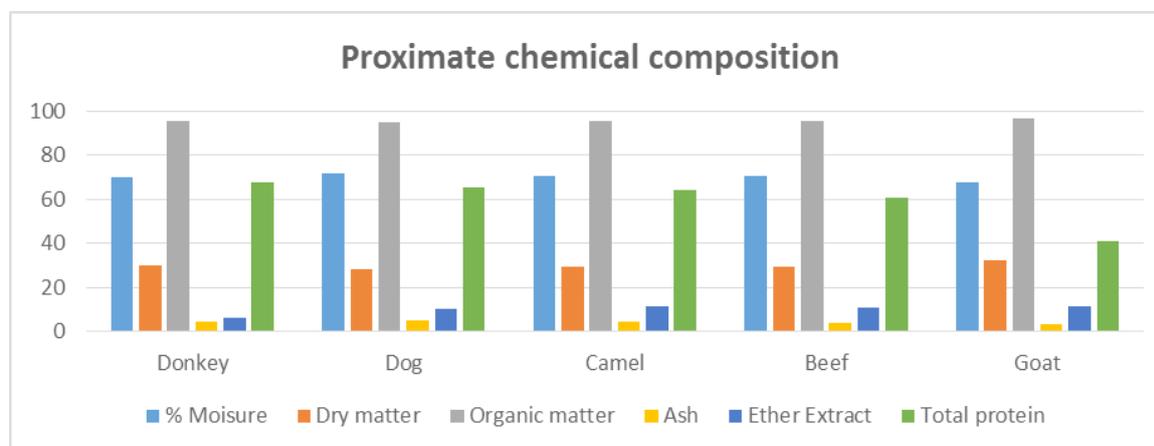


Fig. 6. Proximate chemical composition.

Oleic acid, has an effect on cancer and inflammatory diseases, it also helps in wound healing (Sales-Campos et al., 2013). Linoleic acid can be converted into omega-6 fatty acid like α -linolenic acid (GLA) which is an omega-6 fatty acid and is an essential fatty acid and plays a crucial role in brain function, and normal growth and development (Sales-Campos et al., 2013).

Eicosapentaenoic acid (EPA) and Docosapentaenoic acid (DHA) are an example of omega-3 fatty acids. The dog liver has the highest value of Eicosapentaenoic (19.26%) and Docosapentaenoic acid (4.08%), they play very important role in foetal development and healthy aging (Dunstan et al., 2007 and Krauss-Etschmann et al., 2007) and it is beneficial in prevention and treatment of several diseases (Serhan et al., 2008; Kaur et al., 2011).

Proximate chemical composition

The liver consumption can play a vital role in human nutrition because of its nutritive value. The value of nutrition is measured in terms of the major chemical components such as proteins, fats, carbohydrates, minerals and fatty acids contents (Pearson and Gillett, 1996).

The proximate compositions of livers expressed as dry weight basis are presented in Table 5. The high level of protein (67.47%) and low level of crude fat (6.49%) were determined in donkey liver, while the low level of protein (40.95%) and high level of fat (11.69%) were determined in goat liver ($P < 0.05$). According to the types of liver, protein content differs but not as much as fat content which ranged from 11.69 to 6.49% ($P < 0.05$). The moisture content was almost equal (70-71%) in all liver samples except goat s which had 67.70 % moisture.

Conclusion

In this study, we have realized that camel, beef and goat liver are important sources in terms of essential and nonessential amino acids, long chain omega 6 and omega 3 fatty acids. Although the donkey and dog carcass are not edible in Egypt for religious and cultural reasons, but the liver from donkey has high protein and low fat than other samples, and livers obtained from dog or donkey had the highest amounts of many essential amino acids compared to the studied edible livers. the detection of α -Linolenic acid can be used as a base to differentiate between edible and non-edible livers.

Acknowledgements

Disclosure statement

The authors declare that there is no conflict of interest

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دراسة مقارنة للأحماض الأمينية والأحماض الدهنية والتحليل الكيماوي لكبد الحمار والكلب والجمال والبقر والماعز

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تهدف هذه الدراسة إلى المقارنة بين التركيب الكيماوي لكبد الحمير والكلاب والجمال والبقر والماعز. كل الأنواع محل الدراسة أظهرت اختلافات معنوية ($p < 0.05$) في مفردات التركيب الكيماوي. وقد أظهرت النتائج امتلاك كبد الحمير على نسبة من البروتين (٤٧، ٦٧٪)، وأقل نسبة من الدهن (٤٩، ٦٪) على أساس وزن جاف، في حين امتلك لحم الماعز أعلى نسبة من الدهن (٦٩، ١١٪) مقارنة بالأنواع المدروسة. كما أظهرت النتائج أن الحامض الأميني ليوسين هو أكبر الأحماض الأمينية وجوداً في العينات المدروسة يليه الليسين. كما أظهرت النتائج أن الكبد الجملي به أعلى محتوى مقارنة بالأنواع المدروسة من الحامض الدهني ميرستييك (١١، ٣٪)، والحامض الدهني بالميتيك (٢٩، ٣٣٪)، وأن الكبد البقري به أعلى محتوى من حامض المارجريك (٢٦، ٣٪)، والستياريك (٩٣، ٤٢٪) مقارنة بالأنواع المدروسة. كما أظهرت النتائج عدم احتواء كبد الحمير والكلاب على الحامض الدهني جاما- لينولينك.