FORENSIC IDENTIFICATION OF SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE SAMPLES IN SOME FARM ANIMALS USING GAS LIQUID CHROMATOGRAPHY

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ABSTRACT

We investigated the fatty acid profiles of adipose tissues from cows, sheep and camels in order to identify between the different animal species from the medicolegal point of view. Subcutaneous and perirenal adipose tissues were freshly and randomly sampled from slaughter houses. Fatty acids were transestrified to Fatty Acids Methyl Ester (FAME) and their profile was determined using gas chromatography. The contents of total polyunsaturated oleic fatty acid C18:1 was higher in cows and sheep while camels recorded the lowest levels. However, the saturated stearic fatty acid C18:0 was significantly higher in camels, moderately in sheep and significantly lower in cows (P<0.05). On the other hand, myristic (C14:0) and palmitic (C16:0) fatty acids showed non significant differences in their levels between the three animal species. We conclude that stearic and oleic fatty acids profile could be significantly important from the medicolegal point to identify and discriminate between different animals using adipose tissues and fat depots.

INTRODUCTION

Examination of animal fats is one of the most important steps for species identification in veterinary forensic medicine. Sometimes, only small pieces of fats could be present in the crime scene as the only evidence collected for further forensic analysis. The physical examination of animal fats is not discriminative tool between their species origin, cow's fat tends to be yellowish due to the high levels of beta-carotene pigments while sheep and camel body fats are whitish in color. Since then, chemical analytical methods are required to determine the fatty acids composition of fat samples.

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Fatty acids and glycerin are the main components of any fat so the resulting mixture contains three molecules of fatty acid for each molecule of glycerin. Because of this proportion of acid to glycerin, the chemical compounds found in the fat before it was split are known as triglycerides. While a large variety of fatty acids is found in natural fats, only a few of them are of outstanding importance. These are lauric acid

(C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linolic acid (C18:2), and linolenic acid (C18:3). When the fatty-acid molecule contains the maximum of hydrogen possible, the acid is said to be a saturated fatty acid. It is saturated with respect to hydrogen. Lauric, Myristic, palmitic, and stearic acids are such saturated acids. Their carbon atoms are linked in chain by single bonds. When, however, the fatty-acid molecule does not contain the maximum amount of hydrogen possible, the acid is said to be an unsaturated fatty acid. It is unsaturated with respect to hydrogen. Such unsaturated acids are oleic, linolic, and linolenic acids. Their carbon atoms are linked in chain by one double bond as in case of oleic acid (C18:1) or more double bonds as in linolic and linolenic acids; two bonds (C18:2) and three bonds (C18:3) respectively (Cartwright 1993). Nowadays, the technique of gas chromatography (GC) revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time (Christie 1989). For this purpose, myristic, palmitic, stearic and oleic fatty acids of sampled fats were converted to the simplest convenient volatile derivative as Fatty Acids Methyl Esters (FAME) for further GC quantitative analysis (Murata 1978). The aim of this study was to investigate the fatty acid profiles of adipose tissues from cows, sheep and camels in order to identify between the different animal species from the medicolegal aspect. Fresh fats samples were collected from slaughter houses immediately after slaughtering animals to assess this study.

MATERIALS AND METHODS

Sampling of different fat tissues

Ten samples were taken from each of cows, sheep and camels species in slaughter house. The fat samples were collected from subcutaneous and perirenal fat of each animal species used in this study immediately after slaughtering. The samples were kept in plastic bags and shipped in an insulated ice box to the laboratory.

Total lipid extraction of fat samples

Fat samples were extracted by the procedures similar to the Folch method (1957). Briefly, Chloroform/methanol (2:1, v/v) containing 0.005% butylated hydroxytoluene (as antioxidant) was added (usually 5 ml solvent added to 50-100 _l sample) and mixed vigorously for 1 min then left at 4°C overnight. One ml of 0.9% NaCl was added and mixed again. The chloroform phase containing lipids was collected. The remains were extracted with 2 ml chloroform. The chloroform was pooled and dried under nitrogen and subjected to methylation.

Preparation of Fatty Acids Methyl Esters (FAME)

Fatty acid methyl esters were prepared according to Morrison and Smith method (1964) using 14% Boron Trifluoride /methanol reagent (BF3/MeOH). Lipid samples were mixed with 1 ml hexane in 16 ml glass tubes with Teflon-lined caps. BF3/MeOH reagent (1 ml) was added and the mixture was heated at 90-110°C in a metal block for 1 hour, cooled to room temperature and methyl esters extracted in the hexane phase after addition of 1 ml H2O. Samples were allowed to stand for 20-30 min, and then the upper hexane layer

was removed and concentrated under nitrogen.

Fatty acids profiles

Fatty acid methyl esters were analyzed by gas chromatography using TRACE GC Ultra system (Thermo Electron, Waltham, MA, USA) equipped with a flame-ionization detector (FID). The chromatography utilized a TRACE GC capillary column TR-FAME (30m 0.25 mm I.D., 0.25 μm film thickness, Part No. 260M142P, Thermo Electron). The column oven temperature was held at 150°C for 1 min, increased at 10°C/min to 180°C, then increased to 260°C at 30°C/min and held for 5 minutes. The injector temperature was adjusted at 250°C and the detector temperature at 280°C. The carrier gas used was helium with a flow rate of 1.3 ml/min. Individual fatty acids were identified by comparing the retention time of fatty acid methyl ester (FAME) with standard FAME mixture from Supelco (Product No. 18917 St. Louis, MI, USA). The values of fatty acids are presented as area percentage of total fatty acids.

Statistical analysis :

The results are presented as the mean ± SD (standard deviation). All data were analyzed by one-way analysis of variance (ANOVA) using the software of WINKS SDA Version 6.0 for Windows. Student's t-test was performed to separate differences among means. The differences are considered significant at $P<0.05$.

RESULTS

Fatty acids profile of subcutaneous fat

The results of fatty acid profile of subcutaneous fat of cow, sheep and camel are presented in Table 1. No significant difference in myristic (C14:0) or palmitic (C16:0) fatty acids among the different animals. The saturated stearic fatty acid (C18:0) showed the significant highest value in camels (34.39 ± 2.16) at $P<0.05$ more than the moderate level in sheep and the lowest value were recorded in cows (15.31 ± 1.47).

The same results were found with the total contents of saturated fatty acids, camels showed the highest significant value over the moderate sheep and lowest cow values.

The unsaturated oleic fatty acid (C18:1) was not significantly different in cows (50.94 ± 3.18) and sheep (51.22 ± 2.96) while both animals showed higher significant values than camels (31.04 ± 2.19) at $P<0.05$.

Fatty acids profile of perirenal fat

Table 2 shows the results of saturated fatty acid profile of perirenal fats in different animals. As in subcutaneous fat, myristic and palmitic did not show any significant difference between cow, sheep and camel. Stearic fatty acid recorded the highest values in camels (31.03 ± 1.49) which showed the lowest results of unsaturated oleic fatty acid (31.83 ± 1.91). In contrary, cow's oleic fatty acid level (51.57 ± 2.93) was the highest among other species while it showed the lowest stearic saturated fatty acid level (17.55 ± 0.98). Sheep recorded the moderate results of oleic and stearic fatty acids (41.98 ± 2.57 and 23.76 ± 1.57 respectively) between cows and camels.

No-significant differences were found in myristic and palmitic fatty acids between all

the three species: cow, sheep and camels ($P<0.05$).

DISCUSSION

In our present study, we analyzed subcutaneous and perirenal fats of different animals using the most accurate and rapid technique could be used in a modern lab; Gas Chromatography (GC). The study focused on three saturated fatty acids; myristic (C14:0), palmitic (C16:0) and stearic (C18:0) and the monounsaturated oleic fatty acid (C18:1) in order to establish a reliable method in differentiation between edible fat tissues of different animals. We have used three different animal species; cow, sheep and camel and all samples were collected randomly from the slaughter house immediately after slaughtering animals. All slaughtered cow and sheep were raised on different fattening rations and some green forage while camels were grazing more as the concept of fattening camels is rarely distributed in Egypt. All animals were slaughtered to be a source of meat for human consumption. Forensic examination of adipose tissues and fat of animals is limited to either physical examination, or genetic fingerprinting using Polymerase Chain Reaction (PCR) or chemical analysis. The physical examination depends on the presence of whole or part of the animal to best identify the origin species, while genetic fingerprinting depends on the design or commercial availability of species-specific primers to discriminate between fats of different animals. PCR is a promising and sharp-cut discriminative technique but careful preparation of fat samples is required to avoid possible inhibition of DNA amplification during the polymerase chain reaction. Furthermore, visualization of PCR amplicons requires using toxic fluorescent dyes as ethidium bromide which exerts a possible contamination of environment and dangerous neurotoxicity to test applicants. In this study, chemical analysis using GC revealed non-significant differences of myristic (C14:0) and palmitic (C16:0) fatty acids from subcutaneous and perirenal fat of cow, sheep and camel animals. The most important and significant results from the medicolegal point of view is the proportion between saturated stearic (C18:0) and monounsaturated oleic fatty acids (C18:1). In cows and camels certainly, it was noticed a reverse proportional correlation between stearic and oleic fatty acids. Cows showed highly significant levels (51.26 ± 2.86) of oleic monounsaturated fatty acid (MUFA) and the lowest levels of the saturated stearic fatty acid (16.43 ± 1.28), while in contrary, camels showed the lowest levels of oleic fatty acid (31.44 ± 1.13) and highly significant results in stearic fatty acid (32.71 ± 1.72). This result is a promising tool to discriminate forensically between adipose tissues and fats of two species depending on their chemical analysis of their stearic and oleic fatty acids. On the other hand, sheep fat and adipose tissues could be identified by their highly significant oleic fatty acids levels (46.60 ± 3.16) as in cows, while a moderate level of stearic fatty acid (22.44 ± 1.19) which is significantly higher than cows (16.43 ± 1.28) and lower than camel (32.71 ± 1.72).

It is well known that the fatty acids composition of muscle fat and adipose tissue is dependent on the diet fed to animals **(Doreau and Ferlay, 1994)**, ruminants and other farm animals are either fed on forages only or on forage and concentrates or grains. **Berthiaume et. al (2006)** have pointed in their

study a significant difference in compositions of fatty acids between animals of two beef production systems based on forage finishing or grain-forage diets. **Leat (1978)** observed a greater proportion of mono-unsaturated fatty acids (MUFA) in the fat deposited by fatter animals, while **Duckett et al. (1993)**, who investigated the effects of switching from pasture feeding to concentrate feeding, reported a time-dependent increase in the proportion of C18:1, a marginal difference in the proportion of C14:0 and C16:0, and a time-dependent decrease in C18:0, this agrees with our results obtained from current study. Since most of cows and sheep are usually slaughtered after their exposure to long time of concentrates feeding for fattening purposes, concentrates raised significantly the contents of oleic fatty acid as MUFA in cows and sheep (51.26 ± 2.86 and 46.60 ± 3.16 respectively) due it is higher contents of proteins more than 30%. On the other hand, concentrates and grains feeding resulted in decreasing stearic saturated fatty acid which significantly reached a lowest level in cows (16.43 ± 1.28) and moderately higher level in sheep (22.44 ± 1.19). Camels in Egypt are not exposed to fattening regimes, and they always feed on forages or kept grazing most of the time, this feeding manner can interpret the highest levels of stearic saturated fatty acids (32.71 ± 1.72) in camel's adipose tissue samples. The significant decrease in oleic MUFA (31.44 ± 1.13) in camel's fats could be due to low-level concentrate rations received by these animals.

Table 1 : Fatty Acid (FA) Profile of Subcutaneous Fat of Different Animals.

Animals	Cow	Sheep	Camel
Myristic FA (C14:0)	4.06 ± 0.86^{a}	3.84 ± 0.93^{a}	3.7 ± 0.89^{a}
Palmitic FA (C16:0)	29.62 ± 2.5^{a}	30.67 ± 1.88^{a}	30.29 ± 2.05^{a}
Stearic FA (C18:0)	15.31 ± 1.47^{a}	21.12 ± 1.32^{b}	34.39 ± 2.16^{c}
Oleic FA (C18:1)	50.94 ± 3.18^{a}	51.22 ± 2.96^{a}	31.04 ± 2.19^{b}

Values (mean ± *SD*) in the same row with different superscript letters are significantly different ($P<0.05$), $n=10$

Table 2 : Fatty Acid Profile of Perirenal Fat of Different Animals.

Animals	Cow	Sheep	Camel
Myristic FA (C14:0)	3.82 ± 1.01^{a}	3.25 ± 1.35^{a}	3.54 ± 0.94^{a}
Palmitic FA (C16:0)	33.94 ± 3.14^{a}	27.12 ± 2.63^{a}	33.55 ± 2.13^{a}
Stearic FA (C18:0)	17.55 ± 0.98^{a}	23.76 ± 1.57^{b}	31.03 ± 1.49^{c}
Oleic FA (C18:1)	51.57 ± 2.93^{a}	41.98 ± 2.57^{b}	31.83 ± 1.91^{c}

Values (mean ± *SD*) in the same row with different superscript letters are significantly different ($P<0.05$), *n=10*

Table 3 Mean Values of Total Fatty Acid Profile [Subcutaneous and Perirenal] between Different Animals

	Cow	Sheep	Camel
Myristic Fatty Acid [C14:0]	3.94 ± 0.97^{a}	3.55 ± 1.05^{a}	3.62 ± 1.13^{a}
Palmitic Fatty Acid [C16:0]	31.78 ± 2.74^{a}	28.90 ± 2.07^{a}	31.92 ± 2.11^{a}
Stearic Fatty Acid [C18:0]	**16.43 ± 1.28^{a}**	22.44 ± 1.19^{b}	**32.71 ± 1.72^{c}**
Oleic Fatty Acid [C18:1]	**51.26 ± 2.86^{a}**	46.60 ± 3.16^{a}	**31.44 ± 1.13^{b}**

Mean values in the same row with different superscript letters are significantly different ($P<0.05$), *n=10*. Bold figures show the reverse proportional correlation from the medicolegal point of view between cows and camels.

CONCLUSIONS

This study has resulted in a forensic tool for identification and discrimination between adipose tissues and fat samples derived from different cows, sheep and camels using Gas Chromatography (GC). The forensic tool depends on the reverse proportional correlation of stearic and oleic fatty acids between cows (and sheep) and camels. Cows and sheep showed highly significant levels of oleic fatty acid and the lowest levels of the saturated stearic fatty acid, while camels showed the lowest levels of oleic fatty acid and highly significant results in stearic fatty acid. This correlation could be a promising tool on the path of veterinary forensic medicine to identify and differentiate fat and adipose tissue samples derived from other animals than those used in the current study.

REFERENCES

Berthiaume, R.; I. Mandell, L. Faucitano, and C. Lafreniere. (2006) : Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotants: 1. Feedlot performance, carcass quality, and production costs. J. Anim. Sci. 84:2168-2177.

Cartwright I. J. (1993) : In Biomembrane Protocols: I. Isolation and Analysis, Series: Methods in Molecular Biology, pp 183-195

Christie, W. W. (1998) : Gas chromatography-mass spectrometry methods for structural analysis of fatty acids. Lipids 33:343-353.

Doreau, M. and A. Ferlay (1994) : Digestion and utilization of fatty acids by ruminants. Anim. Feed Sci. Technol. 45:379-396.

Duckett, S.; D. G. Wagner; L. D. Yates; H. G. Dolezal and S. G. May (1993) : Effects of time on feed on beef nutrient composition. J. Anim. Sci. 71:2079-2088

Folch, J.; Lees, M. and Soane, G. H. (1957) : A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem.; 226: 497-509.

Leat, W. M. (1978) : Factors affecting the fatty acid composition of depot fats of cattle and other ruminants. Page 231 in Patterns of Growth and Development in Cattle, Current Topics in Veterinary Medicine. Vol. 2. H. De Boer and J. Martin, ed. Martinus Nijhoff, The Hague, The Netherlands.

Morrison W. R. and Smith L. M. (1964) : Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res.; 53: 600-608.

Murata, T. (1978) : Analysis of fatty acid methyl esters by a gas-liquid chromatography chemical ionization mass spectrometry computer system. Journal of Lipid Research Volume 19, pp 166-171.

الملخص العربى

الاستعراف الطبى الشرعى للدهون المجمعة من تحت الجلد وحول الكلى لبعض حيوانات المزرعة بطريقة الكايموجراف السائل - الغازى

تم دراسة مستوى الأحماض الدهنية للدهون المجمعة من الأبقار، الأغنام، والجمال فى محاولة للاستعراف والتفريق بين حيوانات المزرعة المختلفة من الوجهة الطبية العدلية، تم جمع عينات طازجة بعد الذبح مباشرة وبطريقة عشوائية من دهون مالحت الجلد والدهون المرجودة حول الكلى لحيوانات مذبوحة فى المجازر المختلفة، تم تحضير العينات المختلفة ومن ثم مثيلتها إلى صورة المثيل إستر الخاصة بكل حمض دهنى وأخيراً قياس مستواها باستخدام جهاز الكايموجراف السائل - الغازى، أظهرت الدراسة ارتفاع نتائج الحمض الدهنى الأوليك الغير مشبع فى الأبقار والأغنام ارتفاعاً ملحوظاً بينما سجلت الجمال أقل مستوى لها من حمض الأوليك، كذلك كان مستوى حمض الاستياريك المشبع عالياً إحصائياً فى الجمال، متوسطاً فى الأغنام، وأقل مايكون فى الأبقار، من ناحية أخرى، أظهرت النتائج عدم تأثر مستوى كلاً من أحماض الميرستيك والبالمتيك الدهنية إحصائياً فى كل من حيوانات المزرعة الثلاثة.

نستنتج من هذه الدراسة أن تعيين مستوى أحماض الاستياريك والأوليك فى عينات الدهون قد يكون ذات أهمية ملحوظة من الناحية الطبية العدلية للاستعراف والتفريق بين مختلف حيوانات المزرعة.