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ORIGINAL ARTICLE

Composition of intramuscular fatty acids from sheep living in Steppe zones, TIARET region, ALGERIA

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ABSTRACT
The effect of two distributed diets (pasture in steppe rangelands and a diet based on barley concentrate) on intramuscular lipid composition was studied in sheep. Some samples of the "Biceps femoris" ovine muscles where collected in the spring period (n = 5), and in the winter period (n = 4) from Tiaret local slaughter house.A small difference was observed in the fatty acid profile analyzes, which is probably related to the phenomenon of remanence where sample collection began at the beginning of the seasons, which influenced the intramuscular lipid fraction, which appears to be approximate in the meat of these sheep. C18: 2n6 had the highest content, followed by C20: 4n6 (P <0.001), while there was no significance for the PFA / SFA ratios (0.272 vs 0.339) (P <0.001) and just as many Σn6 / Σn3 (0.051 vs 0.124) for both periods (P <0.001). Discriminatory analysis of intramuscular fatty acids shows that despite the limited effect of these sheep under both regimes, which are characterized by a similar fatty acid profile, their meats are suitable at desirable stages, which is why is in favor of a certain reduction in the risk of having certain health problems in humans. This makes sheep farming on steppe rangelands beneficial, in favor of quality meat production, which meets the demand. It should be noted that even for concentrate feeding, these sheep presented properties more or less similar to each other.
Keywords: *Pasture, Concentrated, Diets, Lamb Meat, Intramuscular, Fatty Acids.*

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INTRODUCTION

Grazing in the steppe areas that extend from the Tellian atlas in the north, to the Saharian atlas in the south, is characterized by a herd composed in the majority of sheep (about 80% of the herd). This steppe zone has an essentially pastoral vocation, which produces sheep meat [1]. There are some varieties of steppe plants that are very valuable to sheep in these areas [2]. The latter author has established a scale of palatability of these steppe plants, ranging from 0 to 10 and a palatability index greater than 5, which has made it possible to form species that have an appreciable pastoral value, such as *Artemisia Herba Alba* and *Atriplex halimus*, which have a specific quality index (Isi) equal to 7. Other plants do not offer the same pastoral interest, and are only consumed if the sheep are forced to do so. On the other hand, there are certain steppe plants such as *Atractylis serratuloides* and *Peganum harmala* that are not at all appreciated by sheep. With the advent of the spring season, steppe plants begin to grow, and pastures become rich and well supplied in these different plant species, both for sheep and other animal species in the steppe zone of the wilaya of TIARET. This spring abundance decreases during the summer period, with the rarity of precipitation, while most of these steppe plants complete their vegetative cycle. Thus, the natural food supply provided by this steppe becomes increasingly less important, and even begins to disappear. In this case, and in order to avoid the problems of underfeeding sheep flocks, the farmers distribute to their herds certain concentrated foods such as cereals. These diets based on steppe plants provide sheep with a lipid level that differs according to the nature of the plants consumed, and whose lipid content in the plant may be of the order of 3%; it is particularly concentrated in the leaves of these plants, these lipids consist of more than 50% of galactolipids (Mono and Digalactosyl Diglycerides), some of which fatty acids may be the factor having the most influence, including 18: 3 n-3, which constitutes 50% of fatty acids totals present at these plants, followed by palmitic acid C16: 0 and linoleic acid 18: 2 n-6 which also constitute a level of 20 and 15% respectively, in addition to about 25% of phospholipidsincluding phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine, with regard to the lipid content in cereal-based diets, the latter amounts to almost 4%, consisting mainly of triglycerides (around 98%). The latter have an unsaturated character, with a predominance of C18: 2 linoleic acid, followed by C18: 1 oleic acid, and C16: 0 palmitic acid [3, 4].

Nowadays, the consumer is interested in the quality of the meat, especially its flavor. According to [5]. and [6], the latter is particularly more intense among sheep living on the pastures of steppe areas, compared to those living in sheepfold, which makes them more appreciable and more demanded by these consumers. According to [7], the flavor is mainly influenced by the variation of intramuscular fatty acids, which differs according to the size and number of intramuscular adipocytes, itself influenced by certain factors, such as diet, age and sex. On the other hand, these fatty acids have a positive impact on the quality of the meat, including its tenderness and juiciness[8].[4] reported that the composition of ingested fatty acids (FA) present in the diet is different from that of absorbed FA in meat, which are not part of the diet.This variation is explained by the high hydrogenation of unsaturated fatty acids at the rumen level, such as conjugated linoleic acids and Trans monounsaturated fatty acids [3]. In ruminants, saturated fatty acids found in meat are considered harmful to human health, as they may increase the incidence of cardiovascular diseases, metabolic syndromes and many other chronic diseases[9]. This fact leads us to say that if we want to reduce their negative impact on the health of consumers, it is imperative to reduce the rate of saturated fat to a threshold of 15% to 30% of total energy intake [10] ; however, there are polyunsaturated fatty acids such as conjugated linoleic acid (C18: 2c9, t11), eicosapentaenoic acid or EPA (C20: 5n-3), and docosahexaenoic acid or DHA (C22: 6n-3) in meat, which are essential and beneficial in reducing the incidence of certain chronic and inflammatory diseases[11].

The aim of this study was to characterize the intramuscular lipid content and to determine their variation in meat, depending on the diet followed, whether in sheep raised in the steppe areas, or those reared in sheep fold.

MATERIAL AND METHODS

Biceps femoris muscle samples from 9 different sheep carcasses ranging in age from 6 to 8 months were collected on different occasions in 2017, from four potential sheep farmers who graze in the large steppe areas of the Tiaret region. Grassing in these steppe areas occurs over two periods: from March to the end of June and from December until the end of February.

The selection of these two collection periods was chosen conceptually to determine and estimate that locally produced lamb meat during the spring period is consistent with the period of time when it occurs; however, meat produced in winter is equivalent to that obtained from sheep fed with concentrate Bravo-Lamas *et al* [12]. According to Aurousseau *et al* [13] and Luciano *et al* [14], concentrated feeding is essentially based on barley. In this experiment, the meat used was cut into equal pieces, taken from the deep muscle region, and preserved at 4 ° C for 7 days for good maturation and then frozen at -20 ° C for the next manipulations. Depending on the nature of the study, and following the doubt about the homogeneity of the sheep flock, as well as its liaison with the nature of the usual consumption of meat, we have voluntary omit to take into account the origin of the sheep and their weight at slaughter Bauchart *et al* [15].

Analysis of methyl esters of fatty acids

The samples were previously minced, lyophilized and then homogenized before the fatty acids were determined. Direct obtention of the fatty acid methyl esters by trans esterification, using the 20% boron trifluoride solution in methanol Rule[16], made it possible to carry out these analyzes, in order to identify and quantify fatty acids by gas chromatography, using 1, 2, 3-tripentadecanoylglycerol as internal standard. The latter is equipped with a SP2330 capillary column of 30 m * 0.25 mm (Supelco, Tres Cantos, Madrid), and an oven that can reach the temperature of 150 to 225 ºC, at a rate of 7 ºC per minute, and the temperature at the injector and detector is 250ºC Tor et al[17].

Statistical analyzes

To make the statistical analysis and to distinguish the possible differences between the intramuscular fatty acids of the biceps femoris muscles of the sheep from the different diets, we did the ANOVA test, followed by the Bonferroni test (see Table below).

Table N° 01: Averages and standard deviation of each sampling method, with the intramuscular fat content of the Biceps Femoris muscle (mg / g fatty acids).

Fatty Acids	Concentrate		Grazing		Signification
	Mean	SD	Mean	SD	
C 10	0,086	0,015	0,071	0,015	***
C12	2,548	0,749	1,880	1,964	***
C 14	1,705	0,361	1,529	0,881	***
C 15	0,347	0,101	0,104	0,085	***
C 16	11,931	3,377	10,967	5,101	***
C 17	1,590	0,576	0,880	0,430	***
C 18	6,610	1,540	7,479	3,910	***
C 20	0,040	0,006	0,047	0,019	***
C 24	0,120	0,026	0,237	0,086	***
C14 :1	0,089	0,028	0,065	0,027	***
C16 :1	1,162	0,437	0,885	0,331	***
C18 :1 cis-11	0,920	0,277	0,705	0,227	***
C18 :1 cis-9	20,318	8,175	17,683	7,516	NS
C18 :1 trans-9	1,705	0,625	0,580	0,350	***
C20 :1	0,056	0,019	0,051	0,012	***
C18 :2n6	4,063	0,993	3,603	0,711	***
C18 :2n6	0,228	0,045	0,313	0,171	***
C18 :3n6	0,047	0,010	0,034	0,021	***
C18 :3n3	0,190	0,062	0,454	0,336	***
C20 :2n6	0,173	0,037	0,213	0,071	***
C20 :3n6	0,135	0,019	0,148	0,028	***
C20 :4n6	1,666	0,190	1,871	0,476	***
C20 : 5n3	0,077	0,049	0,197	0,148	***
C22 : 6n3	0,043	0,051	0,120	0,089	***
SFA	24,980	5,544	23,197	9,826	NS
MUFA	24,252	9,294	19,972	7,846	NS
PUFA	6,684	1,112	7,009	1,351	NS
n6	6,315	1,110	6,184	1,096	NS
n3	0,311	0,096	0,772	0,530	NS
Σn6/Σn3	0,051	0,018	0,124	0,076	NS
ΣPUFA/ΣSFA	0,272	0,048	0,339	0,152	NS

NS: non significant ***$p < 0.001$.
SFA: Saturated Fatty Acids.
MUFA: Mono unsaturated Fatty Acids
PUFA: Polyunsaturated Fatty Acids.

RESULTS AND DISCUSSION

The results of the present study reveal a dominance of the C16 fatty acids, followed by the following predominant fatty acids: C18, C12 and C14 respectively, which are derived from the diet of plant steppes. Regarding the diet based on the concentrate, we found the same fatty acids found in the previous diet, based on steppe plants: C16, C18, C12 and C14.

This study does not give any meaning to the total content of SFA at the level of these meats, and this may explain according to [18] in that sheep meat is naturally rich in SFA, and these results from the biohydrogenation of PUFA contained in the diet.[19] has demonstrated that a high carbohydrate intake from food and accumulated in the rumen leads to a decrease in rumen uptake, which will mitigate the biohydrogenation of polyenic fatty acids. [12] found that the SFA homology of these two regimes suggests that some intrinsic factors that are uncontrollable during sample collection (such as race and sex), as well as other extrinsic factors (such as diet and age at slaughter) play their part. [20] reported that palmitic acid (C16) can disrupt and unbalance plasma fibrinolytic activity; in addition, C14 meristic acid, palmitic acid (C16), and stearic acid (C18) may involve glycoregulation, which may promote the onset of type 2 diabetes in the consumer. This non-significant difference in intramuscular meat SFA from both diets studied is consistent with the results of several studies[21, 22-23-24-12],In contrast, in an experiment that was performed by [25] to determine the effect of feeding for 60 days on the lipid composition of sheep meat, two types of diets were provided to animals: grass grazing and grazing with feed supplement. The results showed that grazing sheep receiving a feed supplement had a high SFA content, compared to those from grass pastures. Despite a significant difference observed for mono-acids for the total content of monounsaturated fatty acids (MUFA), it appears that the latter does not present any significant difference for meats produced with both diets. The same finding was made for C18: 1-c9, which may be due to variation in stearoyl-CoA desaturase (SCD) activity, which will influence its synthesis in intramuscular lipids [26]. These results are in agreement with those of [22] in sheep, and as also cited by [27] for cattle finished pastoral or mixed. Diet is primordial factor that influences the lipid composition of meat and which undergoes microbial actions as soon as it is in the rumen by two processes: lipolysis and hydrogenation [28; 29].

The results reveal that the content of total polyunsaturated fatty acid (PUFA) is not affected by the diet, and this is in concordance with the results published by many researchers [30; 31].In a comparative experiment involving two diets provided to sheep, one herbal and the other concentrated, realized by [32], they demonstrated that there is no difference in the PUFA content of milk for these two diets; however, other authors have found that the PUFA content can be affected by the pasture diet, increasing PUFA-n3 to the detriment of PUFA-n6 compared to the concentrate based diet[13, 33-34].This is explained by the fact that fatty acids from fresh plants are more protected against bio-hydrogenation, as explained by[35], and on the other hand, the proportion of PUFAs is higher in sheep grazing, and this could be explained by a high protection against the actions of biohydrogenation of fatty acids of fresh plants or ensilages. However, [36]have shown that this elevation could also result from secondary metabolites of fresh plants that may inhibit rumen flora.

The α-Linolenic acid 18: 3n 3 (ALA) is the most dominant fatty acid in plants, compared to 18: 2n 6 (AL) [37]. In contrast, fatty acid "AL" is considered as the main fatty acid of the concentrated food, in comparison with "ALA" [38]. Our results report that the most dominant fatty acid in both diets is 'AL', followed by arachidonic acid C20: 4n6 (AA) and ALA in herbal meat, while in sheep fed on concentrate, the AL is followed by the predominance of the other fatty acids: AA, C18: 2-t9, 12 and ALA respectively. Thereby, this could be related to the phenomenon of remanence, according to [13], and that a short period of the concentrate does not completely affect the intramuscular lipid fraction outcome from plants, half of which has already disappeared within the next 20 days, but which changes completely 40 days later. This does not prevent us from thinking that this is probably due to the low number of repetitions in each diet, given the logistical problems encountered in the field.

Other studies have shown that AL and ALA are essential fatty acids, and considerable proportions of these acids flee at saturation, and undergo various metabolic processes, forming long-chain polyunsaturated fatty acids by intervention of desaturation and elongation enzymes. With regard to LA, the latter is metabolized to eicosatrienoic acid C20: 3n6, which in turn is converted to AA. However, ALA is metabolized to EPA, then to DHA [39, 40].

The PUFA content of the long chains obtained, is represented by AA, EPA and DHA, is considerably significant; however, the total PUFA content in meats from both diets has no significance. [41], indicated that the use of alfalfa hay as the only diet given to sheep, in a well-defined daily amount, offered only reduced amounts of PUFA n-3.

[42]have suggested introducing supplements such as whole flaxseed or flaxseed oil, which is naturally protected against biohydrogenation or even to give fish oil in the ration of ruminants, may certainly raise the levels of PUFA n-3 long chain (C20-C22).In a meta-analytic study realized by [43], the latter reports that a reduced PUFA n-3 diet is beneficial for human health. According to [44] an herbal ration allows to improve the fatty acid profile in ruminants, and it is possible to improve by adding PUFA-rich dietary supplements (oils or oleaginous).However, they may be hydrogenated, nonetheless, this addition is only possible if the fodder negatively influences the quality of the meat. This situation evoked the efforts made to protect PUFAs against biohydrogenation, such as the introduction of species rich in secondary metabolites in the diet, as well as encapsulation. Human health is susceptible to some chronic diseases, as reported by [45] that long-chain PUFA-n3 anticipates health disorders, such as cardiovascular diseases, inflammatory diseases and cancer. Furthermore, remarkable results have been reported by [46]: in 19 cohort studies, long-chain PUFAs (ALA, DPA and DHA) were reported to be integrated with a lower density in the risk of fatal coronary artery disease, although DPA has a tendency to be incorporated with a reduced risk in fatal coronary artery disease.

Reports abundantly employees to define the nutritional value of fatty acids to determine a diet with a positive impact on human health, as recommended by [47]; the latter insists on the need for the PUFA / SFA ratio value to be greater than 0.45. Nevertheless, the n-6 / n-3 ratio always remains below 4, and according to [40], the recommended value for the n-6 / n-3 ratio should in no case exceed 4. In the present study, the value of the n-6 / n-3 ratio was for sheep in steppe areas in mean of 0.13, which is consistent with the results of many researchers[23, 48]: 0.25 and 1.77, respectively.

[49]reported a high concentration of PUFA-n3 long chains in sheep fed on herbs, with a decrease in the n6 / n3 ratio. However, the C18: 3-n3 content in our study was 0.46 for the plant-based diet, versus 0.2 for the concentrate diet. Our result reports on the PUFA / SFA ratio, proportions (Grass 0.34 vs 0.28 concentrate) lower than the value recommended by the UK Department of Health. Indeed, these low values are similar to those reported by [50, 51-52-53]. This difference is due in part to the nature of the pastures [54], and on the other hand to the breed of the animal [55]. [56]have also reported that grazing and the sheep breed affect the quality of fatty acids in functional tissues of the animal.
"The authors have declared that no competing interest exists".

CONCLUSION

The results of this study highlighted the incidence of different dietary treatments on intramuscular lipid metabolism, which had a limited effect, not differ in their total composition, namely the SFA, MUFA, PUFA. This can be due to the phenomenon of remanence; whose fatty acid profile seems to be approximate in the muscles. Overall, and given the above, these results show on the nutritional plan that the fatty acids have potential to reduce the risk of being affected by chronic diseases in the human being, and to produce suitable sheep at desirable stages for a health safe and sane, by which sheep farming in steppe rangelands could be recommended.

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