

ORIGINAL ARTICLE

Interest of poly-unsaturated fatty acids n-3 of grazing Herbs on phospholipids, triglycerides, cholesterol and fatty acids of Rumbi Lamb meat

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ABSTRACT

In North Africa, the consumption of lamb meat was and still traditionally prevalent as it provides 50% of animal proteins on the consumer's dish. This trial aims to assess the amount of lipids: triglycerides (TG) and phospholipids (PL), cholesterol and meat fatty acids (FA) from grazing lambs (G) compared to ones on a standard diet (S). For this purpose, 24 lambs of local breed "Rumbi" were used. After rearing for 90 days followed by a slaughter process, samples of Longissimus Dorsi (LD) from carcasses were extracted and analyzed. The results showed that TG and PL were higher by +24.45% and +17.55% ($p < 0.05$) respectively in meat from G than meat from S, and that less cholesterol was observed (-35%). Moreover, the poly-unsaturated fatty acids n-6 (PUFA n-6) were less pronounced in group G showing (3.60 vs 4.64%, $p < 0.05$), in contrast with PUFA n-3 that presented a significant increase (1.40 vs 0.41%, $p < 0.001$), which correlates with the antioxidant factors dominating in the analyzed pasture herbs such as tocopherol (+58.87%), flavonoids (+83.21%) and polyphenols (+26.27%). As a result, the ratio n-6/n-3 came in favor of the grazing lambs G with 2.79 as opposed to S with 12.08, consequently making the nutritional benefit very obvious, besides, no significant difference was seen regarding the carcass yields (49.93 vs 51.23%). Finally, it is recommended to boost this approach and promote the local breed and phylogenetic products aiming to take seriously these opportunities as part of a pilot action for resource diversification.

Keywords: Extensive rearing, Grass, Lamb meat, Lipids, Longissimus Dorsi, Antioxydants.

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INTRODUCTION

Meat has a very variable quality of saturated and unsaturated fats, influenced by several factors such as the animal species, the piece and type of muscle, the farming conditions and the type of diet [1]. Their contents in the muscles of grass-fed lambs, compared to those of lambs fed with concentrate, vary from 10 to 15% [2,3], in favor of unsaturated fatty acids [4], with different distributions between the classes of neutral TG and polar PL lipids [5,6].

The herb promotes the tissue deposition of PUFA with cholesterol-lowering properties, with the simultaneous contribution of foodborne cholesterol, less than 30% [7,8]. Plant resources potentially usable by ruminants include natural herbaceous and shrubby vegetation, composed of perennials, woody species and grasses [9].

This vegetation is generally subdivided into quite specific plant formations such as *Stipa tenacissima* or *Lygeum spartum* in clay soils or *Artemisia herba alba* in fine-textured soils, remt courses (*Arthrophytum scoparium*) and the psamophyte and halophyte steppe [10]. It is important to mention that the particular aptitudes of these phylogenetic resources rich in PUFA and bioactive molecules with an antioxidant

activity that are attractive, undervalued and unrecognized in the southern Mediterranean, have been intelligently developed at the international level and have enabled the development of cultivars that are worthy of interest [11].

The aim of this trial is to assess the impact of lipids, bioactive factors and natural trophic resources of grazing lands in Algeria on the quality of intramuscular adipose tissue issued from lamb meat of local breed reared in the arid climate.

MATERIAL AND METHODS

Animals

Under the supervision of the Laboratory of Food Technology and Nutrition in the university of AbdelHamid Ibn Badis in Mostaganem, Algeria, a group of lambs G (n=12) of local breed (Rumbi) (12.25 ± 2 kg and 90±5 days of age) was raised after weaning exclusively on grass grazing, rotating in arid climate in western Algeria (33°32'40.6 "N 0°16'46.1 "W) during the spring season for three months until the hay season. The grass mainly contained *Aristida pungens*, *Retama raetam* and *Atriplex Halimus*, *Suaeda fruticosa*, *Frankenia thymifolia* and *Salsola vermiculata*. On the other hand, a control group S (n=12) in intensive livestock farming was flattened by a standard feed composed of (55% maize, 27% soya flour, 17% bran and 1% minerals), distributed ad libitum with fodder after an adaptation period of one week. Before weaning, both lots belonged to a herd in the experimental area.

Representative samples of the diets were taken for possible physico-chemical analyses, and weight measurements were performed weekly for the calculation of the Average Daily Gain (ADG) during the test and also at slaughter. Animals judged to comply with the conventional classification grid [12], characterizing weight, fat cover and conformation, are slaughtered in a local commercial slaughterhouse at an age of 180±10 days. Their carcass yield was calculated after cooling at 4 C°. Samples (100g per sample) of the long dorsal muscle (*Longissimus dorsi*) were conditioned and stored at -20°C for further analysis.

Measurements and Analysis

The total lipids (TL) of each sample (meats and diets) were extracted and methylated respectively by the methods of [13] and [14]. The methyl esters of lipid extracts from meat and diets were separated and quantified by a gas chromatograph (Perkin-Elmer Auto System XL) with FID detector.

The lipid classes (TG, PL) in meat were determined by separation on SEP-PAK columns (Waters Corporation, Milford, USA) using the method described by [15]. 80mg of lipid extracts are rinsed in 500 chloroform µl and then introduced into the silica cartridge. 30 ml of solvent A (92 ml/8ml) of petroleum ether and diethylene ether is introduced into the cartridge. The TG content entrained and filtered under a slight vacuum, are collected in a tared Balloon 1. 30 ml of solvent B (methanol) are introduced into the same cartridge. After filtration, the PL are collected in a tared Bag 2.

The cholesterol is determined by the method of [16]. The process consists of reacting the alcohol function of cholesterol with a chloroformic extract to which acetic anhydride and sulphuric acid (catalyst reaction) are added. It is a specific colored reaction of the 3 β-hydroxy-steroids with a 5-6 double bond (λ : 680 nm). A standard curve is drawn.

$$\text{Concentration (mg/ml)} = \frac{\text{OD}}{\text{a}}$$

$$\text{Concentration (mg/100g of tissue)} = \frac{\left(\frac{\text{OD}}{\text{a}}\right) * 9 * 1000}{\text{mg lipids} * \text{TL}}$$

Where: OD: Optical Density,

a: Slope of the standard curve of cholesterol,

9: Total volume of the tube in ml,

1000: to calculate in 1 g TL in relation to the weighed mass in mg.

The tocopherol acetate was measured by HPLC according to [17]. The flavonoids and polyphenols were measured respectively by spectrophotometry at 535nm according to [18], and at 765nm according to [19].

The Malondialdehyde (MDA) in nmol/g is measured by the TBARS test in kinetics [20]. Estimations of the Digestible and Metabolisable energy (DE and ME) of diets were made according to the work of [21] and [22].

Statistical Analysis

The data were analyzed using the software statistical analysis system (SAS) (GLM) procedure of [23] by analysis of variance (ANOVA), followed by a comparison of means with Bonferroni test. The level (p<0.05) was considered the significance level.

RESULTS AND DISCUSSION

Total, Neutral and Polar Lipids

The total lipids (TL) were higher in meat from S at $p < 0.05$ (+26.03%) (Table 1). The TG were very pronounced in both meat types slightly dominated by meat from G (+24.45%). However; the PL showed a dominance of +17.55% in meat G over S at $p < 0.05$ (Figure 1).

The variation in total intramuscular lipids was influenced by diet lipids (4.73 vs 3.28%), by the growth rate of lambs in both lots (0.360 vs 0.180 kg/day), and their live weight at slaughter (48.04 vs 29.84kg, $p < 0.001$) for S and G respectively.

The amount of fat (-26.03%) deposited in grass meat G versus meat S had a slight quantitative influence on carcass yield (49.93 vs 51.23%, $p > 0.05$). This relates to the types of low-energy vegetation compared to the concentrate DE (2339.63 vs 2994.13 Kcal/kg MF) and ME (1885.44 vs 2484.82 Kcal/kg MF) respectively, in accordance with the results of [24] and [25]. High levels of intramuscular lipids are theoretically accompanied by an increase in the proportion of TG, relative to adiposity [26]. The TG/TL disproportion reported in lot G was probably the consequence of mobilizing reserve lipids to meet the energy needs of travel along pasture routes.

Polar fats (PL) are relatively rich in PUFA from 35 to 50% [27], against neutral fats (TG) which are relatively low (from 5 to 15%). The linoleic acid (LA) is preferentially incorporated into PL while the alpha linolenic acid (ALA) is incorporated into both TG and PL. These results are comparative with the observations of [28], and show that the consumption of green fodder also leads to a significant increase in PUFA-LC particularly the EPA and DPA in muscular PL, which can reach 20 to 25%. This is not negligible in human nutrition according to [29], despite the competition between desaturases and elongases which are common for n-6 and n-3 FA.

This significant fraction of PUFA-LC in lean meat is related to the proportion of PL in accordance with [30] who states that the total PUFA-LC (n-6 and n-3) of lean meat represent 2.2 to 4.6% of total fatty acids (TFA), while in fat meat, they represent only 0.7 to 3% of TFA. Their proportion in muscles with oxidative metabolism is therefore lower than in glycolytic muscles [31], which affects the nutritional quality of the lipid component. The authors [32] and [33] made the same findings by comparing several types of muscles.

On the other hand, in animals S and G slaughtered at two different weights (+37.88%) and a different ADG (+50%), the proportion of PUFA n-3 in light lamb muscles G was higher compared to heavy lambs S in agreement with [34], this is due to the difference between the composition of the covering fat and the muscle. These PUFA n-3 variations are likely related to the degree of fattening in the animals, which could influence the proportion of PL in the muscles with a decrease in PUFA proportions, especially n-3 type in lamb meat raised by a concentrated feed.

These projections may be used to reduce adiposity in favor of muscular tissue to meet human nutritional and dietary needs, hence the importance of energy alternatives such as oilseed and pasture grass.

In addition, [35] report that ALA originating from pasture grasses has a glycolipid form characterized by its low sensitivity to hydrolysis at the rumen level unlike the triglyceride form of ALA in oilseeds, which allows it to reduce the intensity of the biohydrogenation process which can be greater than 80% [36,37], and to promote its deposition and synthesis of PUFA-LC.

This beneficial effect of grass consumption on the preferential deposition of PUFA n-3 in the muscles is proportional to the duration of grazing [5], and to the absence of a finishing phase [35]. Similar results were obtained by [38] and [39] on young bulls.

Finally, the exploitation of the relationship between the nature of dietary fat and the quality of the deposited fatty acids promotes the orientation of livestock production towards products with an enhanced health value [40].

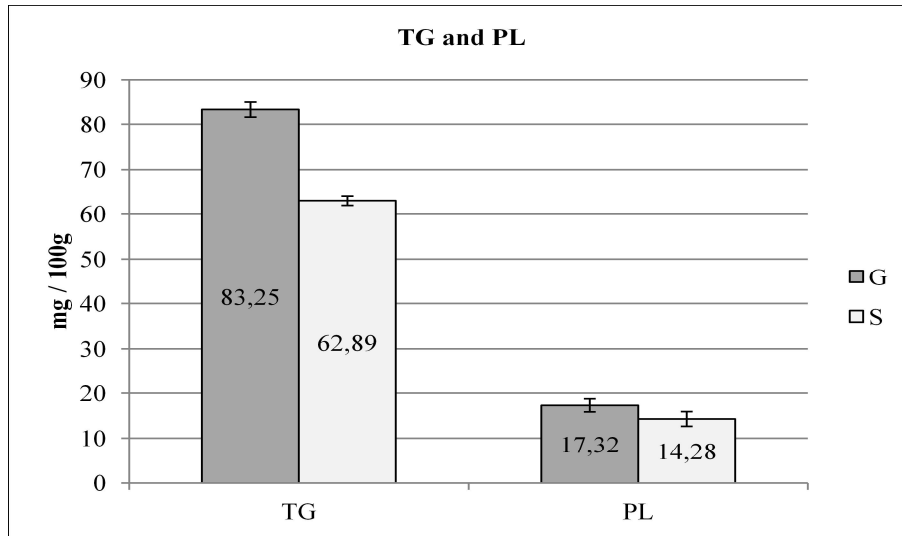


Figure 1: TG and PL in mg/100g of meat

Cholesterol

Grass meat G had low cholesterol levels (-35%) compared to meat S (8.62 vs 13.28mg/100g, $p < 0.05$) (Figure 2), in contrast with the high values reported by [41] (50 and 80 mg/100g), probably related more to the studied muscle than to the amount of food consumed.

The results of the trial agree with some authors who affirm that meat low in TL and high in PUFA, particularly n-3 of meat from light animals, is lean and dietetic [42]. The quantities of PUFA n-3 sufficiently available in the ration reduce cholesterol levels through a decrease in hepatic synthesis of very low density lipoproteins (VLDL), despite the endogenous lipogenesis of cholesterol being the most common steroid with a diverse physiological function [43].

Moreover, the nature of dietary protein and rearing factors have also a significant influence on the quality of meat lipids in livestock, which are currently lower in fat [44]. It should be noted that the lipoproteins of ruminants are of high density (HDL, 80%). They exercise a major role in the transport of cholesterol between the liver and peripheral tissues. Their contents vary according to nutritional conditions and increase proportionally with the fraction of TG or according to the cholesterol of the ration [45].

Indeed, with a good pasture management, the ingested grass remains at a leafy stage with a digestibility greater than 70%, which is a reflection of its morphological composition in favor of the leaves in relation to the stems, which therefore limits slightly the ingestion and optimizes feeding [46]. Under these conditions, the animal can meet its nutritional needs and ensure productivity of sufficient quality for consumers [47].

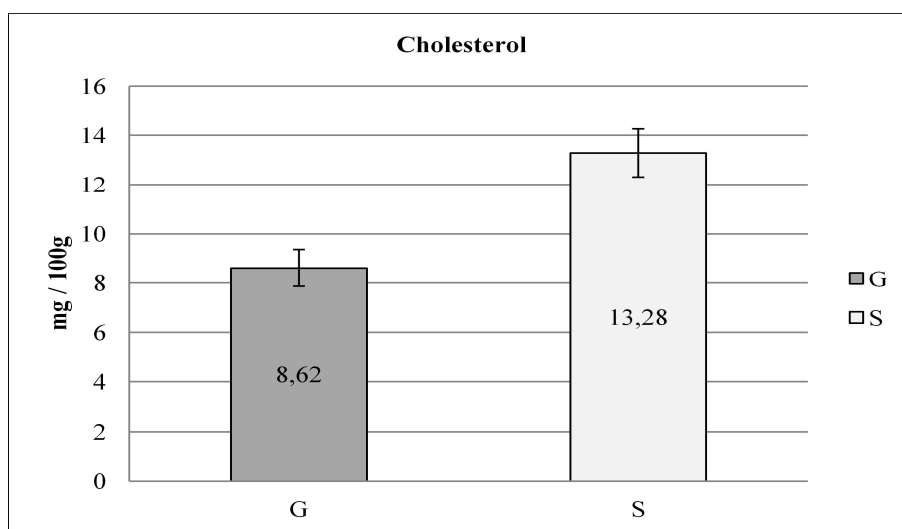


Figure 2: Cholesterol in mg/100g of meat.

Fatty acids

The saturated fatty acids (SFA) studied individually (C16:0 and C18:0) showed no significant difference between the two types of meat. However, mono-unsaturated fatty acids (MUFA) had a lower content in meat G compared to S, in particular oleic acid (C18:1 n-9c) expressing a difference of 15.9% ($p < 0.001$). These results align with those published by [24] and [48].

Regarding PUFA, the ALA was higher in meat G than S (+72.91%) (Table 1) due to the richness of herb in n-3 (+93.73%) (35.17 vs 2.19%) and antioxidant factors respectively tocopherol (1246.83 vs 582.81 μg to $\text{co}/100\text{g}$), flavonoids (8.58 vs 1.44 mg Eq quercitine/ g MS) and polyphenols (9.59 vs 7.07 mg Eq gallic acid / g MS), which provide protection against lipoperoxidation with a low MDA level from T_{60} (19.71 vs 30.18 nmol/g , $p < 0.05$).

Table 1: Fatty acids from meat as a % of identified FA.				
	G	S	SEM	P
TL %	12.16 ^b	16.44 ^a	3.03	<0.05
C14:0	6.93 ^a	2.94 ^b	1.82	<0.01
C16:0	26.65	24.67	1.73	NS
C18:0	18.67	19.23	0.39	NS
C18:1 n-9	35.98 ^b	42.79 ^a	4.82	<0.001
C18:2 n-6	2.74	3.40	0.47	NS
C18:3 n-3	0.96 ^a	0.26 ^b	0.39	<0.001
C20:0	0.17 ^a	0.12 ^b	0.04	<0.01
C20:3 n-6	0.05	0.02	0.02	NS
C20:4 n-6	0.51	0.31	0.14	NS
C20:4 n-3	0.14 ^a	0.00 ^b	0.10	<0.01
C22:4 n-6	0.01 ^b	0.07 ^a	0.04	<0.05
C22:5 n-3	0.21 ^a	0.04 ^b	0.12	<0.001
C22:6 n-3	0.03	0.00	0.02	NS
SFA	54.45 ^a	48.00 ^b	4.56	<0.001
MUFA	40.51 ^b	46.95 ^a	4.56	<0.001
PUFA	5.05	5.05	0.00	NS
n-6	3.59 ^b	4.64 ^a	0.74	<0.05
n-3	1.39 ^a	0.41 ^b	0.69	<0.001
LA/ALA	3.20 ^b	14.31 ^a	7.86	<0.001
n-6/n-3	2.79 ^b	12.08 ^a	6.57	<0.001

a, b Values in same line for the different exponents are significantly different. NS is Non-Significant difference.

The PUFA-LC were represented mainly by C22:5 n-3 Docosa pentaenoic (DPA) with a proportion of 80.95%. This clear dominance in grass meat is in agreement with the results of [49]. The LA content is not significantly different between the two groups, although the sum of the FA n-6 show a difference ($p < 0.05$) in favor of meat S. The reason is probably due to the inhibition of hydrogenation C18:1 to C18:0, despite the richness of the concentrate diet in C18:2 n-6 compared to grass feed (50.99 vs 18.42%).

Consequently, the LA / ALA and n-6 / n-3 ratios decrease much more in meat G compared to S responding to nutritional recommendations for SFA often incriminated in case of excess as a pro atherogenic factor [50].

Other work [51] has shown that FA concentrations are linear in terms of total fat content, but that their slopes are different according to diet. These conflicting bibliographic results are probably related to FA metabolism, season or breeding system.

CONCLUSION

Our study reveals that Algerian lamb meat of local breed issued from grazing in arid areas has been enhanced by the richness of herb in PUFA, this was mainly seen in the n-6/n-3 ratio that was brought closer to the international norms. Moreover, the abundance of antioxidant factors in grass provides a better protection for this meat against lipoperoxydation ensuring a better storage. Therefore, this projection encourages the dynamics of extensive rotational rearing systems, towards developing products with nutritional benefits and supplying a healthier meat to the consumer.

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CONFLICT OF INTEREST STATEMENT

The authors declare no financial and commercial interest.

ETHICAL STANDARDS AND INFORMED CONSENT

“All institutional and national guidelines for the care and use of laboratory animals were followed.”

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