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Trace element content, Amino acid composition and fatty acid profiles and of Timadidite's lamb meat, raised in an agro-silvo-pastoral system of the Moroccan middle-Atlas

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Abstract. Traditional food products are often considered beneficial and healthy Foods. Timahdite sheep breed is the main sheep breed raised in the middle-Atlas in Morocco. The breeding of this local rustic breed plays a crucial socio-economic role in this mountain area. Indeed, it generates a significant economic input for the rural population and plays a role of valuing natural resources in forest areas. Little information is available on Timahdite sheep meat produced in a traditional production system. This study aimed to provide more insights on the quality and nutritional value of Tamahdite's sheep meat, which is in the process of being labeled “Protected Geographical Indication” as a protected product from the Middle Atlas. The longissimus lumborum is used for meat analysis and quality parameter determination. Analyses of amino acid composition show that this meat has a high protein value with a protein digestibility-corrected amino acid score of 325.09 and EAA index of 162.20. The fatty acid profile analysis shows that this meat has polyunsaturated fatty acids (PUFA)/saturated fatty acids and n-6/n-3 PUFA ratios of 0.50 and 9.60, respectively. In addition, the results show that this meat contains 5.70 mg/100g of trace elements, where zinc and iron are the most abundant.

1 Introduction

Meat is a major source of nutrients for humans, especially oligo-elements, essential amino acids (EAA), and fatty acids (FAs). Because of its high nutritional value, meat is a staple food in humans' alimentary diet. Since the past decades, meat consumers are interested in healthy and wholesome products. Consequently, particular attention is paid to local products prepared under traditional production systems, generating a growing demand for labeled sheep meat. The mountainous areas of the middle Atlas located at an altitude of more than 1000 m cover 26% of the surface of Morocco. Despite their spatial and demographic importance, these regions have been relatively neglected in rural development plans, due to hostility from the surrounding environment and development difficulties in comparison with the plain areas. In these regions, extensive rearing, mainly that of small ruminants of local breeds, represents the main, if not the only source of income for rural households, given its biological (rusticity of local breeds) and economic (low cash flow requirements) adaptation to the constraints of the environment.

The Timahdite breed (Bergui) is an autochthon sheep breed from the middle Atlas of Morocco and remains in terms of numbers the dominant sheep breed in this geographical area. It is a breed characterized by its hardiness, its adaptability, its prolificacy, and its performance in fattening and industrial crossbreeding [1]. Aware of this potential and given the agricultural production globalization, the Ministry of Agriculture has given a certain priority to the sheep sector in its rural development programs of the Moroccan economy. Since 1980, the Timahdite breed has been included in the national program for the genetic improvement of sheep. Firstly, the main objective of this program was the conservation of local breeds in their geographic areas called "cradles of pure breeds". Secondly, this program focused on improving the weight production of animals. In this program, the

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characteristics linked to the quality of the carcass and the meat remained unrecognized, no importance was given to the distinctive signs for the sheep meat quality. We chose to analyze meat from Timahdite sheep reared under this typical and traditional system of middle Atlas, because such information is little available and the results will contribute to promote and enhance this meat for potential uses. On the other hand, this study could contribute to the diversification of mountainous areas, preventing them from depopulation. The main purpose of the present study was to determine the amino acid composition, trace-element content and fatty acid profile of Timahdite sheep breed produced under the agro-silvo-pastoral system.

2 Material and methods

The evaluation of the nutritional value of Timahdite sheep meat was carried out on triplicate for each sample and each parameter.

2.1. Animal material

The study was conducted on twelve (n=12) 7-month-old female lambs of Timahdite sheep breed with a pre-determined live weight range between 33 and 37 kg, weaned at 3 months of age. Lamb selection was done with the assistance of an official of ANOC – middle Atlas Morocco and a veterinarian. This study was conducted in the middle Atlas mountain area (El-Hajeb province, Morocco; (longitude −5:49◦. latitude 33.69◦. and altitude 773 m). The sheep farmer is a member of the ANOC- Consortium Dir-El Hajeb; they adopted an annual rhythm of lambing and practiced a semi-extensive breeding system. The Timahdite breed was raised under the agro-silvo-pastoral system, where the pair rangelands/forest was used throughout the year. The diet of the animals was based on pasture when available and the supplementation of 150 to 250 g/day, mainly barley in drought, hunger season, and in some physiological periods (flushing and steaming). The dominant plant species in this geographical area were *Quercus rotundifolia. Stipa tenacissima. Dactylis glomerata. Festuca spp., Cynosurus elegans. Crataegus laciniata. Arrhenatherum elatius,* and *Juniperus phoenicea.*

The *longissimus lumborum* (LL) samples were taken 24 hours post-mortem were trimmed and freeze-dried at –60◦C for 72 h. The meat powder was stored under vacuum in polyethylene bags at −18◦C, until subsequent analyses

2.2 Oligo-element content

After mineralization, the samples were analyzed by atomic absorption spectrometry (Perkin Elmer Atomic Absorption Spectrometer Analyst 200) according to the method described by the International Standard Organization [2].

2.3 Essential amino acid composition

300 mg of freeze-dried meat was dissolved in 10 ml of hydrochloric acid (6 N) with 0.1% of phenol and hydrolyzed for 24 hours at 110°C. Subsequently, the pH was adjusted to 2.2 with NaOH (7.5 N). The norleucine 50M was used as an internal standard. Then, the homogenate was diluted with a citrate buffer. After filtration, it was analyzed using a high performance liquid chromatography (Biochrom 20 Plus amino acid analyzer, Pharmacia. Cambridge. UK) equipped with Sodium-oxidized column cation-exchange resin; Post-column derivatization of the amino acids to ninhydrin; and Spectrophotometric detection at 570 nm. The protein value was assessed according to the method proposed by FAO/WHO/UNU [3], by calculating the chemical index (CI), Protein digestibility-corrected amino acid score (PDCAAS), and index essential amino acids (IEAA). Chemical index = the minimum ratio between the percentages of essential amino acids (EAA) of the tested protein, compared to each of the corresponding EAA present in the pattern protein [3]. PDCAAS = (CI × true digestibility of the meat). The IEAA was determined according to the following equation [4]:

$$\sqrt[n]{\frac{a}{ap} * \frac{b}{bp} * \frac{c}{cp} * \dots \frac{j}{jp}} \quad (1)$$

2.4 Fatty acid composition

The intramuscular fat (IMF) has been extracted by a cold method using the solvents-water mixture (chloroform/methanol/water (2/1/1; v/v)). Afterwards, the extracted IMF was methylated, converted to fatty acid methyl esters (FAME) and analyzed in gas chromatography-FID according to the method described by Ben Moumen, Mansouri [5]. The FAME standard, containing 37 components (Supelco. Bellefonte. PA. USA), was used to identify the different peaks. The lipid indices were calculated from fatty acid profiles and results were expressed as a percent of the total FAME.

3 Results and discussion

Red meats often have a bad reputation with dieticians who deem them too high in saturated fat [6]. Recent studies have shown that certain fatty acids in these meats can have a very favorable effect on human health such as long-chain polyunsaturated fatty acids n-3 and conjugated linoleic acid [7]. In addition, ruminant meat is an important source of protein-rich with essential amino acids and a source of iron and zinc that are much more bio-accessible than those from plants [8, 9].

The results of trace element content, amino acid composition and fatty acid profile of *longissimus lumborum* muscle of Timahdite sheep breed are summarized in Tables 1, 2 and 3, respectively.

3.1 Trace-element content

Red meat is a major source of iron, heme iron with high bio-availability [10]. The previous study reports that the oligo-elements in fresh meat have limited bioavailability and bioaccessibility, except for iron [11]. Sheep meat is a particular source of oligo-minerals to a healthier lifestyle, particularly heme-iron, which consists primarily of erythrocyte [10]. The trace element contents of Timahdite sheep meat are shown in Table 1. Zinc (2.66 mg/Kg) and Iron (2.93 mg/Kg) were the two most abundant trace elements, whereas selenium (0.02 mg/Kg) was the smallest. The obtained results are in the same order than those reported by Polidori, Pucciarelli [12] for Fabrianese PGI-lambs. Then, our outcomes are higher than those reported by Kasap, Kaić [13] in meat for 3 Croatian sheep breeds. This reported difference could be related to the rearing system (pasture availability), the animal's age at slaughter and also to the muscle type [9, 12]. The wealth recorded in iron and zinc for Timahdite lambs meat could be explained by the agro-silvo-pastoral system adopted by the middle Atlas breeders. In this regard, Cabrera and Saadoun (2014) reported that the lamb meat produced under a pasture-based system contains more oligo elements than that of a concentrate-based system.

Table 1. Oligo-element content of Timahdite sheep meats.

Oligo-elements (mg/100g fresh Matter)	**Mean**	**SD**	**Min**	**Max**
Copper	0.09	0.01	0.07	0,10
Zinc	2.66	0.17	2.37	2,9
Iron	2.93	0.8	2.29	4.57
Selenium	0.02	0.00	0.02	0.02
∑ micro-element	**5.70**	**0.86**	**4,76**	**7,25**

3.2 Amino acid composition

The biological importance of proteins is linked to their bio-availability and bio-accessibility. The protein of red meat is one of the major sources of essential amino acids in food [9]. Amino acid composition determined in LL muscle taken from Timahdite sheep breed lambs is presented in Table 2. This analysis conducted by a high-performance liquid chromatography allowed the identification of eight EAA (Valine: 2.73; Methionine: 2.31; Isoleucine: 3.26; Leucine: 4.8; Phenylalanine: 3.40; Histidine: 2.63; Lysine: 4.17: Threonine: 3.6 per 100g of dry matter). Leucine and Lysine were the prevalent EAA found, followed by threonine, glycine and phenylalanine while the lowest values were found in methionine followed by valine, and histidine. Polidori, Pucciarelli [12] obtained similar results in suckling meat of Fabrianese sheep breed, in which the represented EAA were in the same order as that of the present study. The average concentrations of EAA in the tested meat are generally higher than those reported by [12] for Fabrianese PGI-lamb meat. The chemical index, the protein digestibility-corrected amino acid score and IEAA values were 345.84, 325.09 and 162.20, respectively. This high protein value recorded for this typical meat could be explained by the rearing system adopted in the middle-Atlas, which is based on varied fodder resources from agricultural land, natural meadows, and forests. In this way, several authors have reported the beneficial effect of the system based on forest resources particularly oak corns (*Quercus Ilex*), which are rich in protein. The obtained outcomes show that the Timahdite sheep meat presents a veritable source of essential amino acids without restriction in these essential nutrients. As a result, the consumption of this local meat could satisfy the human needs in the indispensable amino acids.

Table 2. Essential amino acid profile of Timahdite sheep meats.

AA (g/100gDM)	**Mean**	**SD**	**Min**	**Max**
Val	2.73	0.33	2,42	3,06
Met	2.31	0.07	2,21	2,39
Ile	3.26	0.10	3,16	3,40
Leu	4.80	0.10	4,69	4,94
Phe	3.40	0.05	3,34	3,47
His	2.63	0.21	2,42	2,87
Lys	4.17	0.31	3,84	4,51
Thr	3.60	0.03	3,54	3,64
CI	345.84	42.40	306,35	388,19
PDAACS	**325.09**	**39.80**	**287,97**	**364,90**
IEAA	**162.20**	**0.67**	**161,47**	**163,00**

AA: Amino acids; **DM**: Dry matter; Val: Valine; Met: Methionine; Ile: Isoleucine; Leu: Leucine; Phe: Phenylalanine; His: Histidine; Lys: Lysine; Thr: Threonine; **PDCAAS**: Protein digestibility-corrected amino acid score; IEAA: index essential amino acids.

3.3 Fatty acid profile

The red meat is a good source of essential fatty acids and their long-chain n-3 polyunsaturated fatty acid (PUFA) metabolites, such as eicosapentaenoic and docosahexaenoic acids that are related to improved health [14]. In the past few years there has also been an increased awareness of the potential benefits of conjugated linoleic acid present in the fats of ruminants, especially vaccenic acid (11t-18:1) [15].

In the present study, oleic acid (C18:1n9: 36.26%), palmitic acid (C16:0: 20.72 %), stearic acid (C18:0: 12.98%) and linoleic acid (C18:2n-6: 11.40%) represent more than 80% of the total fatty acid content of the LL muscle of Timahdite sheep meat. The predominant FA was C18:1. Table 3 presents the results of FA profile of LL muscle of Timahdite sheep breed. In general, the intramuscular FA composition in the present study was similar to that reported by Belhaj, Mansouri [16] for Beni-Guil PGI-lamb meat and by Blasco, Campo [17] for Texel sheep meats and its crossbreeding. The fatty acid sums are given in Table 3. The total saturated fatty acids (SFAs: 40.24%) were the prevalent fatty acids, followed by monounsaturated fatty acids (MUFAs: 39.55%) and polyunsaturated fatty acids (20.18%). The SFA effects on human health is among the most controversial topics in nutrition science. Several researchers have reported the undesirable effects of saturated lipids on health (cardio-vascular diseases). However, numerous studies have shown that some FAs of red meats, such as vaccenic acid and long chain n-3 PUFA, have anti-inflammatory, anticarcinogenic, anti-atherosclerotic, anti-diabetic effects and contribute to the protection against autoimmune diseases. Moreover, other studies have reported the favorable role of certain SFAs, for example, the effect of stearic acid on commercial carcass quality, in particular the aesthetic quality (cover fat firmness).

Table 3. Fatty acid composition of intramuscular fat of Timahdite sheep meat

FAs (%)	Mean	SD	Min	Max
C14:0	2.39	0.69	1.80	3.87
C15:0	0.51	0.07	0.39	0.65
C16:0	20.72	1.25	19.08	23.46
C17:0	1.74	0.43	0.90	2.34
C18:0	12.98	0.77	11.71	14.87
C20:0	0.16	0.05	0.02	0.27
C22:0	0.17	0.03	0.07	0.27
C23:0	0.48	0.22	0.21	0.98
C24:0	1.09	0.30	0.51	1.73
SFAs	**40.24**	**1.86**	**38.13**	**44.51**
C14:1	0.32	0.17	0.04	0.70
C15:1	0.24	0.03	0.16	0.31
C16:1	1.14	0.17	0.93	1.80
C17:1	1.31	0.28	0.77	1.74
C18:1n9	36.26	1.77	32.50	39.10
C20:1n9	0.17	0.03	0.10	0.25
C22:1n9	0.11	0.06	0.02	0.35
MUFAs	**39.55**	**2.12**	**35.36**	**43.55**
C18:2n6	11.40	1.56	7.88	13.99
C18:3n6	0.68	0.30	0.25	1.85
C18:3n3	0.49	0.19	.25	1.12
C20:2	0.35	0.08	0.20	0.52
C20:3n6	0.36	0.07	0.26	0.52
C20:3n3	0.09	0.03	0.03	0.17
C20:4n6	5.52	1.37	3.96	9.12
C20:5n3	0.55	0.20	0.06	1.01
C22:6n3	0.74	0.26	0.25	1.13
PUFAs	**20.18**	**1.21**	**17.93**	**21.96**
PUFA n-6	17.26	1.29	15.36	20.49
PUFA n-3	1.89	0.41	1.00	2.53
PUFA/SFA	0.50	0.03	0.44	0.58
ω-6/ω-3	9.60	3.18	6.36	19.60

FAs: Fatty acids**; SFA**s: Saturated Fatty Acids; **MUFA**s: Monounsaturated Fatty Acids; **PUFA**s: polyunsaturated Fatty Acids.

Myristic, palmitic and stearic acids are the predominant SFAs in the studied meat. Ulbricht and Southgate [18] have reported that the palmitic and myristic acids increase plasma low-density lipoprotein (LDL)-cholesterol [7, 19], whereas C18:0 has a neutral effect regarding the blood cholesterol content [20]. On the other hand, the stearic acid is poorly digested and can be converted to oleic acid by the desaturase enzymes action. The SFA recorded value is lower than those reported for several sheep meats; Budimir, Trombetta [21] for Bergamasca lambs (50.91-55.68%), Belhaj, Mansouri [22] for Beni-Guil breed (49.45%) and Yaranoglu and Ozbeyaz [23] for two Turkish breeds (42.44-44.11%). In the present study, the most abundant PUFAs are Linoleic acid (LA: 11.40%) and Arachidonic acid (ARA: 5.52%). Faria, Bressan [24] and Belhaj, Mansouri [22] reported similar results in the meat for Texel sheep meat (Texel*Corriedale and Texel*Polwarth) and Beni-Guil lamb, respectively. Polyunsaturated fatty acid sum is included in the calculation of the PUFA/SFA and ω-6/ω-3 ratios, to assess the lipid nutritional value [25]. These ratios are involved in certain diseases linked to lifestyle. Nutritional recommendations are to increase the level of PUFA n-3 in the diet to reduce the n-6/n-3 [25]. The obtained values show that the Timahdite sheep breed have an interesting PUFA/SFA ratio (0.50) in respect to the recommended value in human nutrition (0.45); this is due to the relatively high proportion of PUFA (20.18%). These results can be justified by the higher levels of PUFA particularly LA and ARA present in this mountainous local-meat. This recorded ratio is higher than that reported by numerous studies [21, 23, 26]. This richness could be explained by the use of forest resources, especially oak corn which is rich in PUFA n-6 and barely (*Hordeum vulgare*) in case of food supplementation or in finishing period [27, 28]. In addition, oak corn contains high levels of antioxidants and anti-bacterial substances, which reduce FA oxidation and ruminal bio-hydrogenation [29]. In addition, the distribution of barley as the main ingredient influences strongly the meat PUFA content. Sauvant and Bas [30] have reported that the food intake rich in carbohydrates affects the residence time of lipids in the rumen and consequently decreases their bio-hydrogenation. Considering the PUFA n-3, the recorded value is lower than those reported in our study on Beni-Guil sheep breed reared in eastern Morocco, because the breeders of the Ain Beni-Mathar region of Morocco feed alfalfa to their herds as the main ingredient, which is rich in PUFA n-3.

Regarding the ratio of PUFA n -6/PUFA n-3, which should not exceed 4.0, is a better criterion to assess food lipid quality. Previous studies indicated that this ratio plays a significant role regarding the risk of atherosclerosis. [25] suggested that lower PUFA n-6/PUFA n-3 ratio decreases the risk of numerous chronic diseases. Therefore, the recorded PUFA n-6/PUFAn-3 ratio was above the recommended value (9.50). This higher result could be explained by the higher levels of C18:2 n-6 and C20:4 n-6 in this mountainous meat reared under the agro-silvo-pastoral system. The origin of this richness in polyunsaturated fatty acid n-6 is certainly due to the oak acorn and to barley, which are rich in this type of fatty acids [27, 28].

4 Conclusion

The present study provides valuable and complete information on essential nutrients of Timahdite sheep meat. This investigation highlighted that this traditional product of middle-Atlas produced under the typical system of this area (agro-silvo-pastoral) has a high nutritional and biological value due to its richness in essential amino acids, polyunsaturated fatty acids' long-chain and oligo-elements. Our study has provided further scientific insight about the nutritional quality of this mountainous meat, in addition to that already included in the breed standard, related only to the phenotypic traits. Therefore, more researches are necessary for understanding more the effect of age at slaughter, rearing season, feeding system and sex on carcass and meat quality. We are grateful to the Moroccan–Belgian bilateral cooperation program for the financial support of this research through "WBI-Project 1-6" 2015–2017. Our thanks are also to Mr. Bennacer Belhaj, president of the "Dir-El-Hajeb" consortium for their collaborations and the provision of meat samples.

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