

## IMPACT OF GREEN FORAGES IN SPRING, IN THE STEPPE REGIONS OF TIARET "CHEHAIMA AND NAIMA", ON THE FATTY ACID COMPOSITION OF THE "BICEPS FEMORIS" MUSCLE OF LAMBS

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### Abstract

*This work aimed to assess the impact of a diet based on green pastures of steppe rangelands and another based on a concentrate diet, on the composition of the intramuscular lipid fraction of different sections of the biceps femoral muscle of lambs, in two regions of the wilaya of Tiaret, in winter and in spring. The biceps femoral muscle sample collection came from 20 different lamb carcasses (10 samples for each of our two study regions, with 05 samples for each season). These samples were taken on different occasions in large butcher shops. Total SFA content in both regions shows dominance in lambs from the regimen concentrate, that C 16: 0 and C 18: 0 are the most dominant. There is no remarkable difference in total MUFA content, and on individual monounsaturated FA, between the two regimens. In both regions, the most dominant FAs in the intramuscular lipid fraction of pasture lambs are C18: 3n3 followed by C18: 2n6 cis-9, C20: 4n6, C20: 5n3, and C22: 6n3, compared to those from concentrate regimen; however, lambs from Chehaima concentrate regimen had the most dominant FA in their intramuscular fat, C18: 3n6 followed by C20: 4n6, compared to those from pastures of Naima. The values of the ratio n-6 / n-3 correspond to the nutritional recommendations. The feed ratio  $\Sigma$ PUFA /  $\Sigma$ SFA of lambs from pastures in the Chehaima region is the only one that corresponds to the desired value. Based on this research, it is concluded that lambs that have grazed grass in steppe rangelands have the richest meat in omega 3, which is more desirable and benefits for human health.*

**Keywords:** Concentrate, fatty acids, grazing, lambs.

### 1. INTRODUCTION

In response to the rapidly increasing demand for lamb meat by the modern consumer, the current research is more interested now in improving the quality of the meat, often from lambs of green pastures. These meats are rich in polyunsaturated fatty acids (PUFA), and which present relatively simple production processes. Our previous study indicated that the nature of the diet is an essential factor that influences the composition of intramuscular fatty acids (FA) in lambs, whose family FAs n3 are more representative in those fed on steppe plants (Rabah et al., 2019), other studies also

confirm the influence of diet on FA (Díaz et al., 2005; Ružić-Muslić, 2012), however, other intrinsic and extrinsic factors may influence and be responsible for the difference in FA such as sex, age, type of breeding, transport to the slaughterhouse, conservation of meat (De Smet et al., 2003 ; Amin and Mao, 2020). Currently, many ruminant breeders tend to modify their feeding practices by reducing the diet based on concentrate, and are now moving more and more to pasture grass which confers little fat in the ruminant carcass compared to that based on concentrate (Daley et al., 2010). Also, the problems associated with the use of concentrated feed in the diet of lambs, such as the increase in the content of saturated fatty acids (SFA) and cholesterol in meat, that causes diseases in humans, as chronic diseases such as cardiovascular disease, which interfere with insulin absorption and tumor carcinogenesis (Klir et al., 2012), An increased interest in rising the grass ratio in lamb feed (Prache et al., 2019). Other studies have shown that the meat of pasture lambs is an essential source of FA of the PUFA family n-3, and which has a lower fat content in the carcass, and with a lower content of SFA, unlike meat from those from the concentrated feeds (Jutzeler van Wijlen and Colombani, 2010; Ponnampalam et al., 2018). Reducing the intake of SFA and increasing the intake of PUFA-n3, especially long chain polyunsaturated fatty acids (PUFALC), are recommended by epidemiologists (Dias et al., 2017; Saini et Keum., 2018). The influence of different types of diets on the individual FA content of lamb meat has been the subject of several studies (Nguyen et al., 2018; Pannier et al., 2018). Pastures, especially with young plants are characterized by low fiber content and supplied to ruminants a high amount of linolenic acid (C18: 3 n-3) which helps to increase the content of conjugated linoleic acid (CLA) and its precursor, the vaccenic acid (18: 1 trans-11); the following biohydrogenation in the rumen by microbial action, C18: 3n-3 (ALA) and linoleic acid (C18: 2n-6) (LA) from forage plants are converted into oleic acid (18: 1), the precursor of the synthesis of CLA which is increasingly taken into consideration by nutritionists, because it contributes to the good health of the human being (Angeles-Hernandez et al., 2020 ; Budimir et al., 2020), however, these PUFAs contained in forage plants are exposed to several factors that can reduce their levels, such as botanical composition, seasonal variations in climate, and the vegetative stage of the plant (Cividini et al., 2018). In contrast, the animals fed the concentrate show a low content of PUFA, in particular, PUFA n-3 (Nuernberg et al., 2005). It seems of our knowledge, that there are only a few studies that have reported the content and composition of intramuscular FA in lambs that grazed in steppe rangelands (Berrighi et al., 2017; Rabah et al., 2019), which makes it very important to provide scientific evidence serving to discriminate diets that may improve the performance quality of lamb meat. This study aimed to study the effects of grazing in steppe rangelands on the AF profile of lamb meat.

## 2. MATERIALS AND METHODS

The lambs are traditionally reared in the pastures of the steppe, located in the two regions of Chehaima and Naima (Tiaret steppe), during the spring period and are supplemented in winter. During the period from March to the end of June, and then during the period from December until the end of February, lambs are feeding with concentrate in the barns. Someones have reported that the concentrated feed is made up primarily of barley (Luciano et al., 2012). Biceps femoris muscle samples collection was done from 20 different lamb carcasses (10 samples for each region, and with 5 samples for each season), were collected on different occasions from various large butcher shops of the Tiaret region. Before starting the biochemical analyzes to quantify and identify the methyl esters of fatty acids of the intramuscular lipid fraction by transesterification, by mixing internal standard 1, 2, 3-tripentadecanoylglycerol in a solution of boron trifluoride to 20% in methanol (Tor

et al., 2005), the collected samples were first of all lyophilized and then homogenized. The gas chromatography used is equipped by an oven at a temperature of 150 to 225 °C. The fatty acids were separated by a capillary column SP2330 of 30 m \* 0.25 mm (Supelco, Tres Cantos, Madrid).

### ANALYSES STATISTICS

Statistical analyzes were carried out in order to treat the intramuscular fatty acids of the biceps femoris muscles which characterize the sheep of the different diets, for this, an ANOVA test was carried out, followed by a Tukey Post Hoc test.

### 3. RESULTS AND DISCUSSIONS

The intramuscular AFs of the present study, as shown in the table, are of 24 fatty acids (C 10 :0, C 12 :0, C 14 :0, C 15 :0, C 16 :0, C 17 :0, C 18 :0, C 20 :0, C 24 :0, C14 :1, C16 :1, C18 :1 cis-11, C18 :1 cis-9, C18 :1 trans-9, C20 :1, C18 :2n6 cis-9, C18 :2n6 trans-9, C18 :3n6, C18 :3n3, C20 :2n6, C20 :3n6, C20 :4n6, C20 :5n3, C22 :6n3) (Table.1). These fatty acids were detected from the two diets, and each region has significant differences in FA composition. The individual AFs obtained from the grass-fed lambs had lower concentrations than those from lambs feed with the concentrate, of which the most dominant in Chehaima region was the stearic acid (C 18: 0) when compared with the others lambs from the pasture of Naima region, while, the most dominant in Naima region was the palmitic acid (C 16: 0), compared to those from pastures in the same region, these two AFs constitute the largest part of the lamb meat fatty acids, respectively with 18.1% and 22.2% (Wood et al., 2004).

**Table 1: Mean and standard deviation of mean of Intramuscular fat content of the Biceps Femoris muscle of lambs (mg/g fatty acids)**

Fatty acids	Chehaima		Naima		Signification			
	Grazing	Concentrate	Grazing	Concentrate	PCh vs CCh	PCh vs CN	PN vsNC	PN vs CCh
C 10 :0	0.03±0.01	0.05± 0.01	0.1± 0.00	0.07± 0.00	**	***	***	***
C 12 :0	0.23 ± 0.09	0.47± 0.17	2.46± 1.68	1.93± 1.34	-	-	-	*
C 14 :0	0.2± 0.11	0.27± 0.08	0.95± 0.51	1.78± 0.39	-	***	***	**
C 15 :0	0.22± 0.08	0.11± 0.07	0.19± 0.01	0.23± 0.02	*	-	-	-
C 16 :0	8.34± 3.18	13.33± 2.17	5.65± 3.45	11.23± 1.68	-	-	*	**
C 17 :0	1.08± 0.40	1.74± 0.61	0.76± 0.06	1.03± 0.06	*	-	-	***
C 18 :0	6.17± 3.87	9.28± 1.18	3.82± 3.05	7.78± 1.03	-	-	-	**
C 20 :0	0.07± 0.00	0.05± 0.00	0.06± 0.01	0.07± 0.00	***	-	-	-
C 24 :0	0.34± 0.09	0.15± 0.03	0.24± 0.08	0.14± 0.04	***	***	-	-
C14 :1	0.07± 0.00	0.08± 0.00	0.07± 0.01	0.10± 0.00	-	*	-	-
C16 :1	0.88± 0.32	1.29± 0.43	0.87± 0.32	1.07± 0.43	-	-	-	-
C18 :1 cis-11	0.70± 0.22	0.74± 0.24	0.69± 0.22	0.81± 0.24	-	-	-	-
C18 :1 cis-9	16.52± 7.43	20± 7.45	16.51± 7.43	19.49± 7.45	-	-	-	-
C18 :1 trans-9	0.91± 0.24	1.18± 0.20	0.97± 0.24	1.14± 0.20	-	-	-	-
C20 :1	0.06± 0.00	0.06± 0.00	0.08± 0.04	0.11± 0.01	-	*	-	-
C18 :2n6 cis-9	3.64± 0.72	3.47± 2.06	2.53± 1.53	4.2± 0.96	-	-	-	-

C18 :2n6 trans-9	0.33± 0.04	0.23± 0.01	0.24± 0.01	0.29± 0.04	***	-	-	-
C18 :3n6	0.03± 0.02	0.04± 0.01	0.32± 0.06	1.81± 0.09	-	***	***	***
C18 :3n3	1.34± 0.80	0.12± 0.05	1.74± 1.02	0.15± 0.06	***	*	***	*
C20 :2n6	0.15± 0.07	0.2± 0.03	0.23± 0.03	0.18± 0.07	-	-	-	-
C20 :3n6	0.14± 0.03	0.18± 0.01	0.21± 0.03	0.14± 0.01	-	-	-	-
C20 :4n6	1.68± 0.40	1.82± 0.36	1.41± 0.23	1.07± 0.09	-	***	*	-
C20 :5n3	0.23± 0.06	0.19± 0.07	0.22± 0.10	0.09± 0.04	-	*	*	-
C22 :6n3	0.2± 0.04	0.03± 0.04	0.12± 0.02	0.06± 0.02	***	**	-	**
SFA	16.66± 7.09	25.42± 2.80	14.24± 3.47	24.03± 2.86	**	-	**	***
MUFA	19.11± 7.78	23.5± 8.06	19.17± 7.79	23.56± 8.07	-	-	-	-
PUFA	7.74± 0.97	6.25± 2.19	6.93± 1.95	6.02± 0.97	-	-	-	-
n6	6± 0.98	5.91± 2.12	4.84± 1.49	5.76± 1.04	-	-	-	-
n3	1.75± 0.80	0.34± 0.08	0.59± 0.24	0.27± 0.09	***	***	-	-
Σn6/Σn3	0.31± 0.16	0.06± 0.01	0.12± 0.03	0.09± 0.02	**	***	-	-
ΣPUFA/ΣSFA	0.53 ± 0.20	0.24± 0.08	0.31± 0.23	0.25± 0.01	-	-	-	-

SFA: Saturated fatty acids. MUFA: Monounsaturated fatty acids. PUFA: Polyunsaturated fatty acids. \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P < 0.001$ ; - : not significant. PCh: Grazing in Chehaima. CCh: Concentrate in Chehaima. PCh: Grazing in Naima. CN: Concentrate in Naima.

Lambs that are feed with concentrate which have exhibited the highest C18: 0 content, has been explained by (Qie et al., 2020), who have found that the concentrated diet increases the C18: 0 content, due to the high saturation of PUFAs; moreover, the total content of SFA reveals a dominance in lambs feed with concentrate, these results are in agreement with those of (Hajji et al., 2016); On the other hand, not only the diet can influence the chemical composition of the meat, but also others factors related to the animal such as breed and age (Erasmus et al., 2017). Concentrated foods are rich in starch, which will increase the intake of FAs by stimulating new ruminal fermentation pathways that increase the saturation of PUFAs (Harmon and Swanson, 2020). Certain supplementary feeds in the ration of small ruminants can modify and reduce the composition of intramuscular SFA and increase those which are unsaturated, by the inclusion of specific oils or oleaginous and marine products (Raes et al., 2004). It is strongly recommended to decrease the total intake of the SFA content, given its harmful effect on human health, while no evidence is taken into consideration as to the effect of saturated fats which vary according to a specific fatty acid for health (Astrup et al., 2019), some of which are beneficial such as lauric acid (C12: 0) which has antimicrobial properties (Nakatsuji et al., 2019), also, the blood concentration of heptadecanoic acid (C17: 0) which does not affect coronary results (O'Neil et al., 2014). Others are not recommended for human health like myristic acid (C14:0) and C16:0 (Nieto and Ros, 2012). The results of the present study indicated that the lambs obtained from the concentrate diet had no significance in the total content of monounsaturated fatty acids (MUFA) than those fed in the steppe ranges, as well as in individual MUFA. These results are in agreement with those of (Moloney et al., 2018) who studied the FA profile of cattle grazed, feed only with pastures, and those fed on concentrate. (Mateo et al., 2017) did not indicate any remarkable difference in the total content of MUFA and on individual monounsaturated FA in small ruminants fed with the concentrate in stable or grazing in

the rangelands during the spring period. In fact, it could be related to the phenomenon of remanence, according to tests carried out by (Aldai et al. 2011) to study the effects of feeding strategies on the lipid fraction of lambs, revealed that after changes in the ration by substituting grazing of the grass by the concentrated feed, half of the FAs were partially changed from the total fat one month after of the experiment, however, after 2 months were gone completely. Contrary to the results of the present study, (Steffen et al., 2018) revealed that a high content of FA C18: 1 can cause the risk of developing cardiovascular diseases, which does not only come from the diet, but it could be affected by other factors that regulate its plasma concentration, as its de novo synthesis from C18: 0 by intervention of Stearoyl-CoA desaturase-1 (SCD1). In the two regions, no difference was observed in the total content of PUFAs; these results are similar to those found by (Panea et al., 2011), in contrast to (Lobon et al., 2019), who found that the grass-fed lambs having high levels of total PUFAs compared to those from concentrate. For both regions, the most dominant FAs in the intramuscular lipid fraction of pasture lambs are C18: 3n3 followed by C18: 2n6 cis-9, C20: 4n6, C20: 5n3, and C22: 6n3 compared to those from concentrate, however, the lambs in the concentrate diet had the most dominant FA in their intramuscular fat, C18: 3n6 followed by C20: 4n6 in lambs from Chehaima compared to those from the pasture of Naima. The feed can provide small ruminants with FA in varying proportions, the composition of which feeds concentrated in PUFA is dominated by LA which is the precursor of PUFALC n-6, whereas, pastures contribute to the high intakes of ALA, which is the precursor of PUFALC n-3, where the biohydrogenation of these essential FAs is around 80% and 92% for LA and ALA respectively. This process is highly dependent on the rate of passage and the residence time in the rumen (Dugan et al., 2018). LA n-6 and ALA n-3 undergo elongation and desaturation in ruminant tissues by metabolism in n-6 (arachidonic acid (C20: 4 n-6)) and n-3 (eicosapentaenoic acid (C20: 5 n-3), docosapentaenoic acid (C22: 5n-3) and docosahexaenoic acid (C22: 6 n-3) (Jambrak and Skevin, 2017). The fatty acid enzymes (SCD 1) and (SCD 2) often interfere with ALA to the detriment of LA, while a diet rich in LA can interfere with the process of conversion of ALA to EPA, DPA n-3, and DHA; this process of elongation and desaturation is slow, considered inefficient compared to the direct absorption of PUFALCs (ALA, EPA, and DHA) from food which can escape biohydrogenation in the rumen, because they are protected by metabolites secondary constituted in the grass, which can play a protective role against the saturation of PUFALCs in the rumen (Vahmani et al., 2020). The same results were found by (Liang et al., 2017) in the milk of cattle in different regions, indicating that the C18: 3 content is higher in grass-fed animals, while C18: 2 is dominant in those fed with concentrate, however, the latter is susceptible to be oxidized. In this study, the intramuscular lipid fraction of grass-based diet lambs was lower in C20: 4 n6 which is explained by its synthesis from its precursor C18: 2 n-6. The appearance of PUFALC C22: 6 n-3 which had high values observed in pasture lambs compared to those in concentrate, was due according to (Díaz et al., 2011) to the elongation and desaturation of C18: 3 n-3. Feeding practices in free ranges advantageously improve the FA profile; on the other hand, the variability of the individual plants that characterize and dominate in these different rangelands can affect the capacity and retention time of FA in the rumen, thus their composition in antioxidants which protect PUFAs, lead to a considerable accumulation of these FAs, in particular, C18: 3n3, the precursor of C22: 6n3 (Elgersma, 2015). Recent simultaneous studies have described lipogenesis in the rumen, as a function of biohydrogenation, which is conditioned by the selection of ruminal microflora depending on the type of food, the variety of plant species, the amount of fiber and starch consumed by the ruminant which can decrease ruminal pH, which inhibits the activity of certain bacteria and

stimulates the activity of a new bacterial population, and consequently, a modification of ruminal metabolism of FA (Dewanckele et al., 2020 ; Frutos et al., 2020 ; Huyen et al., 2020). A high content of PUFA n-3, in particular PUFALC such as EPA, is inversely correlated with the prevalence in young people suffering from depression (Berger et al., 2017). The n-6 content did not show any difference in all lambs; however, the n-3 content was higher in the lambs that grazed the grass, same results were found by (Belhaj et al., 2020) in the lambs and by (Aldai et al., 2011) in cattle. The values of the ratio n-6 / n-3 are correctly balanced and correspond to the nutritional recommendations of the (British Department of Health, 1994), which have prescribed a value that must not exceed 4. These results are similar to (Alfaia et al., 2009), who found a ratio that suits the recommendations in Pasture lambs, whereas, in lambs from the concentrated diet, they had a ratio that exceeded 4. The highest  $\Sigma$ PUFA /  $\Sigma$ SFA feed ratio was found in lambs from Chehaima pastures, at a value of (0.53). According to (McAfee et al., 2010), the desired  $\Sigma$ PUFA /  $\Sigma$ SFA ratio, must be greater than 0.4. However, the values of this ratio in lambs feed with concentrate (0.24) from Chehaima, as well as those from the Naima region (pasture: 0.31 and concentrate: 0.25) are not close enough to the value desired, this could be due to the degree of saturation of PUFAs by the process of biohydrogenation caused by ruminal bacteria. These values agree with similar results that were reported by (Majdoub et al., 2015; D'Alessandro et al., 2018).

#### 4. CONCLUSIONS

The results obtained in the present experiment suggest that the meat of lambs having access to pasture in the spring period have a desirable intramuscular fatty acid composition for human health; this diet generated a high intake of PUFAs such as ALA, EPA and DHA. The FA profile of pasture lambs in the Chehaima region is characterized by a dominance of C18: 3n3. Little difference in PUFALC was reported compared to those from the concentrate diet, with high content of C18: 2n6 cis-9; the latter according to the literature is dominant in lambs fed the concentrate, probably due to the phenomenon of remanence where the lambs spent a short time grazing on the grasses in the steppe. In the Naima region, dominant FAs for pastures lambs are the C18: 3n3, followed by C20: 4n6 and C20: 5n3; for those in the concentrate feed, we found the C18: 2n6 cis-9 followed by C18: 3n6. In both regions, the total SFA content was higher in the lambs from the concentrate diet, with individual SFA dominated by C 16: 0 and C 18: 0. Regarding the nutritional ratios, n-6 / n-3 in both diets resulted in favorable values for human health, the ratio  $\Sigma$ PUFA /  $\Sigma$ SFA for the two diets did not reach the values recommended by nutritionists, excepted for pasture lambs in the Chehaima region; this is explained by the saturation rate of PUFAs in the rumen.

We can say at the end, the more lambs are allowed to graze in steppe, and for long periods, the more that it will bring to their tissues to have a lower SFA content, and more beneficial, with a very rich accumulation of PUFA. Further analyzes should be carried out to determine to what extent the impact of dietary supplements can alter the lipid fraction of the meat of lambs that graze in steppe zones, and that obtained by those fed the concentrated feed.

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