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ORIGINAL ARTICLE

The Proportion of Grass and Concentrate in the Diet of Ewes Improve the Fatty Acid Composition of Milk

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ABSTRACT

The aim of this work is to study the variability of the nutritional composition of Fatty Acids in sheep's milk from different food systems with different proportions of grass and concentrate. In this study, three distinct groups (P, PC, C) of Ouled Djalel breed were used in different diet systems, the proportion of grass of which varies in each group with a complement of concentrate. The study was carried from March to May 2015. In this context, the total and defatted dry extract, proteins, lactose fat was determined. The lipid extracts are methylated and then passed in gas phase chromatography, for FA analysis. The TDE and lipids content were relatively higher (17.3% and 5.5% respectively) in the group C compared with those in the group P (16.4% and 4.5%) and with group PC (16% and 4.4%). Full-time grazing in the P group resulted in a decrease in the percentage of saturated fatty acids (SFA) and an increase in the proportion of unsaturated fatty acids, including C18: 3n-3 and 0.44 % compared to group (PC) 0.24% and group(C) 0.21%. A similar trend was recorded for C18: 2 cis9 trans11 with a high content in the group containing the greatest amount of grass. The n-6/n-3 ratio decreases with the increase on behalf of total grass in the ration and reaches values lower than 5. The pasture with grass acts in a considerable way on the improvement of the nutritional and dietary quality of the fatty acids of the milk of ewe.

Key words: *concentrate, ewe, fatty acid, food systems, grass, milk.*

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INTRODUCTION

Milk as a whole is a highly nutritious food. Thus, 85% of the world milk production is derived from cattle, followed by milks from other species such as buffalo (11%), goat (2.3%), sheep (1.4%), and camel (0.2 %) [26].Milk consumption has increased in developing countries, but it has fallen in the developed world [46].The production of ovine and caprine milk is prominent in the Mediterranean basin and the Middle East.The sheep in Algeria represent 26 million head in 2016 [48], which can be a promising future for the milk sector in the agri-food sector. In Algeria, milk of ewe after the use in lambs nutrition it is consumed bythe farmers or transformed traditionally [67].Limited studies exists on the health effects of ovine milk, as most of the research focuses on the consumption of bovine milk products due to its global dominance in the dairy market[42]. Ovine milk has a higher protein, lipid, mineral and vitamin content than bovine or caprine milks[42].However, several studies have shown that milk and dairy products are the major source of saturated fatty acids (SFA) in the human diet [10, 32].

Sometimes dairy products are criticized on the composition of milk especially on the profile in fatty acids. Nevertheless, dairy products remain an important source of FA of nutritional interest in human nutrition especially CLA and omega3[35].Milk lipids are a combination of components can have interesting effect son human health, but the diet of ruminants can alter the composition of milk fatty acids (FA)[22].It is interesting to control the diet of the ewe in order to improve the nutritional composition of milk fatty acids.

Currently, food systems are replacing conventional techniques with natural alternatives and in most cases changing the proportion of grass and times of access to animals for these natural resources. The composition of the milk can be considerably modified by changes in the diet, among which the fat content and fatty acid profile are the most variable and sensitive to changes in diet[63, 37, 60]. In fact, several works were led already in this direction, with various feeds, either by using the concentrate, with or without vegetables supplements of oil[45, 59],or the concentrate with diverse supplements such as plant oil, oilseeds, fish oil [43, 46, 64], the concentrate of cereal [30], only or combined and supplementation of concentrate in forage[38].

On the other hand, several studies have shown that grazing is a key factor in obtaining dairy products with a higher proportion of n-3 FA [17, 18, 40, 41].The grass can influence the lipid metabolism of ruminants by increasing some fatty acids such as Omega 3 and remedial acids CLA[12, 56, 39]. The plant compounds of grazing may partially inhibit hydrogenation in the rumen and reduce the loss of FA as local C18: 3 n-3 during digestion process[36]. Furthermore, the notion «images green" and the greater well-being of the animals of pasture are very appreciated by the consumers; consequently, there is an increasing request of the market for the meat and the milk produces in these conditions [57].

The pastures in of North Africa territories as Algeria are considered as an important source of food for the small ruminants seen their wealth and the diversity of forage species [2]with species that resist climatic conditions in arid regions such as: *Artemisia herba-alba*, *Stipatenacissima* and herbaceous plant in wetlands.To support this idea, the present work aims to study the effects of the food system with different proportions of grass with indigenous plants according to different regions on nutritional quality of the ewe milk fatty acids.

MATERIALS AND METHODS

Animals and diets

Three experiments were carried out on sixty ewes of the local breed OuledDjellal(average age 3 years, 40 ± 1.5 kg of weight) whose experiment P=Pasture(N=20)was carried out in the experimental farm in a wet area in north-western Algeria (latitude 36 ° 6'2 "N and longitude 0 ° 25'11" E, altitude 373 m, annual precipitation 401 mm)the animals made the pasture full-time from 08:00 to 18:00,whereas experiment PC=pasture + concentrate(N=20) on an experimental farm in the zone of Naama, on an arid steppe region in southern Algeria (34 ° 55'59.0 "N 0 ° 25'59.0" W altitude 1177 m, annual precipitation 268 mm) in this group the ewes was allowed to graze a natural pasture from 08:00 to 12:00 and then receives a supplement of a standard concentrate feed and the third experiment C=concentrate(N=20) was carried out on a farm in Hmadna in a semi-arid region in the center-west of Algeria (35 ° 54'00 "N, 00 ° 47'00" E, 91 m altitude, annual precipitation 349 mm) the ewes was placed in an enclosure and was given a standard concentrate feed.

Table 1: Composition of the experimental diets.

diets	P	PC	C
Analyzed composition (%)			
dry mater	82.3	88.4	86.5
Total lipids	2.8	4.9	3.2
Ash	7,5	8,4	3
Crude protein	15.3	16.5	12.4
FA analysis (Percentage of the identified FA)			
C14:0	1.73	1.81	1.54
C16:0	45.11	52.48	37.89
C16:1	0.55	0.30	0.28
C18:0	27.15	34.10	19.28
C18:1 n-9c	7.84	3.43	12.24
C18:2 n-9c	9.66	34.61	26.41
C18:3 n-3	7.50	2.26	1.90
SFA	74.34	89.04	58.94
MUFA	8.39	3.73	12.52
PUFA	17.16	6.96	28.31
n-6	9.77	34.85	26.64
n-3	7,50	2,26	1.90
n-6/n-3	1.3	15.42	14.02
PUFA/SFA	0.23	0.07	0.48

P: full-time grazing, PC: pasture + concentrate, C: concentrated feed, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Vegetation surveys, sampling and chemical analysis

Sampling for the plans consisted of choosing about2 kg of raw grass in all grazed areas for further analysis. Pastures consisted of small to medium-sized herbaceous forage in the wetland whose dominant species were *Trifolium repens, Scolymushis panicus, Medicago sativa, Daucuscarota, Malaria sylvestris, Avena sativa*. On the other hand, the species of plants in the steppe grasses were: *Stipatenacissima, Artemisia herba-alba, Stipamaritima, Lygeumspartum, Atriplexhalimus*. After they identified, samples are dried, crushed and stored in bags to darkness in order to further analysis in the laboratory.

For PC experimentation, after entering at after noon the ewes from the pasture the concentrate (60% corn, 22% soybean meal, 17% brain and 1% minerals) was distributed once day it . On the other hand, in experiment C, the ewes were left in the cowshed and received 1.5 kg of the concentrate per day without access to the pasture, and for experiment P the ewes consume the grass exclusively without concentrate. The chemical composition of the experimental diets was shown in Table 1. Water was always available for all groups of animals.

The chemical composition of different diets includes the dry mater and crude ash was analyze dosing respectively the drying oven at 103°C for 24h and aching at 550°C for 4h [4]. The crude protein (CP) was determined according to the Khjeldal method[5]. Lipids were extracted by chloroform-methanol (2:1) according the Folch method [28]. Lipid FA were freed by saponification (NaOH), and then methylated by methanol–BF3 [49].

Milk sampling and analyses

Milk samples come from manual milking in the morning. Milk is collected in sterile vials and stored at 4 ° C with an electric cooler and sent immediately to the laboratory for analysis. The pH values were measured using a previously calibrated pH meter (Hanna H211, Hanna Instrument,Portugal). The density was determined using lactodensimeter (Brouwland), according to the method described by AOAC [6]and the Dornic acidity, carried out by acid-base titration using Dornic soda. The Total dry extract was determined according to the AOAC method [6] by drying at 103 ± 2 ° C. Freezing point, the fat, protein and lactose were determined by infrared analysis using an Milkoscan instrument (FT 120, FossElectric, Hilleroed, Denmark). Defatted dry matter (DDM) was calculated by the difference between the total dry extract and the fat content.

Fatty acid analysis

Total lipid of each sample of milk or diets were extracted by chloroform-methanol (2:1) according the Folch method [28]. Lipid FA were freed by saponification (NaOH), and then methylated by methanol–BF3 [49]. The methylic esters of FA were separated and quantified by gas chromatograph (Perkin -Elmer Auto System XL) equipped with flame ionization detector and a capillary column (30m x 0.25 mm internal diameter). The operating conditions of the gas chromatograph were as follows: temperature injector and detector of 220 and 280°C respectively. The oven temperature was programmed 45 - 240°C, with 20 - 35°C.min-1. Milk of 1µl was injected with bicyanopropyl phenyl silicone as a stationary phase. Hydrogen was used as a conductor gas. FA peaks were identified by comparison with retention times of FA methyl standards. Quantification was made by an internal standard (C7:0) and (C17:0). Fatty acid contents were calculated using chromatogram peak areas and were expressed as gram per 100 g total fatty acid methyl esters.

STATISTICAL ANALYSIS

The data, expressed as means and standard deviations (SD), was analyzed using a statistical analysis system IBM SPSS Software® version 20.0 (2012). The parametric values were compared with a one-way analysis of variance (ANOVA) and Bonferroni test[62].

RESULTS AND DISCUSSION

Diets composition

The content of dry and mineral matter, proteins, lipids and fatty acids of different diets (Table 1) observed variations. This variation is attributed in large part to the nature of the diet composition. The amounts of dietary fatty acids varied according to the proportion of grass in each group. The proportion of C18: 3 n-3 (ALA) was higher in the diet with large grass share (P) than PC and C diets (7.5 vs 2.3 and 2.3and 1.9%) while the amount of C18: 2 n-6 was higher with complementation of the concentrate. The ratio n-6 / n-3 is reversely related to the amount of grass consumed in the diet, it decrease shewhen the grass had increased in each diet with the following ratios: 1.3, 15.42 and 14.02 in the P, PC and C groups respectively. The mineral matter was higher in P and PC than C diets (7.5; 8.4 vs 3%). The same trend applies to proteins. The diets containing grass contain more protein (18 to 24%) than the concentrate.

Milk composition

The results of the physicochemical analyzes (Table 2) showed highly significant effects (P <0.01) of the food system on the density and freezing point. The TDE was higher (P <0.05) in the milk of ewes fed with concentrate (group C) than in the other groups (17.3 vs 16.4 and 15.9%)respectively P and PC groups. The proteins varied according to the food system (P <0.05) by significant values in the samples obtained from a combination of the two foods: pasture grass and the concentrate supplement. The dry extract degreased (DED) and the lactose content are relatively stable, and have no effect of food system.

Table 2: The milk composition of the ewes according to the food system in each feeding system (g / 100g of milk)

Parameter	Diets			significance
	P	PC	C	
pH	6.70±0.98	6.60±0.07	6.55±0.08	ns
Density	1.034±0.04	1.04±0.02	1.04±0.02	P<0.01
Freezing point	-0.55±0.30	-0.53±0.11	-0.53±0.14	P<0.01
Total solids	16.40±1.54	15.93±1.20	17.30±1.63	P<0. 01
Defatted dry matter	11.85±0.19	11.60±0.19	11.75±0.18	ns
Protein	4.50±0.09	4.77±0.09	4.42±0.09	P<0.05
Lactose	4.47±0.72	4.33±0.38	4.44±0.46	Ns

Each value is the average of 20 samples (n = 20) followed by the standard deviation. P: full-time grazing, PC: pasture + concentrate, C: concentrated feed.

Table 3: Fatty acid composition of ewe's milk according to the food system in each feeding system (percentage of identified fatty acid)

Fatty acid (g/100 g FA)	**Diets**			**Signifiance**
	P	PC	C	
Total lipid	4.54±0.95	4.40±0.78	5.53±1.47	P<0.01
C 14 : 0	7.37±0.58	7.02±0.58	8.48±0.58	ns
C 16 : 0	26.94±0.56	26.98±0.56	28.22±0.56	ns
C 16 : 1	1.48±0.07	1.45±0.07	1.72±0.07	P<0.05
C 16 :1 n-9	0.36±0.01	0.28±0.01	0.34±0.01	P<0.05
C 18 : 0	16.21±1.23	15.02±1.23	13,37±1.23	P<0.05
C 18 : 1 cis 9	26,75±2,26	30.37±2.63	26,78±1,89	P<0.05
C 18 : 1t11 VA	1.64±0.11	1.07±0.11	0.82±0.11	P<0.001
C18 :3n-3ALA	0.44±0.03	0.24±0.03	0.21±0.03	P<0.01
CLA c9 t11	0.43±0.08	0.36±0.08	0.39±0.08	P<0.05
C 20 : 0	0.23±0.01	0,14±0,01	0.21±0.01	P<0.01
C 20 :3 n-6	0.04±001	0.12±001	0.10±0.01	P<0.05
C 20 :5 n-3	0.01±0.01	0	0	ns
C 22 :5 n-3	0.13±0.02	0.07±0.02	0.08±0.02	ns
C 22 :6 n3	0.07±0.04	0.12±0.04	0	ns
SFA	62.03±1.57	59.16±1.57	63.17±1.57	ns
MUFA	35.18±1.52	37.85±1.59	34.25±1.59	ns
PUFA	2.45±0.09	2.57±0.09	2.30±0.09	ns
n-6	1.83±0.08	2.13±0.08	1.96±0.08	ns
n-3	0.62±0.05	0.43±0.05	0.34±0.05	P<0.05
n6/n3	2.95±0.82	5.00±0.82	6.33±0.82	P<0.05
IA	1.55±0.21	1.41±0.30	1.77±0.16	P<0.05

Each value is the average of 5 samples (n = 5) followed by the standard deviation. P: full-time grazing, PC: pasture + concentrate, C: concentrated feed, CLA:Conjugated linoleic acid, VA: Vaccenic acid,SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids ns: not significant.
Atherogenicity index = [(12:0 + 4(14:0) + 16:0]/ [(n-6 + n-3) PUFA + 18:1 + MUFA] **[65]**.

Milk fatty acid profile

The composition of milk FA is presented in Table 3. Milk of different system groups with different proportions of herbs has been associated with changes in certain percentages of milk fatty acids. The C16: 0 concentration was stable while that of C18: 0 increased proportionally in the food system with a high proportion of fresh grass with a recorded proportion of 16.21 g / 100 g in the P food system (100% Grass) against the PC system 15.02 g / 100 g and 13.37 g / 100 g and C respectively. Among the MUFA, C18: 1 n-9, was higher (P <0.05) in the milk of PC system 30.37% than the other, P and C groups (26.75%

vs 26.78%).The Vaccenic acid was also higher in the group P (1.64%) than in groups C(1.08%) or PC (0.82%).However, an increase in CLAc9t11 was observed in the diet in which the fresh herb was the main ingredient, with the following proportions: 0.43% in group (P) vs0.36 and 0.39% in(PC) and (C) groups respectively. A similar trend for C18: 3n-3, the high proportion of grass in the full-time grazing group induced an increase in C18: 3n-3 in this group against PC and C with grades 0.24 and 0.21%.

However, no significant effect was detected of the food system on long-chain fatty acids, in particular: C 20: 5 n-3, C 22: 5 n-3, C 22: 6 n-3. The increase in the proportion of grass in the diet had an great effect (P<0,05) on increase of n-3 PUFA level (two times in P than the C group) by higher proportions in the group of ewes grazing of grass with a rate of 0.62% against 0.43 and 0.34% in the PC or C groups PC or C respectively. The variations in n-3 and n-6 PUFA lead to a decrease in the n-6 / n-3 ratio to a value of 2.95 in the (P) group with the high proportion of grass, whereas this ratio was 5 and 6.33 in the PC and C respectively. For the atherogenicity index, this parameter ranged from 1.41 to 1.77 and showed significant differences (p <0.05) between dietary systems in this study.

The feeding system strongly affected the composition of ewe's milk under full-time grazing conditions and with a supplement produced milk with a density and freezing point closer to those found by [44](1040 g / ml) and [55](-570 C°), these two parameters essentially depend on the milk components. On the other hand, the pH of milk remains stable and falls within the range of values found by [67, 1]. These results also show that breeding of ewes installing can increase significantly extracted dry total (EDT).The increase observed in lipids can be attributed to several factors of variation such as the type of agriculture and feeding, as the latter varies according to the plants available during the movement of the ewes and the ingredients in each diet for example: *Trifolium repens, Phleum pratense, Taraxacum officinale* [20].These milk levels were observed in the ranges reported by [47, 31]with 4.7 and 5.9% fat contents in milk.

The food system has a significant influence on the milk fat content, which is explained by the high proportion of lipids in the diet with up to 5%(Wealth of the diet PC and C in lipids) and when the grazing time decreases the ewes do not consume less energy and they mobilize these nutrients what is in agreement with [14]. These values are substantially similar to those obtained by [53, 1].The use of a high proportion of concentrates in diets (> 60% dry matter) can support both milk fat and protein levels during the first months of lactation [25].Lactose is the specific sugar of milk and is one of the important constituents of its dry matter. According to the values reported by the bibliography, this carbohydrate is one of the most stable constituents and undergoes only small variations. These lactose contents are relatively close to those recorded by [58, 7].

Ours results has been shown to have an overall effect of food system on fatty acids in sheep's milk, which is consistent with the results of [19, 21]. At the same time, some researchers have shown that the diet does not affect the proportion of some of fattyacids such as C14: 0 and C16: 0, they have shown that the inclusion of dietary SFA in the diet of ruminants usually reduces the neo synthesis of these fatty acids due to the inhibitory effect of large amounts of circulating long-chain FA on the expression of genes involved in FA [61].Than an increase in the proportion of C18: 1cis 9in the grass-based diet resulting from the mobilization of body reserves in relation to the availability of energy [54].[15, 33]reported that C18: 1 cis9 is more related to the energy balance of cows than the proportion of grass in diets and is therefore a good indicator of animal mobilization that is consistent with our test.

The proportion of grass in the ration increases (P <0.01) C18: 3 n-3 in milk (0.44 vs 0.24 and 0.21% in the PC group and C respectively), these results correspond to those found [21]which highlights the importance of grass. A diet with 60% total grass on grazing increases contents of CLA C18: 2 c9t11. This content is very strongly related to C18: 1 t11 [27].In our study a similar trend was observed for: CLA and vaccenicacid VA with high proportions (CLA: 0.43% and VA: 1.64%) in group P. [34]with grass autumn, have already demonstrated this specific effect of green grass (more than 6% of C18: 1 t11).

Green grazing is an excellent source of Omega 3 and one of the most effective foods for enriching milk with its fatty acid composition towards a healthy spectrum [51, 30].The marked effects of grazing on ALA, VA levels with differentials up to 50%are related to the high ALA content in green pastures, which is partially biohydrogenated in VA in the rumen and then secreted in milk and partially converted to C18: 2 C9, t11 CLA in the mammary tissue by the action of stearoyl-CoA desaturase [8]. The composition of milk FA (Table 3) has been improved by increasing the availability of pasture [51] or by including specific forage species in the grazing [3, 9]. In Italy, where most dairy ewes feed on pastures, concentrations of RA, VA and ALA in milk fat were highest in late winter, early spring when the availability of was the highest and decreased as lactation progressed and pastures availability and quality decreased [52]. The major variation in milk FA composition was achieved by the amount and type of forage, particularly grazing, in the diet of ewes [8].PUFAs in grass are mainly C18: 3n-3, located in chloroplast cells in

association with galactolipids, and are therefore somewhat protected against lipolysis and subsequent biohydrogenation by the plant cell wall [23]. This allows greater absorption of C18: 3n-3, leading to an increase in n-3 PUFA derivatives (C20: 5n-3, C22: 5n-3, C22:6n-3), such as the results observed in the study of [11].
As a result, n-3 PUFAs increased (P <0.05) with the increase (a gain of 55%) in grass share in the diet inversely to the PC and C groups, this variation is attributed to effects of the concentrate which increases the fat content (PUFA n-3, ≥C18: 0) transferred to the mammary gland (Chilliard et al., 2007). In addition, the fat of the concentrate are mainly triglyceride C18 PUFAs, which are more accessible for ruminalbiohydrogenation than lipids in forages [11].
The ratio n-6 / n-3 decreased with the percentage of total grass in the diet, it was very high (up to 6.33) with rations with little grass. It has reached values of less than 5 which confirms the results found by [38]as soon as the diet contained at least 20% of total grass, it is directly related to food according [33]. Although the increase in milk MUFA found in this study is desirable from a human nutrition standpoint [29], the atherogenicity index decreased markedly with a 50%. This index is lower than that found by [66].On the other hand, [13]reported indices of atherogenicity unchanged when the ewes were fed with olive leaves.

CONCLUSION

In conclusion, this work suggests that the food system with a high proportion of grass amended the fatty acid profile of milk, in particular ALA, VA and CLA, in a sense that can be qualified as positive by a point nutritional status in the current state of knowledge. This seems particularly interesting for improving the nutritional properties of ewe milk during periods of full grazing. The choice of the food system to produce some milk constitutes a necessary operation to obtain the best qualities of the milk. This study re-evaluates sheep's milk and the attention of industries for its transformation, which shows the importance of food systems for northern Africa especially those based on grass.

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