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The Season Effect on the Milk Fatty Acid Profile of Oulmes Breed Cattle in Moroccan Oulmes Zone

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Abstract: *In the present research work, we aimed to characterize the physicochemical quality and fatty acid profile of Oulmesbovine milk depending of season behavior. To perform this phenomenon, forty-eight samples were collected and examined in autumn 2013 and winter, spring, and summer 2014. The milk samples were obtained from Oulmes cows from the Middle Atlas region in Morocco. The milk components as milk fat, protein, and ash were observed not affected by seasonal change (3.6, 3.6 and 0.6%, respectively), while the fatty acid profile has found changed considerably between seasons.It was noticed that Myristic, palmitic, stearic, and oleic acid were detected as the major fatty acids in the Oulmes cow milk. The saturated fatty acid(SFA)and monounsaturated fatty acid (MUFA) contentover the four seasons was higher, with a mean of 66.0 and 32.9%, respectively.The essential n-6 and n-3 polyunsaturated fatty acids were not abundant in Oulmesmilk fat. Nevertheless, improving the diet of the animals could may increase n-3 fatty acid content and improve the source of α-linolenic acid in milk fat.*

Keywords: *Oulmes breed, milk, physicochemical, fatty acids profile.*

I. Introduction

It is generally known that cow's milk is an essential part of most daily diets andis a complex mixture of specific bioactive molecules such as proteins, lipids, saccharides, and biologically active substances including immunoglobulins, enzymes, oligosaccharides, hormones, and cytokines (Pouliot and Gauthier, 2006). Despite this, milk developed a bad reputation for contributing to cardiovascular disease because of its high saturated fat content. However, milk must not be marginalized in an average diet since, in addition to calcium and protein; it may alsohelp to prevent some cardiovascular and neoplastic diseases (Lecerf 2008).Some of these benefitsare due tothe fatty acid profiles of milk, some of which cannot be found in any other food.

Nevertheless, cow milk composition differs not only between species but also within species (Pougheon 2001), either through geneticsor farming practices.Cow's milk fat, in particular fatty acid profiles,is also affected by the diet of dairy cows (Palmquist*et al*., 1993; Dewhurst *et al.*, 2006).This composition changes from summer to winter depending on the mode of feeding.Therefore, tracking livestock may be a solution to improvethe nutritional qualities of milk without dietary changes.

Nevertheless, cow feeding can be an important way to improvethe dietary qualities of milk fat.The aim of this workwas to investigate the diet of dairy cows and the fatty acid profiles of the produced milk during different seasons knowing that seasonal fluctuations are quantitatively significant and due mainly to changes in diet (Quist *et al*., 2008).

II. Materials And Methods

1.1 Milk samples

Fresh Oulmes milk was obtained from cows inhabiting theOulmes region during autumn 2013 andwinter, spring, and summer 2014. Oulmes is a rural place located 150 km northwest of Rabat andis in the high mountains of the Middle Atlas and middle hillssituated in the west in theAmazigh area.Ofthe main Moroccan local breeds, Oulmes-Zaerdatesto 1912 and wasrecognized by ministerial decree in 1982. It is an original Moroccan racereared for two purposes, milk and meat production, and it is well adapted to harsh environments (Boujenane, 2002). This region is also well known as a natural reserve of aromatic and medicinal plants(Chatibi, 2011).

Forty-eight milk samples were collected in the early morning. The milk from each cow was collected directly in sterile bottles without preservative and kept at 4°C.On arrival at the laboratory, all milk samples were stored at -20°C untilchemical and fatty acid profiling.

1.2 Chemical analysis

The nitrogen content (*N*) in the milk samples was estimated by Kjeldahl's method,and crudeprotein content was calculated as *N*×6.25. The ash content was obtained by incineration of thesample placed in a muffle furnace at 550°C for 6h(AOAC, 2000). The fat content was determined directly after collecting and processing using an auto-analyzer (LACTOSCAN®MilkAnalyzer) (Konyali, 2010).

1.3 Analysis of fatty acids

Milk fatty acid profiles were analyzed accordingto the procedure described by Akraimet al. (2005) with slight modifications.According to this method, fatty acid methylation is donein two stages: a basic methylation followed by an acid methylation that does not involve isomerization of cis-9, trans-11C18:2 (Duckett*et al*., 2002).

Milk samples were centrifuged at 10,000 x g for 30min at 5°C to harvest milk fat and then methylation was performed with 0.5N methanolicNaOH followed by heating at 90°C for 10min. 14% boron trifluoride (BF3)was then added followed by a heating at 90°C for 10min.After addition of hexane and distilled water, the tubes were placed in a shaker for 10min then centrifuged at 2880 x g for 15min.

1.4 Analysis by gaschromatography

Fatty acid methyl esters (FAMEs) were separated with a gas chromatograph (Varian CP 3380) equipped with a flame ionizationdetector using a Varian CP-Select CB capillary column [50 m × 0.25 mm (i.d.) with 0.39 mm (e.d.)]. Nitrogen was used as a carrier gas;the initial column temperature was 40°C held for 1 min, after which the temperature was increased to 45°Cat rate 1°C/min and from 45°C to 200°C at rate of 6°C/min. The temperature was kept at 200°C for the subsequent 40 min. Injector and detector temperatures were set at 230°C and 250°C, respectively. Fatty acid identification was carried out by comparing their retention times against fatty acid methyl standards. Resultsfor each fatty acid are expressed as a percentageof the sum of total FAs,and saturated FAs, monounsaturatedFAs, and PUFAs werecalculated.

III. Statistical Analysis

Results are expressed as means ± standard deviation (SD). Significant differences between means were tested by ANOVA on arc transformed data followed by Tukey's studentized range test at $P<0.05$.

IV. Results And Discussion

1.5 Chemical composition

The chemical characteristics of the 48 milk samples are shown in Figure 1.There was little variability between seasons.Oulmes breed milk protein ranged between3 and4% (mean 3.6%), as found previously(3.6%) withaLactoscan milk analyzer (Elhamdani*et al*., 2016). This value washigher than that found in Holstein cow milk from theLordegan region of Iran (3.3%); (Mirzadeh*et al*.,2010), Chinese Holstein cows in Northern China(3.1%) (Yang *et al*., 2013), cows in Pakistan (3.3%); (Imran *et al*., 2008), and Cameroon (3.3%); (Ponka*et al*.,2013).

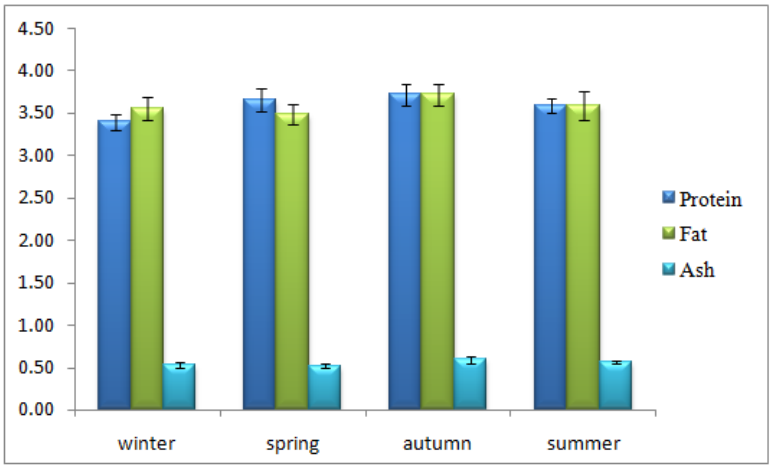

Figure 1. Chemical composition of Oulmescow raw milk during the fourth seasons.

The average fat content rangedbetween3and4.5%, consistent withthe AFNOR value(3%; AFNOR 2001) and similar studiesfrom Brazil (3.6%) (Cofani*et al*., 2009) and local Nguni cattle in South Africa (3.3%; Mapekula*et al*., 2011).Cows get dry food in winter and grass in summer as they are outside for a long time (Fox and Mcsweeney, 2003). Fat variabilitydepends on several factors such as the weather conditions, stage of lactation, and feeding. Milk protein content is alsosubject to seasonal changes and locality and its variabilityis known to behigher than that of fat content (Louwrens*et al*., 2000).

Ash content was 0.5% in spring and autumn (maximumof 0.6%). This is consistent withthe ash content of 0.6% in milk from Cameroon (Ponka*et al*., 2013) and 0.7% in Holstein Friesian cow milk ofSouth-Eastern Spain (Sanz Ceballos *et al*., 2009).The effect of seasonon ash content was small,consistent with results reported previously (Rao and Mishra, 2010).

1.6 Fatty acid profiles

In this phase of search work, the proportions of short-chain FAs (4:0, 6:0, 8:0, 10:0, and 12:0) in milk fat were not affected by the season. This was also the case for medium-chain FAs (14:0, 16:0, and 16:1) in milk fat, the content of which was also similar between the four seasons except on summer (C16:0 = 22,086) and higher in the autumn (C16:0=24.063) compared to spring and winter (23.499 and 23.411).

Table 1. Amount of fatty acids (g/100g) in milk fat of Oulmes breed determined by GC.

	Winter	Spring	Autumn	Summer	SD
	Mean	Mean	Mean	Mean	
C4:0	0.02^{a}	0.04^{a}	0.06^{a}	0.03^{a}	0.04
C6:0	0.50^{ab}	0.68^{a}	0.41^{b}	0.32^{b}	0.16
C8:0	0.76^{b}	1.04^{a}	0.62^{b}	0.79^{ab}	0.38
C10:0	1.84^{ab}	2.06^{ab}	1.55^{b}	2.27^{a}	0.64
C12:0	2.13^{ab}	2.23^{ab}	1.84^{b}	2.72^{a}	0.68
C14:0	9.80^{b}	10.32^{ab}	9.92^{ab}	10.78^{a}	0.94
C16:0	23.41^{b}	23.50^{b}	24.06^{ab}	$22,08^{a}$	1.61
C16:1	1.27^{ab}	1.81^{ab}	1.43^{a}	0.72^{b}	0.64
C18:0	25.13^{b}	22.19^{c}	25.28^{b}	$27,41^{a}$	4.57
C18:1 n-9	32.91^{a}	32.58^{a}	31.34^{a}	$29,34^{b}$	5.35
C18:2 n-6	1.45^{a}	1.62^{a}	0.90^{b}	0.69^{c}	0.38
C20:0	0.22^{c}	0.56^{bc}	1.28^{a}	$1,05^{ab}$	0.56
C24:0	0.54^{c}	1.35^{b}	1.26^{b}	$1,76^{a}$	0.64
SFA	$64,36^{b}$	$63,99^{ab}$	$66,32^{b}$	$69,24^{a}$	2,08
MUFA	$34,17^{a}$	$34,38^{a}$	$32,77^{ab}$	$30,06^{b}$	1,72
PUFA	$1,45^{a}$	$1,62^{a}$	$0,90^{ab}$	$0,69^{b}$	0,38

Fatty acids: C4:0, butyric acid; C6:0, caproic acid; C8:0, caprylic acid; C10:0, capric acid; C12:0, lauricacid; C14:0, myristic acid; C16:0, palmitic acid;C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C20:0, arachidic acid ; C24:0, lignoceric acid, **SD**: standard deviation.
SFA = sum of saturated FA; **MUFA** = sum of monounsaturated FA; **PUFA** = sum of polyunsaturated FA.
[abc]Different letters for the seasons in the same parameterindicatesignificant difference($P<0.05$).

These found results are consistent with those reported by Palmquist*et al*.,(1993), who reported a higher percentage in autumn (C16:0 = 30.78) than in summer (C16:0=28.76). While theC16 content in milk fat was not related to the feed, this fatty acid is partly derived from *de novo*synthesis (Agenas*et al*., 2002;Steinshamn*et al*., 2008).

Milk fat has not always had a good reputation due to its high saturated fatty acid content. However, saturated fatty acids are now regarded as not as"bad" as previously thought and also have very important functions. For instance, butyric acid (C4:0) hasa protective role against colon cancer (Senguptа*et al*., 2006) and the long fatty acids such as myristic acid (C14:0) specificallyacetylates proteinsso are considered active and useful to cellularfunction (Rioux*et al*., 2007).

Likewise, the saturated medium chain fatty acids (caproic acid C6, caprylic acid C8, and capric acid C10) are an interesting group because they play a potential role in adiposity. Further, a study in rats and humans has suggestedthat diets rich in medium-chain fatty acids can help to reduce weight (Tsuji *et al*., 2001).

The majority of 18-carbon fatty acids in milk fat were affected by the season. The mean proportions of stearic acidbeing higher in summer (C18:0 = 27.4) in agreement with Prechet and Molkentin (1999) and Barbano (1990), who reported that the mean proportions of C18:0 were higher in summer and in spring.Moreover, C18:1 and C18:2 were higher in spring (32.6; 1.6) and in winter (32.9; 1.5), respectively, also in concordance with these two studies. Oleic acid (C18:1) and linoleic acid (18:2) are two fatty acids found in large quantities in fresh grass (Boufaïed*et al*, 2003; Jenkins *et al*., 2008) and must be ingested by cows to be secreted in milk. Feeding the cow is therefore of great importance with respect to the composition of unsaturated fatty acids in milk.

Regarding mono-unsaturated fatty acids, there was little palmitoleic acid (C16:1) but a substantial amount of oleic acid (C18:1 n-9), which is considered a positive component of milk. Oleic acid is a precursor of long chain fatty acid derivatives (especially 24 carbons), a constituent of brain structures and particularly myelin. For the cardiovascular system, its neutrality is an important advantage and it is now accepted as a replacement to saturated fatty acids in the diet to reduce cholesterol (Gordon *et al*., 1995).

Furthermore, the concentrations of all 20:0 and 24:0fatty acidswere similar betweenthe four seasons, with higher percentages of both of these fatty acids in the summer. This change can be explained by the grazing period but also by supplementation with concentrate.

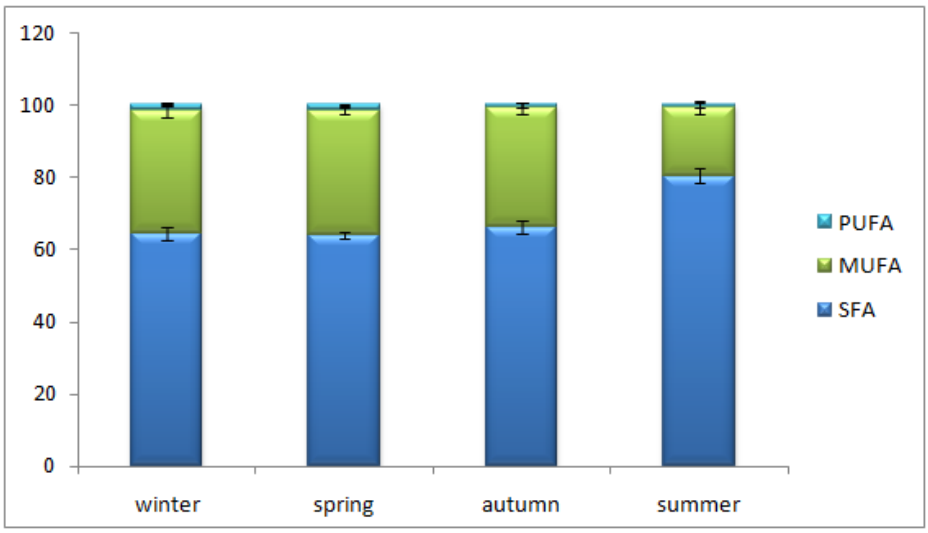

Figure 2: The SFA, MUFA and PUFA percentages of Oulmes bovine raw milk during the four seasons.

In the current study, the mean percentage of SFAsdid not change between seasons (Figure 2).However, the summer value (SFA=69.2) was higherthan that found by Prechet*et al*., (1999) (SFA=56.5) andsimilar to Prechet*et al*., (1999) during the winter (SFA=64.4 vs. 65.2).These percentages are also close to those found by Cortes *et al*.,(2010) for Holstein cow milk (SFA=71.3).

Nevertheless, the mean proportions of MUFAswere higher than those found by Prechet*et al.*,(1999)in the winter (34.2; 19.3) and summer (30.1; 21.4), respectively, and also higher than those reported by Cortes *et al.*, 2010 (16.5).

Theessential polyunsaturated n-6 and n-3 fatty acids were not present at high levels in the milk fat. Nevertheless, improving the diet of animals can increase the n-3 fatty acid content and make milk fat a greater source of α-linolenic acid. Thus, since an excess of n-6 fatty acids is undesirable, the low content of PUFA milk fat can be an asset if care is taken to maintain very low n-6 while increase n-3 in animal feed.

V. Conclusion

This study confirmed that there is a change in milk composition according to the season, in particular fatty acids, and this is probably due to an increased proportion of grass in the diet. The nutritional properties of milk can be improved cheaply by taking advantage of periods beyond full pasture (late winter or autumn). Milk fat has a specific composition that, whencompared qualitatively with "good" vegetable oils that provide polyunsaturated fatty acids (colza, nut), monounsaturated (olive), provides a natural complement to these fats by providing the fatty acids not found in oils.This interesting composition make the Oulmes raw milk a typical food product which could be encouraged as part of normal daily intake.

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