# A survey on the fatty acid composition of cow's milk in different production systems in Khorramabad, Iran with respect to ω-6:ω-3 ratio

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### ABSTRACT

The ratio of  $\omega$ -6:  $\omega$ -3 is connected to the higher risk of non-communicable diseases, thus this ratio is becoming more important than quantity of  $\omega$ -6 and  $\omega$ -3 in human nutrition. In countries like Iran, cow's milk is mainly produced in rural area and less in conventional systems. The lactating cows reared in rural and conventional farms are different in terms of nutrition, daily yields, and proportion of concentrate to forage that these might affect milk fatty acid profiles. Therefore, the aim of this study was to determine the ratio of  $\omega$ -6:  $\omega$ -3 in cow's milk produced in conventional and rural dairy farms in west of Iran, Khorramabad. Twenty bulk milk samples (rural and conventional) were collected from the conventional dairy farms and local milk collection centers. Fatty acid profiles were determined and results showed that conventional milk had higher  $\omega$ -6 (2.1± 0.4) and lower  $\omega$ -3 (0.16 ± 0.03) in comparison with rural milk. The ratio of  $\omega$ -6: $\omega$ -3 in rural milk was significantly lower (8:1) than that in conventional milk (13:1) (p<0.01). In conclusion, milk produced in rural dairy farms had better ratio of  $\omega$ -6: $\omega$ -3, thus rural milk could be healthier for human nutrition in terms of  $\omega$ -3 fatty acids.

**Keywords:** Rural Milk; Conventional Milk; Fatty Acids; ω-6; ω-3; Khorramabad

### **INTRODUCTION**

Cow's milk contains about 3-5% fat that is generally blamed as main unhealthy nutrient, because fat intake has been related to noncommunicable disease; obesity and cardiovascular diseases [1]. In recent decades, it is usually recommended to reduce fat intake and drink low fat milk. On the other hand, milk fat contains fatty acids which are as essential as vitamins, minerals and amino acids for a healthy life. Milk fatty acids are including two special families of fatty acids  $\omega$ -6 and  $\omega$ -3 which are essential for human health [2, 3].

 $\alpha$ -Linoleic acid (C18:2), the main  $\omega$ -6 fatty acid in milk fat, is an essential fatty acid required for the synthesis of arachidonic acid (C20:4, n-6) and eicosanoids [4].  $\alpha$ -Linolenic acid (C18:3,  $\omega$ -3) can be converted to the more biologically active very long-chain polyunsaturated fatty acids (PUFA), EPA and DHA. In addition, the ratio of  $\omega$ -6 to  $\omega$ - 3 fatty acids is thought to be an important parameter determining the nutritional value of milk. Both  $\omega$ -6 and  $\omega$ -3 fatty acids have double bonds in positions greater than C9. Human and other mammals are not able to synthesize essential fatty acids because of lacking the  $\Delta$ -12 and  $\Delta$ -16 desaturase enzymes [1]. Therefore desaturation of fatty acids does not occur at positions greater than C9 and prevents human and mammals to synthesize fatty acids of the  $\omega$ -3 and  $\omega$ -6 families. The  $\omega$ -3 and  $\omega$ -6 fatty acids are considered to be essential for human and must be provided by the diet [3, 5].

From human nutrition point of view, the healthiest ratio between  $\omega$ -6 and  $\omega$ -3 fatty acids is 2:1 to 4:1 (3). It is believed that the unbalanced ratio of  $\omega$ -6: $\omega$ -3 increases prevalence of non-communicable disease. Therefore, attempts to reach the healthy ratio of  $\omega$ -6: $\omega$ -3 in animal products is interest of animal nutritionist. Various

factors including breed, season, geographic location and ration had been reported to affect the composition of fatty acids of cows' milk [6]. For example, the ratio of  $\omega$ -6: $\omega$ -3 in milk of cows fed fresh forage was closer to the recommended ratio for human health [7]. In contrast, it is observed that the ratio of  $\omega$ -6:  $\omega$ -3 in milk produced in conventional dairy farms is about 20:1 which is far from recommended healthy ratio [8].

In countries like Iran, cow's milk is mainly produced in rural areas and less in a conventional system. The lactating cows in rural and conventional farms are different in terms of nutrition, daily yields, and proportion of concentrate to forage. Rations in conventional dairy farms contain usually dry hay supplemented with concentrate, whereas, in rural system production, dairy cows are fed mainly fresh forage with no supplementation of concentrate. It is speculated that fatty acid composition especially  $\omega$ -6 and  $\omega$ -3 of milk produced in rural and conventional system might be different. Therefore the aim of this study was to determine and comparison of fatty acid composition and  $\omega$ -6:  $\omega$ -3 ratio of milk produced in rural and conventional production systems.

## MATERIALS AND METHODS

In spring 2012, twenty bulk milk samples (300 ml) were collected from conventional dairy farms and rural milk collection center in accordance with ISO 707. Milk samples were frozen at -80 °C pending fat extraction [9]. Fat extraction carried out according to ISO 14156, IDF 172; 2001. Samples were heated up to 40°C and thereafter were quickly cooled to 20°C. After the funnel decanter to 100 ml of milk samples, 80ml of ethanol, 20ml 25% ammonia solution and 100ml diethyl ether (Anhydrous,  $\geq 99\%$ ) were added and thoroughly. mixed Then 100ml pentane (Anhydrous,  $\geq$ 99%) was added to the collection without removing the aqueous phase. After the two-phase mixture formation, the aqueous phase was separated from the pentane phase. 100ml of sodium sulfate (Anhydrous,  $\geq 99.0\%$ ) was added to heptane phase. 5 grams of sodium sulfate powder was added to extracted fat and after smoothing, using a rotary evaporator, heptane was removed from the sample [10].

Milk fat was methylated according to ISO 15884, IDF 182; 2002. About 100mg of milk fat dissolved in 5ml of hexane and 0.2ml sodium metoxide solution 2M was added. After 5 minutes (reaction time), 0.5g sodium hydrogen sulfate monohydrate was added to the contents of the tube and the tube was centrifuged at 2000 RPM for 3 min. The upper phase was injected to gas chromatography (Varian 3800) equipped with a capillary column Cp-sil 88 to over 50 meters and the FID detector [11]. Working conditions were set according to standard ISO 15885, IDF 184; 2002. Carrier gas, nitrogen with a pressure of 14psi was used and the injector temperature was 250°C [12]. In order to isolate the exact and complete recovery of fatty acids (particularly short-chain type) temperature program proposed by Kramer et al., (2004) was used: temperature of 45°C (4 min) increased in temperature of 13°C per minute to a temperature of 175°C (held at this temperature for 27 min), increase in temperature of 4°C per minute up to 215°C (kept at this temperature for 35 minutes)[13].

## Statistical analysis

Data analysis was performed using SAS software to compare  $\omega$ -6 and  $\omega$ -3 content of milk samples with t-test (p $\leq$ 0.05) [14].

## RESULTS

Fatty acid profiles of rural and conventional milk as percentage of total milk fatty acids are shown in Table 1. Palmitic acid (C16:0) was the highest among fatty acids in milk in both rural (38.0%) and conventional (37.2%) milk. Oleic acid (C18:1) was the highest unsaturated fatty acid with 22.83% and (23.5%) in conventional and rural milk, respectively. The amount of saturated fatty acids in both rural and conventional milk was similar and was the highest compartments of milk fatty acids with 66.8 and 68.8%, respectively. MUFAs were 27.8 % and 27.0% of milk fatty acids in the rural and the conventional production systems, respectively. However, rural milk had significantly lower PUFAs (1.9%) than conventional milk (2.3%).

The total amount of Linoleic acid (18:2 cis & trans) was accounted for  $\omega$ -6 fatty acids and the amount of Linolenic acid (18:3) were accounted for  $\omega$ -3 fatty acids. The total amount of  $\omega$ -6 in

rural milk were significantly lower (p<0.01) than that in conventional milk (1.6% vs. 2.12%). In contrast, amount of  $\omega$ -3 in rural milk were significantly higher (p<0.01) than that in conventional milk (0.21% vs. 0.16%). Thus the  $\omega$ -6:  $\omega$ -3 ratio in rural milk (8 to 1) was significantly lower (p<0.01) than that in conventional milk (13 to 1)

Table 1. Fatty acid profiles of milk produced in rural and	
conventional production system (Percent of total milk fatty acids)	)

Fatty acid	Conventional		Rural		Signifi
	Mean	SD	Mean	SD	level
4:0	3.8	±2.46	2.8	±1.22	-
6:0	0.3	±0.10	0.3	±0.12	-
8:0	0.6	±0.2	0.5	±0.1	-
10:0	2.1	±0.50	1.8	±0.22	-
12:0	3.1	±0.6	2.7	±0.32	-
14:0	12.2	±1.34	12.0	±0.42	-
14:1	2.5	±0.5	2.6	±0.22	-
16:0	37.2	±3.32	38.0	±1.1	-
16:1	1.6	±0.2	1.7	±0.11	-
SFA					
18:0	8.8	±2.07	8.6	±0.70	-
18:1 trans	0.73	±0.34	0.9	±0.21	-
18:1 cis	22.1	±3.7	22.6	±1.3	-
18:2 trans	0.06	±0.03	0.1	±0.09	*
18:2 cis	2.06	±0.45	1.5	±0.30	**
18:3	0.16	±0.03	0.21	±0.04	**
20:0	0.8	±0.4	1.1	±0.3	*
22:0	0.11	±0.03	0.1	±0.06	-
MUFA	27.0	±3.6	27.8	±1.3	-
PUFA	2.3	±0.43	1.9	±0.3	**
<u>-6ω/-3ω</u>	13.3	±3.7	8.1	±1.56	**

- No significant

\* Significant p<0.05

\*\* Significant p<0.01

#### DISCUSSION

The aim of this study was to corroborate if milk fat composition differs between rural and conventional milk production system. In order to minimize the variables and the conditions, milk samples were collected of Holstein dairy cows that diet contained at least 40% concentrate. Rural milk samples were collected during spring from dairy cows that were kept in a grazing system with ad lib access to fresh forage. It is estimated that approximately 50% of bovine milk fat is synthesized from plasma lipids, of which 88% have dietary origin [15, 16]; therefore, changing the diet can have major effects on the milk fatty

acid content. Milk has maximum percentage of saturated fatty acids during winter and the amount of saturated fatty acids in the both systems in this season was high that are important in terms of nutritional and health risks [17]. The highest amount of saturated fatty acids in both farming systems can because of bacterial population in the rumen of livestock and lack of access to grazing areas in the city of Khorramabad. When the concentrate is used in feed, rumen's microbial activity of Propionibacterium, Streptococcus and Lactobacillus increasing spp. is and biohydrogenation of dietary lipids occurs [18]. Total saturated fatty acids concentration did not differ between management systems. This result was consistent with the results of other researchers that had collected milk samples in the spring [6, 7]. However, concentrations of Myristic acid (C14:0) and Palmitic acid (C16:0), [two fatty acids thought to carry higher CHD risk (1)], were higher in rural and conventional produced milk. The damaging effects of saturated fatty acids might be questioned by some scientists, the general advice to the public is to moderate saturated fatty acids intake [19]. On the other side, the amount of Linolenic acid ( $\omega$ -3) was higher than in rural farming systems. This difference can be due to dietary intake and the use of fresh hay in the feed of the rural farming system. Fresh forage contains one to three percent of the fatty acid which 55 to 65 percent is  $\alpha$ -Linolenic acid [20]. Some researchers have conducted research using green forage (forage to concentrate ratio consumed, 65:35) in spring and summer that have reduced the biohydrogenation of long chain PUFA in the rumen and increases the fatty acids in milk composition [15, 18]. Another reason of increasing the PUFA in milk during the spring and summer is increasing the enzyme activity of unsaturated fatty acids in the mammary glands. The result of the current study was in agreement with results from other researchers that had compared the  $\alpha$ -Linoleic ( $\omega$ -3) in both industrial and organic systems [7, 21-22]. The differences and attributed rations between the amount and ratio of  $\omega$ -6: $\omega$ -3 in rural and conventional dairy farming systems can be used to choose type of breeding (concentrate on industrial system against fresh forage and access to pasture in rural farm) [8, 23] and sampling protocol in different seasons [7]. Fresh forage contains a high percentage of unsaturated fatty acids, with  $\alpha$ -Linolenic acid (C18:3) being the predominant  $\omega$ -3 fatty acid in fresh forage [15]. Current results were consistent with the findings of other researchers that had collected milk samples in the spring [6-7, 21-22, 24-25].

## CONCLUSION

In conclusion, this pilot study showed that the milk content of  $\omega$ -6 and  $\omega$ -3 in rural and conventional production systems are different. It was found that the ratio of  $\omega$ -6: $\omega$ -3 in rural milk (8 to 1) was less than that in conventional milk (13 to 1) that were far from the recommended

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10.ISO 14156:2001 (E), IDF 172:2001 (E) (2001) Milk and milk products- Extraction methods for lipids and liposoluble compound. healthy ratio (2:1 to 4:1). This unbalanced ratio of the milk produced in the country may be associated with an increased incidence of chronic diseases, therefore, more attention and more extensive research in the field of human nutrition and health perspective in Iran is needed.

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