

Effect of Feeding Systems on Bovine Milk Quality in Mexico

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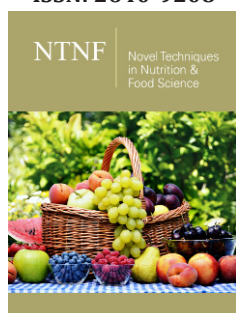
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Abstract

The trial aimed to compare the fatty acid profile of milk from dairy cows under Exclusive Grazing (EG), Supplemented Grazing (SG) or Full Confinement (FC) system. Sampling was performed in 2017 and 2018 on 6707 dairy cows from Querétaro, Tabasco, Colima, Veracruz and Chiapas in Mexico. Among the 84 farms included in the trial, 25.3% were in FC (1699 animals) and fed corn silage, alfalfa hay, tropical forages and commercial balanced concentrate (16% CP; 5 to 7kg/ head/day); in both extensive (EG) and supplemented (SG) grazing there was a mixture of tropical grasses: *Cynodon niemfluensis*, *Muhlenbergia robusta*, *Brachiaria brizantha*, *B. decumbens* and *Echinochloa Polystachya*. Group EG (30%, 2014 heads) was permanently grazing while SG (44.6%, 2944 heads) was also supplied with a commercial balanced feed (18% crude protein; 2 to 3kg/head/day). Average daily milk yield was significantly ($P<0.05$) different among groups: 16.2±2.12kg (FC), 9.5±2.72kg (SG) and 7.2±1.530kg (EG). The breeding system also affected milk fatty acid profile, particularly the ω_6/ω_3 ratio: increasing the amount of concentrate in the diet significantly ($P<0.05$) increased milk ω_6 or decreased ω_3 concentration, thus diminishing the beneficial effects for human health.

Keywords: Milk; Grazing; PUFA; Omega fatty acids; Omega6/Omega3 ratio

Introduction

In recent years, the consumers' demand for foods with high nutritional value has strongly increased [1]. Concerning those of animal origin, it is accepted that animal diet can affect their quality [2-5]; in particular, foods produced by grazing ruminants are recognized by nearly all consumers, and farmers themselves, as high-quality foods [6]. On the other hand, dairy specialization and intensive farming have brought about an increase in the use of concentrates, thus reducing or even eliminating pasture as a feed source in many countries [7]. Indeed, in some areas like the tropics, there is still an abundant source of natural grasses and leguminous trees, that allows to feed cows in silvopasture environments, thus producing high quality milk [8]. Previous studies on the nutritional quality of milk in Mexico demonstrated several benefits of grazing in Zebu cow and goat [8]. In fact, lower content of Saturated Fatty Acids (SFA) and higher levels of ω_3 Polyunsaturated Fatty Acids (PUFA), were observed in milk from grazing animals compared to that from animals in full confinement [9,10]. It has been proven that a lower content of SFA favors human health [6], as well as it has been demonstrated that ω_3 PUFAs, in particular arachidonic acid and Docosahexaenoic Acid (DHA), are able to improve oxidative stress. This is critical, since oxidative stress is characterized by a decrease in the capacity of the endogenous system to act against oxidative attack directed to biomolecules, and it has been associated with different severe pathologies, such as cancer, cardiovascular diseases, type 2 diabetes, hypertension, and neurodegenerative diseases [6].

Other research focused on the importance of ω_6/ω_3 ratio [11], suggesting a value of 4 may prevent cardiovascular diseases up to a 70% reduction in mortality. More recently, the importance of maintaining a ω_6/ω_3 ratio lower than 4 was underlined [12,13] also because, in modern diets it results higher than 10 [14,15]. Thus, milk from grazing ruminants could have beneficial effects of human health, contributing to decrease the ω_6/ω_3 ratio of the diet. Aim of this study was to evaluate, over two years, the effect of grazing on the milk fatty acids profile, particularly the ω_6/ω_3 ratio, by comparing 6707 lactating cows undergoing three different breeding systems in Mexico.

Materials and Methods

Experimental design and treatments:

The experiment was performed on bulk milk of 84 farms (total of 6707 dairy cows, 2nd to 4th parity) in Mexico, for two years: 2017 and 2018. The farms were in Querétaro (Latitude: 20.5931, Length: -100.392 20°, at 1820msnm), Tabasco (18°20' north latitude, 93°15' length, 10 meters over the sea), Colima (latitude 19.2433, length-103.725 19° 14' 36" North, 103° 43' 30" West, over 550msnm), Veracruz (17° 09' latitude, 98° 39' length, 0 meters over the sea) and Chiapas (length: 093°22'51.74" latitude: N17°33'23.51", at 4080msnm). In each farm, the calving's were grouped, thus, most of the cows had the same days in milk; the lactation stages considered were from 30 to 60 days postpartum and 90 to 110 days postpartum. The farms were grouped according to their feeding system: Exclusive Grazing (EG), Supplemented Grazing (SG) or Full Confinement (FC). Groups EG (30.0%, 2014 heads) and SG (44.6%, 2944 heads) grazed on *Cynodon nemfluensis*, *Muhlenbergia robusta*, *Brachiaria brizantha*, *Brachiaria decumbens* and *Echinochloa polystachya* and in one farm in Queretaro on *Lolium perenne*. The animals in EG were permanently grazing while those in SG were supplemented (2 to 3kg/head/day) with commercial balanced concentrates (18% CP). Animals from group FC (25.3%, 1699 heads) were fed corn silage, tropical forages (Sugarcane tops, King grass, *Brachiaria sp*, Star grass), alfalfa hay and commercial balanced concentrate (16% CP; 5 to 7kg/head/day).

Feeds and milk sampling and analysis

In May and August of each year, bulk tank milk samples (100mL) were collected once a day for three consecutive days, in a sterilized plastic falcon tube, refrigerated at 3 °C and transported to the laboratory. An aliquot of each sample was analyzed for fat, protein and lactose (MilkoScan™ 133B, Foss Matic, Hilleroed, Denmark) while another one was refrigerated at 3 °C for 4h, frozen at -21 °C for 48h and then lyophilized.

Contemporary, in EG and SG system pasture samples were collected as follows: grass of four different areas (2.5m² each) was cut at 3cm from the ground; once weighed, 4 representative samples (1kg each, obtained balancing the amount from the 4 different areas) were air-oven dried at 65 °C, milled through a 1mm screen and analysed according to AOAC [16] for Dry Matter (DM, ID 934.01), Crude Protein (CP, ID 984.13), ether extract (EE, ID

920.29); the structural carbohydrates were also determined [17] and nutritive value (UFL=1700 kcal of net energy for lactation) was calculated [18].

Milk fatty acid analyses

Total fat of milk lyophilized samples was separated by a mixture of hexane isopropane (3/2, v/v), according to Hara and Radin [19]. Transmethylation of fatty acids was performed by the base-catalysed procedure described by Christie [20] and modified by Chouinard et al. [21]. FAME were quantified by Gas Chromatography (GC) using a CP-3380 chromatograph equipped with a split injector, Flame Ionization Detector (FID) and auto sampler CP 8400. A DB23 column (30m x 0.25mm i.d.) with a film thickness of 0.25µm was employed. Nitrogen was used as carrier gas at a flow rate of 30ml/min. Temperature's column was held for 1min at 120 °C, then programmed at rate of 10 °C/min to 200 °C and held for 5 °C/min to final temperature of 230 °C; temperature injector and FID were 250 °C and 300 °C, respectively. Integration for each fatty acid was performed by a Varian Star Chromatography Workstation Software. Identification of the peaks was made on the basis of the retention times of standard methyl esters of individual fatty acid (FAME mix C4-C24 #18919-1 AMP). The final concentration of FAME was expressed as mg/100g of milk.

Atherogenic and thrombogenic index

To better characterize the milk nutritional characteristics, the Atherogenic Index (AI) and the Thrombogenic Index (TI) were calculated according to Ulbricht and Southgate (1991):

$$AI = [C12:0 + (4 \times C14:0) + C16:0] / (\omega-3 + \omega-6 + MUFA)$$

$$TI = (C14:0 + C16:0 + C18:0) / [(0.5 \times C18:1) + (0.5 \times \text{other MUFA}) + (0.5 \times \omega-6) + (3 \times \omega-3) + (\omega-3/\omega-6)].$$

where:

C12: 0 = lauric acid, C14: 0 = myristic acid, C16: 0 = palmitic acid, C18: 0 = stearic acid, C18: 1 = oleic acid,

In the equations, C14:0 is considered to be 4 times more atherogenic than other FAs. To the C18:1, the omega 6 PUFA and to the rest of MUFA coefficients of 0.5 have been assigned because they are less anti-atherogenic than the omega 3, to which a coefficient of 3 was assigned.

Statistical analysis

All the data were analyzed using a one-way ANOVA design. Data analysis was carried out using the General Linear Model Procedures (Statgraphics- Centurion), calculated with Statistical Analysis System [22].

Result

Feed analysis

The chemical composition of the diets fed by animals in the different breeding systems is reported in Table 1. The highest content of crude protein and the lowest NDF and ADF percentages were registered in FC while EG showed opposite results. Consequently, the diet nutritive value increased with the increase

of concentrate in the diet (UFL/kg DM: 0.75-0.76 vs 0.78-0.80 vs 0.83-0.87, for EG, SG and FC system, respectively).

Table 1: Diet chemical composition (%DM) and nutritive value (MUF/kg DM) in Exclusive Grazing (EG), Supplemented Grazing (SG) and confinement (FC) system.

	EG	SG	FC
CP	13.4-14.6	13.8-15.0	14.1-15.6
NDF	40.4-44.0	36.1-39.0	31.1-33.3
ADF	28.4-30.2	27.1-29.3	22.5-24.1
UFL/kg DM	0.75-0.76	0.78-0.80	0.83-0.87

CP: Crude Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; UFL: net energy for lactation.

Milk yield

Average milk yield was kg 7.2±1.53 vs 9.54±2.72 vs 16.20±2.12kg, for EG, SG and FC respectively Milk chemical composition was unaffected by treatment (Table 2).

Table 2: Milk chemical composition (g/kg).

	EG	SG	FC
Fat	45.0±1.1	44.7±2.0	44.0±2.2
Protein	35.1±1.0	35.1±2.1	35.0±1.1
Lactose	49.1±1.1	48.4±2.0	49.0±1.0

EG: Exclusive Grazing; SG: Supplemented Grazing; FC: Full Confinement.

Milk fatty acid profile

Myristic (C14:0), margaric (C17:0) and stearic (C18:0) acids, as well as total SFA, were significantly ($P<0.05$) higher in milk from FC than SG and EG. Group EG showed the lowest value of palmitic acid (C16:0) (28.12g/100g) statistically different ($P<0.05$) from both SG (30.00g/100g) and FC (32.13g/100g). The other SFAs were not significantly different among the breeding systems (Table 3).

Table 3: Milk chemical composition (g/kg).

	EG	SG	FC
C4:0	0.60±0.15	0.62±0.16	0.50±0.17
C6:0	0.85±0.21	0.81±0.19	0.70±0.13
C8:0	0.78±0.17b	0.67±0.14a	0.70±0.12ab
C10:0	1.92±0.84	1.71±0.32	1.92±0.23
C12:0	2.44±0.48	2.28±0.44	2.55±0.46
C14:0	9.62±1.06a	9.58±1.42a	11.91±1.23b
C15:0	1.74±0.26	1.70±0.28	1.67±0.47
C16:0	28.12±2.41a	30.00±2.84b	32.13±2.95b
C17:0	1.44±0.21b	1.44±0.23b	1.12±0.24a
C18:0	15.76±1.75ab	14.70±3.87a	17.46±0.82b
C20:0	0.43±0.12b	0.44±0.12a	0.51±0.10ab
SFA	63.71±4.23a	63.96±2.67a	71.17±3.50b

EG: Exclusive Grazing; SG: Supplemented Grazing; FC: Full Confinement; Means with different letters indicate differences ($P<0.05$) among breeding systems

Milk from SG showed the highest concentration, even if not significantly different, of unsaturated fatty acids (UFA): 35.76g/100g vs 35.09g/100g vs 34.62g/100g, for SG, EG and FC, respectively. Concerning the monounsaturated fatty acids (MUFA), milk from EG had the highest concentration (32.35g/100g) compared to FC (31.42g/100g) and SG (32.27g/100g), but, again, the differences were not significant. Similar results were found for the Polyunsaturated Fatty Acids (PUFA) concentration with no statistical difference among the breeding systems. The most representative acid in milk from all the systems was the oleic acid (C18:1) followed by linoleic acid (C18:2) and palmitoleic acid (C16:1) (Table 4). The $\omega6/\omega3$ ratio was significantly ($P<0.05$) different among the systems: group FC had the highest value (6.21:1) followed by SG (3.35:1) and EG (2.07:1). This result was mainly due to the different linolenic acid (C18:3) concentration among the groups. Concerning $\omega6$ FA, a significant lower concentration ($P<0.05$) was found for linoleic acid (C18:2) in milk from EG compared to those from the other systems (Table 4). Both the Atherogenic (AI) and Thrombogenic (TI) indexes were higher for FC than EG and SG, but a statistical difference ($P<0.05$) was seen only for TI (Table 5).

Table 4: Milk unsaturated fatty acids (UFA) profile (g/100g).

	EG	SG	FC
C14:1	1.10±0.46a	1.40±0.40b	1.33±0.25ab
C16:1	1.52±0.49a	2.13±0.68b	1.85±0.26ab
C18:1	29.73±3.66	28.74±2.31	28.24±2.64
C18:2	1.84±0.75a	2.67±1.35b	2.75±0.47b
C18:3	0.90±0.27b	0.82±0.40b	0.45±0.10 a
MUFA	32.35±3.88	32.27±2.22	31.43±2.31
PUFA	2.74±0.96	3.49±1.72	3.20±0.53
UFA	35.09±4.37	35.76±3.18	34.63±2.50
$\omega6/\omega3$	2.07±0.52a	3.35±0.67b	6.21±1.24c

EG: Exclusive Grazing; SG: Supplemented Grazing; FC: Full Confinement; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; UFA: Unsaturated Fatty Acids; $\omega6/\omega3$: Omega6/Omega3 ratio. Means with different letters indicate differences ($p<0.05$) among breeding systems.

Table 5: Milk atherogenic (AI) and thrombogenic (TI) index.

	EG	SG	FC
AI	2.02±0.48	1.99±0.31	2.39±0.25
TI	1.56±0.32 a	1.58±0.19 a	1.89±0.22 b

EG: Exclusive Grazing; SG: Supplemented Grazing; FC: Full Confinement. Means with different letters indicate differences ($p<0.05$) among breeding systems.

Discussion

The breeding system significantly affected milk yield which increased with the increase of concentrate in the animals' diet. Concerning milk chemical composition, fat percentage was higher

when animals had access to pasture but the differences with the full confinement system were not significant. By contrast, milk fatty acid profile was significantly healthier in the grazing than in the full confinement systems. Indeed, Park et al. [22], Jensen [23], Chapkin [24], and Banskalieva et al. [25] discussed that fat and cholesterol have worldwide increased in human's diet, thus becoming a serious health risk due to coronary and vascular problems. According to these authors, the consumption of saturated fatty acids, particularly lauric (C12:0) myristic (C14:0) and palmitic (16:0), are related to hypercholesterolemia due to an increase in plasma Low Density Lipoproteins (LDL) while stearic (C18:0) and oleic acid (C18:1) decrease LDL and increase High Density Lipoproteins (HDL), favoring liver formation of Very Low-Density Lipoproteins (VLDL) that allows cholesterol to be transformed to gall bladder salts. Data in the present trial confirmed this low potential hypercholesterolemic effect of milk from grazing animals; in fact, milk from exclusive grazing had significantly lower contents of both myristic (C14:0) and palmitic (16:0) acids, and milk from supplemented grazing only of palmitic acid than that from full confinement system.

Despite a similar content of oleic acid (C18:1), milk from EG and SG showed 50% higher content of linolenic acid (C18:3) than that from FC group. Some studies reported modifications of milk fatty acid profile as a function of animals' diet: in particular, a decrease of C16:0 and an increase of C18:0 and C18:1 content was observed in cows grazing pasture compared to cows fed a total mixed ration [26,27]. Concerning $\omega 6$ and $\omega 3$ PUFA, it has been shown that milk contents of both linoleic acid (C18:2, $\omega 6$) and linolenic acid (C18:3, $\omega 3$) are affected by the feeding system [28]. Similarly, in the present study, the highest content of linoleic acid was in milk from FC while that of linolenic acid in milk from EG. Decreasing milk $\omega 6/\omega 3$ ratio showed several beneficial effects for human health [5,15], particularly with values lower than 4, since higher levels could modify the beneficial effects of $\omega 3$ [14,15]. In present trial, the relationship between breeding system and milk $\omega 6/\omega 3$ ratio was shown feeding animals with higher quantity of concentrates increased milk yield and $\omega 6/\omega 3$ ratio. Similar results were reported by Salado et al. [29], in a study aimed to evaluate the effects of diets with different levels of concentrate (3.5, 7.0 and 10.5kg/day) on milk yield and quality of grazing dairy cows in early lactation.

These authors registered higher milk yield and protein in groups fed 7.0 and 10.5kg/day than in group fed 3.5kg of concentrate. In contrast, milk fat did not differ among the groups and, even if the potential hypercholesterolemic fatty acids of milk (C12:0 to C16:0) did not change by increasing concentrate intake, linolenic acid decreased and the $\omega 6/\omega 3$ ratio increased in groups fed higher amounts of concentrate. Corazzin et al. [30] evaluated the effect of concentrate supplementation (High: 3.0kg/head/d vs. Low: 1.5 kg/head/d) on milk fatty acid profile of Italian Simmental dairy cows grazing on alpine pasture. Low milk showed higher concentration of linolenic acid and total PUFA than High milk. Recently, Santa-Ana et al. [31] and Galina et al. [32] compared two breeding systems for

goats, full confinement or grazing: milk from animals fed on pasture showed higher PUFAs and MUFAs and lower SFAs with a significant reduction of atherogenic index, thus presumably more beneficial for human health.

Musco et al. [33] evaluated the effects of a feeding strategy (based on the use of outdoor paddocks; forage: concentrate ratio at least 70:30; forage including at least five different herbs; and no silages) in dairy cows on milk yield and chemical composition, and blood metabolic profile, including the evaluation of oxidative stress. These authors reported that the proposed feeding system was able to increase milk quality, mainly in terms of fats quality, without negative effects of animal health. Animals fed higher forage: concentrate diet were able to maintain body homeostasis by changing metabolism despite the low energy diet and they showed a general improvement of oxidative status, probably due to an improvement of the biological antioxidant potential. All these results showed that, even considering the differences among ruminants, management on grazing is the key component to improve omega relationship.

Finally, both milk Atherogenic (IA) and Thrombogenic Index (IT) were affected by breeding system. They take into account the potential effect of each fatty acid on human health, and they were lower for grazing than full confinement animals. Thus, milk from grazing animals should have low probability of increasing the incidence of atheroma and/or thrombus formation [34]. The possibility of producing healthier milk by grazing may be of great importance in those areas, like Mexico, still plenty of natural grasses. Also, further studies in the same areas should confirm the hypothesis that grazing may be also beneficial for animals' health, thus addressing the increasing concerns about animal welfare [35].

Conclusion

The breeding system resulted as a fundamental aspect to determine the nutritional quality of milk, mainly related to its fatty acid profile. Despite an increase of milk yield, the full confinement system showed a worsening of healthier parameters: $\omega 6/\omega 3$ ratio greater than 4:1 and increase of both atherogenic and thrombogenic indexes. Therefore, in response to the consumer demand for foods with high nutritional quality, the grazing systems has to be encouraged and milk from animals with free access to pasture has to be recommended.

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