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Effect of genotype, lactation and climatic factors on fatty acid profile of bovine milk

*Isabel Cristina Acosta-Balcazar[1], Jorge Quiroz-Valiente[2], Lorenzo Granados-Zurita[2], Emilio Manuel Aranda-Ibáñez[1†], Edith Hernández-Nataren[1], Joaquín Alberto Rincón-Ramírez[1], Lorenzo Danilo Granados-Rivera[3]**

[1]*Colegio de Postgraduados, Campus Tabasco, México*
[2]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Huimanguillo, Tabasco, México*
[3]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experiemental General Terán, Nuevo León, México*

**Corresponding author: granados.danilo@inifap.gob.mx; dgr_8422@hotmail.com*
[†]*Deceased*

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Abstract: Milk fat from bovine milk contains fatty acids that may have favourable properties for human health, for example, conjugated linoleic acid (CLA) has nutraceutical activity. This research aimed to know the effects of genotype, days of lactation and climatic factors on the fatty acids (FA) profile of milk and particularly the content of CLA in milk fat. Seventeen first-calving milking cows in early lactation were used for the assessment of milk; 12 were Gyr and five were F1 (Holstein/Gyr) crosses. Sampling was carried out every 15 days, from the beginning to the end of lactation (300 days). Fatty acids were analyzed employing gas chromatography. The genotype did not influence the content of the fatty acid groups: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and CLA. The highest MUFA and PUFA contents were recorded at 100 days of lactation (32.334 and 3.553 g/100 g of FA, respectively), while SFA and CLA had their highest production at 200 days of lactation (63.238 and 1.378 g/100 g of FA, respectively). Regarding the climate, the highest temperature caused a decrease in the CLA content, because temperatures above 30 °C caused a decrease in the grazing time.

Keywords: CLA; Gyr; temperature; tropics

Bovine milk and its dairy products are an important source of essential fatty acids (FA) and vitamins in the human diet and it plays a critical role in the sensory properties of these foods (Ortega et al. 2013). Bovine milk fat differs from goat's milk fat, which has relatively high levels of medium-chain FA (C6:0–C10:0) and may be responsible for its strong taste and lower acceptance by humans (Tolentino et al. 2015). Bovine milk is on average composed of 98% of triglycerides, 70% are saturated FA, 25% are monounsaturated and 5% are polyunsaturated and trans FA (Lindmark Mansson 2008). Scientific data shows that the consumption of abundant amounts of milk or dairy products can be harmful to health due to the high content of saturated FA (FAO 2016), bringing as consequences an increase in the con-

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centration of cholesterol, causing diseases such as type 2 diabetes, cancer, heart disease and obesity (Ortega-Perez et al. 2013). However, some fatty acids such as butyric acid also have favourable properties for human health which seems to exert a protective effect against colon cancer. Oleic and vaccenic acids reduce the risk of cardiovascular disease and conjugated linoleic acid (CLA) has been related to anticancer activity (Martinez et al. 2013). Several factors can alter the lipid and CLA profile of milk, among these the production system, type of diet, breed, use of additives in the diet, level of milk production, lactation stage and time of year can be mentioned (Martinez-Borraz et al. 2010). In Mexico, most of the studies have been carried out in areas of the Altiplano, where the largest dairy basins are located, leaving a gap of information regarding the tropical zones, where the production system that predominates is the dual-purpose one (Roman-Ponce et al. 2013), the feeding system is managed under grazing (Prieto-Manrrique et al. 2017) and weather conditions are rather severe (De Jesus et al. 2016). The objective of this research was to evaluate the effect of factors such as genotype, lactation days (LD) and climatic factors (maximum, minimum and relative humidity) on the FA profile in bovine milk under a semi-intensive production system and in tropical conditions.

MATERIAL AND METHODS

Location

The experiment was conducted in a private production unit, located in the municipality of Paraíso, Tabasco, Mexico (5 m above sea level). The climate in the region is classified as warm and humid, with a rainy period in summer and autumn. The average annual rainfall is 2 295 mm, wet season is from June to October with 70% of the annual rain, and dry season from November to May; the maximum, average and minimum temperatures recorded are 35, 25 and 15 °C, respectively, with 77.4% relative humidity (INEGI 2016).

Animals, experimental design and treatments

Seventeen first-calving milking cows in early lactation were used for the assessment of milk; 12 were Gyr and five cows were F1 (Holstein/Gyr). Milk samples were collected at each milking (4:00 and 16:00) every 15 days for a time of 300 days; for the analysis of days in lactation this time was grouped into three periods (100, 200 and 300 days). Treatments consisted of two genotypes (Gyr vs F1) and 100, 200 and 300 days in lactation. Additionally, the relationship between conjugated linoleic acid concentrations related to temperature and relative humidity was evaluated.

In this study, animals were handled according to the guidelines and regulations for animal experimentation of the Academic Division of Animal Sciences of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (ID project: 945934889).

Feeding

The feeding system was based on pastures, offered to free access, under an intensive rotational system, in 52 paddocks of approximately 2 500 m^2 with an electric fence. The main pastures consumed were as follows: limpo grass (*Hemarthria altissima*, 65%), German grass (*Echinochloa polystachya*, 5%), Bahia grass (*Paspalum notatum*, 10%) and giant star grass (*Cynodon plectostachyus*, 20%); with an occupancy period of 7 h/day/paddock.

In addition, the cows were offered maize silage between 6 kg and 10 kg per cow per day (to complete the amount of biomass available in the paddock), divided between the two milkings, and commercial feed at the rate of 1 kg per 3 kg of milk produced, with averages of 8% and 18% of protein, respectively; they were also provided 50 g of mineral salts per day per cow, all this in order to meet the requirements of the animals and avoid weight loss during the milk production period.

Milk production and composition

Milk samples were collected every 15 days, at two milking times, for 300 days. Sampling was carried out using four Waikato MK (New Zealand) milk meters. A portion of the total volume of milk milked per cow was collected. The milk samples were stored in plastic vials (50 ml) and kept under refrigeration at 4 °C and then analyzed for chemical composition. The variables measured were daily

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milk yield (DMY, kg/day), milk composition (g/kg) and yield (g/day) of fat, lactose, and protein, as well as the fatty acid profile (g/100 g of FA).

Laboratory analysis

A total of 709 samples were analyzed; of which 629 were used to determine the chemical composition and 80 for the fatty acid profile. The determination of fat, protein and lactose in milk was carried out by ultrasound (LactoScan, Nova Zagora, Bulgaria) at the Central Laboratory of the Postgraduate College, Campus Tabasco. Each sample was heated in a water bath until reaching a temperature between 15 °C and 20 °C to optimize the analysis and preserve the useful life of the equipment.

FA extraction

The extraction of FA from milk was carried out according to the methodology of Feng et al. (2004). 50 µl of the lipids extracted from milk were taken and placed in polypropylene tubes, 3 ml of sodium methoxide (0.5 M in methanol to protect the isomerization process of unsaturated FA) were added, and they were stirred for 1 min with vortex. The tubes were placed into a beaker with distilled water, at 80 °C for 10 min, the tubes were removed from the beaker, allowed to cool for 10 min, 3.5 ml of hexane were added to dissolve and to extract only the fat, and 5 ml of 6% potassium carbonate to saponify and release the FA, which were vortexed for 1 min and centrifuged for 5 min at 2 500 × *g*.

Then the hexane fraction, located in the upper part of the tube, was extracted and deposited in polypropylene tubes, which contained 0.5 g of sodium to eliminate moisture excess and 0.1 g of activated carbon to eliminate impurities; they were shaken with vortex and centrifuged at 1 500 × *g* for 5 min. The first phase was then extracted from hexane, and filtered through an Acrodisc (titan 44513-NN, 17-mm green filter and 0.45-µm nylon membrane; Thermo Fisher Scientific, Waltham, MA, USA; to ensure a sample free of impurities) and placed in a vial where it was stored at –5 °C until its analysis by gas chromatography.

The methyl esters of FA were determined through gas chromatography, using a Hewlett Packard 6890 chromatograph with a flame ionization detector (HP, Inc., Palo Alto, CA, USA) and automatic injector (series 53308-02; Supelco Inc., Bellefonte, PA, USA), for which it was necessary to use a capillary column of fused silica (SP-2560, 100 m × 0.25 mm × 0.2 µm film thickness), a FAME Mix C4-C24 standard, cat. No. 18919-1AMP (Supelco Inc., Bellefonte, PA, USA), a specific standard for cis-9, trans-11 and trans-10, cis-12 isomers from Nu-Chek (Elysian, MN, USA) and helium was used as carrier gas. 0.1 µl of the methylated fat sample was injected and the used temperature ramp began with 140 °C for 2.95 min, then it was increasing 1 °C/min up to 210 °C, then it rose 0.70 °C/min up to 235 °C.

Statistical analysis

The statistical analysis consisted of a completely randomized design in an arrangement with repeated measurements, using the MIXED procedure in SAS v9.4 software (SAS Institute, Inc., Cary, NC, USA) and the comparison of least squares means that was carried out through Tukey's test ($P \leq 0.05$).

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \Upsilon_k + \alpha\Upsilon_{ik} + \varepsilon_{ijk} \quad (1)$$

where:

Y_{ijk} – the response due to subject k, treatment j and period i;

μ – the overall mean; α_i is the fixed effect due to the breed treatment, assuming that $\Sigma\alpha_i = 0$;

$\beta_{j(i)}$ – the random effect due to subject i nested within the treatment, $\beta_{j(i)} \sim N(0, \sigma^2\beta)$, $\beta_{j(i)}$ is independent;

Υ_k – the fixed effect due to the period k, $\Sigma\Upsilon_k = 0$;

$\alpha\Upsilon_{ik}$ – the fixed effect of interaction due to treatment i and period k, $\Sigma\alpha\Upsilon_{ik} = 0$;

ε_{ijk} – the random error component, $\varepsilon_{ijk} \sim N(0, \sigma^2\varepsilon)$.

To know the direct influence of the climatic factors (maximum and minimum temperature and relative humidity) a multiple regression model was applied using the REG procedure and the STEPWISE method for the selection of variables.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \varepsilon_i \quad (2)$$

where:

Y – the dependent variable (milk production, fat, lactose, protein, conjugated linoleic acid, etc.);

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$\beta_0, \beta_2, \ldots \beta_n$ – the parameters of the regression equation;
$X_1, X_2, \ldots X_n$ – the independent variables (temperature and humidity);
ε_i – the random error component.

RESULTS

Milk production

The F1 cross had higher milk production (14.07 ± 0.91 kg/day) than Gyr cows (9.33 ± 0.86 kg/day) (Table 1); this meant 33.68% more milk. In both genotypes, milk production was higher during the first 100 days; in F1 cows, the peak of production was up to 15.63 kg of milk per cow per day and in Gyr cows it was 10.76 kg of milk per cow per day.

On the other hand, the minimum temperature was the climatic factor that caused the highest variations in milk production; in this regard, a milk increase of 426 g/day was obtained in Gyr cows and 513 g/day in F1 cows for each degree of an increase in the minimum temperature (Figure 1).

Milk fat was the component of milk with the highest variation. Milk fat was higher in Gyr cows (4.65%) than in F1 cows (4.13%). Milk fat was lower in the first 100 days of lactation, ranging from 37.30 g/kg to 49.60 g/kg at the end of the investigation period.

Furthermore, fat was the only component of milk that varied in relation to climatic factors; according to the linear regression analysis, the lower the temperature, the lower the fat production (0.213 g/kg) in both genotypes (Figure 2). Moreover, the average lactose was 45.45 g/kg in the

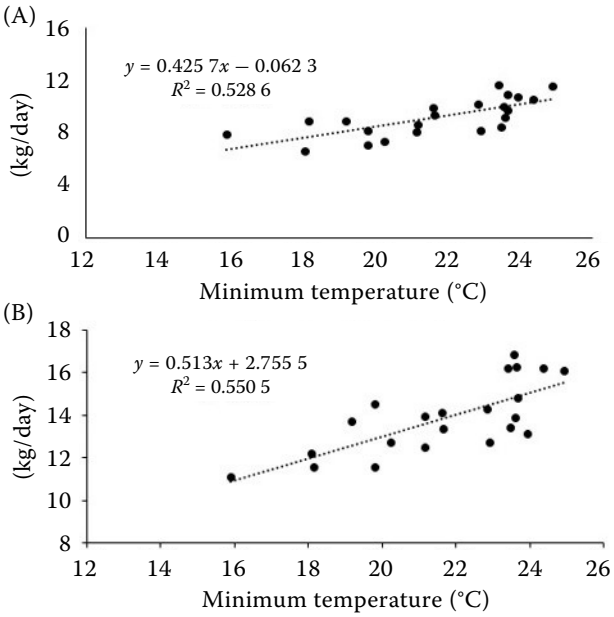

Figure 1. Production of milk related to minimum temperature in Gyr (A) and F1 (B) cows

first 100 days of lactation, increasing by 2.68% towards the next 200 days (46.70 g/kg) and finally decreasing to 46.40 g/kg by the end of the 200-day period. The protein content within 100 days in lactation was 32.30 g/kg, increasing by 2% towards 200 days (32.95 g/kg) and it ended within 300 days with an average of 32.40 g/kg.

Fatty acid profile

Table 2 shows the mean values of the fatty acid profile of Gyr and F1 cows during lactation. There

Table 1. Milk production (kg/day) and milk composition (g/kg of milk) and yield (g/day) of fat, lactose and protein for genotype and days of lactation

Variables	Genotype				*P*-values		
	Gyr	SEM	F1	SEM	genotype	DIM	genotype × DIM
Milk production (kg/day)	9.33	0.63	14.07	0.98	0.001	< 0.000 1	0.740
Production (g/kg of milk)							
Fat	46.55	1.23	41.32	1.90	0.035	< 0.000 1	0.270
Lactose	45.46	0.39	45.07	0.60	0.594	0.001 9	0.954
Protein	31.99	0.21	31.75	0.33	0.559	< 0.000 1	0.899
Yield (g/day)							
Fat	434.36	13.99	581.45	21.67	< 0.000 1	< 0.000 1	0.475
Lactose	424.17	3.78	634.13	5.86	< 0.000 1	0.000 2	0.508
Protein	298.45	2.08	446.74	3.22	< 0.000 1	< 0.000 1	0.344

DIM = days in milk; SEM = standard error of the mean

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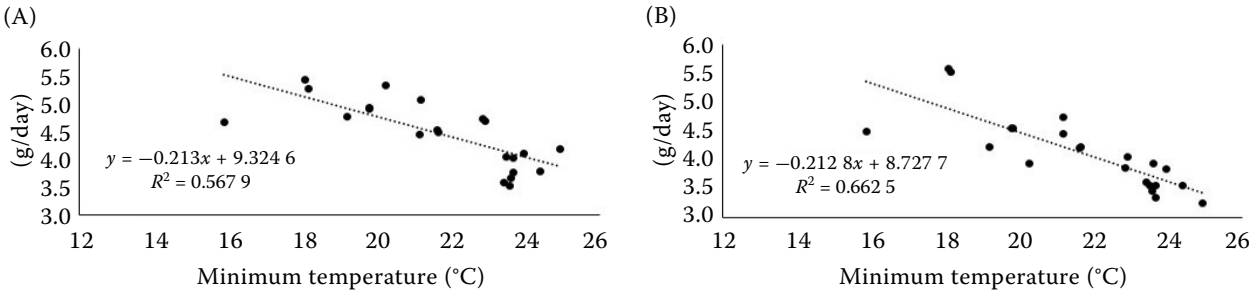

Figure 2. Production of milk fat related to minimum temperature in Gyr (A) and F1 (B) cows

was a trend ($P \leq 0.07$) for SFA to increase in milk from Gyr cows compared to F1 cows. Likewise, a tendency ($P \leq 0.08$) is observed for MUFA to decrease in the milk of Gyr cows compared to F1 cows. There was no difference in the concentration of the cis-9, trans-11 isomer of CLA between the races

Table 2. Fatty acid profile (g/100 g of FA) of milk from Gyr and F1 cows during lactation

Fatty acids	Genotype				*P*-values		
	GYR	SEM	F1	SEM	genotype	DIM	genotype × DIM
Butyric	0.655 ± 0.06	0.044	0.734 ± 0.04	0.044	0.260	0.728	0.295
Caproic	2.133 ± 0.18	0.095	1.896 ± 0.11	0.095	0.131	0.825	0.906
Caprylic	1.418 ± 0.10	0.075	1.176 ± 0.06	0.075	0.064	0.743	0.964
Capric	3.069 ± 0.19	0.165	2.513 ± 0.12	0.165	0.054	0.042	0.595
Undecanoic	0.494 ± 0.06	0.033	0.358 ± 0.04	0.033	0.028	0.027	0.082
Lauric	3.476 ± 0.22	0.162	2.907 ± 0.14	0.162	0.047	0.005	0.681
Tridecanoic	0.217 ± 0.02	0.010	0.217 ± 0.01	0.010	0.984	0.322	0.162
Miristic	11.356 ± 0.44	0.228	10.414 ± 0.27	0.228	0.026	< 0.000 1	0.857
Myristoleic	1.705 ± 0.22	0.107	1.375 ± 0.14	0.107	0.073	< 0.000 1	0.037
Pentadecanoic	0.350 ± 0.04	0.018	0.333 ± 0.03	0.018	0.551	0.016	0.180
Palmitic	30.179 ± 1.02	0.567	29.349 ± 0.64	0.567	0.341	0.000 9	0.021
Palmitoleic	2.452 ± 0.18	0.084	2.352 ± 0.11	0.084	0.340	0.000 2	0.025
Heptadecanoic	0.711 ± 0.05	0.017	0.669 ± 0.03	0.017	0.651	< 0.000 1	0.895
cis-10heptadecenoic	0.298 ± 0.04	0.007	0.335 ± 0.03	0.007	0.011	< 0.000 1	0.221
Stearic	9.213 ± 0.69	0.321	9.774 ± 0.44	0.321	0.264	0.001	0.283
Eladic	3.349 ± 0.28	0.188	3.623 ± 0.18	0.188	0.342	0.008	0.443
Oleic	22.652 ± 1.42	0.920	25.221 ± 0.90	0.920	0.096	0.000 3	0.664
Linolelaidic	0.181 ± 0.02	0.015	0.218 ± 0.01	0.015	0.129	0.000 5	0.004
Linoleic	1.580 ± 0.24	0.098	1.781 ± 0.15	0.098	0.197	0.000 6	0.064
Arachidonic	0.120 ± 0.01	0.006	0.122 ± 0.01	0.006	0.870	0.089	0.760
Linolenic	0.238 ± 0.04	0.014	0.279 ± 0.02	0.014	0.091	0.003	0.219
cis-9, trans-11 CLA	1.254 ± 0.10	0.064	1.309 ± 0.06	0.064	0.565	0.000 4	0.009
Others	2.957 ± 0.19	0.088	3.041 ± 0.12	0.088	0.527	0.495	0.548
ΣSFA	63.397 ± 1.51	0.973	60.496 ± 1.25	0.973	0.079	0.009	0.453
ΣMUFA	30.458 ± 1.32	0.841	32.870 ± 1.17	0.841	0.089	0.015	0.798
ΣPUFA	3.254 ± 0.31	0.193	3.582 ± 0.26	0.193	0.274	0.208	0.023

CLA = conjugated linoleic acid; DIM = days in milk; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SEM = standard error of the mean; SFA = saturated fatty acids

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evaluated. During lactation, differences were found in the concentration of SFA, MUFA and the cis-9, trans-11 isomer of CLA (Figure 3).

Regarding temperature and humidity, they did not affect the concentration of fatty acids in the milk of Gyr and F1 cows. However, a decrease in the concentration of the 18:2-cis-9, trans-11 isomer of CLA was detected with the increase in ambient temperature (Figure 4).

DISCUSSION

The higher milk production observed in F1 cows compared to Gyr cows could be related to heterosis from the crossing of *Bos taurus* animals with *Bos indicus*. In particular, *Bos taurus* transmits

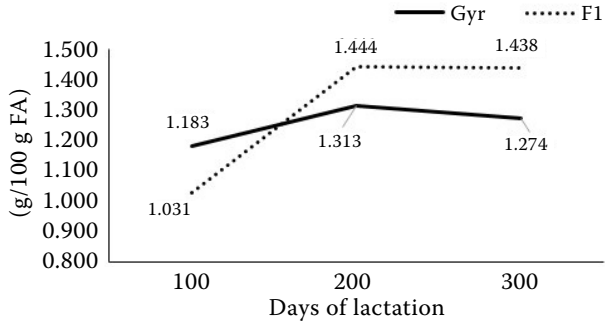

Figure 3. Yield of conjugated linoleic acid in milk during the lactation period

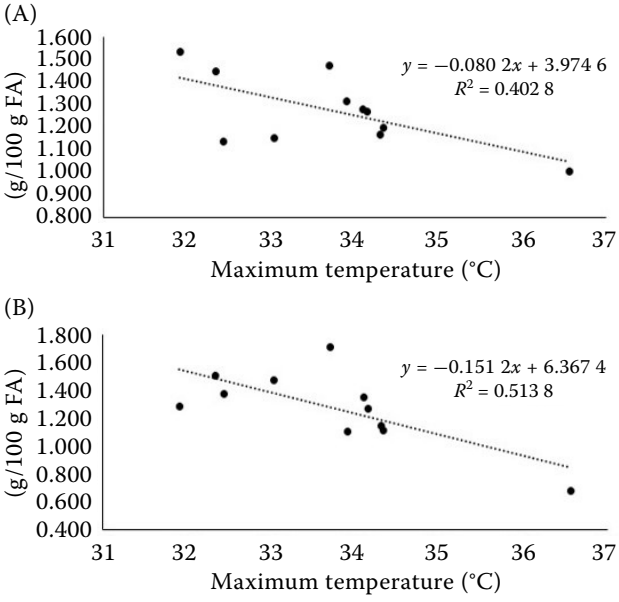

Figure 4. Production of conjugated linoleic acid related to maximum temperature in Gyr (A) and F1 (B) cows

to a crossbreed the ability to improve nutrient metabolism, while *Bos indicus* transmits information on adaptation to the tropical environment.

Therefore, a cow is obtained with a high capacity to take advantage of the available nutrients which is also tolerant to heat stress generated by the tropical climate; consequently, milk production increases (Lopez et al. 2013). Likewise, the fact that 100 days after the start of lactation the highest milk production was observed, independently of the breed, can be explained because this period corresponds to the ascending phase in milk production, a phase characterized by increasing production until reaching maximum or peak production.

On the other hand, temperature has a direct relationship with the production of bovine milk. Animals often exceed the capacity of their normal mechanisms to dissipate the heat they generate. In this regard, cows are homeothermic animals and they try to maintain their temperature at 38 °C, through the control of internal heat production and the gain and loss of external heat (Bernabucci et al. 2014). When the temperatures are high, a stress condition is caused which affects the physiology and homeostasis of the cow, and is reflected in a decrease in voluntary feed consumption and consequently a decrease in milk production (Carabano et al. 2016).

Another remarkable issue is that the Gyr cows showed the highest fat concentration, agreeing with various reports that indicate that *Bos indicus* cows are characterized by having a higher fat content in milk than *Bos taurus* cows. In another aspect, the decrease in fat concentration with an increase in milk production can be explained by the negative energy balance that cows show at the beginning of lactation, since milk production requires a large amount of energy, therefore, the energy consumption is lower than the energy requirement, and consequently decreases the concentration of fat in milk.

The genotype was not a factor that influenced the FA profile, except for undecanoic, lauric and myristic acids, these were found in higher concentrations in the milk of Gyr cows, which meant more than 15.32% of FA in Gyr; while in F1 cows, these same FA represented 13.67% (Table 2). The concentration of this group of FA is similar to that reported by Cerutti et al. (2016) with F1 cows and higher than the report by Renno et al. (2013). Regarding SFA, the values in this study are

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lower than those reported by Lindmark Mansson (2008; 70.0 g/100 g of FA), Castro-Hernandez et al. (2014; 70.0 g/100 g of FA), Roca-Fernandez et al. (2012; 70.0 g/100 g of FA) and Granados-Rivera and Hernandez-Mendo (2018; 70.0 g/100 g of FA). In these studies, pure Holstein cows and different crosses were used, being fed on pastures or supplemented. By including fresh forage in the cow's diet, there is an increase in the content of unsaturated FA (MUFA and PUFA) (Ortega-Perez et al. 2013). This could be the reason for the concentration of MUFA and PUFA in the present study since the cows were grazing between 5 and 6 h during the day. In particular, the CLA concentration did not differ significantly between races, however, such concentration was higher than that reported by Granados-Rivera and Hernandez-Mendo. (2018; 1.0 g/100 g of FA) in grazing cows; it also surpassed that reported by Renno et al. (2013), Castro-Hernandez et al. (2014), Cerutti et al. (2016) and Welter et al. (2016), whose CLA productions were between 0.43 and 0.76 g/100 g of FA with Holstein and Holstein/Gyr cows, grazing and supplemented in tropical countries such as Mexico and Brazil; but lower compared to 1.81 g/100 g of FA reported by Kelly et al. (1998), with Holstein cows housed and supplemented with maize silage. Pastures have higher contents of linoleic acid, CLA precursor in the biohydrogenation process that takes place in the rumen, and linolenic acid, vaccenic acid precursor, which is an intermediary of CLA production in the *de novo* synthesis process in the mammary gland (Ortega-Perez et al. 2013). On the other hand, the fat fraction of tropical grasses may contain more than 60% of these LAC precursors, however, it will fundamentally depend on the soil fertility (Hafla et al. 2013).

Seventy-seven percent of recorded FA showed differences ($P < 0.05$) related to lactation days, as for the groups of SFA. They had an average of 61.159 g/100 g of FA in the first 100 days of lactation, increasing by 3.4% at 200 days and ending with an average of 61.442 g/100 g of FA, that is, 1.67% less than at 200 days. Regarding the MUFA, at the beginning they had an average of 32.334 g/100 g of FA, showing a decrease of 6% at 200 days (30.383 g/100 g of FA) and finally they amounted to 32.189 g/100 g of FA at 300 days. Graphically, it can be observed that there is a negative correlation between the SFA and MUFA groups, because as SFA increased, MUFA decreased; although the highest production occurred in SFA, which reached almost a double content compared that of MUFA. Although the contents of SFA and MUFA during lactation were different statistically, the variations of these FA were very small in percentage terms. The results of other studies consulted are very inconsistent, so it is not clear why this behaviour occurs through lactation. This makes it necessary to carry out an investigation with better control of these aspects. The PUFA group was the only one that did not show any significant differences for lactation (Table 1), which means that the productions of these acids were somewhat constant and even tended to be maintained towards the end of lactation. Regarding the CLA, the result was highly significant ($P = 0.000\ 4$); the production averages in the first 100 days were 1.183 g/100 g of FA for the Gyr breed, that is 14% more than in the F1 crosses, which had 1.031 g/100 g of FA. At 200 days, both genotypes showed an increase, being greater in the F1 cross, which was 40% (0.414 g/100 g of total fatty acids), while in the Gyr breed it was 11% (0.130 g/100 g of total fatty acids). Finally, the Gyr breed had a decrease of 0.039 g/100 g of total fatty acids, which is 3% concerning the average of 200 days and the production of F1 tended to be maintained, since its decrease did not mean even 1%. The variations in this component could be explained by the content of CLA precursors (linoleic acid and linolenic acid) in pastures which is highly variable, since this content will depend on the cutting age, the species, the season and the type of soil. Because of the little information that exists in the tropics, lines of research should be made so that more information can be provided on the production of this fatty acid and its relationship with the factors mentioned here.

Climatic factors (temperature and humidity) did not affect the contents of the three groups of fatty acids present in milk fat, but there were decreases in CLA content when there was an increase in maximum temperature (Figure 4); this could be so because the animals shortened the grazing time when the temperature exceeded 30 °C, causing a low consumption of linoleic and linolenic acids, this decrease was greater in the F1 cross (88.75% compared to the Gyr breed). This phenomenon could be explained due to the genetic material that is modified in the crossing between races, therefore, the animals are more susceptible to variations in temperatures (Santana et al. 2015).

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CONCLUSION

The highest milk production occurred in the F1 (Holstein/Gyr) cross and the production peak occurred in the first 100 days for both genotypes, the minimum temperature being the climatic factor that positively affected milk production.

The fat content in milk was more abundant in the Gyr breed, while the lactose and protein content did not show any variations with respect to the genotype. For both cases (Gyr and F1) the highest fat content occurred in the last 100 days of the lactation period, while the highest lactose and protein content was recorded after 200 days.

The contents of SFA, MUFA, PUFA and CLA had similar behaviours in both genotypes; SFA and CLA were more abundant at 200 days of the lactation period, while AGM and AGP showed a higher content in the first 100 days of lactation.

The climatic conditions did not produce any changes in the content of the fatty acid groups, but they affected the CLA content.

Conflict of interest

The authors declare no conflict of interest.

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