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Full Length Research Paper

The comparison of lactation performance and milk fatty acid composition of Sarabi indigenous and Holstein cows

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The objective of this study was to specify fatty acid content of milk from Sarabi indigenous cows and compare it with milk fatty acid profile of Holstein cows. Ten Holstein and twelve Sarabi confinement cows that have been fed on total mixed ration with corn silage, alfalfa hay and dairy concentrate were used in a completely randomized design. Holstein cows had greater milk production (P<.0001), whereas Sarabi cows on average had higher milk content of fat (P=0.0540), protein (P=0.0340) and lactose (P=0.1794). Milk from Sarabi and Holstein cows had similar content of the *cis*-9 *trans*-11 conjugated linoleic acid (0.28 and 0.30%, respectively) and short and medium chain fatty acids (18.58 and 18.62%, respectively) Sarabi cows numerically produced higher concentrations of saturated fatty acids (60.51 and 59.29%, respectively), and long chain fatty acids (43.32 and 41.26%, respectively), as well as lower concentrations of mono unsaturated fatty acids (20.75 and 21.65% respectively) than Holstein. The percentage of C16:0 main saturated fatty acid in milk fat was not different between the groups. Concentrations of C14:0 and C18:0, second and third prominent milk saturated fatty acids were respectively 9.98 and 9.93% in Sarabi and 10.10% and 9.60% in Holsteins. In summary, despite the slight differences between fatty acid content of milk from Sarabi and Holstein cows, we did not find any statistical significance.

Key words: Breed, Sarabi cow, milk fatty acid, conjugated linoleic acid.

INTRODUCTION

Milk fat is a rich source of bioactive lipids, such as butyric, vaccenic and conjugated linoleic acid, attributed to anti-carcinogenic, anti-diabetic, anti-obesity, anti-atherogenic and immunomodulatory functions in experimental animal models (Shingfield, 2008). In contrast, palmitic, myristic and lauric acids, the major saturated fatty acids in milk fat, generally contribute to an increase in blood cholesterol levels. Public health concerns are driving research into modifying the fatty acid profiles of cow's milk (Glasser et al., 2008), particularly towards reducing the proportion of the aforementioned saturated fatty acids, increasing monounsaturated fatty acids and polyunsaturated fatty acid content and/or enhancing the concentration of bioactive lipids (Shingfield, 2008).

There are two different ways to alter milk fatty acids, supplementation of dairy cow feed or genetic selection. While feed supplementation is the most popular way to improve the nutritional quality of milk, it presents certain disadvantages (Soyeurt et al., 2006). First, this approach ignores the animal genetic effect, even though the effect of genetics on milk components such as milk fat has

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been demonstrated previously. Secondly, this improvement is not permanent as when supplementation is stopped, changes in nutritional quality disappear. The advantages of the genetic approach are linked to these disadvantages: Genetic improvement is permanent and it has the advantage of creating additional value through selection (Soyeurt et al., 2006).

Moreover, Stoop et al. (2008) stated milk fat composition can be changed by means of selective breeding, which offers opportunities to meet consumer demands regarding health and technological aspects. Soyeurt et al. (2006) suggested the possibility of obtaining milk products with improved nutritional quality by choosing the right breed. Maurice-van Eindhoven et al. (2011) also suggested that the genetic features of a population in favor of human health can be modified permanently by making use of the different breeds in the dairy cattle population. Various authors reported a certain effect of breed on milk fat composition but findings were often contradictory, some supporting and others disproving a breed effect on a distinct fatty acid (Bartla et al., 2008).

Nevertheless, the desire to produce milk and dairy products with greater nutritional value has encouraged research on comparisons of indigenous dairy cows milk, in the same way that it has been done among different breeds. Bar et al. (2006) found that the milk gained from the two native, the Whitebacks and Polish Red breed, had the fatty acid ratio most beneficial for human nutrition than Holstein-Friesian varieties. Bartla et al. (2008) while determining the changes of the milk fatty acid profile of Brown Swiss and indigenous Peruvian Criollo cows also observed significant differences for milk yield, fatty acid excretion, milk fat content conjugated linoleic acid and poly unsaturated fatty acids. In addition, Yassir et al. (2012) reported the level of cis-9, trans-11 conjugated linoleic acid in milk fat of Mafriwal was significantly higher ($P<0.05$) than that of the Jersey cows. Myburgh et al. (2012) reporting the milk composition of four African cattle breeds, the Afrikaner, Boran, Nguni and Tuli, and two composite breeds (the Bonsmara and Drakensberger), stated that the nutrient composition of the milk of these cattle, including the dry matter, whey proteins and non-protein nitrogen is lower than that of dairy breeds.

The Sarabi indigenous cow is one of the major regional dual purpose cows in Iran, which has a strong adaption to cold weather, diseases and parasites. The breed was imported from Gharabagh and breeds in Sarab, Mianeh and Ardebil regions of Iran (Khansefid, 2010). Although Sarabi is a dual purpose cow, the milk production of this breed is noticeably higher than other Iranian vernacular cow breeds (Khansefid, 2010). However, there is no information on the fatty acids profile of its milk. The objective of this study was to bridge the existing gap by specifying the fatty acid content of milk from Sarabi cows in comparison with the profile of Holstein cows, a major dairy cow present in Iran.

MATERIALS AND METHODS

Animals and diets

The experiment was conducted at the Research Station of Indigenous Sarabi Cattle Herd, in November and December 2010. Holstein (n = 10; 2 primiparous and 8 multiparous) and Sarabi (n = 12; 5 primiparous and 7 multiparous) cows that have been confinement fed on with corn silage, alfalfa hay and concentrate were used in the experiment. Administration of diet was as follows: half of alfalfa were fed at 08:00, half of concentrate were fed at 10:00, half of corn silage were fed at 12:00, another half of alfalfa were fed at 20:00, and another half of concentrate were fed at 22:00, for *ad libitum* intake (target of 10% orts). Amounts of forage and concentrate offered and refused were recorded daily for each group. Samples of forage, concentrate and their orts were collected weekly, and dried at 105℃ for 24 h for dry matter determination. Samples of individual feed ingredients were oven-dried (60℃), ground, and analyzed for crude protein (CP) and neutral detergent fiber (NDF) content (Table 1). Dry matter intake was determined for each group by weighing the amount fed and orts.

Cows were milked at 0600, and 0170 h daily and, individual weights of milk were recorded at each milking (Table 2). A composite milk sample from morning and evening milking on December 1, 2010 was collected for each cow. One set of samples were used to determine fat, protein, lactose (Dairy scan, jet/1). The second set was stored on ice and were immediately sent to the Chemistry Faculty of Tabriz University for fatty acid analysis. Milk fat was extracted by the method of Bligh and Dyer (1959). The derivation of fatty acids was done by the method of Shanta and Decker (1993). Milk fatty acid was analyzed in a gas chromatograph (Technologies Agilent; model 6890). The fatty acid composition of milk fat is expressed as amount of each individual fatty acid per fat present.

Extraction of lipids

Milk fat was extracted using a modification of the Bligh and Dyer method (1959). In brief, a sample of milk (10 g) was mixed (vortexed for 2 min) with methanol (10 ml) and dichloromethane (5 ml) in a polyethylene centrifuge tube (50 ml). Dichloromethane (5 ml) and NaCl (0.1 g) was then added and mixed (vortexed for 30 s). The mixture was centrifuged (1780 × *g*, 20 min, 0℃) to partition into two distinct solvent layers separated by a white gelatinous layer. The bottom layer (dichloromethane) was collected. A dichloromethane wash (5 ml) was added to the gelatinous layer, mixed (vortexed for 1 min) and centrifuged (1780 × *g*, 10 min, 0℃). Dichloromethane wash was combined with the original extract, and then the lipid samples were stored in amber-coloured glass vials with screw-top lids fitted with PTFE/silicone inserts at −20° until analysed.

Derivation of fatty acids

The fatty acids were derived by Shanta and Decker (1993) method. Frozen samples were melted at 40℃ water bath and were dried using nitrogen injection, and then 2 ml of hexane were added to dried samples. Next, 100 µL of samples were transferred to new tubes and 25 µL of internal standard (heneicosanoic acid) was added to them. This was followed by an addition of 400 µL of methanol and 100 µL methyl guanidine. Samples were dried using nitrogen and boiled for 10 min, after which the tubes were recapped. After cooling the mixture to room temperature, 5 cc of saturated saline were added to the tubes, and then 2 cc petroleum ether were added to the mixture and dried using nitrogen. In the next stage, the mixture was shaken for 3 min and was centrifuge.

Table 1. Ingredient and nutrient composition of diets.

Ingredient (%DM)	Sarabi	Holstein
Alfalfa hay	46.57	47.10
Corn silage	18.41	15.75
Barley grain	5.36	5.66
Wheat grain	5.36	5.66
Corn grain	2.83	3.00
Wheat barn	9.91	10.47
Cottonseed meal	2.49	2.63
Canola meal	1.48	1.56
Rice barn	2.10	2.12
Bagasse	1.76	1.86
Molasses	1.76	1.86
Urea	0.18	0.19
Buffer	0.71	0.74
Premix	0.36	0.38
Dicalcium phosphate	0.18	0.19
Calcium carbonate	0.36	0.38
Salt	0.18	0.19
Nutrient		
CP (%)	13.5	13.7
NDF (%)	35	35
EE (%)	3	3.1
Ca (%)	0.99	1.01
P (%)	0.38	0.39
NEL (Mcal/kg)	1.37	1.38

for 5 min to partition into two distinct layers. Afterward, the bottom layer was omitted and the top layer was dried by nitrogen. Finally, 5cc hexane was added to the mixture and samples were dried again by nitrogen and transferred to freezer.

Experimental design

Lactation performance and fatty acid data were analyzed using a general linear model and lactation period (1st, 2nd and 3rd portion) and lactation number (1st, 2nd and 3rd and greater). Milk yield or fat concentration was used as covariate (SAS Institute Inc., Cary, NC), according to the model:

$y_{ij} = \beta. + \beta_1 x_{ij} + r_i + \varepsilon_{ij}$

Where, y_{ij} is the $_j$th observation in ith group; $\beta.$ is the intercept; β_1 is the regression coefficient; x_{ij} is the appropriate covariate (example parity, lactation period, milk yield or fat content); r_i is the fixed effect of the ith breed and ε_{ij} is the residual error. Tukey's test was used to test treatment means ($P < 0.05$) of the experiment (Table 4).

RESULTS

Milk production and composition

Data on milk yield and composition for each group are shown in Tables 2 and 3. As shown, milk production of Holstein cows was significantly (<0.0001) higher than Sarabi which is not surprising considering the traits of these two groups. The obtained value for Sarabi cows in the current study is in accordance with the results of previous studies (Research Centre of Indigenous Sarabi Cows, 1999; Shariflu and Nikkhah, 2008). Average daily milk production in lactating period for Sarabi is about 7.4 L in an average milking period of 204 days (Khan, 2009). The dispersion of daily milk yield of Sarabi cattle in the Research Centre of Indigenous Sarabi cows (1990) has been from 2 to 18.2 L, with all the averages being in this range. Shariflu and Nikkhah (2008) in a five-year long study in the Hakimieh Station and Agriculture College of Tehran University reported that the overall mean of actual milk yield in the Hakimieh Station and the Station of Agriculture College were 1281.3 and 566.4 and their ranges were 110.7 to 4907.2 and 98.0 to 1880 kg respectively. The adjusted milk yield for 305 days was 1887.78 and 1252.61, with the standard deviations of 999.13 and 539.05 kg.

Regarding the milk composition, in spite of higher values obtained for Sarabi in comparison with Holstein which is not unlikely, milk composition for both Sarabi and Holstein cows were obviously low. Sarabi will usually average 4.3 to 4.9% fat, while Holsteins will generally average 3.2 to 3.8% fat in the milk. The range of milk fat percent for Sarabi has been reported to be between 2.3 to 7.2, and most of averages have been in the range of 4.3 to 4.9% (Research Centre of Indigenous Sarabi cows, 1999). Average fat percent in the Hakimieh station was 4.71, which is close to that of Jersey Breed (Shariflu and Nikkhah, 2008); it have been said Sarabi was used in the establishment of Jersey dairy breed (Khan, 2009).

Moreover, while there are no published reports for Sarabi milk protein and lactose, a value around 3.5 and 5%, respectively was expected. Holstein cows normally have 3.7% fat, 3.1% protein and 4.8% lactose. Milk composition is affected by many environmental, dietary and inherited factors. However, in the case of current study, considering diet ingredients and nutrients, it may be due to underfeeding of some micronutrients. Considering that the milk protein to milk fat ratio is high, the low fat problem may be more critical than protein. Due to the fact that the deficiency of fiber and production of trans-fatty acids in the rumen which are of the main proposed reasons for low milk fat syndrome is unlikely, a deficiency of some micronutrients is possible (Jodie, 2010). Moreover, considering the decline of milk protein along with milk fat, and deficient of bypass protein in diet, the deficiency of methionine and lysine is very likely. There are reports that increasing diet methionine and lysine increases milk fat percent as well as protein (Pisulewski et al., 1996; Christensen et al., 1994).

Milk fatty acid composition

Milk fatty acid composition (g/100 g fat) of Sarabi and Holstein cows is presented in Table 5. There are no

Table 2. Parity, day in milk and lactation period from Holstein (n = 10) and Sarabi (n = 12) dairy cows.

Variable	Holstein		Sarabi	
	Mean	Range	Mean	Range
Parity	2.7	1 - 5	3.5	1 - 7
Day in milk	146	44 - 300	83.75	16 - 205
Lactation period	2	1 - 3	2.3	1 - 3

Milk samples were taken from Holstein (n = 10) and Sarabi (n = 12) dairy cows on the same day, while all cows were consuming the same diet.

Table 3. Dry matter intake of Holstein (n = 10) and Sarabi (n = 12) dairy cows.

Ingredient	DMI (kg/day)	
	Holstein	Sarabi
Alfalfa hay	8.4	5.85
Corn silage	2.8	2.31
Dairy concentrate	6.6	4.4

Table 4. Least squares means for milk production and milk composition of Holstein and Sarabi cows.

Item	Holstein	Sarabi	*P*-value
Milk yield (kg/day)	23.45 ± 1.13	8.63 ± 1.03	<0.0001
Milk fat (%)	2.60 ± 0.20	3.67 ± 0.18	0.0009
Milk fat (kg/day)	0.563 ± 0.05	0.320 ± 0.04	0.0026
Milk protein (%)	2.94 ± 0.03	3.24 ± 0.02	<0.0001
Milk protein (kg/day)	0.693 ± 0.03	0.279 ± 0.03	<0.0001
Milk lactose (%)	4.34 ± 0.04	4.74 ± 0.04	<0.0001
Milk lactose (kg/day)	0.910 ± 0.07	0.409 ± 0.07	0.0001

reports known to the authors comparing the milk fatty acids profile of Sarabi cows, so the similar available records for Jersey that is believed to be offspring of Sarabi, was used. As shown, Sarabi produced higher concentrations of saturated fatty acids, long chain fatty acids and slightly lower concentrations of mono unsaturated fatty acids than Holstein; however, the difference was not significant. The most prominent saturated fatty acids of milk, C16:0, was unaffected by breed and was 30.79 and 29.70% of total fatty acids in Sarabi and Holstein cows, respectively. C14:0 and C18:0, the second and third most prominent saturated fatty acids in milk fat were 9.98 and 9.93% for Sarabi and 10.10 and 9.60% for Holstein cows, respectively. White et al. (2001) also previously reported milk saturated fatty acids of Jersey were higher than Holstein; however, in their experiment milk from Holsteins was lower than milk from Jerseys for C14:0, which was inverse in our study.

Palmquist and Beaulieu (1993) reported that Jerseys produced 13% more stearic acid (C18:0). In contract current experiment, Milk from Sarabi and Holstein cows had similar content of the *cis*-9 *trans*-11 conjugated linoleic acid (0.28 and 0.30%, respectively) and short – and medium-chain fatty acids (18.58 and 18.62%, respectively). Capps et al. (1999) and White et al. (2001) reported that Jerseys produced significantly ($P < 0.05$) higher concentrations of short- and medium-chain fatty acids (C6:0 to C14:0) than Holsteins. In contrast, Palmquist and Beaulieu (1993) reported that Holstein cows produced 8 to 42% more C6:0, C8:0, C10:0, C12:0 and C14:0 compared with Jersey cows when fed total mixed ration (TMR) rations with added fat. In accordance our experiment, White et al. (2001) stated that Holsteins produced significantly ($P < 0.05$) higher concentrations of conjugated linoleic acid (0.56 vs. 0.46% of total fatty acids) than Jerseys. Similarly, Capps et al. (1999) reported that Jerseys produced lower levels of conjugated linoleic acid than Holsteins when fed a total mix ration with added yellow grease.

Further studies may offer explanations of breed differences for fatty acid production (White et al., 2001). Another approach would be to analyze the variations in Δ9-desaturase activity. Endogenous production of unsaturated fatty acids, particularly some mono-unsaturated fatty acids and nearly all conjugated linoleic acids, is regulated by the Δ9-desaturase activity (Soyeurt et al., 2006, 2008). The implication of Δ9-desaturase in the endogenous production of percent (%) mono unsaturated fatty acids in milk fat was suggested by the positive genetic correlations observed between % mono unsaturated fatty acids and the Δ9-desaturase indices (Soyeurt et al., 2008). The Δ9-desaturase activity can be studied for four pairs of fatty acids that represent products and substrates for Δ9-desaturase. These fatty acid pairs were *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-918:1/18:0, and *cis*-9, *trans*-11 conjugated linoleic acid /*trans*-11 18:1 (Kelsey et al., 2003; Soyeurt et al., 2006). Besides breed, dietary affects (Chouinard et al., 1999), seasonal variation (Lock and Garnsworthy, 2003) and individual animal differences (Soyeurt et al., 2008) were observed for Δ9-desaturase activity. Consequently, a cow of a specific breed with a higher Δ9-desaturase activity should produce higher contents of mono unsaturated

Table 5. Least square means for fatty acid content of Sarabi and Holstein cows.

Fatty acid (%)	Holstein	Sarabi	*P*
C6:0	2.07 ± 0.44	1.97 ± 0.31	0.8609
C8:0	1.17 ± 0.26	1.26 ± 0.19	0.7961
C10:0	2.57 ± 0.58	2.63 ± 0.62	0.8220
C12:0	2.75 ± 0.69	2.82 ± 0.55	0.9348
C14:0	10.10 ± 1.54	9.98 ± 1.33	0.9532
C15:0	1.14 ± 0.24	0.94 ± 0.19	0.5585
C16:0	29.70 ± 2.06	30.79 ± 1.74	0.6942
C17:0	0.51 ± 0.28	0.49 ± 0.21	0.9668
C18:0	9.60 ± 1.38	9.93 ± 1.51	0.8781
C16:1	1.06 ± 0.42	0.81 ± 0.31	0.6550
C18:1 trans-11	0.75 ± 0.03	0.72 ± 0.02	0.8578
C18:1	20.41 ± 2.47	19.68 ± 3.7	0.8600
cis-9, trans-11 C18:2	0.30 ± 0.08	0.28 ± 0.42	0.8578
Saturated fatty acids[1]	59.29 ± 4.59	60.51 ± 3.70	0.3941
Mono unsaturated fatty acids[2]	21.65 ± 2.37	20.75 ± 3.56	0.8371
Short and medium chain fatty acids[3]	18.62 ± 1.11	18.54 ± 1.21	0.9648
Long chain fatty acids[4]	41.26 ± 6.14	43.32 ± 6.86	0.8301
Desaturase index			
cis-9 16:1	0.03 ± 0.003	0.02 ± 0.002	0.0739
cis-9 18:1	0.68 ± 0.04	0.67 ± 0.02	0.9230

1-6:0+C8:0+C10:0+C12:0+C14:0+C16:0+C18:0 , 2-C16:1+ 18:1, 3-C6:0–C14:0.

fatty acids and conjugated linoleic acid in milk fat (Soyeurt et al., 2008).

Moreover, our data showed no difference between Sarabi and Holstein for levels of desaturase index, while this index was numerically higher for Holstein than Sarabi. Higher numeric values of desaturase index obtained for Holstein could be an explanation for slightly high content of conjugated linoleic acid and 16:1 in milk fat of Holstein cows in comparison with Sarabi cows. Soyeurt et al. (2008) stated that the high fat content in Jersey cows was in line with the negative value of genetic correlation observed between percent fat and the percent mono unsaturated fatty acids. They also found negative genetic correlations between percent fat or percent protein and the studied desaturase indices and suggested that an increase in *Δ* 9-desaturase activity by some of its products could inhibit the synthesis of milk fat or protein in the mammary gland.

Conclusion

Sarabi cows had lower milk production and higher milk concentrations of fat, protein and lactose than Holstein cows. However, daily yields of milk fat, milk protein and milk lactose were greater for Holstein cows. There were only minor differences in milk fatty acid profiles between Sarabi and Holstein cows, suggesting that incorporation of Sarabi genetics into crossbreeding schemes with Holstein cows would not significantly alter the fatty acid profile and the health benefits of milk.

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