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Effect of Breed on the Fatty Acid Composition of Milk from Dairy Cows Milked Once and Twice a Day in Different Stages of Lactation

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Abstract: The objective of this study was to evaluate the effect of breed on the overall composition and fatty acid composition of milk from cows milked once a day (OAD) and twice a day (TAD) in different stages of lactation. Milk samples were taken from 39 Holstein-Friesian (F), 27 Jersey (J), and 34 Holstein-Friesian × Jersey (F × J) crossbred cows from a OAD milking herd and 104 F and 83 F × J cows from a TAD milking herd in early (49 ± 15 days in milk), mid (129 ± 12 days in milk), and late (229 ± 13 days in milk) lactation. Calibration equations to predict the concentrations of individual fatty acids were developed using mid-infrared (MIR) spectroscopy. There was a significant interaction between breed within the milking frequency and stage of lactation for the production traits and composition traits. Holstein-Friesian cows milked OAD produced milk with lower concentrations of C18:0 in early and mid lactations compared to F × J and J cows. Holstein-Friesian cows milked TAD produced lower concentrations of C18:0 in early lactation and lower concentrations of C16:0 and C18:0 in late lactation compared to F × J. Lower concentrations of these fatty acids would reduce the hardness of the butter when the milk is processed. In the OAD milking herd, F cows were superior for daily milk yield compared to J cows, but Jersey cows produced significantly ($p < 0.05$) higher percentages of fat and a higher concentration of C18:0 fatty acid. The relative concentrations of C18:0 and C18 *cis*-9 in F and J cows milked OAD imply there is no breed effect on the activity of delta-9-desaturase, whereas stages of lactation likely have an effect. These results can be used to assist with selecting breeds and cows that are suitable for either OAD or TAD milking, allowing closer alignment with milk processing needs.

Keywords: breed; dairy cattle; fatty acid; milking frequency; New Zealand; stage of lactation

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1. Introduction

Dairy farming in New Zealand is pasture-based and spring-calving with a traditional practice of twice a day (TAD) milking. In recent years, some farmers have adopted once a day (OAD) milking due to farm management and animal welfare benefits [1,2]. Before 1960, the New Zealand dairy herd was approximately 70% Jersey (J) cows, however; post-1960, Holstein-Friesian (F) cows became very popular [3] due to their greater production of milk solids [4]. Crossbreeding between F and J breeds has been practiced since the 1960s [5]. Cross-bred cows usually have similar productive but better reproductive performance and survival rates compared to the parent breeds due to heterosis [6–8]. The New Zealand dairy herd currently consists of 49.1% Holstein-Friesian × Jersey crossbred cows (F × J), 32.7% F, 8.4% J, and 9.3% other breeds and crosses [4].

Milk composition affects the processability of milk into products such as cheese and butter [9,10]. Milk solid fat content at 10 °C (SFC_{10}), which is influenced by milk fat, and fatty acid composition, determines the hardness (or the spreadability) of butter. The higher the SFC_{10}, the higher the hardness. [11]. Softer butter is preferred mostly due to its ability to be easily spreadable at low temperatures.

Milk fatty acid (FA) composition is affected by genetic variation [11,12], breed [9,12,13], stage of lactation [14–16], diet [17,18], and season [19,20]. Metabolic disorders such as ketosis [21,22] and sub-acute ruminal acidosis [23,24] also affect the fat and FA composition of milk. Among these factors, the breed has the greatest effect on FA composition [25]. Milk from F cows has greater concentrations of unsaturated fatty acid (UFA) and lower concentrations of saturated fatty acid (SFA) relative to J cows. Milk from J cows has higher concentrations of medium-chain fatty acids (SCFA) and medium-chain fatty acid (MCFA) compared to F cows [26].

Several studies have investigated the effects of breed on milk production [3,5,27], and fatty acid composition [4,12,25], and also the effect of stage of lactation on milk fatty acid composition [13,28,29] in cows milked TAD. Few studies have investigated the effect of breed on milk production traits for OAD and TAD milking [5,27]. However, the effect of breed on the FA composition of cows milked OAD in different stages of lactation has not been reported. The objective of this study was to examine the milk composition and FA composition of different breeds of cows milked OAD and TAD in different stages of lactation without control of the feed.

2. Materials and Methods

2.1. Farms and Cows

The experiment took place on No. 1 Dairy farm and No. 4 Dairy farm (latitude: 40°22′35.1″, longitude: 175°36′51.1″), Massey University, New Zealand. The soil type and climatic conditions of both farms are similar. Fresh ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture are used as a predominant feed on both farms. The No. 1 Dairy farm is managed in a OAD milking system with restricted supplementation and a lower stocking rate of 2.4 cows/ha. In contrast, the No. 4 Dairy farm is managed in a TAD milking system with higher supplements and a higher stocking rate of 2.6 cows/ha.

Details of feed and chemical composition of feed of the farms where this study was carried out are provided in Table 1. In early lactation, cows at No. 1 Dairy farm were fed approximately 8 kg DM/cow/day pasture, 4 kg DM/cow/day herb mix crop containing chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and red clover (*Trifolium pratense*), and limited supplements (maize silage, tapioca, and dried distillers grain). Cows at No. 4 Dairy farm were fed approximately 17 kg DM/cow/day pasture, with maize silage, concentrate (soy meal), and dry roughage. In mid-lactation, No. 1 Dairy cows were fed approximately 12 kg DM/cow/day pasture and 4 kg DM/cow/day crop mix without any supplements whereas No. 4 Dairy cows were fed approximately 17 kg DM/cow/day pasture with adequate supplements (maize silage, dried distillers grain, and grass baleage). In the late lactation, cows at No. 1 Dairy farm were fed approximately 4.5 kg DM/cow/day pasture, 3 kg DM/cow/day herb mix crop, with tapioca pallets, concentrate (corn gluten feed pallets), and Lucerne baleage. Cows at No. 4 Dairy farm were fed approximately 10 kg DM/cow/day pasture, with maize silage, concentrate (corn gluten feed pallets), straw, and grass baleage. Feed composition for cows at No. 1 Dairy farm and No. 4 Dairy farm in early, mid, and late lactation are presented in Tables S1 and S2.

Table 1. Dietary feed composition and chemical composition of the feed offered at No. 1 Dairy and No. 4 Dairy during the sampling season in early lactation, mid-lactation, and late lactation in the 2020–2021 production season.

Farm	No. 1 Dairy (OAD [11])			No. 4 Dairy (TAD [12])		
Lactation Stage	Early (September)	Mid (December)	Late (March)	Early (September)	Mid (December)	Late (March)
Feed ingredients (kg DM per cow per day)						
Pasture	8.0	12.0	4.5	17.0	17.0	10.0
Herb mix crop [1]	4.0	4.0	3.0	-	-	-
Maize silage	1.0	-	-	5.0	5.0	2.0
DDG [2]	1.5	-	-	-	1.0	-
Tapioca pellets	1.5	-	1.5	-	-	-
Concentrate [3]	-	-	2.0	4.0	-	1.0
Dry roughage [4]	-	-	-	0.2	-	1.0
Baleage [5]	-	-	4.0	-	1.0	10.0
Feed chemical composition						
ME [6] MJ ME/kg DM	11.89	12.12	9.98	11.36	10.58	11.28
CP [7] % of DM	20.08	19.48	21.91	19.49	19.08	21.27
NDF [8] % of DM	38.18	38.66	39.88	44.13	43.48	45.69
ADF [9] % of DM	20.94	20.38	26.00	20.71	23.60	25.40
SSS [10] % of DM	16.77	14.11	8.79	19.13	17.55	8.59
Lipid % of DM	4.08	4.69	4.54	4.40	4.09	4.77

[1] Herb-mix comprises of chicory (*Cichorium intybus*), plantain (*Plantago lanceolata),* and red clover (*Trifolium pratense*); [2] Dried distillers grain; [3] Grain-based concentrate; [4] Hay and straw; [5] Lucerne baleage in the No. 1 Dairy farm and grass baleage in the No. 4 Dairy farm; [6] ME = metabolisable energy; [7] CP = crude protein; [8] NDF = neutral detergent fibre; [9] ADF = acid detergent fibre; [10] SSS = soluble sugars and starch; [11] OAD = once-a-day milking; [12] TAD = twice-a-day milking; DM = dry matter. Baleage is dried forage, developed from the process of bailing the forage.

The feed samplings were carried out 24 h before each milk sampling using the same method described by Correa-Luna et al. [30]. The samples were freeze-dried, ground, and analyzed with the near-infrared reflectance spectrometry technique for the chemical composition of feed [31]. No specific software was used for feed balancing. The level of feeding and feed composition in each farm was defined by the farm manager, who feeds the cows as a group attempting to satisfy the requirements for metabolizable energy for maintenance, live weight gain, activity, and milk production. The confounding effect of feed and milking frequency was the limitation of this study as the feed provided to cows milked OAD and TAD were slightly different.

One hundred spring calving cows (39 F, 27 J, and 34 F × J) from the OAD milking herd and 187 spring calving cows (104 F and 83 F × J) from the TAD milking herd were used in this study. Cows were of first (54 cows from No. 1 Dairy and 123 cows from No. 4 Dairy) and second (46 cows from No. 1 Dairy and 64 cows from No. 4 Dairy) parity, with no clinical mastitis or metabolic diseases during the sampling period. There is a genetic connection between the two farms as there are common sires on the two farms.

2.2. Sampling of Cows

Selected No. 1 Dairy cows were milked at 6.30 am and the selected No. 4 Dairy cows were milked at 5.30 am and 2.30 pm during early (49 ± 15 days in milk), mid (129 ± 12 days in milk), and late (229 ± 13 days in milk) lactation in the 2020–2021 production season (cows were milked once in each stage of lactation). The Waikato milk meters were used to collect composite milk samples. The samples were stored in the refrigerator at 0–4 °C immediately after collecting until analysis. Preservatives were not added, and all the analyses were carried out within two days of collection.

2.3. Analysis of Milk Samples

The concentrations of fat, protein, and lactose in individual samples were determined by a Milkoscan FT1 (Foss, Hillerød, Denmark). The concentrations of individual fatty acids were predicted using the calibration equations developed in FTIR calibrator software (Foss Analytical, Hillerød, Denmark) using a data set with mid-infrared spectral data and gas chromatography reference values. Milk composition was analysed by following the ISO standard of ISO 9622:2013 [IDF 141:2013].

2.4. Measurement of Body Condition Scores of the Cows

The monthly body condition score of each cow was measured from calving to dry-off date during the 2020–2021 production season, by a single research technician using a 10-point scale [32]. Only cows from No. 1 Dairy farm were weighted (mean = 463 kg, SD = 56 kg) and therefore live weight was not analysed.

2.5. Statistical Analysis

Data were analysed using a MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) statistical software. The following mixed linear model was used to obtain the least-squares means and the standard errors:

$$Y_{ijklmn} = \mu + M_i + B_j\,(M_i) + L_k + S_l + S_lB_j\,(M_i) + \beta_1 d_m + C_m + e_{ijklmn}$$

where

Y_{ijklmn} is the observation n for any of the production traits and composition traits in milking frequency i, breed j, lactation number k, stage of lactation l, and cow m.
μ is the population mean.
M_i is the fixed effect of milking frequency (i = OAD and TAD).
$B_j\,(M_i)$ is the fixed effect of breed j nested in milking frequency i (j = F, F $\times$ J, and J).
L_k is the fixed effect of lactation number (k = 1st and 2nd lactation).
S_l is the fixed effect of the stage of lactation (l = early, mid, and late).
$S_lB_j\,(M_i)$ is the fixed effect of interaction between stage of lactation l and breed j nested in milking frequency i.
β_1 is the regression coefficient of the linear effect of deviation (days) from herd median calving date on trait Y of cow m.
C_m is the random effect of cow (m = 1, 2, . . . , 287) assumed with mean zero and variance $\sigma^2_{\mathbf{c}}$. e_{ijklmn} is the residual random error assumed with mean zero and variance $\sigma^2_{\mathbf{e}}$.

The quadratic effect of deviation from herd median calving date was not included in the model because this effect was not significant. The fixed effect of feed cannot be included in the model because it is confounding with milking frequency. F-values were used to show the level of importance that the fixed effect had on the dependent variables. The F-value is obtained by dividing the mean sum of squares of the effect by the mean error sum of squares.

Monthly records of each cow on each milking frequency were used to model the BCS curves for each cow using a third-order polynomial.

3. Results

The means, standard deviations, and the F-values and associated probabilities for each dependent variable from the analyses of variance are shown in Table 2. For most traits, the F statistics indicated that the stage of lactation, milking frequency, and lactation number explained the majority of the variation. Exceptions were concentrations of C18:0 and LCFA, for which deviation from the median calving date was the most important independent variable. The effect of breed nested in milking frequency and the interaction effect of breed nested in milking frequency and stage of lactation and the deviation from herd median calving date explained smaller, but significant amounts, of variation in the study variables.

Table 2. Means, standard deviations, and F-value for factors affecting production traits, milk composition, and fatty acid composition of cows milked once-a-day (OAD) and twice-a-day (TAD) during the 2020–2021 production season.

Variable	Mean	SD	Milking Frequency	Lactation Number	Breed (MF)	Stage of Lactation	Breed (MF) × S	dmcd
Milk yield (L/cow/day)	17.6	5.8	320.5 ***	207.2 ***	6.7 ***	498.7 ***	6.9 ***	3.1 *
Fat yield (kg/cow/day)	0.84	0.3	58.7 ***	110.6 ***	2.4	153.8 ***	1.2 *	7.2 **
Protein yield (kg/cow/day)	0.67	0.2	146.6 ***	209.9 ***	3.6 *	288.9 ***	4.3 ***	0.7
Lactose yield (kg/cow/day)	0.88	0.32	372.7 ***	175.9 ***	5.2 **	586.3 ***	9.3 ***	3.5
Fat%	4.41	1.1	241.2 ***	2.2	15.8 ***	34.8 ***	8.6 ***	0.2
Protein%	3.92	0.4	236.4 ***	10.1 **	16.8 ***	851.6 ***	5.8 ***	7.8 **
Lactose%	4.95	0.3	127.3 ***	9.9 **	1.6	633.4 ***	11.8 ***	1.6
Fatty acid (% of the total FA)								
SFA [1]	70.0	3.1	5.0 *	71.0 ***	4.6 **	215.4 ***	4.1 ***	3.7
UFA [2]	30.2	2.6	32.5 ***	87.5 ***	3.8 *	168.9 ***	5.1 ***	2.7
PUFA [3]	2.95	0.5	107.1 ***	64.0 ***	2.0	377.2 ***	73.8 ***	0.2
C4:0	4.01	0.3	167.7 ***	9.2 **	0.1	566.6 ***	28.4 ***	11.8 **
C6:0	2.81	0.2	13.4 ***	29.0 ***	1.0	435.8 ***	25.8 ***	1.1
C8:0	1.52	0.2	13.0 ***	28.6 ***	1.0	494.4 ***	23.3 ***	0.2
C10:0	3.42	0.4	45.4 ***	38.3 ***	1.0	283.0 ***	19.2 ***	1.0
C12:0	3.82	0.4	82.2 ***	32.7 ***	0.8	62.2 ***	10.8 ***	9.6 **
C14:0	12.7	1.3	5.3 *	45.3 ***	1.5	191.8 ***	4.9 ***	20.6 ***
C16:0	31.9	2.5	20.8 ***	51.8 ***	1.9	292.6 ***	3.6 ***	1.6
C18:0	13.1	1.5	8.2 **	5.1 *	9.6 ***	13.4 ***	10.1 ***	22.7 ***
C18:1 *cis*-9	20.2	2.8	8.0 **	60.6 ***	1.6	199.7 ***	7.1 ***	17.4 ***
Omega6	1.59	0.4	29.3 ***	19.7 ***	4.1 **	676.6 ***	65.5 ***	0.4
SCFA [4]	8.33	0.6	49.9 ***	24.2 ***	0.4	607.6 ***	32.0 ***	5.2 *
MCFA [5]	20.0	1.8	22.9 ***	47.1 ***	0.9	63.2 ***	6.4 ***	14.3 ***
LCFA [6]	66.7	2.6	63.5 ***	4.8 *	2.6	1.1	7.8 ***	30.0 ***

[1] SFA = Saturated fatty acids; [2] UFA = Unsaturated fatty acid; [3] PUFA = Polyunsaturated fatty acid. [4] SCFA = Short-chain fatty acids (sum of C4:0, C6:0 and C8:0); [5] MCFA = Medium-chain fatty acids (sum of C10:0, C12:0); [6] LCFA = Long-chain fatty acids (sum of C14:0, C16:0, C18:0 and C18:1 *cis*-9 and Omega6). MF- milking frequency; S- stage of lactation; dmcd- deviation from median calving date. Statistical significance is given as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Regardless of the significant ($p < 0.05$) interaction between breed within milking frequency and stage of lactation, the daily yields of milk, fat, protein, and lactose declined from early to late lactation in both cows milked OAD and TAD and for all breeds (Figure 1). Cows milked TAD produced greater yields at all three stages of lactation. Jersey cows mostly produced significantly lower daily yields of milk, protein and lactose compared to F and F × J, but similar levels of daily fat yield in mid-lactation and late lactation. The significant breed within milking frequency and stage of lactation interaction most likely occurred in mid-lactation when between breed means changed in magnitude.

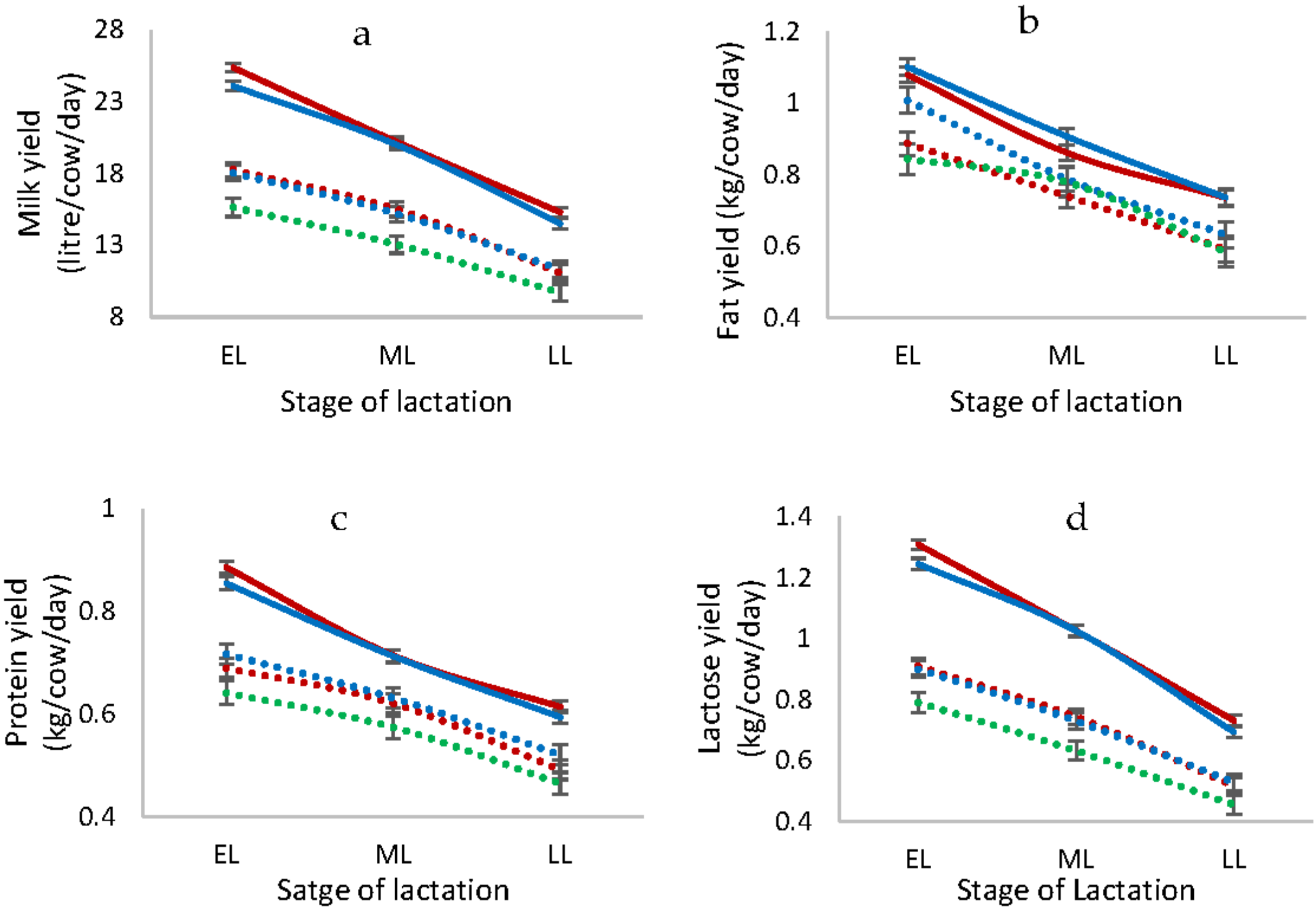

Figure 1. Daily yield of (**a**) milk, (**b**) fat, (**c**) protein, and (**d**) lactose in milk from Holstein-Friesian (. . . .), Holstein-Friesian × Jersey (. . . .) and Jersey (. . . .) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the production season 2020–2021. EL = early lactation (<90 days); ML = mid lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

Figure 2 shows the percentages of fat, protein, and lactose in the three stages of lactation. In cows milked OAD, the percentages of fat and protein significantly ($p < 0.05$) increased, and the percentages of lactose significantly decreased from early to late lactation. Jersey cows produced significantly higher percentages of fat and protein compared to the other two breeds. In cows milked TAD, F cows produced significantly higher fat percentages in early lactation compared to the other two stages of lactation whereas F × J cows produced significantly higher fat percentages in early and late lactation compared to mid-lactation. In early and late lactation, F × J cows produced significantly higher fat percentages compared to F cows.

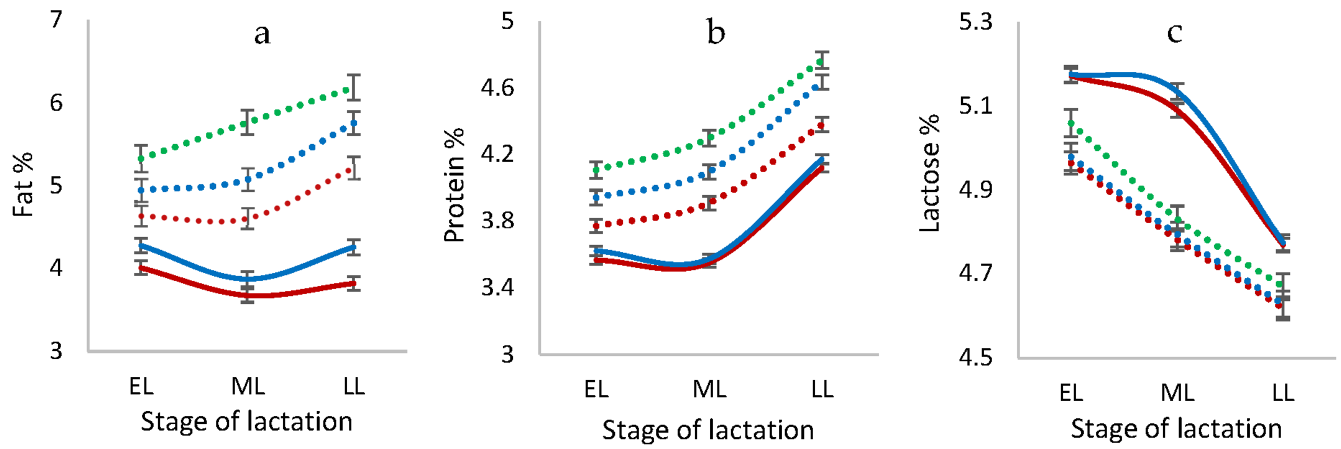

Figure 2. Percentages of (**a**) fat, (**b**) protein and (**c**) lactose in milk from Holstein-Friesian (. . . .), Holstein-Friesian × Jersey (. . . .) and Jersey (. . . .) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the production season 2020–2021. EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

Figure 3 shows the concentrations of C16:0, C18:0, and C18:1 *cis*-9 in three stages of lactation. The concentration of C16:0 was significantly ($p < 0.05$) higher in ML compared to early and late lactation, in cows milked OAD and TAD. In late lactation, J cows produced a significantly higher concentration of C16:0 compared to F × J cows milked OAD, whereas in the cows milked TAD, F × J cows produced a significantly higher concentration of C16:0 compared to F cows. Holstein-Friesian cows milked OAD, and TAD produced a significantly lower concentration of C18:0 throughout the lactation period and the concentration was significantly affected by stages of lactation. Holstein-Friesian × Jersey and J cows milked OAD did not show significant variation in the concentration of C18:0 in mid-lactation and late lactation. The concentration of C18:0 *cis*-9 was significantly higher in early lactation compared to mid and late lactations in cows milked OAD and TAD, in all the breeds.

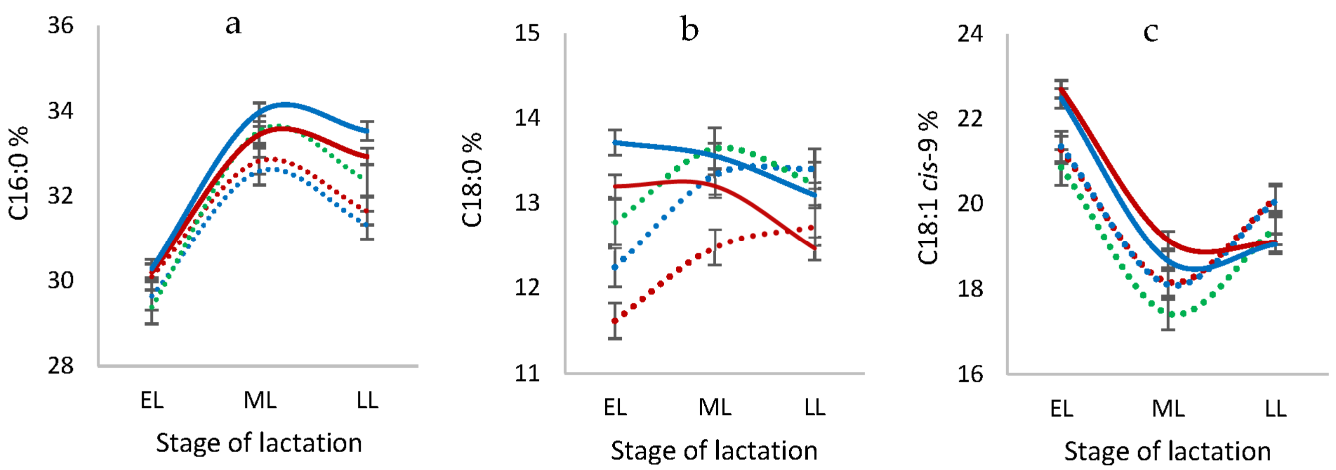

Figure 3. Concentrations of (**a**) C16:0 (**b**) C18:0 (**c**) C18:1 *cis*-9 in milk from Holstein-Friesian (. . . .), Holstein-Friesian × Jersey (. . . .) and Jersey (. . . .) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the production season 2020–2021. EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

The concentration of SCFA varied significantly (p < 0.05) across the three stages of lactation in both milking frequencies (Figure 4). The concentration of MCFA was significantly higher in mid-lactation compared to early and late lactation in cows milked OAD. In late lactation, F × J cows produced a significantly higher concentration of LCFA compared to F cows milked TAD.

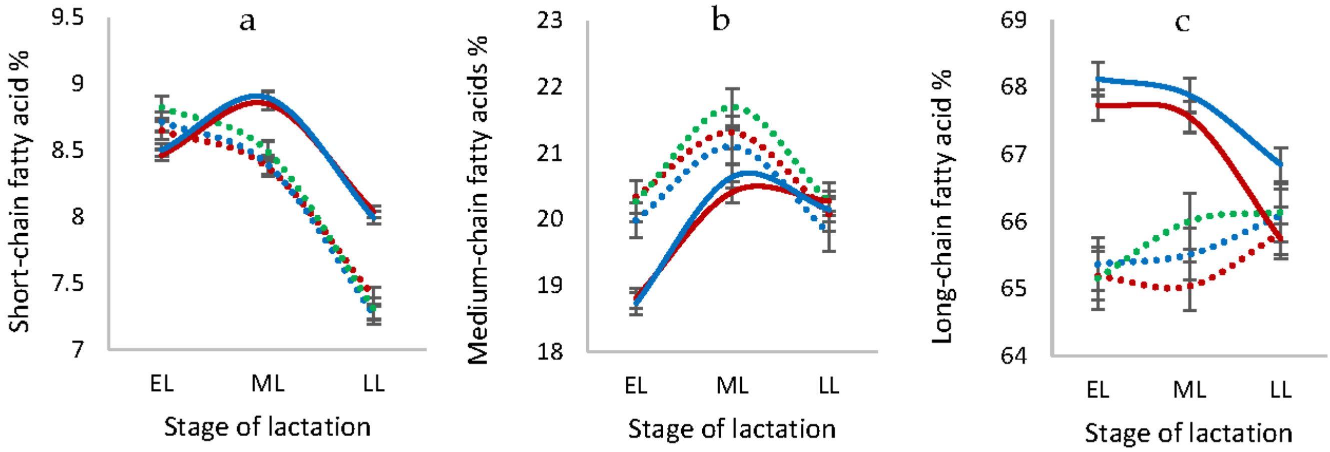

Figure 4. Concentrations of (**a**) short-chain fatty acids (**b**) medium-chain fatty acids (**c**) long-chain fatty acids in milk from Holstein-Friesian (. . . .), Holstein-Friesian × Jersey (. . . .) and Jersey (. . . .) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the production season 2020–2021. EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

Breed effect was significant for daily milk and fat yields, percentages of fat and protein, and concentration of SFA based on the F-values (Table 2). Least-squares means and standard errors for each breed within milking frequency for milk production, composition traits, and FA acid composition are presented in Table 3. In cows milked OAD, J cows showed a significantly (p < 0.05) lower daily milk yield, protein yield, and lactose yield compared to F and F × J cows. Holstein-Friesian × Jersey cows produced a significantly higher daily fat yield. Jersey cows produced significantly higher percentages of fat and protein compared to F cows with F × J cows producing intermediate values. In cows milked TAD, F cows produced significantly higher daily milk yield and F × J cows produced significantly higher fat and protein percentages. The breed effect for most of the FA concentrations was not significant in cows milked OAD and TAD. The concentrations of SFA and C18:0 were significantly higher and the concentration of UFA was significantly lower in J cows milked OAD and F × J cows milked TAD. The results of this study have to be considered with caution due to the confounding effect of feed with milking frequency.

Table 3. Least squares means and standard errors for the production traits, milk composition and fatty acid composition of milk from Holstein-Friesian (F), Holstein-Friesian × Jersey (F × J) and Jersey (J) cows milked once-a-day (OAD) and twice-a-day (TAD) in the 2020–2021 production season.

Variable	Milking Frequency				
	OAD			TAD	
	F	F × J	J	F	F × J
Milk yield (L/cow/day)	14.95 ± 0.39^{a}	14.85 ± 0.40^{a}	12.8 ± 0.46^{b}	20.29 ± 0.25^{a}	19.53 ± 0.27^{b}
Fat yield (kg/cow/day)	0.74 ± 0.02^{b}	0.81 ± 0.02^{a}	0.74 ± 0.03^{b}	0.89 ± 0.01^{a}	0.91 ± 0.02^{a}
Protein yield (kg/cow/day)	0.6 ± 0.01^{ab}	0.62 ± 0.01^{a}	0.56 ± 0.02^{b}	0.74 ± 0.01^{a}	0.72 ± 0.01^{a}
Lactose yield (kg/cow/day)	0.72 ± 0.02^{a}	0.72 ± 0.02^{a}	0.63 ± 0.02^{b}	1.02 ± 0.01^{a}	0.99 ± 0.01^{a}
Fat%	4.82 ± 0.10^{c}	5.26 ± 0.11^{b}	5.76 ± 0.12^{a}	3.83 ± 0.06^{b}	4.13 ± 0.07^{a}
Protein%	4.02 ± 0.04^{c}	4.22 ± 0.04^{b}	4.39 ± 0.04^{a}	3.74 ± 0.02^{a}	3.79 ± 0.02^{a}
Lactose%	4.79 ± 0.02^{a}	4.8 ± 0.02^{a}	4.85 ± 0.03^{a}	5.01 ± 0.01^{a}	5.03 ± 0.02^{a}
Fatty acid (% of the total FA)					
SFA [1]	69.47 ± 0.29^{b}	69.64 ± 0.3^{b}	70.73 ± 0.34^{a}	70.19 ± 0.18^{b}	70.76 ± 0.2^{a}
UFA [2]	30.93 ± 0.24^{a}	30.99 ± 0.25^{a}	30.14 ± 0.28^{b}	29.83 ± 0.15^{a}	29.35 ± 0.16^{b}
PUFA [3]	3.14 ± 0.03^{a}	3.09 ± 0.03^{a}	3.09 ± 0.04^{a}	2.86 ± 0.02^{a}	2.8 ± 0.02^{b}
C4:0	3.83 ± 0.03^{a}	3.84 ± 0.03^{a}	3.84 ± 0.03^{a}	4.11 ± 0.02^{a}	4.12 ± 0.02^{a}
C6:0	2.77 ± 0.02^{a}	2.76 ± 0.02^{a}	2.81 ± 0.02^{a}	2.83 ± 0.01^{a}	2.84 ± 0.01^{a}
C8:0	1.54 ± 0.01^{a}	1.53 ± 0.01^{a}	1.56 ± 0.02^{a}	1.51 ± 0.01^{a}	1.51 ± 0.01^{a}
C10:0	3.61 ± 0.04^{a}	3.54 ± 0.04^{a}	3.58 ± 0.05^{a}	3.38 ± 0.03^{a}	3.34 ± 0.03^{a}
C12:0	4.04 ± 0.04^{a}	3.97 ± 0.04^{a}	4.03 ± 0.04^{a}	3.76 ± 0.02^{a}	3.74 ± 0.03^{a}
C14:0	12.92 ± 0.12^{ab}	12.78 ± 0.12^{a}	13.14 ± 0.13^{b}	12.7 ± 0.07^{a}	12.76 ± 0.08^{a}
C16:0	31.52 ± 0.24^{a}	31.18 ± 0.26^{a}	31.76 ± 0.28^{a}	32.19 ± 0.15^{a}	32.59 ± 0.17^{a}
C18:0	12.27 ± 0.16^{b}	12.99 ± 0.17^{a}	13.22 ± 0.19^{a}	12.96 ± 0.1^{b}	13.46 ± 0.11^{a}
C18:1 *cis*-9	19.85 ± 0.24^{a}	19.84 ± 0.25^{a}	19.24 ± 0.28^{a}	20.31 ± 0.15^{a}	20.07 ± 0.16^{a}
Omega6	1.71 ± 0.03^{a}	1.63 ± 0.03^{b}	1.61 ± 0.03^{b}	1.55 ± 0.02^{a}	1.5 ± 0.02^{b}
SCFA [4]	8.14 ± 0.05^{a}	8.13 ± 0.05^{a}	8.21 ± 0.06^{a}	8.45 ± 0.03^{a}	8.47 ± 0.04^{a}
MCFA [5]	20.58 ± 0.18^{a}	20.29 ± 0.19^{a}	20.74 ± 0.21^{a}	19.83 ± 0.11^{a}	19.84 ± 0.12^{a}
LCFA [6]	65.36 ± 0.26^{a}	65.66 ± 0.29^{a}	65.78 ± 0.31^{a}	67.01 ± 0.17^{b}	67.62 ± 0.18^{a}

[1] SFA = Saturated fatty acids; [2] UFA = Unsaturated fatty acid; [3] PUFA = Polyunsaturated fatty acid. [4] SCFA = Short-chain fatty acids (sum of C4:0, C6:0 and C8:0); [5] MCFA = Medium-chain fatty acids (sum of C10:0, C12:0 and C14:0); [6] LCFA = Long-chain fatty acids (sum of C16:0, C18:0 and C18:1 *cis*-9 and Omega6). [a, b and c] Means with different superscripts between breeds within milking frequency are significantly different ($p < 0.05$).

Figure 5 shows the body condition scores (BCS) measured during the sampling period. The F × J and J cows milked OAD had a similar trend in BCS throughout the lactation period, which was higher than F cows in early and mid-lactation. The cows milked TAD showed lower BCS throughout lactation compared to the cows milked OAD.

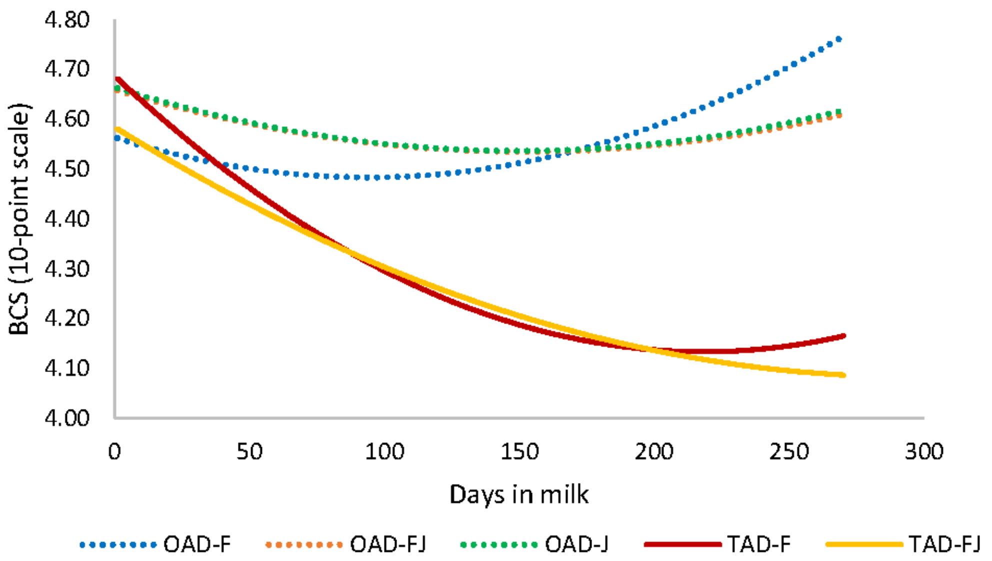

Figure 5. Body condition score of Holstein-Friesian, Holstein-Friesian × Jersey and Jersey cows milked OAD (. . . .) and in TAD (—) on a 10-point scale during the 2020–2021 production season.

4. Discussion

The chemical composition of feed slightly differed between the farms (Table 1). The metabolisable energy contents of the feed provided for cows in both farms were similar (ranging between 10–12 ME MJ/kg DM). Under the grazing conditions of this study, the crude protein content of the feed was higher than the recommended level of 16–17 g/100 g DM [33]. Higher crude protein content can possibly cause a high metabolic load in cows resulting in reduced performance [34]. The NDF content of the feed given in No. 4 Dairy was higher than the recommended level of 35% for cows grazing high-quality pasture [35].

In this study, OAD milking frequency consisted of F, F × J, and J cows but TAD milking frequency only consisted of F and F × J cows. Therefore, the breed was nested in milking frequency for statistical analysis. Milk FA composition affects milk processability especially, butter hardness [11] and cheese coagulation parameters [9]. The lower concentrations of MCFA, C16:0, C18:0, and the higher concentrations of C18:1 *cis*-9 could make the butter softer in early lactation than in mid and late lactations in F and F × J cows milked OAD. This is in agreement with Auldist et al. [15], who reported that the milk from early lactation had lower SCF_{10} than mid or late lactation milk. On the other hand, the higher concentrations of MCFA, C16:0, C18:0, and lower concentration of C18:1 *cis*-9 from milk produced in mid-lactation would give harder butter compared to the milk produced in early and late lactation from cows milked OAD. Cows milked TAD would also expect to show similar characteristics for butter hardness as cows milked OAD but the higher concentration of C18:0 in the early lactation would be likely to increase the hardness of the butter if the milk is processed.

In cows milked OAD, F milk would give softer butter in early and mid-lactation due to lower concentrations of C18:0 compared to F × J and J milk as the concentrations of other important fatty acids: C16:0 and C18:1n *cis*-9 that determine butter hardness [11] were not significantly different across the breeds. In late lactation, F and F × J cows produced significantly lower concentrations of C16:0 compared to J cows, which could lead to lower butter hardness. Overall, this study showed F cows milked OAD would be more likely to produce softer butter in all three stages of lactation. Similarly, in cows milked TAD, in early lactation, F milk showed a lower concentration of C18:0 and in late lactation, lower concentrations of C16:0 and C18:0 compared to F × J cows, which suggested that the butter produced from F milk would be softer than F × J cows in early and late lactation. In mid lactation, these FAs were not significantly different across the breeds. It is also clear that the F cows milked OAD, and TAD could produce softer butter than F × J and J cows regardless of the milking frequencies.

Generally, Holstein-Friesian cows produced lower concentrations of C18:0 compared to J cows, which suggests that butter produced from F cows is likely to be softer than the butter from J cows. Thus, is consistent with MacGibbon, [11] and Mackle et al. [36] who reported that solid fat content at 10 °C was positively correlated with the concentrations of high melting point long-chain saturated fatty acids and negatively correlated with the concentration of C18:1 *cis*-9. Generally, the concentrations of C16:0 and C18:1n *cis*-9 are the key FAs that influence butter hardness [11,36] as they are the predominant FAs.

The rennet coagulation parameters are affected by the stage of lactation [37]. Lucey, [38] reported that milk from late lactation was less suitable for cheese making because of a long clotting time and reduced curd firmness. The reasons proposed were higher milk pH and proteolysis of casein. Auldist et al. [9] reported that the concentrations of C8:0, C10:0, and C12:0 were negatively correlated with curd firming rate (K_{20}) and positively correlated with curd firmness (A_{60}), whereas the concentration of C18:1 *cis*-9 was positively correlated with the curd firming rate. However, Auldist et al. [9] reported that the relationship between milk composition and coagulation parameters was not sufficiently reliable to make predictions about cheese quality. In the current study, higher concentrations of C8:0, C10:0 and C12:0 was observed in early lactation in cows milked OAD and mid-lactation in cows milked TAD (Figures S1 and S2). It is not clear how individual FAs affect milk coagulation parameters. In addition, in this study, the breed did not affect the concentrations of MCFA and C18:1 *cis*-9 in all three stages of lactation.

In the current study, breed effects were significant for some traits. The higher percentages of fat and protein in J cows, lower percentages in F cows and intermediate percentages in F × J cows concurred with Palladino et al. [14] and Lopez-Villalobos et al. [25]. Jersey cows produce milk with higher concentrations of total solids compared with milk produced by F cows [9]. The superiority of the J cows milked OAD for the percentages of fat and protein would be beneficial in the New Zealand payment scheme as Sneddon et al. [39] reported the J cows have a greater milk value per litre of milk. The J cows milked OAD tend to produce significantly greater concentrations of SFA than F cows, with F × J cows producing intermediate values. This is in agreement with Palladino et al. [14] and Lopez-Villalobos et al. [25]. The opposite pattern was apparent for the concentration of UFA, being the highest in the F cows. Similar results were reported by Soyeurt et al. [12] and Palladino et al. [14]. The concentrations of long-chain saturated fatty acids were higher in J cows compared with F cows, as was demonstrated by Auldist et al. [9], who mentioned that the concentrations of long-chain saturated fatty acids were higher and the concentrations of long-chain unsaturated fatty acids were lower in J cows, compared to F cows, as was found in the present study. The higher concentrations of C18:0 and lower concentrations of C18:1 *cis*-9 in J cows suggest the lower level of conversion of C18:0 into C18:1 *cis*-9. DePeters et al., [40], Townsend et al. [41], and Drackley et al. [42] suggested that J cows have less active delta-9-desaturase, which converts C18:0 into C18:1 *cis*-9. In this study, the breed effect on delta-9-desaturase was not observed for cows milked OAD and TAD, whereas the effect of the stage of lactation was observed. Holstein-Friesian cows produced a lower concentration of C18:0 compared to the J cows, which suggests that butter produced milk from F cows is likely to be softer than the butter from J cows, which is in agreement with MacGibbon [11] and Mackle et al. [36].

Milk concentrations of C16:0, C18:0, C18:1 *cis*-9 and MCFA influence butter-making properties [11]. Short- and medium- chain FA are de novo synthesised in the mammary gland. This process is mostly driven by precursors and enzymes [43] and is influenced by energy balance of the cows [13,44] and the feed. Milking frequency affects cow energy balance, with cows milked OAD having improved energy balance compared to cows milked TAD [45,46] (Figure 5). The limitation of this study was the lack of control on the feed. Therefore, further studies should be conducted to study the effect of milking frequency with the control of feed.

5. Conclusions

Holstein Friesians cows are more likely to be producing softer butter compared to other breeds of cows regardless of the milking frequency due to the lower proportion of C18:0 in their milk. Holstein Friesians cows milked OAD tend to produce softer butter in early and mid-lactation whereas they would be more likely to produce softer butter in early and late lactation when they were milked TAD compared to the other breed of cows. Jersey cows milked OAD and F × J cows milked TAD produced higher percentages of fat and protein and higher concentrations of SFA. The relative concentrations of C18:0 and C18 *cis*-9 suggested the breed effect on the activity of delta-9-desaturase is not significant, whereas the effect of stages of lactation could be significant. These results suggest that further studies should be undertaken to examine the effects of milking frequency and feeding levels, with the prospect that better decisions could be made when selecting breeds, individual cows, and feeding levels to improve milk processability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/dairy3030043/s1, Table S1: Chemical composition of individual feed offered at No. 1 Dairy (once-a-day milking) during the sampling season in early, mid, and late lactation in the 2020–2021 production season. Table S2: Chemical composition of individual feed offered at No. 4 Dairy (twice-a-day milking) during the sampling season in early, mid, and late lactation in the 2020–2021 production season; Figure S1: Concentrations of C4:0, C6:0 and C8:0 in milk from F, F × J and J cows milked OAD and F and F × J cows milked TAD during the production season 2020–2021; Figure S2: Concentrations of C10:0, C12:0, C14:0 and omega6 in milk from F, F × J and J cows milked OAD and F and F × J cows milked TAD during the production season 2020–2021.

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Data Availability Statement: Not applicable.

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