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Analysis of Repeated Milk Yields and Composition Traits by Mixed Models for Crossbred Holstein Friesian Dairy Cows

Ewonetu Kebede Senbeta*, Kefelegn Kebede Kefenie and Adane Shegaw Abebe
School of Animal and Range Sciences, Haramaya University, Ethiopia
P. Box 138, Dire Dawa, Ethiopia
**Corresponding author, e-mail: ewonetu2011@gmail.com*
Co-authors' e-mail addresses: kebede123@yahoo.de (Kefenie); asneadane@gmail.com (Shegaw)
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Abstract

This study evaluated the effects of milking month, milking time, and lactation stage on milk yield and composition traits of crossbred dairy cows. Twelve crossbred Holstein Friesian cows with the same parity were purposefully selected and categorized into three groups; each consisting of four. All cows were individually managed for 120 days. Milk yield record was done every day for the morning and evening time whereas milk samples were taken every fifteen-day intervals. Data collected were analysed by PROC MIXED procedure of SAS 9.4. The "morning milkings" in all milking months showed the highest variations for "milk yield" whereas the "evening milkings" showed the highest variations for "milk urea nitrogen" in the 1st and 2nd and for "fat" in the 3rd and 4th months. The main findings in this study were the month-to-month variations in milk composition and milk yield within milking times. Milk fat was the only component that was significantly influenced by milking month, milking time, and lactation stage. Finally, this study concluded that repeated measurements for the respective milking times in milk yield and milk composition at a monthly interval showed variations. Further study should be conducted using repeated measurements at a weekly level for early detection of yield and quality alterations and health disturbances of dairy cows.

Keywords: lactation stage; milk compositions; milking month; milking time; milk yield.

Introduction

Milk yield and milk quality in dairy farms are affected by biological and management factors (Assan 2014, Cinar et al. 2015). Cattle breeders are not interested only in getting more milk, but also in having better milk quality (Bondan et al. 2019). Among milk chemical components, fat and protein are widely used to monitor cows' nutritional status (Bauman and Griinari 2003). For instance, fat content in milk could be an indicator of feeding and milking because it is highly dependent on the amount of fibre in the diet (Bauman and Griinari 2003) and on udder emptying (Tancin et al. 2007). Protein in milk is highly dependent on the genetic capacity of the cow, but can be altered by factors such as the stage of lactation and udder health (Urech et al. 1999). Moreover, the concentration of milk urea is a useful measurement for assessing whether the balance between the cow's intake of protein and energy is correct (Noro and Wittwer 2012). It has been established that fat percentage has more milking-to-milking variations than protein (Quist et al. 2008). Fat and protein percentages may be 0.32 and 0.09 points higher in the evening than in the morning milking (Gilbert et al. 1973). Besides, information on fat and protein levels is used for the planning of mating animals.

The day-to-day variations in milk composition have been described in several studies (Gilbert et al. 1973, Quist et al. 2008). The daily variations in milk yield and composition has been reported to be about 6-8% for milk yield, 5-8% for fat content, 1-2% for protein content, and 1% for lactose content (Svennersten-Sjaunja et al. 2005). These variations are caused by different factors such as milking interval, milking frequency, udder emptying, sampling procedure and frequency, equipment, feeding regime, parity, and stage of lactation (Rook et al. 1992). Regular milk records and analysis are essential for maintaining milk quality improvement in a dairy farm (Bondan et al. 2019). The total outcome and quality of dairy products are highly dependent on the milk yield and its contents such as protein, fat, and lactose. Continuous measurement of these parameters gives information about deviations that could have effects on raw milk quality. Besides, measuring milk composition and milk yield continuously can also be useful in herd management. Because the energy requirements of the dairy cow are highly dependent on milk yield, yield records are essential in calculating the daily ration for each cow.

The repeated measures experiment is a typical design in animal science research (ZoBell et al. 2003), and the analysis refers to multiple measurements made on the same experimental unit, observed either over time. In repeated measures designs, the usual practice is to apply treatments to experimental units in a completely randomised design, and measurements are made sequentially over time. With this type of experimental design, there are two fixed effects (treatment and time) and two sources of random variations (between and within animals). Some of the more common designs in animal sciences include repeated measurements of such things as weight gain, blood parameters, and products of metabolism and digestibility of nutrients. Such measurements are commonly taken on subjects that have been randomly allocated to fixed treatment effects such as feeds, drugs, hormones, etc., with pens or blocks considered as random effects in the design (Platter et al. 2003).

Often, measurements made on the same animal are more likely to be correlated than two measurements taken on different animals, and two measurements taken closer in time on the same animal are likely to be more correlated than measurements taken further apart in time. The primary objectives for repeated measures data are to examine simple factor effects (main effects) and the interaction effects among them. The distinguishing characteristic of the repeated measurement analysis model from other models is the assumption about the error variance and covariance structure (Templeman et al. 2002). With the repeated model, the usual assumptions about error variances being independent and homogeneous are no longer valid (SAS Institute, Inc. 2002). The analysis of repeated measures data, therefore, requires an appropriate accounting for correlations between the observations made on the same subject and possible heterogeneous variances among observations on the same subject over time. Repeated measures of milk data every month within milking times and lactation might be a better method of evaluation in milk yield, milk composition, and cow health than using a cut-off value for daily and lactation stage records. The variations in milk yield and composition have been described in several studies for each stage of lactation using different experimental designs. However, there is limited information that describes the use of repeated measures designs on milk data every month within milking times and lactation. Therefore, the objectives of this study were to evaluate the effects of milking month, milking time, and lactation stage on milk yield and composition traits of crossbred dairy cows using repeated measures designs.

Materials and Methods

Description of the study area

The study was carried out at Haramaya University dairy farm located in Haramaya

District at latitude 9°26' and longitude 42°3'E. The area is elevated at 1980 meters above sea levels and receives 780 mm mean annual rainfall. The mean annual minimum and maximum temperatures are 8.5 and 24.4 °C, respectively.

Experimental animals and their managements

Twelve crossbred Holstein Friesian lactating cows with the same parity were purposefully selected and categorized into three groups; each consisting of four cows based on their lactation stage, i.e. early (birth to 120 days), mid (91-210 days), and late (185-305 days). All cows were housed for 120 days as a single unit in free stalls with concrete flooring cubicles (2.20 m long and 1.20 m wide) that were bedded with rubber mats. The floors of cubicles were scraped three times daily. Lights were left on in the building during night times. The cows were provided with concentrate feed three times at eight hours intervals (7:00 AM, 3:00 PM, and 5:00 PM). All cows were fed concentrate individually at a rate of 0.5 kg per 1 kg of milk following the recommendation of an earlier study (Pandey and Voskuil 2011). Besides, the cows were fed maize silage individually in the morning starting from 8:00 AM until 2:00 PM, hay in the afternoon starting from 3:00 PM until the next morning of 8:00 AM as *ad libitum* throughout the study period, and were provided with tap water *ad libitum*.

Experimental diet ingredients, ration formulation, and analysis

The concentrate ration was mixed on the farm, from grains and by-products such as ground maize, wheat bran, soybean meal, peanut meal, salt, limestone, and ruminant premix at proportions of 56.1, 20.6, 6.7, 14.9, 0.7, 1.0, and 1.5%, respectively (Table 1). Samples (500 g from each feed) were collected once for each feed and the analysis for their nutritional contents was done in duplicate and then the average of the two values was taken. The samples were analysed for *dry matter (DM)*, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and ash. DM and nitrogen (N) were analysed according to the standard methods of AOAC (2000), whereas NDF, ADF, and acid detergent lignin (ADL) were determined by the methods of Van Soest et al. (1991). The experimental feed was analysed at Haramaya University, Animal Nutrition laboratory.

Milk data collection and quality analysis

Milking was done by milking machine twice a day at an equal milking interval at 6:00 AM and 6:00 PM. Morning and evening milk yields for each cow were separately weighed by using a sensitive balance and recorded for 120 days. Milk samples (100 ml each) were collected separately for the morning and evening every fifteen days interval from each cow and analysed in duplicate for each sample. A sample was taken eight (8) times per cow per month for each milking time, i.e. morning and evening time and then the average value was calculated from the values of all animals in the same group. A separate analysis was done for the morning and afternoon milk for fat, protein, lactose, solid-not fat (SNF), total solids (TS), milk urea nitrogen (MUN) by using a milk analyser (Milko-scan) machine at Haramaya University, Dairy Technology laboratory.

Statistical analyses

Descriptive statistics were computed for milk yields, fat, protein, lactose, solid-not fat (SNF), total solid (TS), and milk urea nitrogen (MUN) by using SAS software (version 9.4; SAS Institute Inc., NC). Milk yield and milk composition data were analysed according to a completely randomised design with the repeated measures (milking months) using the PROC MIXED procedure of SAS. Accordingly, the statistical analyses included milking month, milking time, lactation stage, and the interaction between milking month and lactation stage as fixed effects and animal effect nested within milking month and residual as random effects. The following statistical model was used:

$$Y_{ijk} = \mu + LS_i + MM_j + MT_k + (MM \times MT)_{jk} + \varepsilon_{ijk},$$

Where;
Y_{ijk} = dependent variable,
μ = overall mean,
LS_i = fixed effect of i^{th} lactation stage,
MM_j = fixed effect of j^{th} milking month,
MT_k = fixed effect of k^{th} milking time,
$(MM \times MT)_{jk}$ = interaction between j^{th} lactation stage and j^{th} milking month, and
ε_{ijk} = error term ~N (0, σ^2e).

The subject of the repeated measures was defined as cow nested within the treatment, and it was treated as a random effect in the model. When the F ratio was significant, multiple comparisons of least squares means (LSMs) were performed using Bonferroni's test adjustment.

Results and Discussions

The chemical compositions of the feed ingredients used in the formulated ration are presented in Table 1. The DM contents of most ingredients were slightly higher than the acceptable ranges, 88.36-90.72% (Geerts 2014) and this suggested that all ingredients are appropriate for use and storage since high DM contents control the growth of mould in feeds. The CP values of observed oilseed cakes (nouge seed cake, peanut meal, and by-product of oil extraction plants) were higher than values (28-35%) reported for protein sources in Ethiopia (Adugna 2008). This could be due to variations in the used oilseed type, method, and efficiency of extracting oil from seeds, other managements, and environmental conditions. The CP contents of wheat bran were lower than the values (19.47%) observed in the earlier study report (Lemma et al. 2016). Moreover, the evaluated native grass hay CP value was lower than 9.48% (Tufan et al. 2016) and higher than 4.73% (Addisu et al. 2013). The NDF values for the hay and maize silage were higher than 69.9% and the roughage feeds that contain lower than this value of NDF are categorized as poor quality roughage (Singh and Oosting 1992). The CP value of maize silage was in line with the result (8% CP) reported by NRC (1996).

Table 1: Proportion of ingredients used in the experimental ration and their nutritive values

Ingredients	Proportion (%)	Average nutritional values (%)						
		DM	CP	NDF	ADF	ADL	EE	Ash
Ground corn	56.1	89.0	7.1	27.9	3.9	0.6	5.3	2.3
Wheat bran	20.6	93.1	15.3	43.1	9.5	4.2	4.8	3.9
Soybean meal	6.7	93.2	38.5	NE	NE	NE	8.9	8.0
Peanut meal	13.4	94.7	37.3	34.7	13.8	6.3	9.6	6.2
Maize silage	-	94.9	7.7	75.0	41.0	NE	2.3	7.6
Hay	-	95.0	6.7	79.0	43.0	NE	1.3	8.9
Salt	0.7	-	-	-	-	-	-	-
Limestone	1.0	-	-	-	-	-	-	-
Ruminant premix	1.5	-	-	-	-	-	-	-

DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; NE = not evaluated.

The results of least month-to-month variations for the respective milking times in milk yield and compositions showed some differences (Table 2). In all milking months, the highest variations were found for "milk yield during the "morning milking times". The highest variations were found for "MUN" during the 1st and 2nd milking months in the "evening milkings" whereas the highest variations were found for "fat" during the 3rd and 4th milking months in the "evening milking". The month-to-month coefficients of variations in milk yield, fat, protein, SNF, TS, and MUN were found to range from 5.72-

29.64, 7.31-17.82, 4.03-12.11, 1.26-4.42, 2.91-7.92, and 3.45-20.07%, respectively. The main findings in this study were the month-to-month variations in milk composition and milk yield within milking times. Similar findings in this regard were also found in a study carried out by Quist et al. (2008). For practical farming, this means that repeated measurements of each milking time of milk yield and milk composition at the monthly level are useful in early detection of yield and quality alterations and health disturbances and would thus, be helpful in the management of the dairy herd.

Table 2: Mean values, standard deviations, and coefficients of variations in milk yield and compositions within milking month by milking time

MM	MT	Variable	Mean	STD	CV	MM	MT	Variable	Mean	STD	CV
1	Evening	MY (kg)	14.12	0.81	5.72	3	Evening	MY (kg)	13.95	1.67	11.94
		Fat (%)	3.81	0.28	7.31			Fat (%)	4.70	0.84	17.82
		Protein (%)	3.37	0.23	6.96			Protein (%)	3.45	0.22	6.48
		SNF (%)	8.69	0.26	3.00			SNF (%)	8.93	0.26	2.92
		TS (%)	12.47	0.47	3.75			TS (%)	13.67	0.93	6.80
		MUN (mg/dl)	23.01	2.74	11.91			MUN (mg/dl)	28.12	2.53	9.00
	Morning	MY (kg)	14.97	2.86	19.09		Morning	MY (kg)	14.59	3.56	24.42
		Fat (%)	3.55	0.44	12.33			Fat (%)	3.47	0.26	7.45
		Protein (%)	3.44	0.16	4.63			Protein (%)	3.39	0.17	5.08
		SNF (%)	8.78	0.11	1.26			SNF (%)	8.77	0.15	1.73
		TS (%)	12.29	0.57	4.66			TS (%)	12.27	0.41	3.33
		MUN (mg/dl)	23.16	2.88	12.45			MUN (mg/dl)	27.22	0.94	3.45
2	Evening	MY (kg)	14.64	1.48	10.12	4	Evening	MY (kg)	13.09	1.12	8.59
		Fat (%)	3.77	0.28	7.46			Fat (%)	4.12	0.68	16.61
		Protein (%)	3.41	0.14	4.03			Protein (%)	3.44	0.30	8.70
		SNF (%)	8.80	0.12	1.33			SNF (%)	8.87	0.31	3.47
		TS (%)	12.52	0.36	2.91			TS (%)	13.04	1.03	7.92
		MUN (mg/dl)	23.69	4.76	20.07			MUN (mg/dl)	32.99	5.01	15.19
	Morning	MY (kg)	14.93	3.11	20.83		Morning	MY (kg)	13.87	4.11	29.64
		Fat (%)	3.69	0.38	10.40			Fat (%)	3.50	0.54	15.46
		Protein (%)	3.50	0.24	6.73			Protein (%)	3.41	0.41	12.11
		SNF (%)	8.97	0.20	2.26			SNF (%)	8.88	0.39	4.42
		TS (%)	12.64	0.60	4.77			TS (%)	12.40	0.95	7.62
		MUN (mg/dl)	22.39	3.80	16.96			MUN (mg/dl)	31.30	1.37	4.38

MM = Month; STD = Standard deviations; CV = Coefficient of variations; MY = Milk yield; SNF =Solid-not fat; TS =Total solid; MUN =Milk urea nitrogen.

Milking month and milking time had no significant effects on milk yield, protein, and SNF, whereas all these parameters were influenced by the lactation stage (Table 3). Correspondingly, there are results of studies that report that milk yield and components are unaffected by lactation months (Bhoite and Padekar 2002, Sudhakar et al. 2013). Although it was not significant, the milk yield was higher in the morning session than in the evening time. This agrees with results obtained by other authors (Weiss et al. 2002, Fayeye et al. 2013, Tona et al. 2016, Bondan et al. 2019) who attributed the increase in morning milk yields to longer milking intervals. However, Fayeye et al. (2013) noted a significant difference between morning and evening milking sessions for the quantity of milk. This study showed that the lactation stage significantly influenced milk yield and the highest milk yield recorded at an early stage (Table 3). This observation is consistent with Vijayakumar et al. (2017) who reported the highest peak of milk yield in an early stage and then declined in the later stage of lactation. Correspondingly, Sekerden (2002) reported the gradual increase of milk yield from the date of calving and thereafter decreasing up to the end of lactation. Furthermore, Olafadehan and Adewumi (2010) noted a decreasing trend of milk yield as the months in lactation of the cows advanced. This may be due to the changes of hormones causing deterioration of the mammary glands, nutrient requirements of the fetus (unborn calf), and insufficient nutrition for milk production (Vijayakumar et al. 2017). Besides, Wilde et al. (1999) reported a decrease in yield performance at about 3-4 months after calving, which was associated with the next pregnancy and apoptosis of milk-producing cells in the udder.

Milk fat was the only component that was significantly influenced by milking month, milking time, and lactation stage (Table 3). This is in line with Thomas and Sasidharan (2015) who reported a significant influence of months on milk fat. The significant effect of the stage of lactation on fat percentage is in line with the findings by Kayastha et al. (2008). However, in the previous studies, non-significant effects of stage of lactation on fat were reported (Bhoite and Padekar 2002, Sarkar et al. 2006).

Table 3: Effect of milking month, milking time, and lactation stage on milk yield and compositions (least mean squares)

Trait	Milking month				Milking time		Lactation stage		
	1	2	3	4	Morning	Evening	Early	Mid	Late
MY	14.5442[a]	14.7850[a]	14.2683[a]	13.4767[a]	14.5863[a]	13.9508[a]	16.4544[a]	14.4950[b]	11.8562[c]
Fat	3.6775[b]	3.7258[ba]	3.8092[ba]	4.0808[a]	3.5483[b]	4.0983[a]	3.5325[b]	3.7863[b]	4.1513[a]
Protein	3.4050[a]	3.4525[a]	3.4208[a]	3.4233[a]	3.4325[a]	3.4183[a]	3.2625[c]	3.4250[b]	3.5888[a]
SNF	8.7342[a]	8.8858[a]	8.8483[a]	8.8742[a]	8.8492[a]	8.8221[a]	8.6713[b]	8.8362[ba]	8.9994[a]
MUN	23.0858[c]	23.0400[c]	27.6692[b]	32.1475[a]	26.0179[a]	26.9533[a]	26.0000[a]	25.8988[a]	27.558[a]

[abc] Means with the same letter in the row are not significantly different. MY: Milk yield, MUN: Milk urea nitrogen; SNF: Solids-not fat.

The evening milk session revealed higher fat content compared to the morning milk (Figure 1) which is consistent with the findings of Bondan et al. (2019) who reported higher fat content in the afternoon milking than in the morning for cows milked twice a day. This result, however, disagrees with those who reported lower milk fat in the late stage of lactation (Bohmanova et al. 2009). Moreover, many other scholars reported higher fat content during the late lactation than other stages (Stoop et al. 2009). The lower fat contents in the morning and early-stage milk samples were probably related to the effects of milk dilution or larger milk volume, which concurs with the results found by Weiss et al. (2002). The fat content consistently increased with the progress of lactation months (Table 3). This is consistent with Auldist et al. (1998) and Cobanoglu et al. (2017) who noted an increase of milk fat during advanced lactation as positively affected by the lower milk yield. Besides, Yano et al. (2014) reported the richest fat component at the start of lactation. The significant effect of the lactation stage on fat disagreed with the observation of Shibru et al. (2019).

Milk protein and SNF were not significantly influenced by milking month and milking time (Table 3). This contradicts with the earlier findings in studies reported by many scholars as the month had a significant influence on milk SNF component (Thomas and Sasidharan 2015). The stage of lactation was significantly affected all observed milk components except MUN, while the highest values were recorded at the late lactation stage (Table 3). Similar results of significant effects of the lactation stage on all milk constituents except lactose were also reported by Shuiep et al. (2016). The results, however, disagreed with the findings of earlier studies that reported non-significant differences of protein and SNF among the different lactation stages (Gurmessa and Melaku 2012, Gajbhiye et al. 2019). However, significant effects of stage of lactation on SNF content were reported in some cattle breeds (Sarkar et al. 2006, Kayastha et al. 2008). Besides, Sarkar et al. (2006) observed a significant effect of the lactation stage on the protein content of milk. However, Gajbhiye et al. (2019) reported a non-significant influence of stage of lactation on the protein content of milk. This study revealed the highest protein and SNF contents at the late stage of lactation. In agreement with this study results, Auldist et al. (1998) reported higher milk protein values in the late stage of lactation. This study had shown decreased milk yields from early to late lactation in an inverse to the concentrations of fat, protein, and SNF. This agrees with the findings of Pollott (2004) who reported that the increase in concentrations of protein and fat in subsequent stages of lactation had direct effects on the milk TS and SNF. SNF and protein components significantly increased with the stage of lactation (Table 3). This is corresponding to the findings of an earlier study conducted; it was reported that when the milk yield increased in the early period of lactation, the protein content decreased (Cobanoglu et al. 2017).

MUN was not significantly influenced by milking time and lactation stage but was significantly affected by milking month (Table 3). On the contrary, Jilek et al. (2006) reported the influence of lactation stage on the level of MUN but, no variation was found by stage of lactation on the same trait in other research reports so far (Schepers and Meijer 1998). MUN was higher in the evening milking than morning milking time and this is contrary to the observations by Bondan et al. (2019) who reported the highest MUN levels in morning milking sessions for cows milked twice a day. The highest MUN was observed in the 4^{th} month of lactation (Table 3). This is consistent with Arunvipas et al. (2003) who reported that MUN reached peak values in the 4^{th} month. However, Carlsson et al. (1995) reported that MUN reached a maximum between 3 and 6 months of lactation. Furthermore, increased MUN levels as lactation progressed were reported in an earlier study (Hojman et al. 2004). This result agrees with that of Jilek et al. (2006), who reported that MUN rapidly declined after calving, and then gradually rose through to the end of lactation. The reduction of MUN in the first month of lactation is related to the inability of cows to ingest sufficient feed early in lactation, leading to relatively lower protein intake, which is consistent with the earlier study reports (Carlsson et al. 1995, Arunvipas et al. 2003).

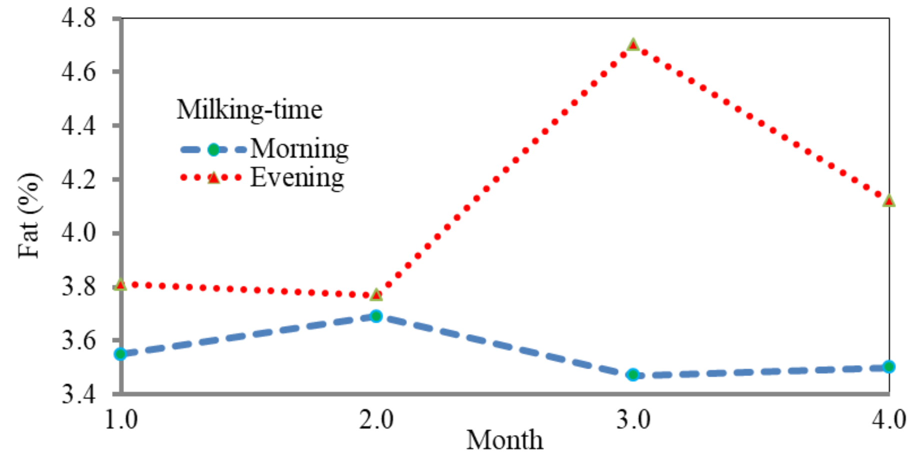

Figure 1: Milking month X Milking time on fat.

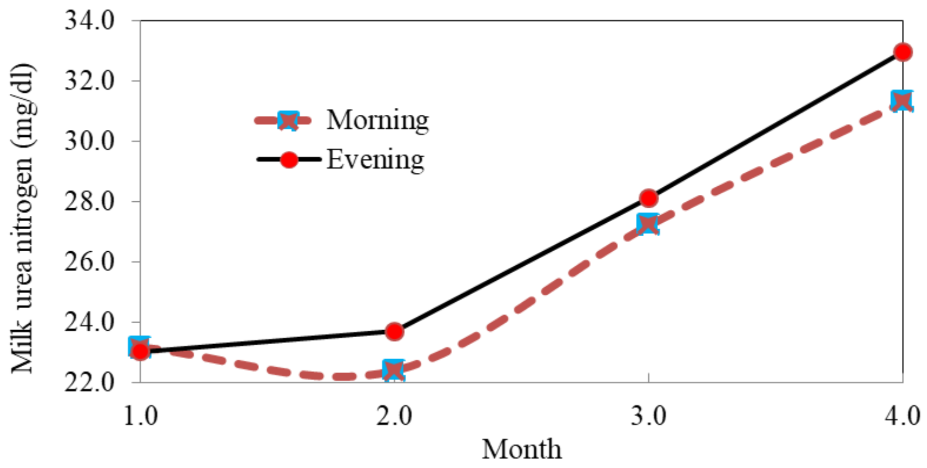

Figure 2: Milking month X milking time on milk urea nitrogen.

There was no significant interaction effect between milking month and milking time on milk yield, protein, and SNF (Table 4). However, a milking month × milking time interaction effect was observed for fat and MUN (Figure 1 and Figure 2). Significantly ($P \leq 0.001$), the highest fat value was obtained for milking month 3 in the evening milking time and the lowest fat value was obtained for milking month 3 in the morning time. It may be inferred that the dilution effect of milk on the percentage components of fat is noticed mainly in the late months of lactation than the first three lactation months. This implied that the milking month x milking time interaction effect was significantly observed at later lactation months than during the early lactation stage.

Table 4: Interaction effect between milking month and milking time (least mean squares)

Trait	Milking month*Milking time interaction							
	1*M	1*E	2*M	2*E	3*M	3*E	4*M	4*E
MY	14.9683[a]	14.1200[a]	14.9267[a]	14.6433[a]	14.5850[a]	13.9517[a]	13.8650[a]	13.0883[a]
Fat	3.5450[c]	3.8100[cb]	3.6867[cb]	3.7650[cb]	3.4667[c]	4.6950[a]	3.4950[c]	4.1233[b]
Protein	3.4383[a]	3.3717[a]	3.4950[a]	3.4100[a]	3.3917[a]	3.4500[a]	3.4050[a]	3.4417[a]
SNF	8.7783[a]	8.6900[a]	8.9733[a]	8.7983[a]	8.7650[a]	8.9317[a]	8.8800[a]	8.8683[a]
MUN	23.1633[e]	23.0083[e]	22.3883[e]	23.6917[de]	27.2183[dc]	28.1200[bc]	31.3017[ba]	32.9933[a]

MY = Milk yield, M = Morning, E = Evening, MUN = Milk urea nitrogen; SNF = Solids-not fat.
[abcde] Means with the same letter in the row are not significantly different.

The highest value for MUN was obtained for milking month 4 in the evening milking time. Contrarily, Bondan et al. (2019) reported the highest MUN levels in the morning milking session for cows milked twice a day. The milking month x milking time interaction effects increased with advanced lactation months (Table 4). This is fully corresponding to those of Arunvipas et al. (2003) who observed an increase of MUN concentrations with months of lactation and reach a peak in the fourth month of lactation. Besides, the results of different authors are consistent with the increase of MUN with lactation month though it reaches a peak in different months. For instance, Jilek et al. (2006) reported maximum MUN content in the 5th month of lactation, Hojman et al. (2004) noted in the 3rd - 6th months of lactation, and Emanuelson et al. (1993) found the highest level of MUN between the 2nd and 3rd months of lactation. The higher MUN levels at the later month of lactation might be related to the good ability of cows' feed intake that leads to a relatively higher protein intake. A significant interaction effect between milking month and milking time was observed and this indicates the adverse effects of milking months on fat and MUN.

Conclusions

The month-to-month coefficients of variations in milk yield, fat, protein, SNF, TS, and MUN were found to range from 5.72-29.64, 7.31-17.82, 4.03-12.11, 1.26-4.42, 2.91-7.92, and 3.45-20.07%, respectively. Milking month and milking time had no significant effects on milk yield, protein, and SNF. Milk fat was the only component that was significantly influenced by milking month, milking time, and lactation stage. Significant milking month × milking time interaction effects were only observed for fat and MUN. Finally, this study concluded that repeated measurements for the respective milking times in milk yield and milk composition at a monthly level showed variations. Further studies should also be conducted using repeated measurements of each milking time of milk yield and milk composition at a weekly level for early detection of yield and quality alterations and health disturbances of dairy cows.

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Conflict of interest

The authors have no conflict of interest to declare.

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