Identification of feeding pattern and their impact on milk fatty acid profiles from traditional dairy cows in Pangalengan Sub-district

D Anzhany¹, Despal²*, T Toharmat², N Rofiah², N Nuraina², A N Hamidah², A Cusiayuni¹

¹Study Program Nutrition and Feed Science, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University. Jl. Agatis Kampus IPB Darmaga 16168, Bogor-Indonesia
²Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Jl. Agatis Kampus IPB Darmaga 16168, Bogor-Indonesia

*Email: despal@apps.ipb.ac.id

Abstract. Fatty acids (FA) profiles especially conjugated linoleic acid (CLA) are commonly used to distinguish milk quality. However, its content is very sensitive and tends to change with a different type of feed. The study aims to identify the relationship between the feeding system and milk FA profile in the Pangalengan sub-district. The feeding system and milk FA profiles were studied using two-step post observatory research. Step one, milk was collected from 27 traditional-dairy farms using purposive random sampling and compare with a large-scale farm. The result shows that FA profiles vary greatly among traditional farms especially for long-chain FA (LCFA) which was found in small concentrations. CLA content was also found higher in traditional farms. Step two, five traditional farms with the highest CLA milk content were observed for their feeding systems. Parameter observed including cow’s specification, feed type used, and feed intake. The best ration produced high milk CLA and production was the ration consisted of 38 % high-quality forages and 62 % commercial concentrate. Thus, it can be concluded that milk from traditional farms was better than milk from large-scale farms. The best milk CLA was produced with a combination of 38 % high-quality forages and 62 % commercial concentrate.

1. Introduction

Lipids content in cow milk is about 3–5 % total nutrient of the milk [1]. Lipids of the triacylglycerides (TAG) group occupy the largest amount of milk fat. Milk fat plays a role as one of determining factors in the milk price as well as a good source of energy [2]. FA composition influences the taste and the melting point in milk [1]. FAs are reported to be more able to distinguish the quality of milk compared to the levels of fat, protein, SNF, and lactose [3]. FAs in milk are very complex compounds and have at least 400 FAs [1]. There are two groups among those FAs such as saturated FAs (SFAs) and unsaturated FAs (UFAs). The content of the SFAs of milk can reach 65 % of the total FA [4,5]. SFA is presumed negatively correlated with the incidence of coronary heart disease (CHD) and atherosclerosis. This issue causes a negative impression on dairy milk. It could be handled by
increasing the concentration of FAs that positively correlated with health. Those FAs are polyunsaturated FAs (PUFA), monounsaturated FAs (MUFA), and conjugated linoleic acids [6].

Conjugated linoleic acid (CLA) is one of the FAs that potential to prevent many diseases. CLA compounds have anti-obesity, anti-carcinogenic, anti-atherogenic, and anti-diabetic properties [6]. Naturally, milk CLA is synthesized in the mammary gland by the activity of Δ9-desaturase as well as in the rumen by isomerization and biohydrogenation of unsaturated FAs [7]. Besides the processes that occur inside the body, the synthesis of milk CLA is influenced by several factors such as feeding system, geographical animal husbandry, seasonal variations and local forage variations in pasture cattle, temperature, and the initial content of the CLA [7].

The fat content and FA profile of the milk are the most volatile nutrients. Based on the factors that affect the content of CLA in milk, the feeding system is one of the primary and most sensitive factors. One of the constraints of dairy farming in Indonesia is to provide sustainable good quality forages. Variations in the quality of feed forages can occur when the type used changes every day. To maintain the productivity of dairy cows, farmers use additional concentrates. The concentrate used can be either a single concentrate or a mixed concentrate produced by a local cooperative. The ratio of forages:concentrates were reported to affect the productivity and quality of dairy products produced [8]. High concentrate ration is often an option. However, the use of diverse concentrates in rations certainly affects the cost of feed. Differences in the composition of milk fats are probably caused by the quality of forage and pasture. High PUFA consumption of pasture forage increases the concentration of oleic acid, vaccenic acid, and CLA in the milk fat [8].

Based on geographical position, the Pangalengan sub-district are mountains and hills which range between 984–1,571 m altitude and are classified as highlands. Pangalengan district has cool air with temperatures ranging from 16–25 °C (BPS 2018). These conditions provide a comfortable environment for dairy cows. The survey method was chosen as the first step in identifying the profile of milk FAs in the region. The survey was conducted due to limitations in the availability of milk FA data, especially milk from traditional farms in Pangalengan. The dairy center area in Pangalengan has a diverse feeding pattern. Thus attracted the attention of researchers to conduct this study which aims to identify the relationship of milk FA profile with the influence of feeding system in the region.

2. Materials and methods

2.1. Milk collecting sample

The survey was conducted in July–August 2020 in five different villages spread across Pangalengan Subdistrict, Bandung Regency, West Java Province, Indonesia. The survey was done by the purposive random sampling. Interview techniques were conducted to collect livestock data and the feeding systems. The survey continued with milk sampling from traditional farms and the large-scale farm (dairy industry) in the same are. Milk sampling was done twice with a two-week time lag. A total of 54 pairs of milk samples were collected from 27 traditional farms and 4 pairs of milk samples were collected from 1 dairy industry. Milk sampling from farms was done twice a day, which is at morning milking and afternoon milking. Milk was collected in a 500 ml plastic bottle. Milk samples were then immediately stored at a temperature of 4 °C until further analysis.

2.2. Milk fatty acid profiles analysis

2.2.1. Analysis of milk fatty acid. Analysis of milk FAs carried out with three stages consists of fat separation, methylation, and identification and injection using gas chromatography (GC), similarly to the method used by Martha et al. [9]. Milk sample separation was done by mixing 10 μl of milk sample with 2 ml of 25 ml H2SO4 solution in 1-liter methanol, then homogenized using vortex for 2 minutes. Samples were stored at a temperature of −20 °C in the freezer for 12–16 hours until analysis. After separation, the samples were ready for methylation. Methylation was done by thawing the samples until it reaches room temperature, then agitated using vortex for 2 minutes and heated using a
hot plate at 85 °C for two hours. After reaching room temperature, the samples were added with 2 ml of saturated NaCl solution and 1 ml of isooctane. The solution mixture was then homogenized for two minutes and centrifuged at 3000 rpm for five minutes. The top layer of the solution mixture, consisting of isooctane and FAME, was transferred into the vial without damaging the dividing layer. The sample was stored at –20 °C until further analysis. FA profiles and milk CLA were quantified using GC (GC-7820A/G4350B, Agilent Technologies). The identification in the GC followed the following conditions by injecting 1 μl of FAME samples. Each sample takes 58 minutes using HP –5 column (30 m length × 0.320 mm diameter (i.d) and film thickness 0.25 μm; Agilent Technologies). Identification of FA profile using authentic standard (Supelco 37 Component FAME Mix; CRM47885, Sigma-Aldrich and, Standard CLA (O5507, Sigma-Aldrich).

2.2.2. Calculation of milk fatty acid indices. Milk FAs indices were calculated using AI, HH, and DI ratio. Atherogenicity index (AI) was calculated using following formula: AI= [C12: 0 + 4(C14: 0) + C16: 0] / Σ(MUFA + PUFA) [10]; HH ratio was calculated using following model: HH= (C18: 1 + PUFA) / (C14:0 + C16:0) [5]; the Δ9-desaturase index was calculated using following model: DI= C14:1/C14:0 [11].

2.3. Sorting and selecting five traditional dairy farms
Observed farms were selected based on the highest-ranking points. Calculation of CLA yield was done using the following formula: CLA yield (g. day−1): milk production × milk fat content × milk CLA content. The sorting was done based on CLA milk yield from two periods of collection. The farms with the lowest points indicates the highest yield CLA ranking. A total of five farms with the lowest ranking points were selected and further observed the feeding system.

2.4. Chemical analysis of the feed
Feed materials were collected from all five farms during the seven-day observation period. Samples of feed forage collected about 1 kg wet every day. While the feed concentrate sample collected about 3 kg wet on the last day of observation. Forage samples were dried in an open space and then further dried at 60 °C until dry. Concentrate samples were also dried at 60 °C to dry. After the feed samples were dried, the samples were finely ground using Ossel E-250G-V2 milling machine and filtered using a 1 mm sieve. The feed samples were then analyzed chemically for dry matter (DM), Ash, crude protein (CP), ether extract (EE), and crude fiber (CF). Analysis of DM, Ash, CP, and EE was done under AOAC 2005 method. Analysis of CP used the Kjeldahl method, and EE analysis used the soxhlet method. The CF analysis was done following the ANKOM200 procedure for CF analysis.

2.5. Observation of nutrient intake and nutrient sufficient
Observation of feed consumption was carried out on five observed farms. Consumption was calculated by calculating the difference between the feed given and the feed left (% DM) during the seven-day observation period. Nutrient consumption consists of consumption of DM, Ash, CP, EE, CF, nitrogen-free extract (NFE), and total digestible nutrient (TDN). The nutrients sufficient were measured by calculating the difference between the feed nutrients consumed against the feed nutrients requirement. The nutrient requirement was calculated based on the nutrient requirement of each cow by referring to the NRC [12].

2.6. Statistical analysis
Each sample was treated as an experimental unit. The differences in milk fatty acid composition between traditional farms and the dairy industry were analyzed using independent sample t-test analysis carried out using SPSS ver. 20.
3. Results and discussion

3.1. Identification of fatty acid profiles

The average milk FA profiles from the dairy industry and traditional dairy farms showed in Table 1. The average milk FAs profile in traditional dairy farms is more diverse than one in the industry.

| Milk FA profiles (100 % milk fat) | Traditional dairy farms | Industrial dairy farms | Sig. 2 Tailed |
|----------------------------------|------------------------|-----------------------|---------------|
| C6:0                             | 1.75 ± 0.35            | 1.91 ± 0.32           | 0.202         |
| C8:0                             | 0.89 ± 0.21            | 1.08 ± 0.12           | 0.017*        |
| C10:0                            | 1.77 ± 0.52            | 2.33 ± 0.26           | 0.004*        |
| C11:0                            | nd                     | nd                    |               |
| C12:0                            | 3.05 ± 0.84            | 3.81 ± 2.51           | 0.095         |
| C13:0                            | 0.01 ± 0.09            | nd                    | 0.658         |
| C14:1                            | 0.99 ± 0.86            | 0.83 ± 0.13           | 0.599         |
| C14:0                            | 9.00 ± 1.76            | 8.81 ± 1.01           | 0.773         |
| C15:0                            | 0.58 ± 0.19            | 0.56 ± 0.43           | 0.845         |
| C15:1, cis-10                    | 0.99 ± 0.19            | 0.92 ± 0.12           | 0.340         |
| C16:1, cis-9                     | 1.43 ± 2.29            | 1.36 ± 0.19           | 0.932         |
| C16:0                            | 25.46 ± 3.51           | 29.83 ± 3.90          | 0.002*        |
| C17:0                            | 0.55 ± 0.15            | 2.54 ± 5.72           | 0.009*        |
| C17:1, cis-10                    | 0.68 ± 0.40            | 1.52 ± 2.64           | 0.026*        |
| C18:0                            | 1.39 ± 1.18            | 1.85 ± 0.29           | 0.275         |
| C18:1, trans-9                   | 6.04 ± 14.11           | 0.27 ± 0.04           | 0.256         |
| C18:1, cis-9                     | 33.67 ± 16.00          | 33.93 ± 4.82          | 0.964         |
| C18:2, trans-9,12                | 3.94 ± 1.42            | 3.63 ± 0.40           | 0.544         |
| C18:2, cis-9,12                  | 5.05 ± 1.79            | 3.57 ± 0.54           | 0.024*        |
| C20:0                            | 0.06 ± 0.13            | nd                    | 0.196         |
| γ-C18:3                          | 0.02 ± 0.07            | nd                    | 0.391         |
| C20:1, cis-11                    | 0.03 ± 0.09            | nd                    | 0.424         |
| C18:3                            | 0.12 ± 0.22            | nd                    | 0.133         |
| C21:0                            | 0.01 ± 0.02            | nd                    | 0.412         |
| C20:2, cis-11,14                 | 0.09 ± 0.12            | nd                    | 0.052         |
| C22:0                            | 0.04 ± 0.13            | nd                    | 0.421         |
| C20:3, cis-8,11,14               | 0.02 ± 0.11            | nd                    | 0.704         |
| C22:1                            | 0.01 ± 0.08            | nd                    | 0.644         |
| C20:3, cis-11,14,17              | nd                     | nd                    |               |
| C23:0                            | 0.02 ± 0.09            | nd                    | 0.606         |
| C20:4, cis-5,8,11,14             | 0.02 ± 0.10            | nd                    | 0.600         |
| C22:2, cis-13,16                 | 0.01 ± 0.08            | nd                    | 0.704         |
| C24:0                            | 0.02 ± 0.10            | nd                    | 0.595         |
| CLA                              | 1.48 ± 0.50            | 1.02 ± 0.35           | 0.014*        |
| SFA                              | 44.59 ± 6.83           | 52.74 ± 3.51          | 0.002*        |
| UFA                              | 54.59 ± 6.84           | 46.79 ± 3.47          | 0.003*        |
| PUFA                             | 10.75 ± 2.19           | 8.22 ± 0.94           | 0.002*        |
| MUFA                             | 43.84 ± 6.69           | 38.57 ± 2.66          | 0.032*        |
| PUFA/SFA                         | 0.25 ± 0.07            | 0.16 ± 0.02           | 0.000*        |
| AI                               | 1.23 ± 0.44            | 1.47 ± 0.08           | 0.133         |
| HH                               | 1.24 ± 0.54            | 0.99 ± 0.10           | 0.205         |
| DI                               | 0.12 ± 0.14            | 0.09 ± 0.01           | 0.662         |
The t-test analysis showed significant differences in seven FAs between the two groups which consist of C8:0, C10:0, C16:0. C17:0, C17:1, cis-10, C18:2, cis-9,12. The total content of short-chain FAs (SCFAs, C6-C8) of milk from the industry was higher than milk from traditional farms. It was known that the industry uses corn silage in its diet. Corn silage was mixed with other feed ingredients and given as a total mixed ration (TMR) to livestock. The study showed that corn silages diet tend to have higher SCFAs and linoleic acid but lower CLA, cis-9, trans-11 compared to grass silages or pasture diets [13]. The corn silages diet was reported to favors C6–C12, C16:1, and C18:2. It may be caused by the result of linoleic acid contained in corn. The content of SCFAs also plays a role in causing rancid flavor in cooled milk [14]. In addition to SCFA, the use of corn silage in rations can also increase the proportion of C17:0 and C17:1, cis-9 [15]. It showed that C17:0 and C17:1, cis-9 FAs found significantly higher in milk from the industry.

The UFA content of both groups was dominated by C18:1, cis-9. That MUFA in milk is mainly composed of C18:1, cis-9 (oleic acid) and C18:1, trans-11 (vaccenic acid/VA) [4,7]. The C18:1, cis-9, and C18:3, n-3 FAs are reported to have anti-cancer and anti-atherogenic properties as well as improving the immune response [16]. Therefore C18:1, cis-9, can be classified as a FA that is beneficial for health. However, an increase in the proportion of C18:1, cis-9 in milk fat should be a concern as it can be one of the markers of negative energy balance (NEB) occurrence [17].

The average content of CLA milk in this study ranged from 0.77–1.48 %. This result was lower compare CLA content from cows fed with supplemented 2 % prill fat which was 1.55 % milk fat [18]. CLA content in milk varies in the range of 0.25–1.8 % [19]. Higher CLA content was found in the average FAs profile from traditional farms (1.48 %). CLA content in milk and its processed products can be influenced by several factors including feeding system, geographical animal husbandry, seasonal variations and local forage variations in pasture livestock, and temperature [7].

CLA was mainly synthesized in the mammary gland of the precursor C18:1, trans-11 through the enzyme activity of Δ9-desaturase [20–22]. The DI ratio could predict desaturase activity in the mammary study. In addition to the ratios shown in the table, there are several other ratios that can be used such as palmitoleic/palmitic (C16:1, cis-9/C16:0), oleic/stearic acid (C18:1, cis-9/C18:0), and CLA/vaccenic acid (CLA, cis-9, trans-11/C18:1, trans-11) [23]. The ratio considered the most reliable is the ratio of C14:1, cis-9/C14:0. Both FAs fully synthesize via de novo pathway in the mammary gland [24]. The ratio considered the most reliable is the ratio of C14:1, cis-9/C14:0. Both FAs fully synthesize via de novo pathway in the mammary gland. Based on Table 1, shows that a higher DI ratio found in milk from traditional farms. The low DI ratio in milk from the industry was in line with the higher SFA content of the dairy industry. The activity of Δ9–desaturase in the mammary gland was low, followed by an increasing proportion of SFA [5]. However, the ratio of suspected desaturase activity may be erroneous due to differences in substrates of desaturase enzymes and also differences in the length of FA chains [5].

The AI ratio appears to replace the previous ratio that was considered incapable of guessing the atherogenicity of a food [10]. The higher both AI and HH ratio was found in milk from traditional farms. The high AI ratio in food, including milk, was considered more harmful to health [23]. There is no standard for both ratios yet. But this study has compared these results on others investigation and found that this study has slightly lower AI and HH content [25]. The AI in dairy products ranges from 1.42–5.13 with dietary treatment as the most influencing factor [25]. HH in meat and dairy products ranges from 1.27–2.786; 0.32–1.29, respectively [25].
3.2. Sorting and selecting five traditional dairy farm

Table 1 shows that milk from traditional farms has better milk quality than the industry based on its FA profile. Milk from the traditional farms contains higher CLA, PUFA, and MUFA. CLA is one of the good FAs that have the potential as a functional food. CLA yield has been calculated on each milk produced by traditional farms. CLA yields from two periods of collection time were sorted by ranking. The CLA yield (g.day\(^{-1}\)) ranges from 0.25–4.32 g.day\(^{-1}\). Five farms with the highest CLA yield produced more than 3 g.day\(^{-1}\) and been observed further.

3.3. Nutrient consumption and nutrient sufficient

Feed types used and its nutrient content are shown in Table 2. The highest consumption of DM was found in P1 and P5 farms. Both farms have different ratios of forages:concentrates. That the consumption of DM increased in rations with concentrate balance reaching 60 % of total feeding [12]. However, ration with a higher percentage of concentrates in this study was not always accompanied by high consumption of DM. In addition to the concentrate ratio, DM consumption can be influenced by feed quality, body weight, milk production rate, livestock health, feeding frequency, and environmental temperature [12]. The amount of feed provided also influenced consumption. The quality of forage given differs between farms, so it can be one of the factors that influence the consumption of DM rations.

Table 2. Nutrient ration intake from five different observed dairy farms.

| Feed consumption (% BK) | Observed Farms |
|-------------------------|----------------|
|                         | P1 | P2 | P3 | P4 | P5 |
| Grasses and herbs       | 18 | 35 | 6  | 38 | 54 |
| Agricultural by-product | 29 | 15 | 20 | 6  | 6  |
| Corn silage             |    | 21 |    |    |    |
| Commercial concentrate  | 48 | 29 | 38 | 62 | 40 |
| Tofu waste              | 5  | 21 | 9  |    |    |
| Others concentrates     |    |    |    | 6  |    |
| DM (kgDM.day\(^{-1}\))  | 14.72 ± 2.37 | 9.67 ± 1.29 | 10.09 ± 1.36 | 10.96 ± 0.82 | 18.29 ± 1.18 |
| CP (kgDM.day\(^{-1}\))   | 2.55 ± 2.57  | 1.02 ± 0.18  | 1.19 ± 0.13  | 1.51 ± 0.11  | 1.97 ± 0.52  |
| EE (kgDM.day\(^{-1}\))    | 0.63 ± 0.05  | 0.44 ± 0.09  | 0.34 ± 0.04  | 0.47 ± 0.04  | 1.64 ± 0.09  |
| CF (kgDM.day\(^{-1}\))    | 2.40 ± 0.47  | 1.74 ± 0.28  | 2.63 ± 0.32  | 1.56 ± 0.13  | 4.05 ± 0.28  |
| Ash (kgDM.day\(^{-1}\))   | 1.61 ± 0.57 | 1.25 ± 0.22 | 2.12 ± 0.26 | 1.14 ± 0.10 | 2.59 ± 0.16 |
| NFE\(^a\) (kgDM.day\(^{-1}\)) | 8.49 ± 1.32 | 5.17 ± 0.68 | 3.81 ± 0.69 | 6.28 ± 0.47 | 6.61 ± 0.27 |
| TDN\(^b\) (kgDM.day\(^{-1}\)) | 8.51 ± 0.97 | 5.35 ± 0.72 | 4.79 ± 0.65 | 6.62 ± 0.49 | 8.85 ± 0.72 |

\(^a\)NFE = calculated using following formula: NFE = 100 – (% Ash + % CP + % EE + % CF).
\(^b\)TDN = calculated using formula proposed by Wardeh (1981): TDN= -14.8356 + 1.3310 (% CP) + 0.7923 (% NFE) + 0.9787 (% EE) + 0.5133 (% CF).

Nutrient sufficiency was calculated based on nutrients requirements by referring to the NRC [12]. Calculation of nutrient sufficiency was carried out on the content of DM, CP, and TDN of rations which shows in Table 3. Nutrient sufficiency in P2 and P3 farms indicates negative values. Nutrient adequacy in P1 and P5 farms shows positive value with considerable excess nutrients. The sufficiency of nutrients in the treatment of P4 rations shows a positive value with fairly low excess nutrients. Therefore, the nutrient sufficiency in P4 farm considered as the best compared to four other farms.
Table 3. Nutrient sufficient of ration from the five different observed dairy farms.

| Nutrient content | Observed Farms |
|------------------|----------------|
|                  | P1     | P2     | P3     | P4     | P5     |
| DM consumption   | 14.72 ± 2.37 | 9.67 ± 1.29 | 10.09 ± 1.36 | 10.96 ± 0.82 | 18.29 ± 1.18 |
| CP consumption   | 2.55 ± 2.57 | 1.02 ± 0.18 | 1.19 ± 0.13 | 1.51 ± 0.11 | 1.97 ± 0.52 |
| TDN consumption  | 8.51 ± 0.97 | 5.35 ± 0.72 | 4.79 ± 0.65 | 6.62 ± 0.49 | 8.85 ± 0.72 |
| DM requirement   | 10.70   | 11.04   | 11.82   | 10.41   | 11.19   |
| CP requirement   | 1.39    | 1.45    | 1.45    | 1.29    | 1.48    |
| TDN requirement  | 6.33    | 6.53    | 6.99    | 6.14    | 6.60    |
| DM sufficient    | 4.03    | -1.37   | -1.73   | 0.55    | 7.10    |
| CP sufficient    | 1.16    | -0.44   | -0.26   | 0.22    | 0.49    |
| TDN sufficient   | 2.18    | -1.18   | -2.20   | 0.48    | 2.25    |

Nutrient requirement was calculated based on NRC for dairy cattle 2001.
Nutrient sufficient was calculated based on difference between nutrient consumption and nutrient requirement.

3.4. Milk production and milk quality from observed dairy farms

The average milk production of the five farms is quite diverse. Table 4 shows that the highest milk production was found in P4 followed by P5, P3, P1, and P2. The average milk production of each cow in the previous survey was at 15.73 ± 3.07 (data not shown). Based on nutritional factors, the amount of milk produced is strongly influenced by the lactose of milk. Haile-Mariam and Pryce [26] reported that the correlation between milk production and lactose production was close to 1. Milk is isotonic which is shown by lactose secretion that affects water transportation to the alveoli of the udder gland. That the secretion of water into milk would increase as lactose synthesis increases and directly increases milk production [27].

The quality of milk was influenced by three factors including genetic, environmental, and physiological [28]. Based on the survey, that all cattle are Holstein-Friesian dairy cattle and are in the second–third birth period, except for P4 farm who have only given birth once. The five cows were in the lactation period between the third-fifth month when the interview was conducted. All five cows were not in a state of illness when observations were done.

The FA profiles of the five farms were quite diverse. FA concentration C6:0–C14:0 higher in P1, P3, and P4 farms i.e. rations with higher concentrate use. The highest C18:1, trans-9, and lowest C18:1,cis-9 found in rations containing tofu waste and corn silage (P3). The highest CLA was found in the P2 followed by P4. FAs C18:2,trans-9,12 and C18:2,cis-9,12 were higher in rations with more forage percentages i.e. in farm P2 and P5 rations. P3 contains more diverse LCFA s including C20:2,cis-11,14, C20:0, C20:4,cis-5,8,11,14, and C24:0. Differences in the profile of milk FAs indicate the influence of starch, fiber, and lipid components of cow’s feed on the quality of milk. Starch was thought to affect the biohydrogenation process of the rumen and the composition of milk FAs. That rations with higher digestible starch content lead to increased pH of rumen and concentrations of C4:0–C16:0 and lower C18:1,cis-9 and C18:1,trans [29].
Table 4. Milk FA profiles from five different observed dairy farms.

| FA profiles (100 % milk fat) | Observed farms |
|-----------------------------|--------------|
|                             | P1   | P2   | P3   | P4   | P5   |
| C6:0                        | 1.98 | 1.74 | 2.50 | 1.88 | 0.98 |
| C8:0                        | 1.14 | 0.90 | 1.29 | 1.09 | 0.57 |
| C10:0                       | 2.57 | 1.91 | 2.49 | 2.20 | 1.33 |
| C11:0                       | nd   | nd   | nd   | nd   | nd   |
| C12:0                       | 3.31 | 2.89 | 4.92 | 3.62 | 2.24 |
| C13:0                       | nd   | nd   | nd   | nd   | nd   |
| C14:1                       | 1.24 | 0.93 | 1.31 | 1.23 | 0.79 |
| C14:0                       | 10.08| 9.98 | 11.44| 9.94 | 8.74 |
| C15:0                       | 0.63 | 0.71 | 0.44 | 0.54 | 0.71 |
| C15:1, cis-10               | 1.00 | 1.04 | 0.90 | 0.95 | 1.22 |
| C16:1, cis-9                | 1.43 | 1.09 | 2.10 | 1.77 | 1.16 |
| C16:0                       | 27.86| 26.19| 34.40| 26.14| 25.56|
| C17:0                       | 0.38 | 0.53 | 0.36 | 0.53 | 0.61 |
| C17:1, cis-10               | 0.45 | 0.54 | 0.54 | 0.53 | 0.69 |
| C18:0                       | 1.00 | 0.84 | 0.86 | 1.11 | 0.93 |
| C18:1, trans-9              | 0.16 | 0.17 | 0.23 | 0.11 | 0.15 |
| C18:1, cis-9                | 37.71| 39.96| 28.40| 39.95| 40.72|
| C18:2, trans-9,12           | 4.23 | 4.09 | 3.17 | 3.24 | 5.90 |
| C18:2, cis-9,12             | 3.98 | 4.71 | 2.35 | 3.15 | 6.02 |
| C20:0                       | 0.02 | nd   | nd   | nd   | 0.19 |
| γ-C18:3                     | nd   | nd   | 0.71 | nd   | nd   |
| C20:1, cis-11               | nd   | nd   | nd   | nd   | nd   |
| C18:3                       | nd   | nd   | nd   | 0.14 | nd   |
| C21:0                       | nd   | nd   | 0.10 | nd   | nd   |
| C20:2, cis-11,14            | 0.02 | nd   | 0.69 | nd   | 0.13 |
| C22:0                       | nd   | nd   | 0.35 | nd   | 0.34 |
| C20:3, cis-8,11,14          | nd   | nd   | nd   | nd   | nd   |
| C22:1                       | nd   | nd   | nd   | nd   | nd   |
| C20:3, cis-11,14,17         | nd   | nd   | nd   | nd   | nd   |
| C23:0                       | nd   | nd   | nd   | nd   | nd   |
| C20:4, cis-5,8,11,14        | nd   | nd   | 0.21 | nd   | nd   |
| C22:2, cis-13,16            | nd   | nd   | nd   | nd   | nd   |
| C24:0                       | nd   | nd   | 0.16 | nd   | nd   |
| CLA                         | 0.83 | 1.85 | 0.89 | 1.01 | 0.89 |
| SFA                         | 48.96| 45.69| 59.22| 47.14| 42.20|
| UFA                         | 51.04| 54.39| 40.78| 52.66| 57.80|
| PUFA                        | 9.05 | 10.65| 7.31 | 8.11 | 13.07|
| MUFA                        | 41.98| 43.74| 33.47| 44.55| 44.73|
| PUFA/SFA                    | 0.18 | 0.23 | 0.12 | 0.17 | 0.31 |
| IA                          | 1.40 | 1.27 | 2.09 | 1.32 | 1.09 |
| HH                          | 1.13 | 1.30 | 0.70 | 1.21 | 1.47 |
| DI                          | 0.12 | 0.09 | 0.11 | 0.12 | 0.09 |
| Milk production L.day⁻¹     | 12.4 ± 0.9| 11.5 ± 1.2| 14.7 ± 1.1| 20.2 ± 0.4| 15.0 ± 0.4|

nd = not detected on this analysis

The highest milk CLA produced from P2 which ration consisted of 35 % grass and herbs, 15 % agricultural by-product, 29 % commercial concentrate, and 21% tofu waste. However, the cow was in a NEB. While P4 also produced a high CLA and C18:1, cis-9 among the five farms observed but
followed with positive energy sufficiency. In contrast to others *trans*, FAs, CLA has health benefits such as anti-obesity, anti-carcinogenic, anti-atherogenic, and anti-diabetic [6]. CLA has 28 positional and geometric isomer variations [30]. Approximately 75–90 % CLA was derived from linoleic acid and α-linolenic acid [31]. However, that an increase in the proportion of fresh forages or fiber in rations affected the increase of UFA and CLA in milk fats [16]. In other words, the quality of rations, especially forages, plays a role in changes in the profile of milk FAs. In this case, relatively stable forages quality was thought to affect the quality of nutrients and FAs of milk. Another PUFA that attracts attention is γ-C18:3 FAs (gamma-linolenic acid, GLA) which are only found in milk from P4. GLA was successfully detected with a considerable percentage of 0.71 %/100 % milk fat. GLA is a compound with anti-inflammatory activity through the formation of dihomogamma linolenic acid (DGLA) [32]. GLA is classified as a LCFA. Synthesis of LCFA in milk is influenced by feed FAs [33]. The presence of GLA in milk from P4 was thought caused by differences in biohydrogenation processes in rumen so that FAs are successfully absorbed in the digestive organs after rumen. However, GLA on milk is not much discussed.

The AI ratio was higher in farms with a higher percentage of concentrates in their rations. The AI range of the five farms was 1.09–2.09. The studies were similar to the general AI range for dairy products ranging from 1.42–5.13 [25]. The ratio of HH of the five farms was not much different, which is in the range of 0.70–1.47. The HH range on this study was slightly higher than its general range on dairy products which range between 0.32–1.29 [25].

4. Conclusions
It can be concluded that milk from traditional farms was better than the industrial farm. It also shown a higher CLA content found in traditional farms. The investigation of feeding systems from five observed farms showed that feeding system affects milk quality. The best CLA in milk was produced from cows fed with a combination of 38 % high-quality forages and 62 % commercial concentrate.

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