Effect of different altitudes on milk fatty acid and conjugated linoleic acid (CLA) profiles

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Abstract. Geographical position and feed quality of farms in highland and lowland, particularly in the tropical region, cause the differences in milk quality. One of the factors that influenced milk quality is the fatty acid (FA) profile of milk. The objective of this study is to compare the FA profiles of milk produced by farms in highland and lowland regions. Milk sampling was done by random purposive method at two regions which have different altitudes. Milk sampling was conducted with 28 farmers whose cows were fed botanically diverse forages and with commercial mix concentrate of dairy cows. The FA profiles of the milk samples were analyzed using Gas Chromatography (GC-7820A/G4350B, Agilent Technologies). Individual FAME was identified by comparison to standard mixture Supelco 37 Component FAME Mix and standard CLA (O5507, Sigma-Aldrich). The FA profiles were statistically analyzed for normality test and t-test carried out by SPSS ver.22. There were nine significantly different FA profiles consisting of C6:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1-trans9, C18:2-trans, C18:2-cis, and C18:2-CLA. The milk from the lowland region has higher PUFA content, especially CLA content, and also has lower SFA content. The conclusion is milk from highland and lowland regions consists of different FA profiles.

1. Introduction

Common dairy cattle’s breed in Indonesia is Friesian – Holstein. This breed is not an indigenous breed from Indonesia. However, this breed had been bred for a long time so that it adapts to the Indonesian tropical climate [1]. There is an indicator named temperature-humidity index (THI), which is used to determine the comfortability of the environment combined between temperature and humidity. There are four categories of THI, such as < 71 comfort zone, 72 ≤ THI ≤ 79 mild heat stress, 80 ≤ THI ≤ 80 moderate heat stress, and 90 ≤ THI ≤ 97 severe heat stress[2]. In a tropical climate like Indonesia, naturally, THI can reach comfort zone level in the highland region. Pangalengan sub-district is categorized as highland (1200 masl.) with a daily temperature ranging between 16 – 25 °C. Although milk production in tropical highland [3]–[6] was higher than lowland [1], it does not eliminate the
possibility for having a dairy farm in the lowland region, for example, dairy farms in Bogor and Jakarta city, even though both cities have moderately high temperature and humidity.

Although highland environmental conditions support the comfortability of cows, limited access can be an obstacle. Another obstacle can be in the form of marketing efforts for dairy products to the direct market which is hard to meet. Therefore, dairy farming in the lowlands remains popular [7]. Dairy farms in Tanah Sareal sub-district, Bogor City is an example of lowland farm that sells their dairy milk directly to users. Apart from differences in geographical factors, it is suspected that there are differences in the quality of milk produced in the highlands and lowlands. According to Collomb et al., [8], different botanical forages in the highlands have higher PUFA content that implies an increased PUFA content in milk. Therefore, it is known that the quality of forage and fiber significantly affects the formation and quality of milk fat. The total fat content, however, is not sufficient to differentiate milk quality. Analysis of the fatty acid profile of milk can be a reference in determining the appropriate type of milk processing. Some of the fatty acids in milk do not change even though they are processed, while some do.

Until nowadays, the pricing factor of cow's milk is only based on total solid (TS) and total plate count (TPC) caused by feed fiber and hygiene. If the assumption that there is a difference in the fatty acid profile of milk is proven, giving a fair price based on milk fatty acids can be an option. Therefore, in this study, an analysis of the fatty acid profiles of milk from the highland and lowland regions was carried out. In this study, farms located in Pangalengan District, South Bandung Regency, were selected to represent the highland, while farms in Tanah Sareal District, Bogor City, and Cibungbulang District, Bogor Regency, represent the lowland. This study aims to distinguish the qualities of milk produced from the highland and lowland based on their fatty acid profiles.

2. Materials and methods
2.1. Survey of traditional dairy farms
Milk samples were collected from May – September 2020 at Pangalengan sub-district (984 masl – 1571 masl), West Bandung regency, and also at Tanah Sareal sub-district (± 200 masl), Bogor city, and Cibungbulang sub-district (± 350 masl), Bogor regency. The survey was conducted using a random purposive method which involved 35 commercial dairy farms. Of these 35 dairy farms, 21 were located on the Pangalengan highland and 14 others were located on the Tanah Sareal and Cibungbulang lowland. The chosen dairy farms were selected based on their feeding systems. Each farm used different botanical forages and commercial mix concentrates. All the cow's herd consists of Friesian – Holstein cows. The daily regimen from each farm was documented from July – August 2020. The mean daily regimen from both regions is shown in Table 1.

2.2. Milk sampling
Milk sampling was conducted from July – August 2020 using a random purposive method. Milk collecting time differs between and within the regions, but all the farms did milking twice a day. Fresh milk is collected at 5.00 a.m. – 8.00 a.m. as morning milk and 1.00 p.m. – 5.00 p.m. as afternoon milk. Morning and afternoon milk were collected in a 500-ml bottle directly from the cow’s teats in the middle of the milking process. Fresh milk was stored at 4 °C, then the nutrient composition was immediately analyzed and the milk fat was prepared for methylation. After preparation, the milk samples were kept at – 20 °C in a freezer until the analysis.

2.3. Analysis of milk’s nutrient and fatty acid compositions
Each milk sample from morning and afternoon milking was analyzed separately for nutrient composition analysis and fatty acid profile analysis. The nutrient composition of the milk was analyzed using Lactoscan. The existing nutrient compositions of the milk sample in this paper are fat, solid non-fat (SNF), lactose, and protein.

Milk sample preparation was done by mixing 10 µL milk sample with 2 ml of 25 ml H2SO4 on 1-liter methanol, then homogenized using vortex for two minutes. After that, the samples were kept at

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Table 1: Mean daily regimen from both regions

| Time          | Morning Milk | Afternoon Milk |
|---------------|--------------|----------------|
| 5.00 a.m.     |              |                |
| 8.00 a.m.     |              |                |
| 1.00 p.m.     |              |                |
| 5.00 p.m.     |              |                |
–20 °C in a freezer for minimum 12-16 hours until analysis. After preparation, the samples were thawed until it reached room temperature, then heated using a hot plate at 85 °C for two hours. Rest the sample until it reached room temperature, then mix with 2 ml saturated NaCl solution and 1 ml isooctane. The solution was homogenized for two minutes, then centrifuged at 3000 rpm for five minutes. Take the upper layer of solution that consists of isooctane and fatty acid methyl ester (FAME). Samples were kept at –20 °C until analysis of fatty acid profile. The milk fatty acid profile and CLA were quantified using gas chromatography (GC-7820A/G4350B, Agilent Technologies) by injecting 1µl of sample FAME. Analysis of each sample was executed for 58 minutes using HP – 5 column (30 m length x 0.320 mm diameter (i.d) and 0,25 µm film thickness; Agilent Technologies). Identification of fatty acid profile was using authentic standard (Supelco 37 Component FAME Mix; CRM47885, Sigma-Aldrich and, Standard CLA (O5507, Sigma-Aldrich).

2.4. Statistical analysis
Each farm was treated as an experimental unit. Each data was collected on a single occasion. The milk nutrient and fatty acid composition were analyzed for normality test of unstandardized residuals using a Kolmogorov Smirnof method. The differences between the two regions were analyzed using independent sample t-test analysis carried out using SPPS ver.22.

3. Results and Discussion
3.1. Feed composition
The chosen farms are using different botanical forages as well as commercial mix concentrates as the feed basis. The mean of forages: concentrates ratio are shown as the percentages of total feed (DM basis). The forages: concentrates ratio from lowland regions was higher than the highland region. The lack of forages during the dry season could be one of the factors behind this feeding system. Although the statistical analysis was done on milk quality from the farms that were classified to have the same feeding system in both regions, the feeding regimens varied between and within each region. This variation is inevitable in this study of commercial dairy farm, but the result should be considered as a brief picture of the differences in the fatty acid quality of milk from regions with different altitudes.

One of the factors affecting milk quality is the different composition of feed that also indirectly affected milk fatty acid quality. Some studies have been done to explain the effect of type of forages on milk fatty acid profile. The type of forages from different altitudes was also believed to be able to increase conjugated linoleic acid (CLA). Collomb et al., [2] reported that different botanical forages in highland (1275-2120 masl) farms were higher in PUFA. The implication of this forages quality increased the PUFA content on milk including its CLA content. Although, in general, the feeding regimens from both regions consist of different botanical forages and commercial mix concentrates, there were differences in the amount of feed offered. This study cannot show the nutrient content from each region because of the analysis limitation. Therefore, the feeding regimen was classified based on how many percentages of forages and concentrates were offered. The feeding regimen in each region was similar to the classification of the feeding system mentioned by Hanus et al., [9]. According to Hanus et al. [9], the feeding regimen was divided into an organic system (OS) and a conventional system (CS). The organic feeding system is described as “grazing, with a reduced amount of conserved forages (but a higher ratio of this type of feed than concentrates) and a small proportion of grain concentrates compared to CS.” Milk fatty acid profile from the lowland region has lower C16:0 and hypercholesterolaemic FAs (C12:0+C14:0+C16:0), but higher C18:1trans, C18:2-cis, and C18:2-CLA. This is similar to cows that were fed with OS.
Table 1. Feeding system and number of farms.

| Item                      | Feeding system |       |       |
|---------------------------|----------------|-------|-------|
|                           | Pangalengan    | Bogor |       |
| Number of farms (n)       | 21             | 14    |       |
| Feed offered (%/cow per day) |                |       |       |
| Forages                   | 52             | 71    |       |
| Concentrates              | 48             | 39    |       |

1 Amount of different botanical forages from each regions
2 Amount of commercial mix concentrates from each regions

3.2. Nutrient composition of the milk
The production and nutrient composition of the milk are shown in Table 2. Based on the statistical analysis, there were no significant differences in milk composition between the two different regions. The nutrient composition of the analyzed milk consists of fat, solid non-fat (SNF), lactose, and protein. Indonesia has a standard published by the National Standardization Agency of Indonesia (BSN). As the mean result is compared against the standard [10], the milk quality reaches the quality standard, except for the SNF in both regions. The absence of lactose as one of the nutrient standards was due to the constant value and similarity of lactose content within the same species.

Table 2. Milk production and composition of two groups from different altitudes.

| Parameter                      | Pangalengan | Bogor | Sig. (2-tailed) | SNI 2 |
|--------------------------------|-------------|-------|-----------------|-------|
| Fat (%)                        | 4.08 ± 1.25 | 4.49 ± 1.46 | 0.205           | Min. 3.0 |
| Solid Not Fat (SNF) (%)        | 7.58 ± 0.80 | 7.68 ± 0.76 | 0.612           | Min 8.0 |
| Lactose (%)                    | 4.16 ± 0.44 | 4.22 ± 0.42 | 0.625           | -     |
| Protein (%)                    | 2.79 ± 0.29 | 2.82 ± 0.28 | 0.712           | Min. 2.7 |

Analysis were done at Dairy Nutrition Laboratory, Nutrition and Feed Technology Department, Animal Science Faculty, IPB University.

1 Means data from both regions
2 SNI code= SNI 01-3141-1998 [10].

3.3. Fatty acid profile of milk fat
The result of the fatty acid profile calculation is dominated by unsaturated fatty acid (UFA). Percentage of UFA/100% milk fat were 54.54% ± 10.65 on highland and 62.99% ± 8.06 on lowland. The result showed that milk from both regions is good for health due to higher UFA and lower SFA [11], [12]. The SFA believed as FA with a putative negative effect (C12:0 – C16:0). The average of the fatty acid profile from the two regions are presented in Table 3. Based on the t-test analysis, the fatty acid profiles between the two regions were significantly different. The ten significantly different fatty acids consist of short-chain fatty acid (SCFA), medium-chain fatty acid (MCFA), and long-chain fatty acid (LCFA).

The significantly different LCFA consists of six types of fatty acids, which are C16:0, C18:0, C18:1-trans9, C18:2-trans, C18:2-cis, and C18:2-CLA. The fatty acids were higher from the milk in the lowland region than the highland region. The C18:2-CLA content was higher in the lowland which is 1.93%/total milk fat (±867 mg/L milk). This result was higher than previous research which reported that CLA content in the Bogor Regency was around 637 – 528 mg/L milk [13]. Ferlay et al., [14] reported that mountain natural grassland-based cows feed produced higher C18:1-trans9 fatty acid compared with concentrates-based cows feed. Even though cows from highland consumed varied highland botanical forages, but forage: concentrate ratio in the lowland region was higher than the highland region.
Table 3. Fatty acid profiles of two group from different altitudes.

| Fatty Acid Profile, % of total milk fat | Pangalengan | Bogor | Sig. (2 tailed) |
|----------------------------------------|-------------|-------|-----------------|
| **C6:0**                               | 1.68 ± 0.43<sup>b</sup> | 1.35 ± 0.39<sup>a</sup> | 0.002<sup>*</sup> |
| **C8:0**                               | 0.85 ± 0.25 | 0.75 ± 0.38 | 0.202 |
| **C10:0**                              | 1.61 ± 0.57<sup>b</sup> | 1.32 ± 0.62<sup>a</sup> | 0.045<sup>*</sup> |
| **C11:0**                              | nd          | 0.06 ± 0.30 | 0.223 |
| **C12:0**                              | 2.38 ± 0.86<sup>b</sup> | 2.05 ± 1.11<sup>a</sup> | 0.001<sup>*</sup> |
| **C13:0**                              | 0.04 ± 0.20 | 0.07 ± 0.36 | 0.631 |
| **C14:0**                              | 1.04 ± 1.82 | 0.45 ± 0.30 | 0.097 |
| **C14:1**                              | 8.15 ± 2.45<sup>b</sup> | 5.41 ± 1.55<sup>a</sup> | 0.000<sup>*</sup> |
| **C15:0**                              | 0.52 ± 0.29 | 0.79 ± 1.17 | 0.158 |
| **C15:0, cis10**                       | 0.89 ± 0.28 | 0.82 ± 0.40 | 0.368 |
| **C16:1, cis9**                        | 1.76 ± 5.08 | 0.89 ± 0.39 | 0.370 |
| **C16:0**                              | 23.91 ± 6.09<sup>b</sup> | 18.47 ± 3.55<sup>a</sup> | 0.000<sup>*</sup> |
| **C16:1, trans9**                      | 0.54 ± 0.23 | 1.64 ± 5.19 | 0.172 |
| **C17:0**                              | 0.70 ± 0.32 | 0.83 ± 0.45 | 0.160 |
| **C18:0**                              | 1.55 ± 2.26<sup>a</sup> | 2.45 ± 0.81<sup>b</sup> | 0.047<sup>*</sup> |
| **C18:1, trans9**                      | 39.06 ± 10.35<sup>a</sup> | 44.48 ± 8.49<sup>b</sup> | 0.024<sup>*</sup> |
| **C18:1, cis9**                        | 4.11 ± 2.16<sup>a</sup> | 5.68 ± 3.02<sup>b</sup> | 0.014<sup>*</sup> |
| **C18:2, cis9**                        | 5.06 ± 1.67<sup>a</sup> | 7.06 ± 1.87<sup>b</sup> | 0.000<sup>*</sup> |
| **C18:2, cis9**                        | 0.06 ± 0.16 | 0.72 ± 3.82 | 0.261 |
| **γ-C18:3**                            | nd          | nd       | -               |
| **C20:1, cis11**                       | 0.07 ± 0.21 | nd       | 0.088 |
| **C18:3**                              | 0.16 ± 0.31 | 0.09 ± 0.25 | 0.283 |
| **C21:0**                              | 0.01 ± 0.04 | nd       | 0.189 |
| **C20:2, cis11,14**                    | 0.15 ± 0.23 | 0.11 ± 0.37 | 0.627 |
| **C22:0**                              | 0.06 ± 0.24 | 0.08 ± 0.44 | 0.774 |
| **C20:3, cis8,11,14**                  | nd          | nd       | -               |
| **C22:1**                              | nd          | nd       | -               |
| **C20:3, cis11,14,17**                 | nd          | nd       | -               |
| **C23:0**                              | 0.04 ± 0.20 | nd       | 0.272 |
| **C20:4, cis5,8,11,14**                | 0.05 ± 0.23 | 0.06 ± 0.34 | 0.810 |
| **C22:2, cis-13,16**                   | nd          | nd       | -               |
| **C24:0**                              | 0.05 ± 0.24 | nd       | 0.258 |
| **CLA**                                | 1.31 ± 0.46<sup>a</sup> | 1.93 ± 0.85<sup>b</sup> | 0.000<sup>*</sup> |

| ΣSFA | 41.89 ± 9.07<sup>b</sup> | 35.16 ± 7.68<sup>a</sup> | 0.000<sup>*</sup> |
| ΣUFA | 54.54 ± 10.65<sup>a</sup> | 62.99 ± 8.06<sup>b</sup> | 0.000<sup>*</sup> |
| ΣMUFA | 43.71 ± 8.83<sup>a</sup> | 48.05 ± 7.15<sup>b</sup> | 0.033<sup>*</sup> |
| ΣPUFA | 10.83 ± 2.69<sup>a</sup> | 14.93 ± 3.16<sup>b</sup> | 0.000<sup>*</sup> |
| PUFA/MUFA | 0.25 ± 0.05<sup>a</sup> | 0.31 ± 0.09<sup>b</sup> | 0.000<sup>*</sup> |
| C16:1-cis9/C16:0 | 1.72 ± 10.87 | 0.06 ± 0.10 | 0.425 |

<sup>1</sup> Means data from both regions.

* Showing differences from the same row and different column if Sig. (2 tailed) < 0.05.

<sup>a,b</sup> Means from the same row but with different superscripts differ (P < 0.05; T-test significant difference).

nd = not detected on this analysis.

Analyses were done at Dairy Nutrition Laboratory, Nutrition and Feed Technology Department, Animal Science Faculty, IPB University.

SFA= saturated fatty acid; UFA= unsaturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid.
The C16:0 has a higher concentration from milk in highland than in lowland. The C16:0 fatty acid can be synthesized by both de novo or feed derivatization [15]. In this study, palmitic acid (C16:0) is the highest contributor to SFA from the total SFA in both highland and lowland milk. Conversion of C16:0 and C18:0 to C16:1 and C18:1 are affected by Δ9-desaturase (stearoyl coenzyme-A desaturase/SCD) activity [16]. The C16:1-cis9/C16:0 for highland milk (1.72 ± 10.87) was higher than lowland milk (0.06 ± 0.10). This ratio can be used to reflect the activity of Δ9-desaturase [17]. Thus, the SCD activity in the highland was higher than in the lowland.

The significantly different SCFA consists of C6:0 while the significantly different MCFA consists of C10:0, C12:0, and C14:0. Both SCFA and MCFA of milk from the highland region were higher than the lowland regions. The short-medium chain fatty acid (C1 – C15) were synthesized by de novo pathway in the mammary glands. Linoleic acid and its biohydrogenation product have a potent inhibitory of C10 – C14 fatty acid synthesized [18], [19]. In line with this study, the amount means of C18:2-cis, C18:2-CLA, and C18:1-trans in the lowland milk were higher, which was followed by the lower amount means of C10:0, C12:0, and C14:0 in lowland milk. The short-medium chain fatty acid synthesis may be affected by the quantity of concentrates given. Concentrates of any kind given in high number, notably on the onset of lactation, could increase de novo synthesis of short-medium chain fatty acid and SFA [15].

In this study, the total SFA was significantly different between the two different altitudes. SFAs content in milk is usually mentioned as promoting the adverse effects of milk consumption such as cardiovascular disease. A meta-analysis done by Lamarche et al. [20] showed that there is a neutral association between milk intake and coronary heart disease (CHD), stroke, type 2 diabetes, and fractures. It is also reported that the consumption of SFAs from dairy did not increase smaller LDL particles that were correlated with the increased risk of CHD [21]. However, studies about milk’s SFA as health lipid indices need further explanation. Behind the possible negative side, SFA may improve the quality and storability of milk due to the oxidation stability of the fatty acids [15].

4. Conclusion
Based on milk fatty acid profiles, it can be concluded that milk produced from lowland is high in poly unsaturated fatty acids (PUFA), especially conjugated linoleic acid (CLA), low in saturated fatty acid (SFA). Therefore, it can be categorized as a healthy milk and as good as milk produced from cow kept in comfort zone (highland).

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