Original Research Paper

Estimation of Milk Fatty Acids Health Index as Milk Value Added Determinant using FT-NIRS

[1]**Despal Despal,** [2]**Dwitamy Anzhany,** [1]**Idat Galih Permana,** [1]**Toto Toharmat,** [1]**Rika Zahera,** [1]**Noerhayati Rofiah,** [1]**Norma Nuraina and** [1]**Atikah Nur Hamidah**

[1]*Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Indonesia*
[2]*Student Program in Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Indonesia*

Abstract: The current milk price system based on Total Plate Count (TPC) and Total Solids (TS) are less sensitive in determining milk quality. Milk fatty acids profiles reflected milk quality for human health. However, their determination using Gas Chromatography (GC) is impracticable to be included as a daily price decision determinant. The study aimed to find a model for milk value added based on milk fatty acids profiles that reflected milk quality for human health measured by pre-calibrated rapid Fourier Transform Near-Infrared Reflectance Spectroscopy (FT-NIRS) method. Two hundred fifty-six samples of milk were collected from 3 dairy farm areas. Samples were analyzed using a Milkotronic milk analyzer for fat, protein and lactose contents and Gas Chromatography (GC) for fatty acids. The data were inputted into the FT-NIRS spectrum for calibration. The regression model to calculate milk value-added that can be used as a bonus system was developed after classifying and weighting of Milk Fatty Acid Index (MFAI) determined based on expert judgment. The results showed that milk fatty acids profiles vary greatly. Eight parameters (CLA, C16:0, SFA, MUFA, LCFA, PUFA, C18:2 trans9, 12 and H/H) can be detected accurately using FT-NIRS and used in milk value-added calculation. Simpler equation was used Y = 16.38307 + 5.395582 CLA + 0.695062 PUFA - 0.0244 C18:2, trans 9, 12 with R^2 = 0.950 and was validated insignificantly different as calculated from the 8 parameters. It is concluded that the milk processing industry can use milk fatty acids generated from FT-NIRS to add value to milk collected from smallholder farmers.

Keywords: Fatty Acid, Milk Value, Milk Fatty Acid Index, Smallholder

Introduction

In tropical countries, dairy cattle farming was developed as a government program characterized by smallholder farmers and organized by a cooperative (Moran, 2005). The average milk production, 4100 L per cycle, was smaller than the large-scale dairy farm, which produced 7000-10000 L cycle[-1] (Despal *et al.*, 2017). Although milk production was lower in smallholder dairy cattle farming, its milk components were higher because of a negative correlation between milk production and milk components (Husvéth *et al.*, 2010). Therefore, it is necessary to add the value of the milk components.

Currently, milk price is determined by Total Plate Count (TPC) and total solid consisting of milk fat, lactose and protein. Although milk fat is the most varied component (Hasanah *et al.*, 2017), it is less appropriate to

be used as a value-added determinant because it cannot express health benefits and tends to be labelled as villains in human diets (Salles *et al.*, 2019). Milk fat quality as described by milk fatty acid profiles needs to be explored.

Fatty acids as milk value-added determinants have been introduced in Germany and the Netherlands (Coppa *et al.*, 2014). Around 430 distinctive milk fatty acids were identified in milk items, shifting from 4 to 26 carbons chain length (both even and odd, in a straight or branched chain). The saturation degree of the milk fat presented numerous geometrical isomers in cis and trans design. So far, no single technique can separate and quantify all the fatty acids due to their small quantity. Only 14 fatty acids were found with a concentration above 1% (Amores, 2019). Saturated Fatty Acids (SFAs) have been related to an expansion in cardiovascular risk, obesity and some cancers. Notwithstanding, just C12:0, C14:0 and C16:0

appear to be unhealthy, while C18:0, oleic acid and short-chain SFA have been reported to affect human health positively. C4:0 shows beneficial in inhibiting cancer cell growth; C6:0, C8:0 and C10:0 can reduce body fat (González-Martín *et al.*, 2020).

Milk fat is one of the most complex natural fat because of its fatty acid composition (Amores, 2019). Therefore, detailed fatty acid profiles are necessary to demonstrate the benefit of milk fat to human health (Martha *et al.*, 2019). Besides fatty acid saturation, the Atherogenicity Index (AI) (Ulbricht and Southgate, 1991) and Hypocholesterolemic/Hypercholesterolemic (H/H) ratio (Santos-Silva *et al.*, 2002) was calculated from the fatty acid profiles that were used as an indicator to describe the benefits of milk fatty acids for human health.

The conventional method for determining fatty acid is Gas Chromatography (GC) (Amores, 2019). It included steps for lipid extraction, fractionation, methylation, separation and analysis by GC (Martha *et al.,* 2019). However, this method is time-consuming, requires solvent, sophisticated equipment and skilled labour. It is not possible to use such a method to measure daily milk fatty acid value-added. A rapid and accurate technique is needed to measure milk fatty acids. Near-Infrared Spectroscopy (NIRS) technology have been developed that allows a multi-parametric analysis of organic compound in less than one minute without sample preparation and solvent requirement (Despal *et al.*, 2020).

Determination of milk fatty acid in milk collected by the cooperative and sent to the Milk Processing Industry (MPI) are possible using either laboratory NIRS or handheld NIRS. Although the equipment instalment price is high, it is more efficient and practical. The Accuracy of NIRS is dependent on the database used. Although, milk fatty acids detection using NIR spectroscopy have been shown successfully by González-Martín *et al.* (2020), Núñez-Sánchez (2016) and Bergamaschi (2020), but development local database, improved NIRS prediction accuracy (Despal *et al.*, 2020). So far, there is no tropical milk fatty acid database available. Therefore, this study aims to develop a milk fatty acids database and prediction model to determine the health index of milk fatty acids.

Materials and Methods

Sample Preparation

Two hundred fifty-six Holstein Friesian (HF) milk samples were collected from 3 dairy cattle production areas in the West Java Province of Indonesia (116 samples from Pangalengan District of Bandung Regency, 70 samples from Cibungbulang District of Bogor Regency and 70 samples from Kebon Pedes, Bogor Municipality). All samples were collected from traditional farms, except for 16 samples in Pangalengan were collected from a large farm. The cows were 3.5 - 4.5 years old (2nd - 3rd

lactations), mid-lactation (90 – 150 days in milk) with milk production ranging from 8.0 – 20.4 L per head d^{-1} in traditional farms and 22.7 - 34.5 L per head d^{-1} in the large farm. The cows in the traditional farms were fed different proportions of Napier grass, agricultural waste product, corn silage, concentrate and tofu waste with 45 - 60% forage from total feed offered of 9.67 - 18.29 kg DM and nutrient contents of 10.54 - 17.32% CP, 3.37 - 8.67% EE, 14.23 - 26.06% CF, 10.4 - 14.1% ash, 47.8 - 60.4% TDN. The feeds were distributed equally into two feeding frequencies. On the large farm, the cows were fed *ad libitum* using TMR. It consisted of 40 - 45% forage with different proportions of corn silage, Napier grass, concentrate, alfalfa, wheat straw and premix to fulfil the requirement of cows from different milk production groups.

About 500 mL of each sample were collected from morning and afternoon milking in a plastic bottle container, three times in each location with 2-week intervals to cover seasonal variation. Milk samples were collected directly from the four teats in the middle of manual milking from the traditional farmers. Milk from the large farm was sampled from the bulk tank after a group of cows were milked using a milking machine. The samples were collected from healthy cows whose milk was collected by the cooperative and tested regularly. The TPC contents in the milk tested ranged from 2.5 x 10^5 - 7.5×10^5 CFU mL^{-1} in the traditional farms and <2.5 x 10^5 CFU mL^{-1} in milk from the industry. Milk samples were stored in the chilled container during transportation and sent to the laboratory for milk components analysis using a Milkotronic milk analyzer and milk fatty acids using GC and FT-NIRS.

Milk Component Analysis

Milk component analysis, including fat, protein and lactose, were analyzed using Milkotronic milk analyzer serial I-17-817 made in Bulgaria. The 256 samples were examined in duplo. The Milkotronic milk analyzer was cleaned using warm water before each usage. Milk samples were poured into a 10 mL vial and the milk analyzer sensor hose was inserted into the milk-filled vial. The reading of milk components (fat, protein and lactose) was done automatically and the data were printed in the form of a receipt accordingly.

Fatty Acids Analysis using GC

Milk samples were homogenized before analysis by shaking for 5 min at room temperature. Lipid extraction, methylation process and GC condition used in this analysis were similar to Martha *et al.* (2019). The amount of 100 μL milk sample was pipetted into a screw-cap tube. Then 2 mL of H_2SO_4 2.5% in methanol was added. The tube was agitated for 2 min with vortex. Lipid extraction was run by letting the tube overnight in a -20°C freezer. After extraction, the methylation process was conducted by warming the tube in a 75°C water bath for two hours.

The amount of 2 mL of a saturated NaCl solution and 1 mL isooctane was added. The tube was agitated for 30 s and centrifuged at 2000 rpm for 5 min. 1 mL isooctane layer was collected from the upper layer for later injected to G.C.

An Agilent G4350B gas chromatograph equipped with a Flame Ionization Detector (FID) and HP-5 capillary column (30 m length, 0.320 mm diameter, 0.25 μm film thickness) from Agilent Technologies, Palo Alto, CA, USA was used for separation and quantification of milk Fatty Acids (FA). The temperature for the injector and detector was set at 250°C. The carrier gas used was helium with flowrate 1.3 mL minute^{-1}. Initially, the temperature was programmed at 35°C for 2 min and then increased to 100°C with 30°C per minute, then increased to 195°C with 10°C per minute with 5 min hold, then increased to 205°C with 7°C per minutes and 9 min hold, increased again to 240°C with 3°C per minute and 7 min hold. Once the GC was ready, a 1 μL prepared sample was injected with a split ratio of 10:1. Data were recorded in a computer output device installed with GC Chem Station Software integrator version B.03.02 (Agilent Technologies).

Fatty Acids Estimation Using FT-NIRS

Buchi NIRFlex N-500 Solids Cell (made in Switzerland) was used to collect the spectrum. The FT-NIRS was warmed up for approximately 15 min and, after that, automatically run System Suitability Test (SST) using NIRSware operator. After completing SST, external and internal references were scanned. After completing the references measurement, FT-NIRS is ready to be used to collect the sample spectrum. Scanning was done triplicates for each sample. The collected spectra were inputted with chemometric results with the help of the NIRS ware Management Console. The calibration and validation models were carried out by NIRCal V5.6 using the Partial Least Squares (PLS) regression and validation set. The collected spectra were automatically divided into 2/3 for calibration and 1/3 for validation using block-wise methods.

The calibration and validation process produced a comparison between chemometric and NIRS prediction values. The database resulting from the calibration and validation can be used as standard references for subsequent measurement after external validation. The best models generated were selected based on the smallest Standard Error of Calibration (SEC), STANDARD error of Prediction (SEP), the highest calibration coefficients (R^2) and Residual Predictive Deviation (RPD). RPD is a ratio between SD to SEP. External validation was conducted by measuring samples using new calibration and validated with chemometrics results. The comparison values of the standard error of prediction to the Standard Error of Laboratory (SEP/SEL) were calculated.

Milk Value Added Predicting Model Using Milk Fatty Acids Profile

The prediction of milk value added based on milk fatty acid profiles was conducted using a similar model as described by Coppa *et al.* (2017), consisting of Milk Fatty Acid Index (MFAI) classes definition, weighting procedure to calculate MFAI, regression and simulation. The MFAI was grouped based on health benefit (atherogenicity index (AI), Hypocholesterolemic/Hypercholesterolemic (H/H) ratio and Conjugated Linoleic Acid (CLA)), length of fatty acid chains (Short-Medium (SMCFA) and Long-Chain (LCFA) Fatty Acids) and Fatty acid Saturation (saturated (SFA), Monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids). The total SFA was calculated by summarizing all the fatty acids which have not formed a double bond chain. The total MUFA was also calculated by summarizing all the fatty acids, consisting of one pair of the double bond of carbon atoms. The total PUFA was calculated by summarizing all the fatty acids, consisting of more than one pair double bond of carbon atoms.

The LCFA was calculated from all the fatty acids whose carbon atoms are more than 15 (≥C16). The SMCFA was calculated from all the fatty acids which carbon atoms less than 16 (≤C15). The Atherogenicity Index (AI) was calculated using the following formula described by Ulbricht and Southgate (1991) where:

$$Atherogenicity\ index\,(AI)$$
$$=\left[C12:0+4\,(C14:0)+C16:0\right]\backslash\sum\,(MUFTA+PUFA).$$

While H/H ratio was calculated according to Chen and Liu (2020) where:

$$H\,/\,H\ ratio$$
$$=\left(cisC18:1+\sum PUFA\right)/\,(C12:0+C14:0+C16:0).$$

All classes were calibrated and selected according to FT-NIRS ability in predicting the classes. The parameters were selected based on R^2>0.5, RPD>1.5 and RPL (SEP/SEL) <1 criteria. The weighting procedure to calculate MFAI was based on expert judgement. The weight of each class was decided after judgement from five experts. Data normalization to achieve the same weight was calculated using formula $1/\sqrt{(\sum x..^2)}$ as Hwang and Yoon (1981) recommended. Normalized data were calculated by multiplying the normal weight with parameter values. Milk value added was calculated using the formula:

$$Milk\ value-added=\frac{\max value}{(\max value-\min value)}\times(observrved\ value\min value).$$

Correlation prior to regression was conducted to find predictor parameters to be included in the model.

Regression model were generated from model $Y = b_0 + b_1X_1 + b_2X_2 + … + b_nX_n$, where Y is milk value-added, b_0 is intercept, $b_1, b_2,… b_n$ are regression coefficient, $X_1, X_2, …, X_n$. $X_1, X_2, … X_n$ are milk fatty acids classes used in the model. The simulation model was tested using ten independent sample sets.

Research Design and Data Analysis

Data from this field explorative and laboratory research were Analyzed using Variance (ANOVA) analysis from SPSS version 20 to compare traditional and large farms. The calibration and validation of FT- NIRS databases were conducted using partial least squares from NIR Cal V5.6 from Buchi. Milk value added was predicted using the regression model from SPSS version 20.

Results

Milk Production and Component

Milk production and components from traditional and large farms are shown in Table 1. The table shows that the average milk production in traditional farms is significantly lower than the large farm (14.9 vs 28.7) L day^{-1}. However, the milk components were not significantly different (3.92 vs 4.08% fat, 7.55 vs 7.58% SNF, 4.16 vs 4.16% lactose and 2.77 vs 2.78% protein). The table also shows a considerable variation in milk production and fat content compared to SNF, lactose, or protein contents. The average milk production found in traditional farms (14.9 L day^{-1}) was higher than the national average milk production (13 L day^{-1}) (Tasripin *et al.*, 2020).

Total solid (fat + SNF) found in traditional farms was 11.47%, lower than total solid reported by Dann *et al.*, (2014), reaching up to 12.4%. The average lactose content in the traditional farm was 4.16%, showing a typical lactose content in Holstein Friesian (HF) cow's milk. Meanwhile, the average milk protein content in traditional farms was 2.77% lower than average HF's milk protein (Despal *et al.*, 2017).

Milk Fatty Acid Profiles

The milk fatty acids composition from traditional farms compared to the large farm is shown in Table 2. The table shows a considerable variation within the milk from traditional farms, as shown by the Standard Deviation

(STD). Nineteen milk fatty acids can be separated and analyzed using the GC method in this study. Several other milk fatty acids were found in very small proportions and only in a few milk samples. The table shows some milk fatty acids were different significantly in milk from traditional farms and large farms. Harmful saturated fatty acids (C10:0, C12:0, C16:0, C17:0) were significantly lower in milk from traditional farms than milk from the large farm, while healthy unsaturated fatty acids (C18:1, *cis*-9 and C18:2, *cis*-9,12) were higher. This condition was shown by the lower AI and higher HH index.

The C18:1 cis9 and C16:0 were the milk sample's primary fatty acids, which account for more than 63% of the total fatty acids. The average Saturated Fatty Acids (SFA) were about 40%, while unsaturated was 60%. More than 79% of the fatty acids found in the milk sample consisted of Long-Chain Fatty Acids (LCFA). Less than 21% of them were short and medium chains. Conjugated Linoleic Acids (CLA), one of the functional milk components, was found slightly higher in milk from traditional farms (1.360%) than the large farm (1.02%).

Milk Fatty Acids Estimation Using FT-NIRS

The estimation of milk fatty acids using FT-NIRS is shown in Table 3. R^2, RPD and PRL values determine the accuracy of FT-NIRS calibration and validation. It is shown that R^2 calibration for all fatty acids was more than 0.5 except for C15:0, C17:0, C17:1cis10 and C18:0. The data shows a strong relationship between the original and prediction value. The RPD>1.5 found in C16:0, C18:2, trans9,12, SFA, MUFA, PUFA, LCFA, CLA, AI and H/H parameters shows the ability of FT-NIRS in estimating the milk fatty acids. The validation failed to improve the prediction accuracy. R^2 and RPD found in this study were lower than the result found by Despal *et al.* (2020) for forage quality estimation or Coppa *et al.* (2014) in fresh and thawed milk.

External validation values of the model are shown in Table 4. The table shows that prediction error relative (PRL) <1.0 for all fatty acids except C17:1, cis10, C18:0, C18:2, cis9,12 and AI. The PRL was the ratio between Standard Error Procedure (SEP) to Standard Error Laboratory (SEL) values (Yang *et al.*, 2017). PRL <1.0 reflected the ability of FT-NIRS in predicting milk fatty acids composition.

Table 1: Milk production and component (N = 256)

| Parameters | Traditional Farms (N = 240) | | Large Farm (N = 16) | | |
	Avg	STD	Avg	STD	P
Milk yield Lday^{-1}	14.9	3.13	28.7	6.00	0.000
Fat (%)	3.92	1.31	4.08	0.92	0.740
Solids-not fat (%)	7.55	0.57	7.58	0.14	0.900
Lactose (%)	4.16	0.29	4.16	0.08	0.977
Protein (%)	2.77	0.19	2.78	0.05	0.960

N = Total number of observations; Avg = average; STD = standard deviation; P = P-Value

Table 2: Milk fatty acid content (N = 256)

Fatty acids	Traditional farm (N = 240)		Large farm (N = 16)		P-value
	AVG	STD	AVG	STD	
Caproic acid (C6:0) (% Milk fat)	1.598	0.567	1.914	0.320	0.118
Caprylic acid (C8:0) (% Milk fat)	0.862	0.491	1.083	0.122	0.206
Capric acid (C10:0) (% Milk fat)	1.586	0.659	2.332	0.258	**0.002**
Lauric acid (C12:0) (% Milk fat)	2.773	1.165	3.805	2.514	**0.018**
Myristic acid (C14:0) (% Milk fat)	7.173	3.262	8.812	1.009	0.158
Pentadecanoic acid (C15:0) (% Milk fat)	0.639	0.465	0.563	0.427	0.648
Palmitic acid (C16:0) (% Milk fat)	23.42	4.920	29.83	3.897	**0.000**
Heptadecanoic acid (C17:0) (% Milk fat)	0.620	1.643	2.542	5.723	**0.004**
Octadecanoic acid (C18:0) (% Milk fat)	2.029	1.655	1.853	0.285	0.764
C15:1, *cis*-10 (% Milk fat)	0.767	0.441	0.920	0.118	0.329
Myristoleic acid (C14:1) (% Milk fat)	1.425	2.217	0.827	0.133	0.447
Palmitoleic acid (C16:1) (% Milk fat)	1.043	1.991	1.362	0.187	0.651
cis-10 heptadecanoic (C17:1, *cis*-10) (% Milk fat)	0.916	4.727	1.523	2.636	0.718
cis-9 oleic acid methyl ester (C18:1, *cis*-9) (% Milk fat)	41.68	8.488	33.93	4.821	**0.011**
trans 9 elaidic acid methyl ester (C18:1, trans 9) (% Milk fat)	0.358	0.811	0.273	0.036	0.768
C18:2, *cis*-9,12 (% Milk fat)	6.035	2.521	3.569	0.536	**0.006**
Linolenic acid (C18:3) (% Milk fat)	0.072	0.192	0.000	0.000	0.288
C18:2, trans 9,12 (% Milk fat)	4.343	3.069	3.631	0.395	0.513
SFA (% Milk fat)	40.94	8.408	52.74	3.511	**0.000**
MUFA (% Milk fat)	46.22	7.921	38.84	2.680	**0.009**
PUFA (% Milk fat)	11.94	3.986	8.220	0.938	**0.009**
SMCFA (% Milk fat)	16.85	4.740	20.26	1.721	0.043
LCFA (% Milk fat)	82.25	4.842	79.54	1.688	0.115
Conjugated Linoleic Acid (CLA) (% Milk fat)	1.360	0.694	1.020	0.350	0.169
PUFA/SFA	0.312	0.144	0.157	0.025	**0.003**
AI	0.962	0.479	1.465	0.075	**0.005**
H/H	1.828	0.698	0.990	0.102	**0.001**

AVG = average; STD = standard deviation; CV = coefficient of variation; SFA = short chain fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SMCFA = short-medium chain fatty acids; LCFA = long chain fatty acids; AI = atherogenicity index; H/H = Hypocholesterolemic to hypercholesterolemic ratio

Table 3: FT-NIRS calibration using data generated from the conventional method

Parameters	Calibration							Validation						
	N	Mean	Range	SD	SEC	R^2	RPD	N	Mean	Range	SD	SEP	r^2	RPD
Lactose	120	4.195	3.399-4.86	0.242	0.168	0.675	1.442	60	4.210	3.565-4.742	0.203	0.177	0.556	1.145
Protein	120	2.808	2.328-3.272	0.180	0.116	0.710	1.548	60	2.799	2.244-3.199	0.182	0.165	0.554	1.102
SNF	120	7.658	6.173-8.99	0.507	0.298	0.743	1.700	60	7.643	6.403-8.734	0.458	0.446	0.515	1.027
Add Water	120	8.026	0-24.025	5.092	2.889	0.757	1.763	60	7.955	0-21.188	4.701	2.913	0.697	1.614
Salt	120	0.618	0.477-0.729	0.041	0.028	0.688	1.485	60	0.622	0.515-0.701	0.033	0.030	0.533	1.085
Fat	120	4.063	0.085-7.305	1.562	0.574	0.858	2.720	60	4.015	0.085-7.305	1.376	0.572	0.822	2.406
C6:0 (% Milk Fat)	120	0.058	0-0.098	0.019	0.014	0.629	1.303	60	0.058	0.005-0.1	0.019	0.015	0.615	1.260
C8:0(% Milk Fat)	120	0.031	0.003-0.059	0.010	0.009	0.544	1.091	60	0.030	0.009-0.057	0.009	0.009	0.516	1.038
C10:0 (% Milk Fat)	120	0.052	0.002-0.084	0.021	0.016	0.627	1.263	60	0.053	0.002-0.103	0.016	0.013	0.601	1.219
C12:0 (% Milk Fat)	120	0.096	0-0.209	0.044	0.039	0.561	1.130	60	0.094	0-0.214	0.041	0.042	0.490	0.979
C14:0 (% Milk Fat)	120	0.233	0-0.396	0.087	0.067	0.629	1.302	60	0.256	0-0.402	0.087	0.076	0.565	1.155
C14:1 (% Milk Fat)	120	0.030	0-0.35	0.052	0.042	0.605	1.238	60	0.039	0-0.278	0.046	0.051	0.446	0.902
C15:0 (% Milk Fat)	120	0.030	0.012-0.071	0.010	0.011	0.455	0.913	60	0.029	0.012-0.05	0.009	0.011	0.458	0.750
C15; cis10 (% Milk Fat)	120	0.031	0-0.07	0.014	0.012	0.561	1.131	60	0.030	0-0.059	0.012	0.013	0.475	0.944
C17:0 (% Milk Fat)	120	0.022	0-0.092	0.009	0.011	0.366	0.760	60	0.022	0-0.092	0.007	0.012	0.305	0.627
C17:1; cis10 (% Milk Fat)	120	0.027	0-0.081	0.013	0.013	0.489	0.978	60	0.026	0-0.081	0.011	0.014	0.385	0.773
C18:0 (% milk fat)	120	0.118	0-0.754	0.059	0.084	0.301	0.697	60	0.103	0-0.754	0.055	0.076	0.385	0.725
C16:0 (% Milk Fat)	120	0.820	0.018-1.407	0.259	0.151	0.746	1.715	60	0.814	0.018-1.288	0.245	0.155	0.703	1.588
C16:1 (% Milk Fat)	120	0.031	0-0.076	0.014	0.013	0.530	1.061	60	0.031	0-0.059	0.013	0.013	0.489	0.976
C18:1, cis9 (% Milk Fat)	120	1.847	0.034-3.821	0.683	0.470	0.679	1.454	60	1.773	0.034-3.821	0.632	0.473	0.627	1.337
C18:2, trans9,12 (% Milk Fat)	120	0.312	0.004-1.117	0.166	0.083	0.799	1.991	60	0.317	0.004-1.117	0.163	0.099	0.738	1.638
C18:2, cis9,12 (% Milk Fat)	120	0.239	0-0.592	0.111	0.087	0.623	1.285	60	0.236	0-0.592	0.102	0.089	0.560	1.141
CLA (% Milk Fat)	120	0.060	0-0.195	0.038	0.024	0.574	1.572	60	0.060	0-0.195	0.038	0.025	0.556	1.561
SFA (% Milk Fat)	120	1.487	0.037-2.363	0.486	0.260	0.778	1.874	60	1.455	0.037-2.373	0.463	0.254	0.745	1.828
MUFA (% Milk Fat)	120	1.981	0.037-3.912	0.754	0.441	0.728	1.707	60	1.914	0.037-3.595	0.713	0.443	0.685	1.610
PUFA (% Milk Fat)	120	0.625	0.011-1.416	0.263	0.128	0.809	2.055	60	0.622	0.011-1.416	0.261	0.133	0.793	1.958
SCMFA (% Milk Fat)	120	0.483	0.012-0.802	0.164	0.112	0.681	1.461	60	0.473	0.094-0.759	0.130	0.115	0.547	1.133
LCFA (% Milk Fat)	120	3.578	0.073-6.224	1.233	0.511	0.853	2.413	60	3.478	0.073-6.196	1.237	0.502	0.856	2.464
AI	120	1.426	0.895-5.501	0.747	0.449	0.735	1.664	60	1.470	0.799-6.052	0.811	0.481	0.771	1.686
H/H	120	1.382	0.318-2.036	0.678	0.429	0.714	1.582	60	1.381	0.498-2.027	0.601	0.441	0.714	1.363

N = total number of observations; SD = standard deviation; SEC = standard error of calibration; R^2 = coefficient determination of calibration; RPD = residual predictive deviation; SEP = standard error of prediction; r^2 = coefficient determination of validation

Table 4: External validation of independent sample set

Parameters	FT-NIRS		Conventional methods		T-Test	R	SEP	SEL	PRL (SEP/SEL)
	AVG	STD	AVG	STD					
Lactose (%)	4.248	0.191	4.187	0.236	0.003	0.841	0.128	0.276	0.464
Protein (%)	2.867	0.147	2.796	0.157	0.000	0.880	0.069	0.183	0.376
SNF (%)	7.939	0.319	7.626	0.429	0.000	0.861	0.223	0.483	0.462
Add Water (%)	6.850	4.007	7.559	5.396	0.100	0.859	2.854	6.106	0.467
Salt (%)	0.629	0.028	0.620	0.037	0.004	0.823	0.021	0.042	0.498
Fat (%)	3.786	1.027	3.838	1.215	0.612	0.824	0.688	1.580	0.435
C6:0 (% Milk Fat)	0.058	0.013	0.074	0.035	0.001	0.471	0.031	0.047	0.661
C8:0(% Milk Fat)	0.033	0.006	0.041	0.014	0.002	0.087	0.015	0.024	0.637
C10:0 (% Milk Fat)	0.058	0.012	0.065	0.027	0.033	0.498	0.023	0.042	0.551
C12:0 (% Milk Fat)	0.101	0.031	0.114	0.059	0.124	0.366	0.056	0.095	0.584
C14:0 (% Milk Fat)	0.254	0.062	0.128	0.163	0.000	0.043	0.172	0.240	0.718
C14:1 (% Milk Fat)	0.050	0.026	0.225	0.177	0.000	-0.42	0.188	0.218	0.859
C15:0 (% Milk Fat)	0.028	0.009	0.029	0.022	0.709	0.481	0.019	0.024	0.806
C15;cis10 (% Milk Fat)	0.031	0.011	0.001	0.006	0.000	0.342	0.010	0.012	0.843
C16:0 (% Milk Fat)	0.792	0.178	0.928	0.349	0.004	0.508	0.301	0.427	0.705
C16:1 (% Milk Fat)	0.030	0.009	0.016	0.021	0.000	0.554	0.017	0.052	0.332
C17:0 (% Milk Fat)	0.026	0.008	0.009	0.015	0.000	0.416	0.014	0.020	0.663
C17:1;cis10 (% Milk Fat)	0.025	0.007	0.150	0.496	0.100	-0.28	0.498	0.074	6.734
C18:0 (% milk fat)	0.145	0.055	0.131	0.067	0.194	0.332	0.064	0.061	1.063
C18:1, cis9 (% Milk Fat)	1.822	0.514	1.820	0.834	0.986	0.616	0.657	0.970	0.677
C18:2, trans9,12 (% Milk Fat)	0.287	0.103	0.281	0.166	0.812	0.331	0.165	0.614	0.269
C18:2, cis9,12 (% Milk Fat)	0.259	0.076	0.037	0.105	0.000	-0.11	0.136	0.117	1.163
CLA (% Milk Fat)	0.056	0.020	0.055	0.029	0.794	0.156	0.027	0.032	0.842
SFA(% Milk Fat)	1.479	0.322	1.511	0.546	0.211	0.604	0.449	1.097	0.409
MUFA (% Milk Fat)	1.806	0.492	1.912	0.753	0.006	0.660	0.595	1.778	0.334
PUFA (% Milk Fat)	0.574	0.164	0.401	0.309	0.000	0.322	0.300	0.576	0.520
SCMFA (% Milk Fat)	0.506	0.112	0.609	0.221	0.000	0.750	0.154	0.367	0.419
LCFA (% Milk Fat)	3.225	0.842	3.214	1.026	0.685	0.779	0.644	1.494	0.431
AI	1.322	0.232	0.783	0.382	0.00	-0.01	0.698	0.555	1.258
H/H	1.652	0.338	1.880	0.830	0.053	0.068	0.875	2.415	0.362

R = coefficient correlation; SEL = standard error of laboratory; SEP = standard error of prediction

Table 5: Value-added from each parameter and its correlation to total milk value added (% total milk fatty acids value-added)

Parameters	Average*	Standard deviation	Coefficient correlation**
C16:0	2.56	0.57	-0.27
C18:2, trans9,12	0.71	0.55	0.51*
SFA	5.20	1.30	-0.37
MUFA	4.54	1.02	0.04
PUFA	6.87	2.74	0.69*
LCFA	4.92	1.67	0.38
CLA	7.81	3.61	0.81*
H/H	7.41	3.52	0.37
Total	32.63	5.14	

*the value-added was calculated from standardized value; **correlation coefficient each parameter to total price bonus

Milk Value-Added Model

Milk fatty acid profiles included in the milk value-added prediction model based on FT-NIRS results were C16:0, C18:2, trans9,12, SFA, MUFA, PUFA, LCFA, CLA and H/H parameters. Each parameter based on the expert judgment weighed 20, 5, 10, 10, 10, 20, 5 and 20, respectively. While normalizing weight for the parameter was 0.040, 0.003, 0.002, 0.001, 0.001, 0.005, 0.012 and 0.032, respectively.

Milk value-added calculated from normalized data shows the average milk fatty acids value added was 32.63% (ranged from 19.13% to 48.95%). This value was calculated based on an assumption of 20% top of the current scheme. The average value added from each parameter is shown in Table 5. It can be seen from the table that C16:0 and SFA have a negative correlation to the total value-added. The higher milk value-added comes from CLA content, H/H index and PUFA content, which shows the smallholder farmer's healthy milk quality.

Discussion

Milk Production and Component

The average milk production used in traditional farms were 14.9 L day⁻¹. The results were higher than the average Holstein Friesian performance in different agroecosystems in Indonesia (Kusmayadi *et al.*, 2019) because dairy cattle performance in West Java province on average was higher than other areas in Indonesia. The production was lower than the milk production of Holstein Friesian kept under a large scale dairy farming system in the same area, which can produce 20 - 29 L per day (Tasripin *et al.*, 2020) or more than 23 L per day in the temperate area (Könyves *et al.*, 2017). Low milk production of smallholder dairy farms in the tropical area due to heat stress (Despal *et al.*, 2017) and low forage quality used (Hasanah *et al.*, 2017; Lestari *et al.*, 2015). The addition of high-quality forage such as Mung beans' sprout improved milk production (Zahera *et al.*, 2015).

The average total Solid (SNF + fat) content in the milk sample was 11.48%. It was lower than the total solid content in regular HF milk (12%). This study's low total solid value is due to the low protein content found in the milk (<2.8%). However, the average milk fat found in the milk from traditional farms (3.92%) and the large farm (4.08%) was higher than that reported by Dann *et al.*, (2014), which found an average of 3.57%. The milk samples' high-fat content was due to the high fibre used in ration and low milk produced in the traditional farms (Despal *et al.*, 2017). A negative correlation between milk fat and protein has been reported by de Jager and Kennedy (1987). Low protein in milk from the traditional farm is related to the low quality of protein in concentrate used in the ration (Despal *et al.*, 2017). While in the large farm, low protein content in the milk is probably due to high milk produced (Husvéth *et al.*, 2010).

Milk Fatty Acid Profiles

The health index of milk fatty acids expressed by the ratio of PUFA/SFA found in the milk from traditional farms was 0.312, higher than the value reported by Nantapo *et al.* (2014). The higher PUFA/SFA value is found in smallholder dairy farm milk due to the high proportion used of forage (50%) in the ration (Lestari *et al.*, 2015), which contain high PUFA (Collomb *et al.*, 2001). The average LCFA found in the milk from traditional farms was 82.25% which shows a great body storage mobilization (Collomb *et al.*, 2001) due to low nutrient content in the feed, primarily forage (Despal *et al.*, 2020). The average CLA content in the milk sample was 1.360%, higher than the average content of CLA in cow's milk (0.34 – 1.07%) (Fritsche *et al.*, 2000). High CLA content in smallholder dairy farm milk due to high proportion of forage used in ration. According to Lahlou *et al.* (2014), high CLA content in smallholder dairy farm milk was also related to the high proportion used of tofu waste in concentrate which contained high linoleic acid (Damanik *et al.*, 2018) as a precursor for CLA synthesis (Fiore *et al.*, 2021). The Lower AI index and higher H/H ratio found in the milk from traditional farms were caused by high unsaturated fatty acid content in the milk due to high forage used in the ration (Lestari *et al.*, 2015). Good health index of milk fatty acids expressed by AI and H/H values reflected that milk collected from smallholder dairy farmers were healthy milk and should be valued more.

High milk fatty acid variations found can be used as milk value-added determinants. High coefficient variations are shown in almost all individual fatty acids, particularly C14:1, C18:1 trans 9 and C18:3. The fatty acid group with the highest coefficient variation (standard deviation/mean) was AI, CLA, H/H and PUFA. The variation of milk fatty acids can be resulted from variation in feeding (Nudda *et al.*, 2014), primarily forage to concentrate ratio (Soita *et al.*, 2005) and lipid supplementation (Thering *et al.*, 2009).

Milk Fatty Acids Estimation Using FT-NIRS

RPD is the ratio of prediction to the deviation used in Partial Least Square regression (PLS). The RPD, as calculated from the SD to SEP ratio, represented the FT-NIRS model's ability to predict a substance (Williams and Sobering, 1993). According to Williams (2004), RPD <1.5 indicates an unusable prediction. The 1.5 <RPD <2.0 found in most fatty acids tested represented the ability of prediction to distinguish between high and low values. RPD of more than 1.5 was found in total fat, C16:0, C18:2, trans9,12, CLA, SFA, MUFA, PUFA, LCFA, AI and H/H. RPD >2.0 was found in total fat, PUFA and LCFA represented a relevant prediction of FT-NIRS. In general, short-chain and single fatty acids produced lower RPD compared to long-chain fatty acids and fatty acids profiles such as MUFA, PUFA, LCFA, AI and H/H. Lower RPD value was caused by SEP value closed to SD value.

In this condition, the FT-NIRS calibration process was insufficient to predict the value generated from conventional methods (Baillères *et al.*, 2002). Therefore, improvement of such databases is needed. Lower prediction accuracy of milk fatty acid was found in this study due to the considerable variation of milk collected from smallholder dairy farmers, which came from different feeding, location, management systems and seasons (Despal *et al.*, 2017).

The PRL <1 found in this study showed that the model could be used in milk fatty acid prediction. The low PRL value found in this study was caused by the SEP closely matching the SEL. If the SEP value was much more than the SEC, it might indicate too many wavelengths in the models that do not represent the substrate being model (Ozaki *et al.*, 2007). Based on FT-NIRS calibration results, total fat, CLA, C16:0, SFA, MUFA, LCFA, PUFA, C18:2 trans 9, 12 and H/H can be detected

sophisticatedly with $R^2 > 0.5$, RPD > 1.5 and PR<1. However, the value-added milk model used only CLA, C16:0, SFA, MUFA, LCFA, PUFA, C18: 2 trans 9, 12 and H/H parameters because total fat has been used in the current price system.

Milk Value-Added Model

The C16:0 and SFA have a negative coefficient in milk value-added calculation because the C16:0 and SFA have been associated with increased cardiovascular risk. CLA, PUFA and C18:2,trans9,12 were positively and strongly correlated to the total milk value-added. The fatty acid(s) were also have been found to have a positive correlation to human health (Salles *et al*., 2019). To estimate the milk value-added from real data generated from FT-NIRS, the model requires simplification. Based on the correlation of the parameters to the total milk value-added, it can be seen that CLA, PUFA and C18:2,trans9,12 have a strong correlation to the total value-added with a coefficient correlation of more than 0.5. Therefore it is included in the prediction model. Regression between total milk value added (Y) with CLA, PUFA and C18:2,trans9, 12 value from FT-NIRS found equation milk value added (Y) = 16.38307 + 5.395582 CLA + 0.695062 PUFA – 0.0244 C18:2,trans9, 12 with R2 = 0.95. The T-test validation model found insignificant differences between milk value-added calculated from the eight parameters as shown in Table 5 with milk value-added calculated from the model (P = 0.381). The milk processing industry can use this model to calculate milk value-added given to smallholder farmers by using instant CLA, PUFA and C18:2, trans9, 12 data generated from the pre-calibrated FT-NIRS instrument.

Conclusion

From this study, it can be concluded that the health index of milk fatty acids from a smallholder dairy farm reflected healthy milk and therefore needed to be rewarded. The CLA, PUFA and C18:2, trans 9, 12 generated by the pre-calibrated FT-NIRS instrument can be used as the milk processing industry's basis for milk value-added determination to complement the current price system. Suggested formula to calculate milk value added (Y) is Y = 16.38307 + 5.395582 CLA + 0.695062 PUFA - 0.0244 C18:2, trans 9,12.

Acknowledgement

Funding

Author's Contributions

Despal: Designed, conceived and supervised the experiment, did funding acquisition, wrote the original draft and reviewed also edited the manuscript.

Dwitamy Anzhany: Did data curation and formal analysis, investigated the experiment, reviewed and edited the manuscript.

Idat Galih Permana: Supervised and visualized the experiment, did funding acquisition and resourced.

Toto Toharmat: Supervised and validated the experiment, did funding acquisition and reviewed also edited the manuscript.

Rika Zahera: Did formal analysis, arranged the methodology, acted as a project administrator, developed the software, visualized the data, reviewed, end edited the manuscript.

Noerhayati Rofiah: Did formal analysis, supervised and validated the experiment results, acted as a project administrator, arranged the methodology, reviewed and edited the manuscript.

Norma Nuraina: Did data curation and formal analysis, investigated the experiment, reviewed and edited the manuscript.

Atikah Nur Hamidah: Did data curation and formal analysis and investigated the experiment.

Ethics

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

Amores, G. (2019). Total and free fatty acids analysis in milk and dairy fat. Separations, 6, 1-22. doi.org/10.3390/separations6010014.

Baillères, H., Davrieux, F., & Ham-Pichavant, F. (2002). Near infrared analysis as a tool for rapid screening of some major wood characteristics in a eucalyptus breeding program. Ann. For. Sci., 59, 479–490. https://doi.org/10.1051/forest:2002032

Bergamaschi, M. (2020). Chemometric authentication of farming systems of origin of food (milk and ripened cheese) using infrared spectra, fatty acid profiles, flavor fingerprints and sensory descriptions. Food Chem., 305, 1-10. doi.org/10.1016/j.foodchem.2019.125480

Chen, J., & Liu, H. (2020). Nutritional indices for assessing fatty acids: A mini-review. Int. J. Mol. Sci., 21, 1-24. doi.org/10.3390/ijms21165695

Collomb, M., Bütikofer, U., Sieber, R., Bosset, J.O., & Jeangros, B. (2001). Conjugated linoleic acid and trans fatty acid composition of cows' milk fat produced in lowlands and highlands. J. Dairy Res., 68, 519-523.
doi.org/10.1017/S0022029901004885

Coppa, M., Revello-Chion, A., Giaccone, D., Ferlay, A., Tabacco, E., & Borreani, G. (2014). Comparison of near and medium infrared spectroscopy to predict fatty acid composition on fresh and thawed milk. Food Chem., 150, 49-57. doi.org/10.1016/j.foodchem.2013.10.087

Coppa, M., Revello-Chion, A., Giaccone, D., Tabacco, E., & Borreani, G. (2017). Could predicting fatty acid profile by mid-infrared reflectance spectroscopy be used as a method to increase the value added by milk production chains? J. Dairy Sci., 100, 8705-8721.
doi.org/10.3168/jds.2016-12382

Damanik, R.N.S., Pratiwi, D.Y.W., Widyastuti, N., Rustanti, N., Anjani, G., & Afifah, D.N. (2018). Nutritional Composition Changes during Tempeh Gembus Processing. IOP Conf. Ser. Earth Environ. Sci., 116, 1-10. https://doi.org/10.1088/1755-1315/116/1/012026

Dann, H.M., Tucker, H.A., Cotanch, K.W., Krawczel, P.D., Mooney, C.S., Grant, R.J., & Eguchi, T. (2014). Evaluation of lower-starch diets for lactating Holstein dairy cows. J. Dairy Sci., 91, 7151–7161.
doi.org/10.3168/jds.2014-8341

de Jager, D., & Kennedy, B.W. (1987). Genetic Parameters of Milk Yield and Composition and Their Relationships with Alternative Breeding Goals. J. Dairy Sci., 70, 1258-1266.
doi.org/10.3168/jds.S0022-0302(87)80139-X

Despal., Permana, I.G., Toharmat, T., & Amirroennas, D.E. (2017). Pemberian Pakan Sapi Perah, 1st ed, IPB Press, Bogor.

Despal., Sari, L.A., Chandra, R., Zahera, R., Permana, I.G., & Abdullah, L. (2020). Prediction Accuracy Improvement of Indonesian Dairy Cattle Fiber Feed Compositions Using Near-Infrared Reflectance Spectroscopy Local Database. Trop. Anim. Sci. J., 43, 263–269. doi.org/10.5398/tasj.2020.43.3.263

Fiore, E., Lisuzzo, A., Tessari, R., Spissu, N., Moscati, L., Morgante, M., Gianesella, M., Badon, T., Mazzotta, E., Berlanda, M., Contiero, B., & Fiore, F. (2021). Milk fatty acids composition changes according to β-hydroxybutyrate concentrations in ewes during early lactation. Animals, 11, 1–11.
doi.org/10.3390/ani11051371

Fritsche, J., Fritsche, S., Solomon, M. B., Mossoba, M. M., Yurawecz, M. P., Morehouse, K., & Ku, Y. (2000). Quantitative determination of conjugated linoleic acid isomers in beef fat. Eur. J. Lipid Sci. Technol., 102, 667–672.
doi.org/10.1002/1438-9312(200011)102:11<667::aid-ejlt667>3.3.co;2-e

González-Martín, M. I., Vivar-Quintana, A. M., Revilla, L., & Salvador-Esteban, J. (2020). The determination of fatty acids in cheeses of variable composition (cow, ewe's and goat) by means of near infrared spectroscopy. Microchem. J., 156, 104854. doi.org/10.1016/j.microc.2020.104854

Hasanah, U., Permana, I. G., & Despal. (2017). Introduction of complete ration silage to substitute the conventional ration at traditional dairy farms in Lembang. Pakistan J. Nutr., 16.
doi.org/10.3923/pjn.2017.577.587

Husvéth, F., Galamb, E., Gaál, T., Dublecz, K., Wágner, L., & Pál, L. (2010). Milk production, milk composition, liver lipid contents and C18 fatty acid composition of milk and liver lipids in Awassi ewes fed a diet supplemented with protected cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid (CLA) isomers. Small Rumin. Res., 94, 25–31.
doi.org/10.1016/j.smallrumres.2010.06.003

Hwang, C.L., & Yoon, K.P. (1981). Multiple Attribute Decision Making Methods and Applications: A State-of-the-Art Survey. Springer-Verlag, Berlin.

Könyves, T., Zlatković, N., Memiši, N., Lukač, D., Puvača, N., Stojšin, M., Halász, A., & Miščević, B. (2017). Relationship of temperature-humidity index with milk production and feed intake of Holstein-Frisian cows in different year seasons. Thai J. Vet. Med., 47, 15–23.

Kusmayadi, T., Hasan, M., Herawati, E., & Tanuwiria, U.H. (2019). Performances Holstein Frisien (F.H.) dairy Cow in different agricultural ecosystem. J. Phys. Conf. Ser., 1402, 1–6.
doi.org/10.1088/1742-6596/1402/3/033044

Lahlou, M.N., Kanneganti, R., Massingill, L.J., Broderick, G.A., Park, Y., Pariza, M.W., Ferguson, J.D., & Wu, Z. (2014). Grazing increases the concentration of CLA in dairy cow milk. Animal, 8, 1191–1200.
doi.org/10.1017/S1751731114000998

Lestari, D.A., Abdullah, L., & Despal. (2015). Comparative study of milk production and feed efficiency based on farmers best practices and national research council. Media Peternakan, 38, 110–117. /doi.org/10.5398/medpet.2015.38.2.110.

Martha, R., Despal., Toharmat, T., Rofiah, N., & Anggraeni, D. (2019). Comparison of Extraction Methods for Fatty Acid and Conjugated Linoleic Acid Quantification in Milk. IOP Conf. Ser. Mater. Sci. Eng., 546, 1–4. https://doi.org/10.1088/1757-899X/546/4/042022

Moran, J. (2005). Tropical Dairy Farming, Tropical Dairy Farming. Landlink Press, Collingwood. doi.org/10.1071/9780643093133

Nantapo, C.T.W., Muchenje, V., & Hugo, A. (2014). Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian × Jersey cross cow milk under a pasture-based dairy system. Food Chem., 146, 127–133. doi.org/10.1016/j.foodchem.2013.09.009

Nudda, A., Battacone, G., Neto, O.B., Cannas, A., Helena, A., Francesconi, D., Atzori, A.S., & Pulina, G. (2014). Invited review feeding strategies to design the fatty acid profile of sheep milk and cheese. Revista Brasileira de Zootecnia, 43, 445–456.

Núñez-Sánchez, N. (2016). Near Infrared Spectroscopy (NIRS) for the determination of the milk fat fatty acid profile of goats. Food Chem., 190, 244–252. doi.org/10.1016/j.foodchem.2015.05.083

Ozaki, Y., Morita, S., & Du, Y. (2007). Spectral Analysis, in: Ozaki, Y., McClure, W., Christy, A. (Eds.), Near-Infrared Spectroscopy in Food Science and Technology. John Wiley & Sons Inc., New Jersey, pp. 47–52.

Salles, M. D'Abreu, L.F., Junior, L.C.R., César, M.C., Guimarães, J.G.L., Segura, J.G., Rodrigues, C., Zanetti, M. A., Pfrimer, K., & Netto, A.S. (2019). Inclusion of sunflower oil in the bovine diet improves milk nutritional profile. Nutrients, 11, 1–15. doi.org/10.3390/nu11020481

Santos-Silva, J., Bessa, R.J.B., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. Livest. Prod. Sci., 77, 187–194. doi.org/10.1016/S0301-6226(02)00059-3

Soita, H.W., Fehr, M., Christensen, D.A., & Mutsvangwa, T. (2005). Effects of corn silage particle length and forage: Concentrate ratio on milk fatty acid composition in dairy cows fed supplemental flaxseed. J. Dairy Sci., 88, 2813–2819. doi.org/10.3168/jds.S0022-0302(05)72961-1

Tasripin, D.S., Christi, R.F., & Biyantoro, D.D. (2020). Produksi susu dan lama laktasi sapi perah friesian holstein di PT Ultra Peternakan Bandung Selatan. Compos. J. Ilmu Pertan., 2, 25–29. doi.org/10.37577/composite.v2i1.184

Thering, B.J., Graugnard, D.E., Piantoni, P., & Loor, J.J. (2009). Adipose tissue lipogenic gene networks due to lipid feeding and milk fat depression in lactating cows. J. Dairy Sci., 92, 4290–4300. doi.org/10.3168/jds.2008-2000

Ulbricht, T.L.V., & Southgate, D.A.T. (1991). Coronary heart disease and dietary factors. Lancet, 338, 985–992. doi.org/10.1016/0140-6736(92)91558-P

Williams, P. (2004). Near-infrared technology - getting the best out of light: A short course in the practical implementation of near-infrared spectroscopy for the user. PDK Projects, Inc., British Columbia.

Williams, P.C., & Sobering, D.C. (1993). Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. J. Near Infrared Spectrosc., 1, 25–32. doi.org/10.1255/jnirs.3

Yang, Z., Nie, G., Pan, L., Zhang, Y., Huang, L., Ma, X., & Zhang, X. (2017). Development and validation of near-infrared spectroscopy for the prediction of forage quality parameters in *Lolium multiflorum*. Peer J., 2017, 1-20. doi.org/10.7717/peerj.3867

Zahera, R., Permana, I. G., & Despal, (2015). Utilization of mungbean's green house fodder and silage in the ration for lactating dairy cows. Media Peternakan, 38(2), 123-131.