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## Research Article

# Regional Variation Accuracy Detection of Natural Grass Multi-Species as Dairy Cattle Forage using FT-NIRS

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## Abstract

**Background and Objective:** Natural grass is a basic forage for dairy cattle in Bogor Regency and Municipality of Indonesia. Its quality variation might influence dairy cattle performance. Therefore rapid detection is needed to be able to adjust with other ration ingredients. This study aims to compare natural grass quality between 4 districts in Bogor and develop an accurate local Near-Infrared Reflectance Spectroscopy (NIRS) database. **Materials and Methods:** About 4 kg of each 100 natural grass samples from 10 villages belonging to 4 districts have been collected, dried and ground. Proximate analysis (Dry Matter (DM), ash, Crude Protein (CP), Ether Extract (EE), Crude Fibre (CF)), van Soest analysis (neutral and acid detergent fibre (NDF and ADF)), *in vitro* digestibility (DM, OM, NDF and ADF digestibility), energy partition (gross, digestible, metabolizable and net energy for lactation (GE, DE, ME and NEL)) and forage value for dairy cattle (UFL and RFV) have been calculated. The NIRS spectrum has been collected, calibrated, validated internally and externally. **Results:** The results show that natural grass varied greatly, especially DM, CP, NDF, ADFD, GE and RFV values. The natural grass quality from the Bogor Barat district was higher than in other districts. The NIRS detected natural quality accurately ( $R^2 > 0.5$ , RPD  $> 1.5$ , SEP/SEL  $< 1$ ) except for CP and NDFD. **Conclusion:** It is concluded that the high variation of natural grass quality in Bogor can be detected rapidly using a pre-calibrated FT-NIRS database.

**Key words:** Accuracy, dairy cattle, database, FT-NIRS, natural grass, multi-species, quality

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Tropical dairy cattle depend on forage availability<sup>1</sup>. However, cultivated forage only provided up to 70% of dairy cattle forage requirements due to land scarcity and slower plant growth during the dry season<sup>2</sup>. The remaining is fulfilled from natural grass and agricultural byproduct. The natural grass is an important forage and its utilization increase during the dry season and in dense populated dairy cattle area<sup>3</sup> such as in the urban dairy farming area of Bogor Regency and Municipality. Natural grasses consisted of a multi-species mixture such as *Panicum repens*, *Cynodon dactylon* Pers, *Leersia hexandra*, *Brachiaria mutica*, *Cyperus rotundus* L. and *Tricholaena rosea*<sup>2</sup>.

The natural grass quality consumed by dairy cattle is directly related to the quality and quantity of milk produced<sup>4</sup>. The quality is influenced by species composition<sup>5</sup> and soil fertility, which are affected by climatic conditions and land-use intensity<sup>6</sup>. The different climatic conditions and land-use intensity between districts in Bogor Regency and Municipality resulted in the dairy cattle's different natural grass quality values. By knowing the quality of natural grass in a particular area, a dairy farmer can collect the grass from the desired location.

Natural grass is usually collected by dairy farmers daily and fed to the cattle freshly. The variation quality of the natural grass needs to be balanced with concentrate to guarantee a sufficient daily nutrient supply for dairy cattle<sup>2</sup>. Therefore, it was essential to analyze the forage quality. Conventional and classical methods in forage quality analysis were complex, expensive, needed skilled labour and time-consuming<sup>7</sup>. Therefore, it cannot acquire the forage nutrient content timely<sup>4</sup>. Near-Infrared Reflectance Spectroscopy (NIRS) has been widely used in forage analysis<sup>8</sup>, including analysis of mineral content<sup>9,10</sup>, botanical composition<sup>11-13</sup>, chemical composition<sup>5,6,8,14,15</sup>, digestibility<sup>16,17</sup> and anti-nutrition<sup>4</sup>. Fourier Transform Near-Infrared Reflectance Spectroscopy (FT-NIRS) is a highly efficient, rapid and modern NIRS instrument that enables qualitative and quantitative analyses, including improvements in signal-to-noise ratio, spectral resolution, wavelength accuracy and a reduction of time scan<sup>8</sup>.

The NIRS accuracy in detection forage quality depends on the database used in the calibration process<sup>18</sup>. It relies heavily on the extent to which the calibration set represents the samples to be predicted<sup>19</sup>. This accuracy can be challenging to achieve with natural products such as natural grass, which are inherently complex and affected by many sources of variability<sup>6</sup>. To increase prediction accuracy, providing calibration and validation sets that belong to the closed population is essential.

The initial database is developed mainly from temperate natural pastures with different species and compositions to tropical grass. Local databases for common dairy fibre feed sources have been developed<sup>15</sup> but still need some improvements, especially for complex organic substances such as NDF and ADF. Efforts to improve the quality of dairy fibre feed estimation using single species failed to increase the prediction accuracy<sup>7</sup> due to the considerable variation in napier grass quality used. Moreover, digestibility estimation of dairy fibre feed source using NIRS initial database that produces better performance prediction in dairy ration formulation was unavailable. Therefore, this research aimed at studying the regional quality variation of tropical natural grass multi-species mixture as dairy cattle forage and improving its accuracy determination using FT-NIRS local database.

## MATERIALS AND METHODS

**Study area:** The study consisted of field and laboratory observations. Field observation was conducted in 10 villages belonging to 4 districts of Bogor Municipality and Regency, West Java. While laboratory observation was conducted at Dairy Nutrition Laboratory, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Indonesia, from February-July, 2021.

**Sample preparations:** One hundred natural grass samples were collected from 10 villages belonging to 4 districts in Bogor Regency and Municipality (3 villages in Bogor Barat district of Bogor Municipality, two villages in Rancabungur, four villages in Darmaga and one village in Ciampea districts of Bogor Regency). For each village, ten samples of natural grass that were closed to the cattle farm were collected. For external validation, ten completely independent sets of natural grass samples from different dairy cattle areas in West Java (2 samples from Lembang District of West Bandung Regency, three samples from Parung Kuda District of Sukabumi Regency, two samples from Cibungbulang District of Bogor Regency and three samples from Pangalengan District of Bandung Regency) was used.

The fresh samples were weighted and dried in open sun drier for two days and continued in Eyela NDO 400 (made in Japan) oven at 60°C for 48 hours. The dried grasses were ground using a Huayi FFC 15 (made in Japan) blender at medium speed and strained using a 1 mm screen. The samples were stored in a plastic container for further analysis.

**Chemical analysis:** Proximate composition of samples including Dry Matter (DM), ash, Crude Protein (CP), Ether

Extract (EE), Crude Fibre (CF) were analyzed according to AOAC<sup>20</sup>. An Eyela NDO 400 (made in Japan) oven was used to determine DM content. A Nabertherm N 50 (made in Germany) was used to determine ash content. Soxhlet and Kjeldahl systems from Gerhardt Instruments (made in Germany) were used to determine EE and CP. Crude Fibre (CF) and cell wall fraction, including Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), was analyzed following method Ba 6a-05, 15 and 14 from Ankom 200 (Ankom Technology Corp., Macedon, NY) fibre analyzer based on AOCs<sup>21</sup>.

**Digestibility measurement:** Digestibility of the grasses was determined using two-stage methods similar to Riestanti *et al.*<sup>22</sup>. Rumen fluid as an inoculant source was collected from fistulated dairy bull kept in the field laboratory of Dairy Nutrition, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University. The fermentation stage was conducted by incubating a 0.5 g sample with 10 mL rumen liquor and 40 mL buffer in a 100 mL centrifuge tube at a 39 shaker water bath under anaerobic conditions. The fermentation lasted for 48 hrs and was then terminated by adding 2 drops of HgCl. The supernatant was removed and the feed residue was added with 50 mL of 2% HCl-pepsin in the second stage. The tube was incubated for 48 hrs at a 39 shaker water bath under aerobic conditions. After the enzymatic digestion, with the help of a vacuum pump, the feed residue was filtered with a predetermined weight of Whatman paper no 41. The residue was dried in an oven at 105 to determine its DM and then incinerated in a furnace oven at 600 for 4 hrs to determine ash residue. *In vitro* DMD and OMD were calculated by subtracting DM and OMD residues from samples. The parallel sample residue was further analyzed for NDF and ADF to calculate NDF and ADF digestibility (NDFD and ADFD).

**Energy partition and natural grass value for dairy cattle:**

Energy partition (Gross Energy (GE), Digestible Energy (DE), Metabolizable Energy (ME) and Net Energy for Lactation (NEL)), Forage Unit For Lactation (UFL) and Relative Forage Value (RFV) were calculated<sup>18,23,24</sup>. It was calculated using the following formula:

$$GE \text{ (MJ kg}^{-1} \text{ DM)} = 0.0188 \text{ OM (g kg}^{-1} \text{ DM)} + 0.0078 \text{ CP (g kg}^{-1} \text{ DM)} \quad (1)$$

$$DE \text{ (MJ kg}^{-1} \text{ DM)} = (1.0087 \text{ OMD} - 0.0377) \times GE \text{ (MJ kg}^{-1} \text{ DM)} \quad (2)$$

$$ME \text{ (MJ kg}^{-1} \text{ DM)} = 0.87 \text{ DE (MJ kg}^{-1} \text{ DM)} \quad (3)$$

$$NEL \text{ (MJ kg}^{-1} \text{ DM)} = 0.463 + 0.24 \times \frac{ME}{GE} \quad (4)$$

$$UFL \text{ (Kcal kg}^{-1} \text{ DM)} = \frac{NEL \text{ (MJ kg}^{-1} \text{ DM)}}{1700 \times 1000 \times 0.239} \quad (5)$$

$$RFV = 0.775 \times \frac{120}{NDF} \times 88.9 - 0.799 \times ADF \quad (6)$$

**FT-NIRS data acquisition:** The modular FT-NIR spectrometer solids cell (BUCHI; NIRFlex N-500 made in Switzerland) was pre-warmed for 15 min before use. The System Suitability Test (SST) was run automatically to verify the NIRS performance. The external and internal references were run using the application of the NIRSware operator after inserting an external reference (provided by BUCHI) into the external reference holder.

Fifty grams of dried grass mash were put in a 100 mm diameter Petri dish and distributed evenly. The dish was put into the dish holder of the FT-NIRS and a ruminant dried forage database was selected from internal applications of the NIRSware operator (NIRSID). The near-infrared light at various wavelengths (800-2500 nm or 12500-4000 cm<sup>-1</sup>) were sent into the sample, allowing for sample identification by penetrating the sample up to several millimetres deep. The scanning was done 3 times for each sample to get the average result and spectra.

**Calibration and validation models:** Database development was done by calibration and validation of chemical, digestibility, estimate energy and forage values into the collected spectra with the help of NIRSware. The process produced a comparison between the measured and calculated values of the natural grass with the FT-NIRS prediction values. A block-wise method was chosen to separate the collected spectra into 2/3 for calibration and 1/3 for validation. Partial least square regression was used to develop the calibration model, while a validation set was chosen to develop the validation model. External validation was conducted to test the models. The models were selected based on the Standard Error of Calibration (SEC), Standard Error of Prediction (SEP), calibration coefficient (R<sup>2</sup>) and Residual Predictive Deviation (RPD). A model is considered acceptable if SEC and SEP minimum, while R<sup>2</sup>>0.5 and RPD>1.5. The RPD is the ratio between Standard Deviation (SD) to SEP. External validation was conducted using the newly developed (NIRSND) database and the result was

validated with the measured and calculated forage quality and value results. The comparison between SEP to Standard Error Laboratory (SEL) was calculated.

## RESULTS

**Natural grass quality for dairy cattle:** Natural grass quality comparisons between regions are shown in Table 1. The quality varies considerably, especially for DM, ash, CP, NDF, ADFD, GE and RFV, resulting in an insignificant difference between the districts.

The lowest and the highest value were 15.10±3.88-18.63±6.91% for DM, 15.13±4.37-17.88±4.12% for ash, 9.17±2.26-12.97±8.81% for CP, 63.74±3.84-66.37±1.57% for NDF, 29.18±17.07-47.49±21.10% for ADFD, 16.45±0.12-16.89±1.25 MJ kg<sup>-1</sup> DM for GE, 91.57±3.08-99.71±8.74 for RFV.

The EE, CF, ADF, DMD, OMD, NDFD, DE, ME and UFL parameters were significantly different between the districts due to varying quality between districts being higher than within districts. In general, natural grass from the Bogor Barat district has higher quality than the other districts. Although the CF (29.44%), NDF (65.06%) and ADF (30.54%) contents in natural grass from Bogor Barat district were higher than the other districts, DMD (56.27%), OMD (55.45%), NDFD (54.49%), DE (8.82 MJ kg<sup>-1</sup> DM), ME (7.67 MJ kg<sup>-1</sup> DM), NE<sub>L</sub>

(4.40 MJ kg<sup>-1</sup> DM) and UFL (0.62 Kcal kg<sup>-1</sup> DM) parameters were higher than other districts (<53.24%, <53.64%, <51.81%, <8.35 MJ kg<sup>-1</sup> DM, <7.26 MJ kg<sup>-1</sup> DM, <4.14 MJ kg<sup>-1</sup> DM and <0.58 Kcal kg<sup>-1</sup> DM, respectively).

### Accuracy determination of the natural grass using FT-NIRS:

Calibration and validation of the natural grass quality using FT-NIRS are shown in Table 2. The table shows that all parameters can be estimated accurately using FT-NIRS (R<sup>2</sup>C>0.5, RPD>1.5), except for the CP (R<sup>2</sup>C = 0.462 and RPD = 1.363) and ADFD (RPD = 1.160). The highest R<sup>2</sup>C was found in DM (0.87) and ash (0.86) parameters, while the lowest was found in CP (0.46). RPD>2 was found in DM, ash, CF and GE, while RPD<1.5 was found in CP and ADFD. Validation slightly improves the accuracy of DM and ADF parameters.

External validation results are shown in Table 3. The table indicates that the t-test results between measured and estimated values using NIRSND are not significantly different except for DM, CP, ADF, DMD and NDFD. The results indicated that the model could accurately predict natural grass sample quality from other places. The Standard Error Laboratories (SEL) found in this study are low in chemical and energy partition parameters but higher in digestibility and RFV. The Prediction Error Relative (PRL) or ratio of SEP/SEL necessary to evaluate the accuracy of the models was <2 for all parameters, except for DMD.

Table 1: Natural grass quality comparisons between regions

Parameters	Districts				p-value
	Darmaga	Ciampea	Bogor Barat	Ranca Bungur	
DM (%)	18.63±6.91	15.10±3.88	18.56±2.42	17.02±3.88	0.475
Ash (% DM)	15.13±4.37	17.88±4.12	15.22±3.84	15.73±2.61	0.598
CP (% DM)	11.96±10.88	12.97±8.81	12.11±5.92	9.17±2.26	0.537
EE (% DM)	5.00±1.86 <sup>b</sup>	6.09±1.98 <sup>ab</sup>	5.10±1.61 <sup>ab</sup>	6.68±2.93 <sup>a</sup>	0.014
CF (% DM)	27.05±3.23 <sup>b</sup>	29.23±0.31 <sup>ab</sup>	29.44±2.89 <sup>a</sup>	27.74±2.27 <sup>ab</sup>	0.012
NDF (% DM)	65.52±6.02	66.37±1.57	65.06±4.84	63.74±3.84	0.559
ADF (% DM)	28.22±3.68 <sup>ab</sup>	29.52±1.09 <sup>ab</sup>	30.54±2.78 <sup>a</sup>	26.13±3.00 <sup>b</sup>	0.000
DMD (%)	52.03±5.87 <sup>ab</sup>	45.84±3.34 <sup>b</sup>	56.27±5.02 <sup>a</sup>	53.24±6.71 <sup>a</sup>	0.006
OMD (%)	51.74±6.38 <sup>ab</sup>	46.12±3.16 <sup>b</sup>	55.45±5.46 <sup>a</sup>	53.64±6.10 <sup>a</sup>	0.021
NDFD (%)	48.94±8.39 <sup>b</sup>	49.48±0.96 <sup>ab</sup>	54.49±4.40 <sup>a</sup>	51.81±7.21 <sup>ab</sup>	0.023
ADFD (%)	43.65±19.06	29.18±17.07	47.49±21.10	35.39±31.49	0.219
GE (MJ kg <sup>-1</sup> DM)	16.89±1.25	16.45±0.12	16.88±0.62	16.56±0.53	0.484
DE (% GE)	0.48±0.06 <sup>ab</sup>	0.43±0.03 <sup>b</sup>	0.52±0.06 <sup>a</sup>	0.50±0.06 <sup>a</sup>	0.021
DE (MJ kg <sup>-1</sup> DM)	8.18±1.28 <sup>ab</sup>	7.03±0.54 <sup>b</sup>	8.82±1.07 <sup>a</sup>	8.35±1.17 <sup>ab</sup>	0.046
ME (MJ kg <sup>-1</sup> DM)	7.12±1.11 <sup>ab</sup>	6.11±0.47 <sup>b</sup>	7.67±0.93 <sup>a</sup>	7.26±1.02 <sup>a</sup>	0.046
NEL (MJ kg <sup>-1</sup> DM)	4.03±0.71 <sup>ab</sup>	3.28±0.30 <sup>b</sup>	4.40±0.62 <sup>a</sup>	4.14±0.66 <sup>ab</sup>	0.040
UFL (K cal kg <sup>-1</sup> DM)	0.57±0.10 <sup>ab</sup>	0.48±0.04 <sup>b</sup>	0.62±0.09 <sup>a</sup>	0.58±0.09 <sup>ab</sup>	0.040
RFV	95.44±14.30	91.57±3.08	92.87±9.48	99.71±8.74	0.224

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, DMD: Dry matter digestibility, OMD: Organic matter digestibility, NDFD: Neutral detergent fibre digestibility, ADFD: Acid detergent fibre digestibility, GE: Gross energy, DE: Digestibility energy, ME: Metabolizable energy, NEL: Net energy for lactation, UFL: Forage unit for lactation and RFV: Relative forage value. Mean value with a different superscript in the same row show a significantly different at p<0.05

Table 2: Calibration and validation of measured and calculated data using FT-NIRS

Parameters	Calibrations										Validations									
	N	Mean	Range	SD	SEC	R <sup>2</sup> C	RPD	N	Mean	Range	SD	SEC	R <sup>2</sup> C	RPD						
DM (%)	184	90.412	86.172-93.64	1.390	0.500	0.871	2.780	92	90.410	86.172-93.64	1.402	0.500	0.873	2.807						
Ash (% DM)	193	15.167	7.791-24.411	3.405	1.296	0.855	2.628	97	15.157	7.791-24.411	3.408	1.297	0.855	2.627						
CP (% DM)	180	9.609	3.916-17.432	2.841	2.084	0.462	1.363	89	9.593	3.916-17.432	2.779	2.096	0.433	1.326						
EE (% DM)	170	5.129	1.61-11.79	1.812	1.198	0.563	1.512	85	5.129	1.61-11.79	1.817	1.286	0.499	1.413						
CF (% DM)	190	27.684	21.7-36.092	2.874	1.373	0.772	2.093	95	27.684	21.7-36.092	2.882	1.530	0.719	1.884						
NDF (% DM)	189	64.847	55.143-77.49	4.420	2.814	0.595	1.570	93	64.937	55.143-77.49	4.410	2.838	0.589	1.554						
ADF (% DM)	199	28.260	19.681-38.811	3.509	2.175	0.616	1.614	98	28.175	19.681-34.747	3.448	2.135	0.617	1.615						
OMD (%)	199	53.443	41.52-67.27	5.740	3.283	0.673	1.748	98	53.279	41.52-65.99	5.631	3.350	0.646	1.681						
NDFD (%)	177	53.142	35.47-68.10	5.821	3.868	0.559	1.505	88	53.157	39.99-66.61	5.851	3.945	0.545	1.483						
ADFD (%)	194	51.802	35.47-68.10	6.192	3.795	0.625	1.632	96	51.547	35.47-68.10	6.095	4.115	0.545	1.481						
GE (MJ kg <sup>-1</sup> DM)	149	45.473	25.03-81.65	9.738	8.397	0.556	1.160	72	44.906	25.03-79.44	9.526	8.465	0.476	1.125						
DE (MJ kg <sup>-1</sup> DM)	176	16.753	14.55-18.42	0.727	0.357	0.760	2.038	89	16.751	14.55-18.42	0.729	0.369	0.744	1.975						
ME (MJ kg <sup>-1</sup> DM)	174	8.321	6.43-10.21	0.907	0.558	0.726	1.625	86	8.366	6.58-10.22	0.869	0.595	0.686	1.461						
NEL (MJ kg <sup>-1</sup> DM)	174	7.239	5.40-9.632	0.926	0.485	0.726	1.909	86	7.254	5.40-9.632	0.923	0.517	0.686	1.785						
UFL (K cal kg <sup>-1</sup> DM)	174	4.113	2.91-5.74	0.611	0.323	0.721	1.892	87	4.113	2.91-5.74	0.613	0.356	0.662	1.722						
RFV	175	0.581	0.41-0.81	0.088	0.046	0.729	1.913	87	0.581	0.41-0.81	0.088	0.050	0.675	1.760						
RFV	168	95.396	77.2-118.49	9.631	5.829	0.634	1.652	84	95.495	77.2-118.49	9.757	5.945	0.629	1.641						

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, DMD: Dry matter digestibility, OMD: Organic matter digestibility, NDFD: Neutral detergent fibre digestibility, ADFD: Acid detergent fibre digestibility, GE: Gross energy, DE: Digestible energy, ME: Metabolizable energy, NEL: Net energy for lactation, UFL: Forage unit for lactation and RFV: Relative forage value

Table 3: External validation statistics of nutrient contents on tropical dairy forages

Parameters	Measured		NIRSND		t-test	R	SEL	SEP	SEP/SEL
	Mean	SD	Mean	SD					
DM (%)	90.25±0.74		89.05±0.78		0.000	0.158	0.584	0.400	0.686
Ash (% DM)	11.2±2.02		12.08±1.88		0.075	0.147	1.917	2.547	1.329
CP (% DM)	10.51±1.53		15.22±1.28		0.000	0.297	1.574	0.250	0.159
EE (% DM)	29.17±3.21		30.29±2.76		0.200	-0.228	2.709	3.315	1.224
CF (% DM)	6.64±2.54		6.09±1.59		0.344	-0.095	2.783	2.932	1.053
NDF (% DM)	59.03±3.04		60.01±3.24		0.131	0.396	5.850	3.133	0.536
ADF (% DM)	32.20±2.88		29.38±1.62		0.012	0.312	2.892	1.036	0.358
DMD (%)	46.08±9.26		53.98±4.79		0.000	0.255	2.497	9.277	3.715
OMD (%)	52.88±7.61		53.02±4.75		0.806	0.965	5.764	3.488	0.605
NDFD (%)	43.26±10.12		52.80±2.42		0.000	-0.045	2.661	0.552	0.207
ADFD (%)	33.62±12.46		31.71±7.76		0.478	0.026	3.111	0.773	0.249
GE (MJ kg <sup>-1</sup> DM)	16.56±0.69		16.59±0.51		0.754	0.653	0.715	0.486	0.679
DE (MJ kg <sup>-1</sup> DM)	8.22±1.37		8.15±0.95		0.958	0.499	1.116	0.544	0.487
ME (MJ kg <sup>-1</sup> DM)	7.15±1.19		7.09±0.82		0.958	0.498	0.971	0.473	0.487
NEL (MJ kg <sup>-1</sup> DM)	4.07±0.77		4.03±0.55		0.961	0.448	0.627	0.294	0.469
UFL (K cal kg <sup>-1</sup> DM)	0.57±0.11		0.57±0.08		0.963	0.269	0.088	0.042	0.476
RFV	95.82±10.73		94.59±8.02		0.759	0.344	7.373	6.461	0.876

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, DMD: Dry matter digestibility, OMD: Organic matter digestibility, NDFD: Neutral detergent fibre digestibility, ADFD: Acid detergent fibre digestibility, GE: Gross energy, DE: Digestible energy, ME: Metabolizable energy, NEL: Net energy for lactation, UFL: Forage unit for lactation and RFV: Relative forage value

## DISCUSSION

The insignificant difference of natural grass DM, ash, CP, NDF, ADF, GE and RFV values between the region might be caused by the high variation in the nutrition values within the region. It might be due to the complex mixture of the botanical composition<sup>6</sup>, which marked different in seasonal growth pattern<sup>5</sup>, indicated the developing stage of the plant community<sup>13</sup> and influenced by the environment. The complex mixture composed of natural grass species may provide greater yields<sup>5</sup> but with smaller CP, *In vitro* True Dry Matter Digestibility (IVTDM) and greater NDF concentrations<sup>25</sup>. The DMD and OMD value of natural grass in Bogor varies from 45.8-56.3%. The values were similar to the digestibility of grasses reported by Yang *et al.*<sup>26</sup> but lower than the feed digestibility values (60-70%) found by White *et al.*<sup>27</sup>.

The higher quality of natural grass from the Bogor Barat district is influenced by climatic conditions and land used intensity<sup>6</sup>. Higher rain intensity and land fertility might be influenced by the microclimate resulting from urban forests in the district. Trees in the forest affected the spatial redistribution of precipitation and the fluxes of carbon and nutrients within forest ecosystems and landscapes<sup>28</sup>.

An average tropical dairy cow weight 417 and 12 kg milk (4% FCM)<sup>29</sup> at first lactation consumed 12.98 kg DM rations required 20.25 Mcal daily NEL or 1.56 Mcal kg<sup>-1</sup> DM or 6.52 MJ kg<sup>-1</sup> DM NEL<sup>30</sup>. The NE<sub>L</sub> of the natural grass ranges from 3.28 MJ kg<sup>-1</sup> DM in the Ciampea district to 4.40 MJ kg<sup>-1</sup> DM in the Bogor Barat district. Utilization of 50% of the natural grass in ration fulfilled 25.1-33.7% of the NE<sub>L</sub> requirement. The NEL of natural grasses found in Bogor was lower than the hay from species-rich mountainous grasslands in Switzerland, ranging from 4.53-5.63 MJ kg<sup>-1</sup> DM<sup>31</sup>.

The UFL value is calculated based on net energy. It represents the energy value of forage for dairy cows compared to barley<sup>8</sup>. The UFL value of natural grass found in Bogor ranged from 0.48-0.62 Kcal kg<sup>-1</sup> DM. The UFL value was lower than the natural grass samples harvested in the main hills and mountains area of Tuscany (Italy) with UFL 0.51-1.31 Kcal kg<sup>-1</sup> DM<sup>8</sup>. It might be due to different altitudes and latitudes. Environmental deviations caused by latitudinal and altitudinal gradients greatly influenced the plant community diversity's spatial distributions, hence affecting its quality<sup>32</sup>.

The RFV is useful in the market for comparing hays<sup>23</sup>. The RFV value in Bogor was higher than the local forage OI Joro Orok in Nyandarua county of Central Kenya<sup>33</sup>. The RFV is an index of the relative value of a forage that combines the NDF and ADF value into a single index. The index 100 is made

relative to the alfalfa hay quality at its full bloom, containing 41% NDF and 53% ADF. Based on its RFV, natural grass from Bogor can be categorized as the grade 3 forage with 11-13% CP, 41-42% ADF, 54-60% NDF and 56-57% DMD<sup>23</sup>.

In general, accuracy detection found in this research was lower than Parrini<sup>8</sup> and Mwendia *et al.*<sup>34</sup>. The lower accuracy found in this study was due to more heterogenous, such as different location<sup>12</sup>, more complex mixture species<sup>5</sup> and the difficulties in achieving the representativeness within the natural grass<sup>19</sup>. Heterogeneous characteristics of natural grass were due to multi-species mixture found in the natural grass<sup>8</sup> including *Panicum repens*, *Cynodon dactylon* Pers, *Leersia hexandra*, *Brachiaria mutica*, *Cyperus rotundus* L. and *Tricholaena rosea* species<sup>2</sup>. The complexity of the natural grass species mixture is due to the different growth rates of the species<sup>6</sup>, which are affected by biotic and abiotic factors, such as nutrient availability, stage of maturity, topography and climatic conditions. The heterogenization of landscape and biodiversity causes difficulties in achieving natural grass representativeness. It has resulted from less intensification of land use<sup>6</sup>.

The lower accuracy was also found in the digestibility parameters ( $R^2 = 0.56-0.67$ ), which involved biological processes, such as microbial fermentation in rumen<sup>35</sup>. The result was comparable to the raw spectrum ( $R^2 = 0.59-0.61$ ) reported by Samadi *et al.*<sup>17</sup>. However, the authors successfully increased the accuracy using spectra correction Standard Normal Variate (SNV). Energy partitions calculated using chemical and digestibility data produced better prediction accuracy ( $R^2 > 0.7$ ). In contrast, RFV accuracy was lower ( $R^2 = 0.63$ ) than energy partitions. It might have resulted from the lower ADF and NDF accuracy from where the RFV value was calculated.

External validation showed that the DM, CP, ADF, DMD and NDFD could not be measured using the developed database due to the significant difference between the chemical and biological measurements with the NIRS. The significant difference between chemical and NIRS results on CP was due to low R<sup>2</sup>C (0.46) and RPD (1.36), while on ADFD, due to low RPD (1.16) in the calibrated model. The significant difference in DMD was due to higher SEP/SEL (PRL) in the digestibility. Digestibility involves a biological process that varies significantly due to microbial activity involved in the digestion process<sup>35</sup>.

The study implies that variation of natural grass between and within the regions should be adjusted with the reformulation of the ration. The ability of the newly developed FT-NIRS natural grass database to rapidly detect the quality variation can be used by dairy farmers to reform the ration.

However, some parameters (DM, CP, ADF, DMD and NDFD) cannot be detected accurately and still need improvement. It is recommended to increase the number of natural grass samples for improvement of the prediction accuracy.

### CONCLUSION

It is concluded that natural grass in Bogor Regency and Municipality varies greatly in DM, CP, NDF, ADFD, GE and RFV values. Natural grass from the Bogor Barat district is better than other districts, especially in EE, CF, ADF, DMD, OMD, NDFD, DE, ME and UFL parameters. The natural grass quality can be detected rapidly using the pre-calibrated database FT-NIRS, except for DM, CP, ADF, DMD and NDFD. It is suggested to improve the DM, CP, ADF, DMD and NDFD prediction accuracy using more significant sample numbers.

### SIGNIFICANT STATEMENT

This study compares the quality of natural grass from different regions in Bogor Regency and Municipality, West Java Province of Indonesia. This study shows a high variation of natural grass between and within the region. It needed daily adjustment to fulfil the nutrient requirement of dairy cattle. This study will help the researcher uncover the area with better natural grass quality and advise farmers to collect the grass from the desired area and adjust the quality variation by reformulating the ration offered. The NIRS natural grass database's success will help users detect natural grass quality from the different dairy farming areas in West Java Province within a second.

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