



## Identification of Smallholder Dairy Farm Rations for Improving Milk Production, Milk Protein and Nitrogen Utilization in Friesian Holstein Cows across Altitudinal Zones

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### ABSTRACT

Milk production and quality in Indonesia, particularly protein content, remain below national standards. Variations in altitude influence production through differences in microclimate and ration composition. Ration composition determines the availability of essential nutrients for milk synthesis, including sulfur (S) and zinc (Zn), as well as nitrogen utilization efficiency, as indicated by milk urea nitrogen (MUN). This study aimed to explore smallholder dairy farm rations across different altitudes and identify the most effective ration for improving milk production and milk protein while considering MUN. Feed and milk samples were collected from smallholder dairy farms in Pangalengan (a highland area) and Bogor (a lowland area). The quantity of feed offered to the cows and the milk production were measured. Feed samples were analyzed for proximate composition and mineral content (S and Zn). Milk samples were analyzed for quality parameters and MUN. The results showed that altitudinal zones and each ration type affected milk production, milk protein and MUN. The most effective rations were found in the highland groups (H1 and H2), composed of Napier grass and cooperative concentrate (H2) and an additional cassava waste (H1). These rations resulted in significantly higher ( $P < 0.05$ ) milk production and milk component yields, particularly protein yield. Meanwhile, milk composition, including protein percentage, was not significantly affected. MUN values were also closest to the optimal range. In conclusion, a simple ration consisting of 44.88% DM Napier grass and 55.12% DM cooperative concentrate, both readily accessible, was effective in improving milk production and protein yield in both highland and lowland areas.

**Key words:** Dairy cattle, Ration management, Milk production, Milk protein, MUN, Altitude.

### INTRODUCTION

Milk represents a key source of livestock-derived protein and plays an essential role in meeting national nutritional requirements. In Indonesia, the demand for animal protein continues to rise in parallel with population growth. However, domestic milk production has not kept pace with this increasing demand. Beyond the issue of supply shortages, milk quality also remains a challenge, particularly with respect to its relatively low protein content (Christi et al. 2022; Apriliana et al. 2025).

Milk protein is a critical nutrient due to its essential

physiological functions. Furthermore, milk protein serves as an essential source of immunoglobulins for calves (Ostertag and Hinrichs 2023). Therefore, optimizing milk protein content is of considerable importance for improving both human nutrition and livestock productivity (Abugaliev et al. 2025; Sanjulián et al. 2025).

One of the key factors influencing both milk production and milk protein is variation across altitudinal zones. Indonesia's diverse geographical conditions create differences in the temperature-humidity index (THI), which directly affect dairy cattle productivity (Becker et al. 2020). Higher THI levels are associated with heat stress,

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which reduces feed intake and consequently exerts negative effects on milk yield and milk protein content (Becker et al. 2020). In addition to its effects on thermal conditions, altitudinal variation is also associated with differences in locally available feed resources.

Altitudinal variation also shapes local ration composition, particularly in smallholder dairy systems where feed resources and ration choices are highly dependent on local availability. Smallholder dairy farms, which dominate the national dairy sector, typically exhibit considerable variability in feeding practices, particularly in the types and formulations of rations provided to dairy cows (Anzhany et al. 2022). These differences in ration composition, influenced by both altitude and farmers' resource availability, contribute to inconsistent nutrient supply, including variations in essential macro- and micronutrients required for milk synthesis, such as Zn and S. Such inconsistencies often lead to suboptimal milk yield and milk protein. Different rations can also affect nutrient availability and nitrogen utilization efficiency. Milk urea nitrogen (MUN) is widely recognized as a reliable indicator of nitrogen utilization efficiency in dairy cattle (Zhao et al. 2025). Therefore, evaluating MUN is essential to assess the status of nitrogen utilization in cattle, particularly in relation to protein adequacy and the synchronization of protein with energy supply.

This study investigated the diversity of rations used in smallholder dairy farms across different altitudinal zones. The primary objective was to identify the most effective ration for improving milk production and quality, with particular emphasis on milk protein. In parallel, nitrogen utilization efficiency was evaluated to ensure that improvements in production were achieved in a sustainable manner.

## MATERIALS AND METHODS

### Exploration

The highland site was situated in Pangalengan Subdistrict, Bandung Regency (1,410m a.s.l.) and the lowland site was located in Bogor, encompassing Tanah Sareal Subdistrict (175.68m a.s.l.) and the Kawasan Usaha Peternakan (KUNAK II) (222.2m a.s.l.). The lowland exploration included 28 dairy cows from 28 farms, whereas the highland exploration included 32 dairy cows from 32 farms. The cows used were Friesian Holstein in early lactation ( $\leq 90$  days postpartum). No treatment was applied to the animals, because this study employed an observational cross-sectional design. The cows were maintained under commercial farm conditions and supervised by veterinarians from each cooperative. Rations provided on each farm were documented. Milk production measurement and sampling were conducted twice daily, during the morning and afternoon milking sessions. Representative feed and milk samples were collected from each farm for further analysis.

### Measurement of THI

Temperature and humidity were recorded using a data logger (RHT 10, EXTECH Instruments, China). THI was calculated using the equation proposed by Moran (2005):

$$THI = T - (0.55 \left[ \frac{100 - Rh}{100} \right] (T - 58))$$

Where T is air temperature ( $^{\circ}$ F) and Rh is relative humidity (%).

### Milk quality and MUN measurements

Milk quality was analyzed using a Lactoscan Milk Analyzer (Milkotronic Ltd., Bulgaria; EN ISO 9001:2008). MUN was determined in two stages, consisting of sample preparation following Broderick and Reynal (2009) and spectrophotometric measurement according to Astuti et al. (2011). Absorbance was measured at 578nm with a spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific).

### Nutrient composition analysis of feed samples

Feed proximate composition was analyzed using an FT-NIR Spectrometer Solids Cell (NIRFlex N-500, BUCHI, Switzerland) which was calibrated with a local feed database. Dry matter (DM) content was determined by sequential drying of feed samples, first in a 60 $^{\circ}$ C oven and subsequently at 105 $^{\circ}$ C until constant weight. Nitrogen-free extract (NFE) was calculated by difference, obtained by subtracting the percentages of ash, CP, EE, and CF from 100. Total digestible nutrients (TDN) were estimated using the equation proposed by Wardeh (1981).

### Mineral content analysis

For mineral analysis, 2g of dried feed sample was subjected to wet ashing using a mixture of concentrated nitric and perchloric acids, followed by heating until a clear solution was obtained. The digested solution was then filtered and subsequently diluted using distilled water, after which the mineral concentrations were analyzed. Zinc was analyzed using an Atomic Absorption Spectrophotometer (AAS; AA-6880, Shimadzu, Japan) at a wavelength of 213.85nm. Sulfur (S) concentration was determined separately using a UV-Vis spectrophotometer at a wavelength of 423nm. The calculation was based on absorbance readings using the AOAC (2000).

### Study design and data analysis

The comparison of THI values was analyzed using an independent samples t-test. The most commonly used feeding management practices were analyzed using one-way ANOVA, followed by Duncan's multiple range test to identify the most effective practices. In Table 2, statistical analysis was applied only to nutrient composition and nutrient intake variables. Ration composition and nutrient adequacy values were presented descriptively. Moreover, rarely used feeding management practices were presented descriptively to illustrate the diversity of feeding management practices in smallholder farms.

Prior to correlation analysis, normality testing was conducted to determine whether the variables met the assumptions for parametric analysis. Normality was assessed using the Kolmogorov-Smirnov test. The results of the normality test were then used to guide the selection of the appropriate correlation method.

Variables that met normality assumptions were analyzed using Pearson's correlation coefficient, whereas non-normally distributed variables were analyzed using Spearman's rank correlation. Correlation analysis was then performed to determine the relationships between nutrient parameters and milk performance. All statistical analyses were conducted using SPSS statistical software version 26.

## RESULTS

## Temperature-Humidity index across altitudinal zones

The THI data for the two altitudinal zones are presented in Table 1. Significant differences ( $P<0.05$ ) were observed between the lowland and highland sites, with THI values consistently higher in the lowlands during all measurement periods (morning, noon, afternoon, night, and early morning). A clear diurnal pattern in heat stress intensity was observed across measurement periods. According to Moran (2005), heat stress level can be classified as follows:  $THI<72$ , no stress;  $72<THI<78$ , mild stress;  $78<THI<89$ , severe stress;  $89<THI<98$ , very severe stress;  $THI>98$ , cattle dead. In the lowland site, THI values indicated severe heat stress from morning until late afternoon (80.48–84.59), followed by mild stress during the night and early morning (77.34 and 73.96). This pattern suggests that cows experienced continuous discomfort throughout the day. In contrast, cows in the highland site experienced no heat stress

during most periods, except at noon when THI reached 79.33, indicating a short peak of severe stress.

## Variation in feeding ration composition and its effects on milk performance in dairy cattle across altitudinal zones

The rations presented in Table 2 represent the most commonly adopted practices among farmers in both altitudinal zones. These differences directly influenced the ration nutrient composition, nutrient intake, and milk performance. Significant differences ( $P<0.05$ ) were observed in the ration nutrient composition. Most nutrient intake parameters also differed significantly ( $P<0.05$ ) between altitudinal zones (Table 2). Nutrient composition and intakes of nutrients were significantly higher in the highland groups than in the lowland. Overall, the highest nutrient compositions and intakes were consistently observed in H2, though the differences with H1 were not statistically significant.

**Table 1:** Variation in temperature-humidity index (THI) and heat stress levels of dairy cows across altitudinal zones

Time	THI		Heat stress level	
	L	H	L	H
Morning(6-10 am)	80.48±3.10 <sup>b</sup>	70.04±2.03 <sup>a</sup>	Severe	No
Noon (11-2 pm)	84.59±0.48 <sup>b</sup>	79.33±1.71 <sup>a</sup>	Severe	Severe
Afternoon (3-6 pm)	81.45±2.09 <sup>b</sup>	70.34±0.52 <sup>a</sup>	Severe	No
Night (7-11 pm)	77.34±0.79 <sup>b</sup>	68.53±0.62 <sup>a</sup>	Mild	No
Early morning (12-5 am)	73.96±1.19 <sup>b</sup>	67.27±0.35 <sup>a</sup>	Mild	No

Note: Values (mean±SD) having different superscript letters in a column differ significantly ( $P<0.05$ ); L= Lowland; H= Highland.

**Table 2:** Variation in ration composition across altitudinal zones

Ration Composition (%DM)	Feeding pattern			
	Lowland		Highland	
	L1	L2	H1	H2
Napier grass	50.59±13.07	21.61±7.03	43.73±11.06	44.88±15.29
Rice straw	-	22.75±11.63	-	-
Commercial / cooperative concentrate	36.39±16.14	43.12±12.76	51.75±10.54	55.12±15.29
Tofu waste	13.02±5.14	12.52±10.35	-	-
Cassava waste	-	-	4.51±2.44	-
Nutrient composition of rations (%DM)				
Ash	12.24±1.81	12.47±1.17	11.78±1.47	11.70±1.86
Crude protein	10.37±0.92 <sup>a</sup>	10.73±2.41 <sup>a</sup>	13.27±0.72 <sup>b</sup>	14.13±1.20 <sup>b</sup>
Ether extract	6.06±0.94 <sup>b</sup>	6.24±1.60 <sup>b</sup>	3.50±0.24 <sup>a</sup>	3.95±0.23 <sup>a</sup>
Crude fibre	21.77±2.62 <sup>b</sup>	22.29±2.95 <sup>b</sup>	17.81±1.48 <sup>a</sup>	18.19±3.08 <sup>a</sup>
NFE	49.57±2.65 <sup>ab</sup>	48.27±3.33 <sup>a</sup>	53.63±2.08 <sup>c</sup>	52.03±3.44 <sup>bc</sup>
TDN	55.31±1.38 <sup>a</sup>	55.64±1.68 <sup>a</sup>	57.90±1.70 <sup>b</sup>	58.40±2.40 <sup>b</sup>
Sulfur	0.48±0.07 <sup>b</sup>	0.45±0.09 <sup>b</sup>	0.29±0.12 <sup>a</sup>	0.32±0.14 <sup>a</sup>
Zinc	0.030±0.002 <sup>a</sup>	0.028±0.004 <sup>a</sup>	0.033±0.003 <sup>b</sup>	0.034±0.001 <sup>b</sup>
Nutrient intake (kg DM head <sup>-1</sup> day <sup>-1</sup> )				
DM	12.31±4.01 <sup>a</sup>	15.16±3.83 <sup>ab</sup>	16.36±4.55 <sup>ab</sup>	18.23±6.68 <sup>b</sup>
Ash	1.48±0.47 <sup>a</sup>	1.87±0.43 <sup>ab</sup>	1.94±0.60 <sup>ab</sup>	2.11±0.72 <sup>b</sup>
Crude protein	1.26±0.37 <sup>a</sup>	1.60±0.45 <sup>a</sup>	2.17±0.62 <sup>b</sup>	2.60±0.72 <sup>b</sup>
Ether extract	0.74±0.74 <sup>ab</sup>	0.96±0.43 <sup>b</sup>	0.57±0.16 <sup>a</sup>	0.71±0.22 <sup>ab</sup>
Crude fibre	2.65±0.92 <sup>a</sup>	3.66±1.28 <sup>b</sup>	2.92±0.82 <sup>ab</sup>	3.22±0.84 <sup>ab</sup>
NFE	6.07±2.31 <sup>a</sup>	7.30±2.12 <sup>ab</sup>	8.75±2.45 <sup>b</sup>	9.59±4.13 <sup>b</sup>
TDN	6.75±2.36 <sup>a</sup>	8.38±2.16 <sup>ab</sup>	9.46±2.63 <sup>b</sup>	10.70±4.21 <sup>b</sup>
Sulfur	0.060±0.02	0.067±0.02	0.048±0.03	0.055±0.03
Zinc	0.0037±0.001 <sup>a</sup>	0.0042±0.001 <sup>ab</sup>	0.0054±0.002 <sup>bc</sup>	0.0062±0.002 <sup>c</sup>
Nutrient adequacy (kg DM day <sup>-1</sup> )				
TDN	-2.78±0.89	-3.38±0.85	-3.30±0.95	-3.52±1.09
Crude protein	-0.30±0.22	-0.36±0.41	-0.02±0.14	+0.11±0.32
n	10	12	14	9

Note: Values (mean±SD) having different superscript letters in a column differ significantly ( $P<0.05$ ); L1= Lowland ration 1; L2= Lowland ration 2; H1= Highland ration 1; H2= Highland ration 2; DM= Dry Matter; NFE= Nitrogen-free extract; TDN= Total digestible nutrient; n= number of farmers using the ration; Nutrient adequacy calculations were performed with reference to the NRC (2001) requirements for lactating dairy cows.

The different rations also had a significant effect on milk performance (Table 3). Milk production, milk component yield, MUN, and MUN yield were significantly ( $P<0.05$ ) higher in the highland groups (H1 and H2) compared with the lowland groups (L1 and L2). Overall, H2 consistently demonstrated superior performance across most milk component yields, except for fat yield, which was highest in H1.

The rations presented in Tables 4 and 5 represent rations that were less frequently adopted in the lowland and highland areas, respectively. The rarely adopted feeding patterns also influenced milk performance. As shown in

Tables 4 and 5, wide variation was observed among individual farmers across altitudinal zones.

### Correlation between nutrient parameters and milk performance

The correlations between nutrient parameters and milk performance are presented in Table 6. Dry matter, ash, crude protein, NFE, TDN and zinc were positively correlated with milk production, protein yield and MUN yield. Among these parameters, only crude protein and zinc showed significant correlations with MUN. Meanwhile, no nutrient parameter was significantly correlated with milk protein content.

**Table 3:** Effects of feeding patterns on dairy cow milk performance across altitudinal zones

Parameters	Feeding pattern			
	L1	L2	H1	H2
Milk production and nitrogen utilization				
Milk production (L day <sup>-1</sup> )	10.67±4.98 <sup>a</sup>	12.64±3.92 <sup>a</sup>	18.71±4.66 <sup>b</sup>	20.20±5.46 <sup>b</sup>
MUN (mg dL <sup>-1</sup> )	4.99±2.64 <sup>a</sup>	5.46±2.36 <sup>a</sup>	7.85±2.01 <sup>b</sup>	7.47±1.85 <sup>b</sup>
MUN yield (mg day <sup>-1</sup> )	583.37±467.6 <sup>a</sup>	727.87±440.2 <sup>a</sup>	1441.87±398 <sup>b</sup>	1552.99±680.6 <sup>b</sup>
Milk nutrient components (%)				
Fat	3.36±0.41	3.43±0.55	3.56±0.82	3.71±0.68
Solid non-fat	7.25±0.50	7.37±0.30	7.16±0.38	7.28±0.30
Lactose	3.99±0.28	4.05±0.17	3.94±0.21	4.00±0.16
Protein	2.65±0.18	2.69±0.11	2.62±0.14	2.66±0.11
Milk component yield (g day <sup>-1</sup> )				
Fat	375.77±209.9 <sup>a</sup>	452.97±187.8 <sup>a</sup>	1169.67±493.5 <sup>c</sup>	780.18±298.01 <sup>b</sup>
Solid non-fat	812.56±430.4 <sup>a</sup>	953.72±297.3 <sup>a</sup>	961.46±354.01 <sup>a</sup>	1509.39±431.5 <sup>b</sup>
Lactose	446.76±236.7 <sup>a</sup>	524.21±163.8 <sup>a</sup>	751.77±181.63 <sup>b</sup>	830.22±237.47 <sup>b</sup>
Protein	296.74±157.0 <sup>a</sup>	348.55±109 <sup>a</sup>	499.51±120.87 <sup>b</sup>	551.73±157.68 <sup>b</sup>
n	10	12	14	9

Note: Values (mean±SD) having different superscript letters in a column differ significantly ( $P<0.05$ ); L1= Lowland ration 1; L2= Lowland ration 2; H1= Highland ration 1; H2= Highland ration 2; MUN= Milk Urea Nitrogen; n= number of farmers using the ration.

**Table 4:** Lowland rations rarely used by farmers

Ingredient (%DM)	L3	L4	L5	L6	L7
Napier grass	15	27	-	25	-
Pakchong grass	-	15	-	-	-
Maize grass	-	16	-	-	-
Field grass	-	-	-	-	-
Market waste	-	-	12	-	-
Maize husk	-	-	-	-	42
Rice straw	20	18	-	-	-
Commercial cons.	44	24	39	31	30
Rice bran	-	-	-	-	-
Tempe waste	14	-	-	-	-
Tofu waste	7	-	49	16	29
Brewers' Spent grain	-	-	-	28	-
Milk production and nitrogen utilization					
Milk production (L day <sup>-1</sup> )	18.00	14.00	15.75	18.00	11.15
MUN (mg dL <sup>-1</sup> )	8.84	8.02	2.21	8.54	1.63
MUN yield (mg day <sup>-1</sup> )	1591.2	1122.8	348.08	1570.18	181.75
Milk nutrient components (%)					
Fat	3.27	2.84	2.81	2.94	2.33
SNF	7.34	7.58	8.03	7.60	8.11
Lactose	4.04	4.19	4.41	4.18	4.46
Protein	2.68	2.77	2.94	2.78	2.97
Milk component yield (g day <sup>-1</sup> )					
Fat	603.49	408.18	455.12	529.69	267.35
SNF	1354.63	1089.44	1300.57	1394.48	930.55
Lactose	745.60	602.21	714.26	766.94	511.75
Protein	494.60	398.12	476.17	510.06	340.78
N	1	1	1	2	1

Note: L3–L7 = rations from lowland farms; cons= concentrate; MUN= Milk Urea Nitrogen; SNF= Solid Non-Fat; DM= Dry Matter; n= number of farmers.

**Table 5:** Highland rations rarely used by farmers

Ingredient (%DM)	H3	H4	H5	H6	H7	H8	H9	H10
Napier grass	-	20	29	51	28	-	27	-
Field grass	51	-	-	-	-	-	-	32
Maize cob	-	14	71	-	-	-	-	-
Carrot leaves	-	-	-	-	-	35	-	-
Silage	-	-	-	-	-	-	24	-
Coop. cons.	47	66	-	19	49	56	49	68
Cassava waste	2	-	-	-	-	9	-	-
Tofu waste	-	-	-	-	23	-	-	-
Potato peel	-	-	-	30	-	-	-	-
Milk production and nitrogen utilization								
Milk production (L day <sup>-1</sup> )	25.00	10.00	13.50	12.00	24.00	18.00	17.00	20.00
MUN (mg dL <sup>-1</sup> )	5.35	4.14	6.82	7.20	7.20	7.09	8.99	6.30
MUN yield (mg day <sup>-1</sup> )	1337.50	414.00	918.80	864.00	1728.00	1276.20	1528.30	1260.00
Milk nutrient components (%)								
Fat	3.35	2.77	3.44	3.30	3.36	3.20	5.23	3.36
SNF	7.04	7.48	7.48	7.28	7.24	6.99	7.57	7.30
Lactose	3.87	4.11	4.11	4.00	3.98	3.84	4.16	4.01
Protein	2.70	2.74	2.74	2.66	2.65	2.55	2.76	2.67
Milk component yield (g day <sup>-1</sup> )								
Fat	858.19	284.27	476.32	406.12	826.42	589.82	910.97	688.83
SNF	1803.49	767.64	1035.40	895.92	1780.74	1288.38	1318.56	1496.57
Lactose	991.41	421.79	569.25	492.26	978.92	707.78	724.60	822.09
Protein	660.94	281.20	378.82	327.36	651.79	470.01	480.74	547.38
n	1	1	2	1	1	1	1	1

Note: H3-H10 = rations from highland farms; Coop. cons= Cooperative concentrate; MUN= Milk Urea Nitrogen; SNF= Solid Non-Fat; DM= Dry Matter; n= number of farmer.

**Table 6:** Correlation between nutrient parameters and dairy cows performance

Parameter nutrient	Milk performance				
	Milk production	Protein content	Protein yield	MUN	MUN yield
Dry matter	0.300*	-0.060	0.298*	0.160	0.297*
Ash	0.288*	-0.132	0.274*	0.152	0.287*
Crude protein	0.452**	-0.036	0.453**	0.356**	0.497**
Ether extract	-0.041	0.067	-0.026	-0.122	-0.051
Crude fibre	0.073	-0.077	0.066	0.024	0.070
NFE	0.319*	-0.110	0.294*	0.141	0.285*
TDN	0.337**	-0.040	0.337**	0.196	0.339**
Sulfur	-0.153	-0.125	-0.136	-0.137	-0.141
Zinc	0.399**	-0.174	0.362**	0.256*	0.394**

Note: \* and \*\* indicate significance at P<0.05 and P<0.01, respectively. NFE= Nitrogen-free extract; TDN= Total digestible nutrient; MUN= Milk Urea Nitrogen.

## DISCUSSION

The Temperature–Humidity Index (THI) is a critical indicator of thermal stress in dairy cattle, as cows possess a relatively narrow thermal comfort zone. In tropical regions, such as Indonesia, heat stress is a major factor contributing to reduced dairy cow productivity (Asmarasari et al. 2023). Heat stress reduces feed intake as an adaptive mechanism to lower the internal heat production (heat increment) generated during metabolic processes (Becker et al. 2020; Tao et al. 2020). The decline in feed intake directly limits nutrient availability for milk synthesis, thereby reducing milk production and quality, including protein content, and ultimately lowering milk component yields such as protein yield.

In addition to heat stress, differences in milk performance across altitudinal zones were also strongly related to the variation in ration composition (Table 2). Anzhany et al. (2022) demonstrated that rations significantly affect rumen fermentation profiles, which in turn influence milk performance. The higher milk production observed in the highland rations, particularly in H2, was primarily associated with the greater DM intake,

which increased the total nutrient supply available for milk synthesis (Hansen et al. 2022). In addition, the elevated CP content of these rations further supported higher milk yield. Dairy cows generally produce more milk when provided with diets containing higher protein levels. Protein contributes to the supply of glucogenic nutrients used for lactose synthesis, thereby increasing milk volume (Chiogna et al. 2021). A previous study has confirmed that high-protein rations can enhance milk production (Hansen et al. 2022). Highland rations not only provided greater CP but were also balanced with higher levels of TDN and NFE, thereby maximizing dietary protein utilization by rumen microbes. In addition, the higher ash content in the highland rations may have supported milk production by providing essential minerals involved in enzymatic and metabolic processes related to milk synthesis. Zinc, as one of the essential trace minerals, was also higher in the highland rations and has been reported to increase milk production. Zinc has been reported to increase milk production (El-Hamd et al. 2023). By contrast, the higher temperatures in the lowland reduce feed quality (Moyo and Nsahlai 2021) and feed intake (Becker et al. 2020) which together contribute to lower milk performance. These

findings were further supported by the correlation analysis, which revealed that several nutrient parameters, including DM, CP, ash, TDN, and Zn were positively associated with milk production and protein yield (Table 6).

The sulfur content of rations in the lowland farms was higher because these rations contained greater proportions of rice straw and tofu waste. Rice straw naturally accumulates sulfur during paddy cultivation (Doberman and Fairhust 2002), whereas tofu residue originates from soybeans, which contain sulfur-containing amino acids (Wang 2022). At the same time, cows in the lowland were exposed to higher THI values and more prolonged heat stress, which reduced their feed intake and milk production. Consequently, higher sulfur values and lower milk yield occurred in the same environmental context, producing an apparent negative trend. However, the lack of statistical significance in the correlation indicates that sulfur itself did not exhibit a direct relationship with milk production in this study.

The MUN values in the highland rations were closer to the optimal range, reflecting the higher CP supply provided by these rations, as shown in Table 2 (Munyaneza et al. 2017). Although MUN levels across all rations remained below the optimal range, the relatively higher values in H1 and H2 indicate improved CP adequacy, supported by the nutrient adequacy results in Table 2. The significantly higher MUN yields observed in H1 and H2 resulted from the combined effects of elevated MUN levels and greater milk production in these rations.

Milk quality parameters are strongly influenced by milk production. A well-documented phenomenon, the dilution effect, explains the inverse relationship between milk production and milk composition (Manuelian et al. 2022). In this study, cows in the lowland produced less milk due to heat stress, which increased the concentration of milk solids, whereas cows in the highland produced more milk, thereby diluting them. These opposing effects account for the absence of significant differences in milk quality despite clear differences in yield. Among the milk quality components, protein warrants particular attention because of its essential contribution to nutritional value. In this study, the mean protein content in both altitudinal zones remained below the minimum standard of 2.8%. As noted by Pszczolkowski and Apelo (2020), milk protein synthesis depends not only on substrate availability but also on cellular signaling mechanisms that regulate amino acid utilization efficiency and coordinate energy, insulin, and stress regulation. The complexity of these molecular processes helps explain why improving milk protein content is particularly challenging.

Although no significant differences in milk composition were observed between altitudinal zones, the marked variation in milk production resulted in significantly different values of milk component yield. Previous studies have similarly reported that milk component yield tends to increase in parallel with higher milk production (Hansen et al. 2022). Milk specific gravity also contributes, as greater density reflects a higher concentration of solids and thereby enhances component yields. Among the components, protein yield warrants particular attention because of its central role in determining milk's nutritional value. Since component yield is jointly determined by milk volume, density, and

composition, enhancing milk protein concentration would proportionally increase protein yield and, in turn, strengthen the overall nutritional quality of milk.

The substantial variability in milk performance observed under the rarely adopted feeding patterns reflects the heterogeneity of feed resources available in smallholder systems. Unlike the more commonly adopted patterns, which rely on widely available feedstuffs, these less common rations are shaped by localized agricultural by-products and therefore have limited applicability. Nevertheless, variations in feed intake and composition, even at a small scale, contributed to differences in milk production, milk protein content, and MUN. Although used by only one or two farmers, these rations illustrate the potential of locally available feed resources to support milk production, improve milk protein levels, and enhance nitrogen utilization efficiency under specific farm conditions.

### Conclusion

The combination of Napier grass and cooperative concentrate at a ratio of 44.88:55.12% DM was the most effective ration. This ration produced the highest milk production and milk protein yield, with MUN values approaching the optimal range. Although its performance was not significantly different from the ration containing cassava waste (H1), the simpler composition of H2 makes it more practical and applicable for smallholder farms. These findings suggest that a ration consisting solely of Napier grass and concentrate, both readily accessible feed ingredients, could also be implemented in lowland to improve milk production and protein yield.

### DECLARATIONS

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**Data Availability:** All relevant data supporting the findings of this study are included in this article.

**Ethics Statement:** No invasive procedures or experimental treatments were performed on the animals. All measurements (milk collection, feed sampling, and THI recording) followed routine farm practices and were supervised by cooperative veterinarians. As the study was observational, ethical approval was not required.

**Author's Contribution:** Idat Galih Permana and Despal conceived and designed the research concept. Tri Wahyu

Apriliana collected the data. Ruslan Abdul Gopar and Insan Mujahid Afnan assisted in data collection and field observation.

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