



# Qualitative Analysis of the Effect of Cattle Housing Conditions on the Protein Content of Raw Milk Using the Biuret Method

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## ABSTRACT

Whole cow's milk is a source of animal protein whose content can be influenced by environmental factors, such as temperature, altitude, feed type, and barn sanitation. This study aims to compare the protein content of raw cow's milk from the Lembang and Pangalengan regions in West Java using the Biuret method for qualitative analysis. Samples were collected from six locations: Maribaya, Parongpong, Cikole, Margamekar, Margamukti, and Jalan Raya Pangalengan. Test results showed that all samples tested positive for protein, indicated by the formation of a purple color. Samples from Lembang produced a darker purple color with an absorbance of approximately 8,000 ppm, while samples from Pangalengan showed a lighter purple color with an absorbance of 1,000–4,000 ppm. These results indicate that differences in the cows' living conditions can affect milk protein content. The Biuret method proved effective for detecting protein in raw cow's milk.

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## 1. INTRODUCTION

Whole cow's milk is one of the most widely consumed sources of animal protein among Indonesians. The primary protein components in milk consist of casein (approximately 80%) and whey (approximately 20%), each of which has nutritional value and biological functions that are important for the human body (Adi, et al., 2020). Therefore, the quality of milk protein serves as a key indicator in assessing the quality of milk as a food product.

Milk quality, including its protein content, is not only determined by the cow's genetics but is also significantly influenced by the environmental conditions of the dairy farm. Factors such as ambient temperature, farm elevation, type of feed provided, as well as barn sanitation and cleanliness play a crucial role in the milk production process and its composition. Differences in environmental conditions between one dairy farm location and another can lead to significant variations in the protein content of the milk produced (Sari, et al., 2021; Nurhayati & Lestari, 2022).

The Lembang and Pangalengan regions in West Java are two long-established dairy farming hubs in Indonesia. These two areas have distinct environmental characteristics in terms of temperature, elevation, feed types, and barn management. Lembang is situated at an elevation of 1,000–1,312 meters above sea level, while Pangalengan is higher, ranging from 1,410–1,500 meters above sea level. These differing conditions are believed to contribute to variations in the protein content of the milk produced in these two regions.

One of the commonly used methods for the qualitative detection of protein is the Biuret method. This method is based on the reaction between  $\text{Cu}^{2+}$  ions and peptide bonds in a basic environment, which produces a violet-colored coordination complex. The intensity of the violet color formed is directly proportional to the number of peptide bonds contained in the sample; thus, the darker the violet color, the higher the protein content (Fitriany & Santoso, 2022).

The purpose of this study is to determine the protein content of raw cow's milk from the Lembang and Pangalengan regions based on the results of the Biuret test, and to compare the two sets of results in light of differences in the environmental conditions for the cows at each location.

## 2. METHODS

The research was conducted through systematic laboratory testing across two distinct schedules: the first phase took place on May 8, 2026, from 11:00 to 13:30, and the second validation phase was executed on May 18, 2026, from 14:00 to 16:00. Both analytical sessions were carried out at the Physiology Laboratory, Study Program of Biology, Universitas Pendidikan Indonesia.

### 2.1. Materials and Equipment

The operational apparatus and chemical reagents employed in this biochemical analysis are compiled below in **Table 1**.

**Table 1.** Experimental equipment and materials.

Equipment	Materials
Test tubes & test-tube racks	Pure cow's milk sample (Lembang & Pangalengan)
Micropipettes & blue tips	Distilled water
Graduated cylinders & stirring rods	Casein standard powder
Thermometer	Sodium Hydroxide (NaOH 10%)
Petri dishes & Dropper pipettes	Copper Sulfate ( $\text{CuSO}_4$ 1%)

## 2.2 Casein Standard Dilution and Gradient Preparation

A reference baseline was established by executing a serial dilution of a 1% stock casein solution to generate specific concentration benchmarks, measured in parts per million (ppm). Standard solutions were prepared at thresholds of 10,000 ppm, 8,000 ppm, 6,000 ppm, 4,000 ppm, 2,000 ppm, and 1,000 ppm by mixing the stock solution with precise quantities of distilled water to achieve a uniform final volume of 2 mL per reference tube. Each dilution profile was then mixed with 2 mL of pre-formulated Biuret reagent, thoroughly homogenized, and incubated at room temperature for 10–15 minutes to allow complete development of the color complex.

## 2.3 Biuret Qualitative Protocol for Milk Samples

The qualitative analysis of the pure milk samples followed a structured biochemical sequence based on the Biuret reaction (Anderson, 2002; Biology Online, 2023). For each sample, a clean test tube was charged with 3 mL of raw milk. Subsequently, 3 mL of 10% NaOH solution was introduced to establish the required strongly alkaline medium, which is essential for the reaction to proceed (Mohr, *et al.*, 1994; Thermo Fisher Scientific, n.d.). The mixture was carefully swirled, followed by the addition of 0.1 mL (or approximately a few drops) of 1% CuSO<sub>4</sub> solution to provide the necessary Cu<sup>2+</sup> ions (Bettelheim & Landesberg, n.d.). The test tubes were gently agitated to achieve complete homogeneity and left to rest for a reaction window of 10–15 minutes to allow for full color development (Simons, 2020; Stefanescu, *et al.*, 2017). The final color transitions were observed, recorded, and cross-referenced with the pre-established casein standard scale to infer relative concentration ranges.

## 3. RESULTS AND DISCUSSION

### 3.1. Sample Data

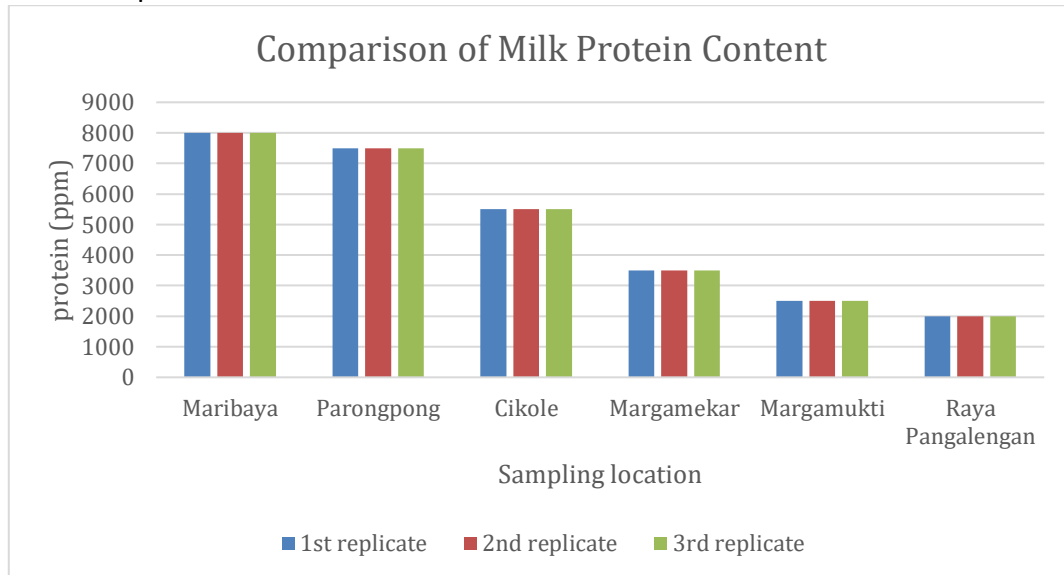
This study used six samples of raw cow's milk collected from two different regions. The following **table 2** summarizes the characteristics of each sample based on location, temperature, elevation, feed type, and housing conditions.

**Table 2.** Milk sample data by location and ambient temperature.

No	Sample location	Elevation (masl)	Type of sample	Ambient temperature (°C)	Type of cattle feed	Cage cleanliness
1	Lembang	1.200	Sample A (Maribaya)	27	Grass and food from KPBS	Pretty Clean
2	Lembang	1.000	Sample B (Parongpong)	25	Tofu pulp, Cassava pulp	Fairly Clean and Well Maintained
3	Lembang	1.312	Sample C (Cikole)	29	Grass	Clean Coop and Well Maintained
4	Pangalengan	1.410	Sample A (Margamekar)	24	Grass, Pulp	Fairly Clean and Well Maintained
5	Pangalengan	1.500	Sample B (Margamukti)	24	Tofu pulp (Concentrate), Grass, Carrot	Clean Coop and Well Maintained
6	Pangalengan	1,410	Sample C (Pangalengan)	25	Grass, Pulp	Clean and Well-Maintained Barn

### 3.2 Results of the Dilution of the Casein Standard Solution

A series of casein standard solutions was successfully prepared at six different concentrations, ranging from 10,000 ppm to 1,000 ppm (**Figure 1**). Each tube displayed a purple color of varying intensity: the higher the casein concentration, the deeper the resulting purple color. Visual observations revealed a clear color gradient ranging from deep purple at a concentration of 10,000 ppm to pale light purple at a concentration of 1,000 ppm. This standard series was subsequently used as a reference for visually estimating the protein content of samples.



**Figure 1.** Protein content of fresh cow's milk samples from different locations

### 3.3 Results of the Biuret Test on Milk Samples

All six milk samples tested yielded positive (+) results in the Biuret test, as indicated by the formation of a purple color in the solution following the addition of NaOH and CuSO<sub>4</sub> reagents. This confirms that all whole milk samples from Lembang and Pangalengan contain protein (peptide bonds). The complete test results are presented in **Table 3**.

**Table 3.** Biuret test results for all milk samples.

Sample Location	Volume (mL)	Absorbance (ppm)	The Result Color
Maribaya (Lembang)	3 mL	~8.000 ppm	Dark Purple
Parongpong (Lembang)	3 mL	6.000–8.000 ppm	Dark Purple
Cikole (Lembang)	3 mL	5.000–6.000 ppm	Light Purple
Margamekar (Pangalengan)	3 mL	3.000–4.000 ppm	Light Purple
Margamukti (Pangalengan)	3 mL	2.000–3.000 ppm	Light Purple
Sample C (JI Raya Pengalengan)	3 mL	1,000 ppm	Light Purple

Based on **Table 3**, there is a fairly striking difference in color intensity between the samples from Lembang and Pangalengan. Samples from Maribaya (Lembang) produced a dark purple color with the highest estimated absorbance of around 8,000 ppm, followed by Parongpong with 6,000–8,000 ppm, and Cikole with 5,000–6,000 ppm. Meanwhile, the

samples from Pangalengan produced a light purple color with lower absorbance, ranging from 1,000–4,000 ppm.

### 3.4 The Effect of Environmental Factors on Protein Content

The difference in protein content detected between the Lembang and Pangalengan samples can be explained by several environmental factors. First, environmental temperature. The temperature at the Lembang sampling site ranged from 25–29°C, while in Pangalengan it was slightly lower, at 24–25°C. Although the difference is not significant, higher temperatures generally increase the metabolic rate of cows, which can affect the protein synthesis process in milk. However, excessively high temperatures can also cause heat stress, which actually reduces milk quality (Handayani & Saputra, 2023). The relationship between ambient temperature and milk protein content is well-documented, with studies consistently showing that elevated temperatures negatively impact milk protein synthesis through multiple physiological pathways (Gao, *et al.*, 2017; Gorniak, *et al.*, 2014). Research has demonstrated that when temperature increases from 6.2°C to 31.3°C, milk protein can significantly decrease by approximately 4.09% (Gao, *et al.*, 2017). This decline is attributed to heat stress-induced alterations in amino acid metabolism, where more amino acids are diverted for maintenance and immune responses rather than for milk protein synthesis (Gao, *et al.*, 2017). Additionally, heat stress has been shown to activate the plasminogen-plasmin system in the mammary gland, which can lead to the breakdown of  $\beta$ -casein, further reducing milk protein content (Silanikove, *et al.*, 2009; Lim, 2022).

The physiological impact is compounded by the fact that heat stress suppresses genes involved in milk protein synthesis while upregulating heat shock proteins, effectively reducing the mammary gland's capacity to produce milk components (Gao, *et al.*, 2017; Thammacharoen, *et al.*, 2022). Given that Lembang's temperature range (25–29°C) approaches or exceeds the thermoneutral zone for dairy cattle, the cows at this location may experience mild to moderate heat stress, potentially contributing to the observed lower protein levels compared to the cooler Pangalengan site (Gorniak, *et al.*, 2014; Bernabucci, *et al.*, 2014). Second, the factor of elevation. The elevation of Pangalengan (1,410–1,500 m above sea level) is higher than that of Lembang (1,000–1,312 m above sea level). The cooler temperatures at higher elevations can affect feed intake and cattle metabolism, which ultimately impacts the composition of the milk produced (Wibowo & Yuliana, 2021). Lower environmental temperatures generally lead to increased feed consumption in dairy cattle as a compensatory mechanism to maintain body heat, which may consequently elevate milk fat and protein synthesis (Rhoads, *et al.*, 2009). Furthermore, elevation-related differences in ambient temperature and atmospheric pressure have been associated with variations in the efficiency of rumen fermentation, thereby influencing the bioavailability of key nutrients such as volatile (Lestari, *et al.*, 2020).

Third, the type of feed. Sample A from Lembang (Maribaya) was fed a variety of grasses and supplementary feed from KPBS (South Bandung Livestock Cooperative), which likely contained high-protein concentrates. This is consistent with the high protein content of the milk produced. In contrast, cows in Pangalengan generally received only grass and byproducts with more limited variety, except in Margamukti, where they also received tofu residue, concentrates, and carrots. Better feed quality was found to have a positive correlation with higher milk protein content (Sari, *et al.*, 2021).

Fourth, barn hygiene. Barn conditions across all study sites were generally clean and well-maintained. However, there was a slight difference between Maribaya, which was rated “fairly clean,” and several Pangalengan sites rated “clean and well-maintained”. Good barn

hygiene plays a role in preventing bacterial contamination that can break down milk protein (Nurhayati & Lestari, 2022; Kurniasih & Pradana, 2022).

Overall, the results of this study are consistent with Biuret's theory, which states that the higher the protein concentration, the more intense the resulting purple color (Fitriany & Santoso, 2022; Prasetyo & Wulandari, 2022). The color gradation observed in the casein standard series (10,000 ppm = dark purple, 1,000 ppm = light purple) provides an adequate reference for qualitatively and visually estimating the protein content of samples.

#### 4. CONCLUSION

Based on the results of the study, the following conclusions can be drawn. First, all samples of whole cow's milk from the Lembang area (Maribaya, Parongpong, Cikole) and Pangalengan (Margamekar, Margamukti, Jl. Raya Pangalengan) tested positive (+) in the Biuret test, as indicated by the formation of a purple color in each sample. This proves that all samples contain protein (peptide bonds).

Second, there were significant differences in color intensity and estimated protein content between samples from Lembang and Pangalengan. Samples from Lembang, particularly Maribaya, produced a dark purple color with the highest absorbance (~8,000 ppm), while samples from Pangalengan produced a light purple color with lower absorbance (1,000–4,000 ppm). This indicates that milk from Lembang has a higher protein content compared to milk from Pangalengan.

Third, these differences in protein content are influenced by the cows living environment, including ambient temperature, elevation, feed quality and type, and barn sanitation. Thus, the Biuret method has proven to be an effective and reliable qualitative method for analyzing differences in protein content in raw milk based on the environmental factors that influence it.

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#### 6. AUTHORS' NOTE

The authors declare that there are no conflicting financial or institutional interests regarding the execution and publication of this research paper. This manuscript is an original academic compilation and is entirely free of plagiarism.

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