Milk Production, Quality Parameters, and Bacterial Colony Counts of Raw Milk from Murrah Buffaloes Reared in Mixed Crop-Livestock Systems

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Abstract: This research aimed to assess the quantity and quality of raw Murrah buffalo milk in a mixed crop-livestock farming system in North Sumatra, Indonesia. The study was conducted at the Sumber Ternak Abadi livestock farm in Pagar Merbau District, North Sumatra, Indonesia, established in 2012 within an oil palm plantation. The study observed 40 lactating Murrah buffaloes. Variables included milk production and quality metrics such as total plate count in colony-forming units per ml, water content percentage, total solids, fat content, and pH. Results demonstrated a milk yield standardized to 305 days ranging from 1,200.78±490.25 to 1,505.71±589.73 kg/head/lactation. The total plate count was $2.1 \times 10^5 \pm 0.32$ CFU/ml, total solids were 16.87% (w/w), fat was 5.7% (v/v), and pH was 6.73. The results confirmed that the raw Murrah buffalo milk from the observed farm meets the Indonesian National Standard for milk quality. A significant positive correlation was found between water content and total bacterial colony count ($r = 0.82$, $p < 0.01$), suggesting that higher water content in milk correlates with increased bacterial colonies.

Keywords: Livestock, milk quality, Murrah buffalo, raw milk.

INTRODUCTION

The Murrah buffalo, a distinguished water buffalo (*Bubalus bubalis*), is esteemed for its impressive milk production capabilities. With its roots traced to the Punjab regions of India and Pakistan, this breed stands as one of the globally recognized and sought-after buffalo breeds, primarily notable for its superior milk yield and quality. The domestic water buffalo represents an essential livestock species in numerous developing nations. This is particularly evident among small-scale farmers integrated into mixed crop-livestock systems, such as those in Indonesia [1]. With their remarkable milk yield, adaptability to diverse environments, and resilience in less-than-ideal conditions, these animals represent valuable contributors to regional food security and livelihoods.

Buffalo milk is distinguished by its enriched nutritional profile, with caloric content nearly double that of cow's milk, and is rich in essential minerals, including calcium, magnesium, potassium, and phosphorus [2]. Nevertheless, realizing the full potential of the Murrah buffalo is contingent upon the consistent quality and safety of the milk they produce. Milk quality is intimately linked to the management practices employed throughout livestock production. As consumers grow increasingly conscious of their food sources, the dairy industry's dedication to milk quality assurance, spanning the entirety of the production journey, has intensified. Consequently, there is an expanding interest in understanding the factors influencing milk quality and the methodologies to enhance it.

Our present study investigated the quantity and quality of raw milk produced by Murrah buffaloes in a mixed farming system in Deli Serdang, North Sumatra, Indonesia. Located within an oil palm plantation, the livestock farm "Sumber Ternak Abadi" has been operational since 2012. As of 2022, the farm housed a total buffalo population of 93. Among these, 40 buffaloes were lactating, most in the third (mid-lactation) and fourth (late-lactation) stages. Manual milking methods are employed at this farm. Factors such as the cleanliness of milking facilities, the quality of water sources, and the training level of the staff have a profound impact on milk quality, especially in terms of bacterial counts.

In raw buffalo milk, the nutrient-rich composition provides fertile ground for rapid microbial growth. It is multidimensional to assess milk quality, considering its nutrient profile and microbial presence. A myriad of intrinsic and extrinsic factors can influence microbial proliferation in milk. Pathogenic bacteria may find their way into the milk from infected animals, contaminated environments, or during various post-collection stages, such as storage, transport, and processing [3]. Ensuring rigorous milk handling procedures is paramount to curbing bacterial growth. Emphasis on hygiene at every stage, such as milking, collection, and

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Table 1: Nutrient Composition of Ration Ingredients

No.	Diet	Nutrient (%)				
		Dry Matter	Crude Protein	Crude Fibre	Crude Fat	Ash
1.	Forage	37.91	11.19	30.08	2.23	8.41
2.	Concentrate	40.40	9.75	16.46	2.41	5.52

transportation, can stave off potential contaminants. Proper storage and processing techniques are also of utmost importance [4]. The pH level of the milk is another critical factor, with lower pH levels potentially fostering microbial growth due to the acidic environment they favor [5].

Our research wants to document the production process and examine the bacterial colony count within Murrah buffalo milk. The insights garnered from this study not only furnish a foundational dataset pivotal for future research endeavors aiming at amplifying yield and hygienic processing but also delve deep into the physicochemical attributes like water content, total solids, fat percentage, and pH. It provides us with some essential but basic information on milk quality. This study aims to analyze Murrah buffalo milk's production process and quality at the Sumber Ternak Abadi farm in Deli Serdang, focusing on bacterial colony count and physicochemical properties like water content, total solids, fat percentage, and pH. It also seeks to identify the relationship between the number of bacteria in the milk and its pH and water content as indicators of milk safety quality. The findings are expected to provide essential baseline data for future research to enhance milk yield and hygienic processing and understand the nutritional advantages of buffalo milk.

MATERIAL AND METHODS

Research Setting and Sample Collection

The study was conducted at the Sumber Ternak Abadi Ranch in the Pagar Merbau District of Deli Serdang Regency, North Sumatra, Indonesia. The tropical climate of North Sumatra, characterized by warm temperatures and high humidity, can directly affect buffalo physiology and, consequently, milk production. Oil palm plantations are known to have specific microclimates, with the dense canopy leading to cooler ground temperatures and higher humidity. This area has an altitude of 0-500 meters above sea level, an average temperature of 27°C and humidity of 84%, and rainfall ranges from 1600-1900 mm per year [6]. Such conditions influence the buffaloes' diet, as the type and quality of available forage differ from open pastures. Based on researchers' observations, the farm currently uses a total mixed ration of forage (weeds and palm leaves) and concentrate consisting of oil palm meal, cassava peel, cassava pulp, coconut pulp, and additional minerals (Table **1**). Furthermore, the proximity to oil palm operations could expose the buffaloes to specific farming practices, fertilizers, or pesticides that might indirectly affect milk quality. Out of the 93 buffaloes present at the ranch, 40 were in the lactation phase, consisting of 18 buffaloes in the third (mid) lactation and 22 buffaloes in the fourth (late) lactation, offering a substantial sample size that accurately represents the lactating population of the farm.

Evaluation of Milk Quantity and Quality

Milk Production Measurement

This evaluation lasted a 30-day and was subsequently standardized to a lactation period of 305 days [7]. Daytime milking outputs were noted in liters per buffalo daily and then converted to the standard 7% Fat Corrected Milk (FCM). The calculations involved are [8]:

a. Milk production 7% FCM = (0.265×milk production (kg/day) + (10.5×fat production)

b. Milk yield (kg/day) = milk yield (l/day)×milk specific gravity

c. Fat yield = % fat × milk yield (kg)

d. 305-day standard milk production = 7% FCM milk production × 30 days/month of production (%)

Quality Indicators

The quality of the raw Murrah buffalo milk was discerned by evaluating parameters such as microbial load, chemical composition, and pH levels. This entailed using both survey methodologies and exhaustive laboratory evaluations, targeting indicators like the total plate count, water content percentage, fat content, and pH.

Laboratory Analysis

Sample Preparation

Milk samples were collected sterilely during daytime milking to minimize contamination. Post-collection, each sample was stored in a sterile container, homogenized, and duly labeled.

Microbial Analysis

In this research, we conducted a detailed microbiological test using the Total Plate Count (TPC) method, guided by the normative reference of SNI ISO 4833-1:2015. This standard provides a systematic approach to enumerate microorganisms in products intended for human or animal consumption. The process began with the aseptic collection of milk samples from buffaloes. Extreme care was taken to prevent contamination, and the samples were promptly stored in sterile containers. Following collection, the samples underwent serial dilution. This step involved systematically reducing the concentration of bacteria in the samples to a range suitable for counting. The process was repeated to achieve further dilutions such as 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, starting with a 10^{-1} dilution. For each dilution, 1 ml of the milk sample was mixed with 9 ml of Buffered Peptone Water (BPW), as recommended by the ISO standard.

After preparing the dilutions, 0.1 ml from each was spread onto Petri dishes containing Plate Count Agar (PCA). The inoculated dishes were then incubated at 30°C for 72 hours. This specific temperature and duration are ideal for promoting the growth of mesophilic bacteria, which are typically present in milk. Post-incubation, the grown bacterial colonies on each plate were counted using a colony counter. These counts were then used to calculate the concentration of bacteria in the original milk samples, expressed in colony-forming units per milliliter (CFU/ml).

Chemical Analysis

To evaluate water content percentage, a designated volume of milk was placed in a pre-weighed flat dish and subsequently dried in an oven set at 102°C. This process continued until the sample achieved a consistent weight. The water content was then determined by subtracting the weight of the dried sample from its original weight. The resultant weight, representing the evaporated water, was then converted into a percentage relative to the original volume of the sample. Milk samples underwent centrifugation to assess the total solids and fat content at 3000 rpm for 15 minutes, separating the cream from the rest of the sample. The isolated cream was then weighed to ascertain the fat content. Concurrently, the weight of the remaining liquid was utilized to determine the total solids and the evaporation of its water content [9].

On-Site Testing

Immediately after the sample collection, the milk's pH and water content were gauged using calibrated laboratory instruments. A pH meter (GL-PH818) was used for pH assessment, while the gravimetric method [9] was employed for water content measurement. For pH measurement, milk samples were initially left to sit at room temperature for about 30 minutes, ensuring a uniform consistency before any measurements were taken. Subsequently, a pH meter was calibrated using standard buffer solutions to ensure accuracy. During the measurement process, the electrode of the pH meter was carefully immersed into the milk sample. The pH value was then recorded once the reading on the meter stabilized.

Data Preparation

Before conducting statistical analyses, the collected data underwent a rigorous preparation process. This involved data cleaning to address any inconsistencies or errors, handling missing values, and appropriate data transformations when necessary. The milk samples were collected from 40 lactating buffaloes.

Descriptive Statistics

We utilized descriptive statistics to assess data related to milk production, pH measurements, milk water content, and bacterial counts. By determining mean values and standard deviations, we offered an insight into our dataset's central tendencies and variability [10].

Hypothesis Testing

We tested hypotheses to address specific research queries and hypotheses aligned with our study goals. The t-test was utilized to analyze the difference in milk production averages between the two lactation stages. A significance level (α) of 0.05 was established for these tests.

The t-Test

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Where:

$\bar{x}_1$ and $\bar{x}_2$ are the sample means of the two groups

s_1 and s_2 are the sample standard deviations of the two groups

n_1 and n_2 are the sample sizes of the two groups

Correlation Analysis

We conducted correlation analyses to understand the relationships among our primary metrics—bacterial count, pH, and water content. These analyses revealed the degree and direction of associations between these variables and set the stage for our subsequent regression analysis. Through regression, we explored the functional relationships between these primary variables, gleaning insights into how a shift in one could predict or influence changes in another.

Statistical Significance

We used a predetermined alpha level (α) 0.05 to determine statistical significance. For results to be considered statistically significant, p-values below this threshold were considered.

Software Used

All statistical analyses were conducted using Microsoft Excel sheets, using the usual mean value and standard deviation formulas.

RESULTS AND DISCUSSION

The research findings shed light on the quality aspects of Murrah buffalo milk within traditional farming practices. The study delved into crucial parameters, including moisture content, pH levels, and total bacterial colonies in the milk. These parameters are important as they directly impact the safety, shelf life, and overall quality of dairy products derived from buffalo milk. Moisture content, one of the parameters examined, directly affects the milk's texture and nutritional content. Additionally, pH levels influence the stability of the milk and its suitability for various processing methods.

Furthermore, the concentration of bacterial colonies in milk is a crucial indicator of hygiene and safety, with higher colony counts potentially indicating compromised milk quality. By exploring these parameters in Murrah buffalo milk from traditional farms, the research contributes to a better understanding of the milk's overall quality, thereby offering insights that could inform practices to ensure safer and more nutritious dairy products for consumers. Fresh buffalo milk's physical and chemical quality is influenced by nationality, feed, feeding system, milking frequency, milking method, seasonal changes, and lactation period [11].

Table **2** shows that the average milk production of Murrah buffalo is 5.71 ± 2.13 kg/head/day of lactation III and 4.67 ± 1.63 kg/head/day of IV lactation. The average production of Murrah buffalo milk 7% FCM was 4.52 ± 1.84 kg/head/day of lactation III and 3.63 ± 1.41 kg/head/day of IV lactation, while the average milk production. Furthermore, Figure **1** shows a standardization of 305 days, namely 1,505.71 ± 589.73 kg/head/lactation III lactation and 1,200.78 ± 490.25 kg/head/lactation IV lactation. The t-test analysis found that the milk production for lactation III and IV showed results that were not significantly different (P>0.05). There is no difference in the milk production of Murrah buffaloes in lactation III and IV because the Murrah buffaloes in lactation III and IV have entered the 3rd to sixth lactation months, so milk production has begun to decline. Milk production will increase from the first to the second lactation month and decrease again in the third lactation month until it enters the dry phase of the cage. The lactation months affect milk production as lactation months increase, and milk production will increase from the first to the second lactation month and decrease again in the third lactation month until it enters the dry phase [12]. River buffaloes usually produce between 1,500 and 4,500 liters of milk per lactation [1].

Table **3** shows that the average water content of Murrah buffalo milk is 83.13%, total solids are 16.87%, fat 5.70% (v/v), and pH 6.73. This result is quite good

Table 2: Milk Yield of Murrah Buffalo

Lactation stage	Daily milk yield (L/head/day) ± Sd	Daily milk yield (Kg/head/day) ± Sd	Milk yield in7% FCM (Kg/head/day) ± Sd
III	5.04 ± 2.04	5.71 ± 2.13	4.52 ± 1.84
IV	4.04 ± 1.57	4.67 ± 1.63	3.63 ± 1.41

P>0.05, no significant difference between the yield of the 3rd and 4th lactation period.
Sd: standard deviation.

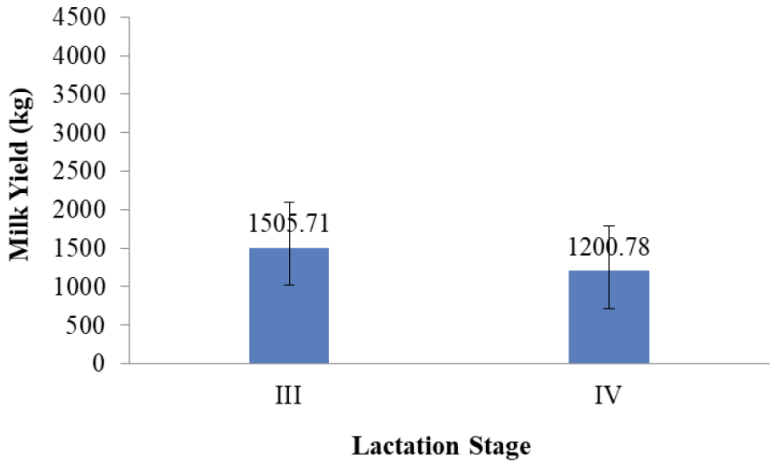

Figure 1: Estimated milk yield of Murrah buffalo for a lactation period (305 days).

Table 3: Milk Quality of Murrah Buffalo

No.	Variable	Mean (%)	Standard Deviation
1	Total solids (%)	16.87	0.02
2	Moisture content (%)	83.13	0.02
3	Fat content (%)	5.70	
4	pH	6.73	0.16
5	Total bacterial colony (CFU/ml)	2.1×10^5	0.32

because it is included in the normal pH category range of 6.3 – 6.8. If the pH is below 6.3, the milk has likely been damaged by acid-forming bacteria such as *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus thermophiles* [13].

Table **3** shows that the average bacterial colony of Murrah buffalo milk at Sumber Ternak Abadi Farm is 2.1×10^5 CFU/ml. The total bacterial colonies resulting from this study were lower than the standards set by the Indonesian National Standard, where the maximum microbial count for dairy was 1×10^6 CFU/ml [14]. Farmers' sanitation and hand hygiene caused the low number of bacterial colonies in this study in milking because milking was done after cage sanitation. If hand sanitation is not done correctly, it will cause a source of contamination to fresh milk. *Escherichia coli* contamination was mainly due to farmers' lack of attention to sanitation and personal hygiene [15].

One of the things that can affect the quality of milk is the milking interval. Milking on the Sumber Ternak Abadi farm is done only once daily. The milking is usually done twice daily, in the morning and evening [16]. The exact time interval between milking in the morning and evening will give relatively little change in milk composition, while different milking time intervals will produce different milk compositions. In addition, environmental factors such as different cage temperatures between morning and evening can also affect the microbiology contained in the milk.

The cleanliness of the environment at Sumber Ternak Abadi Farm is quite good because the cleanliness of the stables is considered, and the stables are pretty clean; livestock manure is rarely found around the stables, and the farm is also far from residential areas. This is according to the requirements determined by the Directorate General of Livestock [17] for dairy livestock; dairy farms should not be located in the city center and residential areas at a distance of at least 250 meters from residential areas. Another cleaning training that needs to be done is the cleanliness of the cage and milking equipment [18]. Where on this farm, the cage is cleaned by washing the floor of the cage by spraying high-pressure water. In this way, the leftovers of stale and smelly food are washed clean so that the milk is not contaminated by the dirt in the cage. Good cage hygiene management can reduce microbial growth. Another thing to note is the cleanliness of the milking equipment. All equipment used on this farm is cleaned before and after milking so that the growth of microbes is minimized.

Table 4: Correlation Analysis of the Variables

No.	Variables	r value	p-value
1.	Moisture content and total bacterial colony	0.82	<0.01
2.	pH and total bacterial colony	-0.67	<0.05

A correlation analysis (Table **4**) assessed the relationships among the variables: water content (%), pH, and total bacterial colony count (CFU/ml). The results revealed several interesting patterns. There was a significant positive correlation between water content (%) and total bacterial colony count ($r = 0.82$, $p < 0.01$), indicating that higher water content in the milk was associated with a higher count of bacterial colonies. Additionally, a significant negative correlation was observed between pH and total bacterial colony count ($r = -0.67$, $p < 0.05$), suggesting that higher pH levels were associated with lower bacterial colony counts.

A regression analysis was carried out to understand further the impact of water content (%) and pH on the total bacterial colony count. The regression model yielded the equation: Total Bacterial Colony Count = 1.25 x Water Content (%) - 0.59 x pH + 1.02. This equation suggests that an increase of 1% in water content is associated with a 1.25 unit increase in bacterial colony count. In contrast, a decrease of 1 unit in pH is associated with a decrease of 0.59 in bacterial colony count. The coefficient of determination ($R^2 = 0.74$) indicates that approximately 74% of the variability in total bacterial colony count can be explained by changes in water content and pH.

These results highlight the importance of water content and pH in influencing the microbial quality of the milk. However, it is essential to note that these findings are based on the current dataset and conditions, and further research with a larger sample size and controlled experiments may be needed to confirm and generalize these relationships.

CONCLUSION

A yield of buffalo milk is retrieved around 1,200.78±490.25 to 1,505.71±589.73 kg/head/lactation. The conclusion from the quality of Murrah buffalo milk at Sumber Livestock Farms is that it is quite good and follows the Indonesian National Standards. Water content and total bacterial colony count showed a significant positive correlation. The coefficient of determination ($R^2 = 0.74$) indicates that approximately 74% of the variability in total bacterial colony count can be explained by changes in water content and pH. Buffalo milk contributes significantly to the livelihoods of many small-scale farmers. It provides a source of nutrition for these communities and can also be sold for income generation.

ETHICAL CONSIDERATIONS

We maintained a high regard for animal welfare throughout the research. Data was collected with minimal disturbance to the Murrah buffaloes, ensuring their well-being. The study adhered to ethical guidelines for research involving animals.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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