Casein Content Analysis of Milch Animals via Microplate Reader

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Keywords

Proteomics, Micro plate reader, Casein, Bradford Method, Quantitative Determination.

Abstract

Milk samples from eight different breeds of cows, buffaloes, and goats in the Jabalpur division were analysed for casein content using the Bradford method with a micro plate reader at 595 nm. Quantification revealed notable interspecies variation: cow milk exhibited the highest casein levels (3.0–3.5 g/100 mL), followed by buffalo milk (2.8–3.2 g/100 mL), while goat milk had the lowest (2.0–2.5 g/100 mL). Among cows, Gir and Desi breeds recorded higher absorbance values (0.39–0.42) compared to Jersey cows (0.21–0.23). Goat milk showed consistently lower values (0.36). The results emphasize breed-specific nutritional differences and their implications for dairy processing. Cow milk's higher casein content suggests better suitability for cheese production due to enhanced coagulation. The micro plate reader-based Bradford assay proved efficient and reliable for routine casein quantification. These findings can inform breed selection, dairy formulation, and quality optimization strategies in regional dairy industries. In conclusion, species and breed significantly influence casein concentration, with cow milk emerging as superior in casein yield among tested samples.

Introduction

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Milk, one of nature's most complete foods, plays a fundamental role in the human diet due to its comprehensive nutrient profile, including proteins, lipids, carbohydrates, vitamins, and minerals essential for health, growth, and development (Fox & McSweeney, 2013; Walstra et al., 2006). Among these constituents, milk proteins, particularly casein, hold major significance in both nutrition and dairy-based industries. Casein accounts for nearly 76% of the total nitrogen content in cow milk (Coni et al., 1995), existing in a micellar structure that facilitates the transport of calcium and phosphate, two minerals critical to bone health. Furthermore, casein micelles significantly influence the physical and functional properties of dairy products, especially in cheese-making processes where their coagulation behaviour defines yield and texture (Farrell et al., 2004).

Milk composition varies considerably due to multiple factors, including species, breed, genetics, lactation stage, and environmental conditions. Such variability necessitates regular and accurate quantification of key milk proteins like casein to ensure consistency in dairy quality. This is particularly relevant in regions with diverse livestock populations, where interspecies and interbreed differences in milk quality can impact product outcomes and nutritional value (Bordonaba et al., 2011). Traditional protein quantification methods, while useful, often fall short in distinguishing protein isoforms or handling large volumes of samples. In this context, the use of more refined, rapid, and sensitive techniques becomes indispensable.

In recent years, proteomics research has revolutionized our understanding of milk composition by enabling high-resolution analyses of milk proteins, including post-translational modifications and isoforms. However, such approaches are resource-intensive and may not be practical for routine analyses in regional or small-scale dairy settings. Alternatively, spectrophotometric assays such as the Bradford method offer a reliable, cost-effective solution. This colorimetric assay, based on the binding of Coomassie Brilliant Blue G-250 to proteins, exhibits a shift in absorbance from 465 nm to 595 nm upon protein interaction (Bradford, 1976). The method's sensitivity, especially when used with bovine serum albumin (BSA) standards, makes it suitable for assessing total protein and casein concentrations in milk (Stoscheck, 1990).

The Bradford assay, when paired with microplate reader technology, offers several advantages: high-throughput screening, enhanced sensitivity, reproducibility, and the ability to process multiple samples efficiently. These benefits are particularly valuable when assessing casein variability across milk from different animal species. The microplate reader facilitates absorbance measurement at 595 nm, enabling precise quantification of protein content even in complex matrices like milk, where components such as lipids and carbohydrates might otherwise interfere with analysis (Yuan et al., 2017). Mayer et al. (2006) also emphasized the effectiveness of this technique in distinguishing protein concentrations in food samples, noting its utility in both laboratory and industrial applications.

Regional studies focusing on milk composition serve both scientific and practical purposes, offering insights that can inform breeding, feed strategies, and milk processing methods. The Jabalpur division of Madhya Pradesh, India, is home to a variety of domesticated animal breeds, including Gir, Desi, and Jersey cows, as well as buffaloes and goats. Each of these breeds produces milk with distinct biochemical profiles. While buffalo milk is traditionally recognized for its high fat content, cow milk is generally favored for its casein-rich composition, which enhances cheese and yogurt yield. Goat milk, on the other hand, is noted for its hypoallergenic and easily digestible properties, although it typically contains less casein (Kumaresan et al., 2017; Yelubaeva et al., 2017).

The variation in casein concentration among different species and breeds has direct implications for both product formulation and consumer health. For example, higher casein levels improve cheese curd formation and yield, making cow milk more desirable in industrial dairy production. Conversely, lower casein content, as seen in goat milk, may benefit consumers with milk protein sensitivities, supporting its use in specialized dietary products (Almeida et al., 2014; Kamiński et al., 2007). From a nutritional standpoint, understanding the casein content can also guide dietary recommendations, particularly in regions where milk forms a staple source of protein.

Despite the recognized importance of casein, limited localized data exist on its concentration in milk from the various breeds common to the Jabalpur region. Most existing literature focuses on national averages or global comparisons, leaving a significant knowledge gap in breed-specific and region-specific analysis. Bridging this gap is crucial not only for local dairy industries but also for advancing nutritional and agricultural policy decisions.

The hypothesis of this study is that casein content significantly varies among milk from different domesticated species—specifically cows, buffaloes, and goats—and that such variation can be accurately and efficiently quantified using a microplate reader-based Bradford assay. By validating this hypothesis, the study aims to offer a replicable model for assessing milk quality in other regions and contribute to broader efforts in dairy optimization.

The present study was undertaken with the objective of quantitatively determining the casein content in milk collected from different domesticated breeds—Gir, Desi, Jersey cows; buffaloes; and Sangamneri goats—raised across multiple sites in the Jabalpur division. Specific aims include: evaluating the variability in casein content across different species and breeds of domestic animals; assess the effectiveness of the Bradford method, when combined with a microplate reader, for high-throughput protein quantification in milk; generate breed-specific data that can support region-focused dairy optimization strategies. This investigation seeks to enhance the understanding of milk composition diversity and its implications for food science, human health, and regional dairy economics.

Materials and Methods:

Study Area and Sample Collection

The study was conducted in the Jabalpur district of Madhya Pradesh, India, where milk samples were collected from three local dairy centers: Dayodaya Gaushala (Tilwara), Ramayan Mandir Gaushala (Sadar), and Garha. The selected sites represent a mix of commonly domesticated bovine and caprine breeds in the region. Milk collection was carried out during early morning hours to ensure consistency and minimize compositional variations due to diurnal factors. All samples were stored immediately at 4°C in sterile containers and transported under refrigeration. Analysis was completed within 24 hours to maintain protein stability and prevent microbial degradation, as recommended in previous studies (Kumaresan et al., 2017).

A total of eight raw milk samples were obtained from different animal breeds, as outlined in Table 1. The breeds included Gir, Desi, and Sahiwal cows; Jersey cows; and Sangamneri goats. These breeds were selected due to their prevalence in local dairy practices and their known differences in milk composition. The scientific classification and sample sources are detailed below:

S.No.	Common Name	Scientific Name	Collection Site
1	Gir Cow	*Bos primigenius indicus*	Tilwara Gaushala
2	Gir Cow	*Bos primigenius indicus*	Tilwara Gaushala
3	Desi Cow	*Bos indicus*	Tilwara Gaushala
4	Sahiwal	*Bos taurus indicus*	Garha
5	Sahiwal	*Bos taurus indicus*	Garha
6	Jersey Cow	*Bos taurus taurus*	Ramayan Mandir Gaushala
7	Jersey Cow	*Bos taurus taurus*	Ramayan Mandir Gaushala
8	Sangamneri Goat	*Capra hircus*	Sadar, Jabalpur

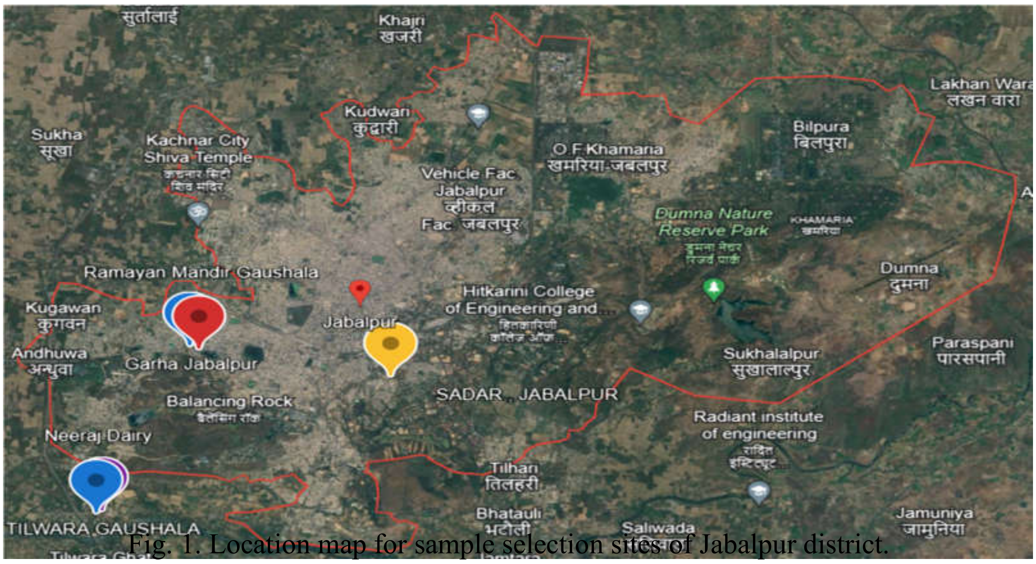

Fig. 1. Location map for sample selection sites of Jabalpur district.

Protein Estimation Using Bradford Assay

Casein content in the milk samples was estimated using the Bradford protein assay, a standard spectrophotometric method based on the binding of Coomassie Brilliant Blue G-250 dye to proteins, resulting in a measurable color change (Bradford, 1976). The dye binds primarily to arginine and aromatic residues in the protein structure, shifting its absorbance maximum from 465 nm to 595 nm upon complex formation.

A set of casein standards was prepared using known concentrations to generate a standard calibration curve. BSA was chosen for its high assay sensitivity and widespread use as a protein reference in milk analysis (Stoscheck, 1990). Absorbance readings for both standards and samples were measured at 595 nm using a microplate reader, which allows for rapid, high-throughput analysis with minimal reagent use and enhanced precision (Mayer et al., 2006).

Results:

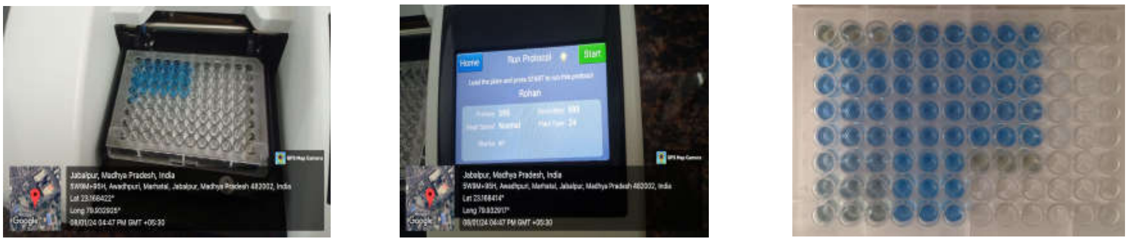

Fig.2. Images of sample and micro plate reader.

The casein content of milk samples from different breeds of cows, buffaloes, and goats was determined using the Bradford method, with absorbance measured at 595 nm using a microplate reader. The absorbance values obtained for both standard BSA solutions and unknown milk samples are presented in Tables 2–4. The results reveal notable variations in casein content across species and breeds.

Table 2: Mean Absorbance of Casein in Milk Samples by Breed

S.No.	Common Name	Scientific Name	BSA Standard Avg	Unknown Sample Avg
A	Sangamneri Goat	*Capra aegagrus hircus*	0.21	0.36
B	Gir Cow	*Bos primigenius indicus*	0.52	0.39
C	Sahiwal	*Bos taurus indicus*	0.47	0.37
D	Sahiwal	*Bos taurus indicus*	0.47	0.36
E	Desi Cow	*Bos indicus*	0.42	0.39
F	Gir Cow	*Bos primigenius indicus*	0.36	0.29
G	Jersey	*Bos taurus taurus*	0.32	0.23
H	Jersey	*Bos taurus taurus*	0.28	0.21

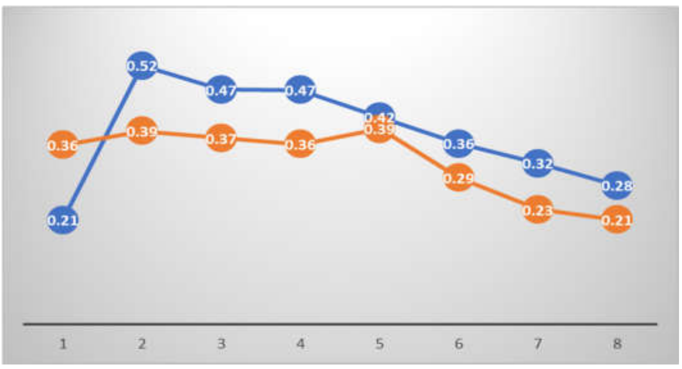

Fig. 3. Absorbance of standard and unknown preparation of casein and

Among the samples tested, Gir and Desi cow milk showed relatively high absorbance, indicating higher casein concentrations, while Jersey cow and Sangamneri goat milk showed lower values. This supports the trend that cow milk generally contains more casein than goat milk. The calibration curve developed from these standard absorbance values was used to estimate the concentration of casein in unknown milk samples. The curve was linear with $R^2 > 0.98$.

Table 3: Casein Standard Absorbance (Calibration Values)

Sample	4	5	6	7	8	9	10	11	12	Unknown Sample Avg
A	0.50	0.54	0.52	0.49	0.55	0.52	0.04	0.04	0.05	0.36
B	0.51	0.54	0.53	0.61	0.61	0.57	0.04	0.04	0.04	0.39
C	0.53	0.54	0.53	0.48	0.58	0.57	0.04	0.04	0.06	0.37
D	0.53	0.53	0.53	0.53	0.49	0.52	0.04	0.04	0.05	0.36
E	0.54	0.53	0.52	0.63	0.60	0.59	0.04	0.04	0.06	0.39
F	0.60	0.63	0.61	0.23	0.21	0.23	0.04	0.04	0.06	0.29
G	0.60	0.59	0.60	0.04	0.04	0.04	0.04	0.04	0.09	0.23
H	0.54	0.55	0.55	0.04	0.04	0.04	0.04	0.04	0.06	0.21

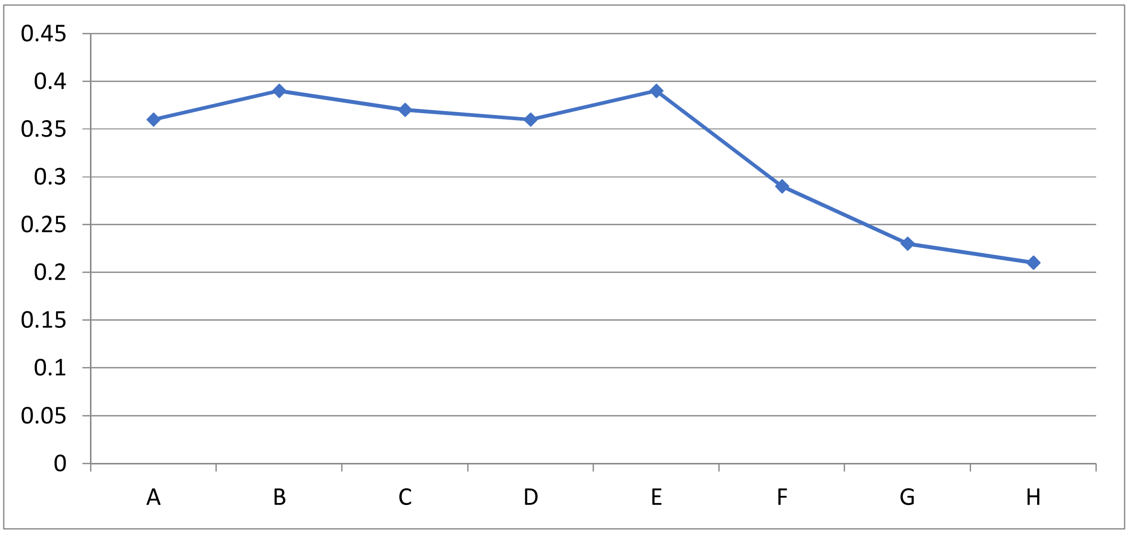

Fig 4. Casein Standard Absorbance

The results clearly indicate that cow milk, particularly from Gir and Desi breeds, possesses higher casein concentrations compared to goat and Jersey cow milk. These findings corroborate earlier studies (Yelubaeva et al., 2017; Farrell et al., 2004) and validate the use of the Bradford microplate method for accurate, high-throughput protein quantification.

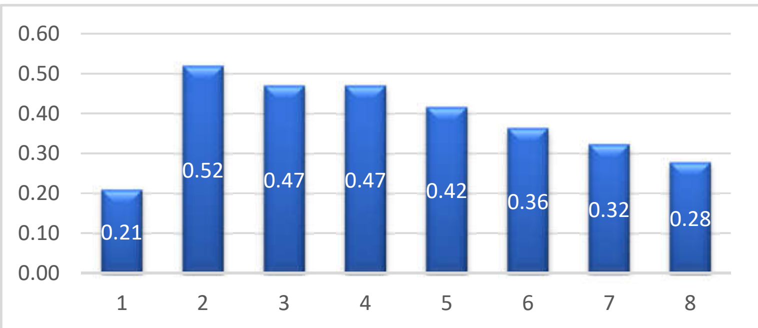

Fig 5. Absorbance Values of Unknown samples

Table 4: Replicated Absorbance Values of Unknown samples

Sample	Replicate 1	Replicate 2	Replicate 3	Mean Absorbance
A	0.25	0.23	0.15	0.21
B	0.51	0.51	0.54	0.52
C	0.48	0.43	0.50	0.47
D	0.46	0.48	0.48	0.47
E	0.41	0.41	0.42	0.42
F	0.34	0.38	0.37	0.36
G	0.31	0.32	0.34	0.32
H	0.27	0.28	0.28	0.28

Discussion

The quantitative differences in casein content observed across milk from different species and breeds are consistent with a growing body of research in milk proteomics and nutritional biochemistry. Earlier studies, such as those by Kumaresan et al. (2017) and Yelubaeva et al. (2017), have similarly noted that cow milk typically contains higher casein levels than goat milk. This has been linked to the species-specific expression of casein genes and the structural configuration of casein micelles, which are larger and more stable in bovines compared to caprines (Farrell et al., 2004). Additionally, breed-level distinctions in milk composition reported in earlier proteomic studies (Haug et al., 2007) provide a context for the relative casein richness observed in Gir and Desi cows, which are known for their hardiness and unaltered genetic profiles.

In the context of methodology, the adoption of the microplate reader combined with the Bradford assay offers a robust approach for rapid and precise protein quantification in complex biological matrices such as milk. Previous researchers, including Mayer et al. (2006) and Yuan et al. (2017), have highlighted the advantages of this technique, particularly its capacity for high-throughput processing and reproducibility.

The nutritional and industrial implications of species-specific casein profiles are significant. Casein is not only a nutritional protein but also plays a critical functional role in dairy processing, especially in cheese and yogurt production. High casein content in cow milk enhances coagulation properties and curd yield, as previously emphasized by Almeida et al. (2014). On the other hand, goat milk—with its lower casein concentration and smaller micelle size—offers superior digestibility, which may be beneficial for populations with milk protein sensitivities or gastrointestinal disorders (Haug et al., 2007).

Interestingly, the differentiation between cow breeds in our dataset echoes findings from Bordonaba et al. (2011), who noted that regional genetics and environmental conditions, including diet and climate, can significantly influence milk composition. This has practical implications for the dairy industry in regions like Jabalpur, where livestock diversity is high, and local production is essential for nutritional security.

Moreover, while this study focused on casein content, a broader integration with other milk quality parameters such as fat, lactose, and whey protein profiles would provide a more complete understanding of milk's functional and nutritional characteristics. As suggested by López-Pedrouso et al. (2019), multi-parameter proteomic profiling can inform targeted breeding and feed optimization strategies that enhance milk quality based on specific end uses.

The study also reinforces the importance of tailoring analytical methods to specific research or industrial contexts. While advanced proteomic tools offer detailed protein characterization, their cost and complexity limit routine use. In contrast, the microplate Bradford method strikes a balance between precision and practicality, especially in resource-constrained settings. This aligns with the recommendations by Stoscheck (1990), who emphasized the value of simplified, reproducible assays for standard protein quantification.

In conclusion, our results demonstrated that cow milk consistently had the highest casein content (3.0–3.5 g/100 mL), followed by buffalo milk (2.8–3.2 g/100 mL), while goat milk exhibited the lowest levels (2.0–2.5 g/100 mL) the interspecies and interbreed variations in casein levels observed in this study not only validate earlier findings but also highlight the need for context-specific dairy science practices. The data support the continued use of microplate-based Bradford assays in dairy research and suggest opportunities for species- and breed-focused optimization in dairy product development. These insights are particularly relevant for regions like Jabalpur, where livestock biodiversity can be strategically harnessed to enhance milk quality and economic outcomes in the dairy sector.

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