

Characterization of Milk Proteins from Vechur and Kasargod Dwarf Breeds of Cattle using SDS-Polyacrylamide Gel Electrophoresis

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Abstract: Vechur and Kasargod Dwarf cattle are indigenous to the state of Kerala, India. Vechur cattle is registered as an indigenous breed as per National Bureau of Animal Genetics Resources (NBAGR) whereas Kasargod Dwarf is labelled as 'non-descript'. Limited information is available on the compositional characteristics of milk from these cattle. In the present study, milk proteins from Vechur and Kasargod Dwarf cattle were characterized to evaluate the protein fragments. The electrophoretic pattern of these milk samples showed the presence of seven protein bands, viz., Lactoferrin (Lf) (76 KDa), immunoglobulin (Ig) (66 KDa), α -S1caseins (α -S1-CN) (23KDa), α -S2caseins(α -S2-CN) (21KDa), β -casein(β -CN) (24KDa), β -lactoglobulin(β -Lg)(18KDa) and α -lactalbumin (α -La) (14 KDa).

Keywords: SDS-PAGE, Vechur, Kasargod Dwarf, Milk characterization

1. Introduction

Vechur cattle, which derived its name from its place of origin, is the smallest of all the Indian cattle breeds described. The basis for the evolution of Vechur cattle was the heavy rain and hot humid climate of the area coupled with low input availability (Ravi *et al.*, 2006).The breed was on the verge of extinction in the 1980s when a massive crossbreeding programme was undertaken by the Government to increase the performance of non-descript cattle in the country. This breed is listed by the Food and Agriculture Organization of the United Nations in their Domestic Animal Diversity Information System. The cattle are also placed in the breed map of cattle listed by the National Bureau of Animal Genetic Resources, ICAR, India (NBAGR, 2001).

Kasargod Dwarf cattle are native to Kasargod, the northernmost district in Kerala. This variant of cattle is also found in Mangalore, Coorg, and some other parts of Karnataka. These are well known for their low feed to milk ratio and resistance to hot and humid climates. Despite these facts, the breed is not included in the list of native breeds of India documented by the National Bureau for Animal Genetic Resources, Karnal, Haryana. Conservative initiatives are undertaken by the farmers' organizations in the state to increase the number of pure Kasargod Dwarf cattle.

During the past few years, investigation studies of genetic polymorphism in bovine milk proteins have gained increased attention, owing to the rising interest in the bioactive proteins of milk. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis is commonly used for high resolution analytical separation of complex proteins like milk proteins. Milk proteins are commonly classified into two categories: Caseins (80% of total proteins), which are insoluble proteins and whey proteins (20%) that are soluble proteins. The caseins are further fractionated as α -s1 α -s2, β and κ . These four casein fractions can be separated using SDS- PAGE in the presence of a reducing agent, giving four bands

corresponding to α - s1 α -s2, β and κ caseins in the increasing order of their electrophoretic mobility.

2. Methods

Fresh raw milk was obtained from Vechur and Kasargod Dwarf cows, conserved in the livestock farm, Kerala Veterinary and Animal Sciences University, Mannuthy. The milk was defatted by centrifugation at 5000g for 30 min at 5°C. The defatted milk was then acidified to pH4.6 using 1NHCl followed by centrifugation at 5000g for 10min. The casein and whey fractions were separated and were freeze-dried and stored at -20°C until further analysis. In the present study, SDS PAGE was used to evaluate the casein and whey protein fractions of Vechur and Kasargod Dwarf milk. The reagents used and procedure followed are explained below:

3. Reagents

- **Acrylamide Solution 30%:** Exactly 29.2 g of acrylamide and 0.8 g of bisacrylamide were dissolved in double distilled water and volume was made up to 100 mL. It was stored at 4°C in amber colour bottle.
- **Electrode buffer (5X):** Exactly 7.5g of Trisbase, 2.5g of SDS and 36g of Glycine were dissolved in 300 mL of double distilled water and pH was adjusted to 8.3 with concentrated HCl. Final volume was made up to 500 mL with double distilled water. Working electrode buffer was prepared by taking 100 mL of electrode buffer (5X) and diluting it to 500 mL, it was stored at 4°C
- **10% SDS Solution:** Exactly 10 g of SDS was dissolved in 75 mL double distilled water and volume made up to 100 mL. It was stored at room temperature in plastic container till further use.
- **0.5MTrisHCl (pH6.8):** Exactly 6.05g of Trisbase was dissolved in 60 mL double distilled water and pH was adjusted to 6.8 with concentrated HCl. Volume was made to 100 mL and stored at 4°C.
- **1.5MTrisHCl (pH8.8):** Exactly 18.15g of Tris base was dissolved in 60 mL double distilled water and pH was

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adjusted to 8.8 with concentrated HCl. Volume was made to 100 mL and stored at 4°C.

- **10% W/V Ammonium Per Sulphate (APS):** APS solution was always prepared fresh by dissolving 100 mg of APS in 1mL double distilled water.
- **Anode Buffer (0.2M Tris, pH 8.9):** Exactly 96.88 g of Tris base was dissolved in 1000 mL of double distilled water and pH was adjusted to 8.9 with concentrated HCl. Total volume was made up to 4000 mL and was stored at 4°C.
- **Cathode Buffer (1M Tris, 1M Tricine, SDS):** Exactly 12.11 g of Tris base, 17.92 g of Tricine (SRL) and 1.0 g of SDS were dissolved in 300 mL of double distilled water and volume was made up to 1000 mL. The pH of the solution was around 8.2 ± 0.2 . The buffer was stored at 4°C.
- **2X Sample Buffer:** Sample buffer was prepared by dissolving the substances given below and final volume was made to 20 mL with double distilled water and stored at 4°C till further use.

Table 1: Composition of Sample buffer

Sl No	Components	Resolving gel 15%	Stacking gel 6%
1	Distilled water (mL)	5.75	8.75
2	Acrylamide: 30% (mL)	12.5	2.03
3	Tris HCl: 1M, 6.8 pH, (mL)	-	3.9
4	Tris HCl: 1.5M, 8.8 pH, (mL)	6.25	-
5	SDS: 10% (μL)	250	156
6	APS: 10% (μL)	250	156
7	TEMED (μL)	10	31

- **Staining Solution:** Exactly 100 mg of Coomassie Brilliant Blue – G250 (0.025%) was dissolved in a solvent mixture containing methanol, acetic acid and water in the ratio of 5:4:1. The staining solution was filtered and stored at room temperature.
- **Destaining Solution – 10% Acetic Acid:** Exactly 10 mL of glacial acetic acid was made up to 100 mL with double distilled water just before use.
- **Gel Solutions:** Gel solution was prepared as described in the Table 2. APS and TEMED were added at the last just before casting the gel.

Gel Preparation: The separating gel was 15%, and the stacking gel was 4%. All solutions were stored at 4°C. The electrophoresis was performed in Bio-Rad Mini-PROTEAN electrophoresis system gel electrophoresis unit. The gel mixtures were gently poured in the casting modules. After filling, the separating gel (8 cm deep) was carefully overlaid

with 1-2 mm deep layer of distilled water to allow a truly flat surface and protect the top of the gel mixture from atmospheric oxygen. After polymerization, the distilled water was replaced by the spacer gel (2 cm deep). The stacking gel was added for about 3 cm deep and soon after adding the combs were inserted. After polymerization of the stacking gel, the comb was removed, and wells were rinsed with cathode buffer.

Sample Preparation: Casein solutions were diluted by adding an equal volume of 2X sample buffer (1:1) and heated at 100°C for 5 min, centrifuged and immediately stored at 4°C. Wide range molecular weight standards from 10 kDa to 250 kDa (Sigma-Aldrich) were used as standards. Samples and standards were applied under the cathode buffer

Table 2: Composition of Gel Solutions

Sl. No	Substance	Quantity
1	Glycerol (SRL)	1.60 mL
2	10% SDS	3.20 mL
3	β – Mercaptoethanol (Hi-Media)	0.80 mL
4	0.5 M Tris-HCl (pH 6.8)	1.0 mL
5	Distilled water	1.0 mL
6	Bromophenol blue (Hi-Media)	2.5 mg

Run Conditions: Electrophoresis was performed at room temperature using constant voltage. Voltage was kept constant at 40V until the samples completely left the stacking gel and in the separating gel it was increased to 60V, and then voltage was maintained constant (90-100V) until the tracking dye reached the bottom of the gel.

Staining with Coomassie Brilliant Blue R-250: Immediately after the end of electrophoresis, gels were removed from the plates and the gel containing the marker and samples was cut and placed in a fixative solution containing 50% methanol and 10% acetic acid. After 30min, the fixative solution was replaced by a staining solution containing 0.1% Coomassie Brilliant Blue R-250, where the gels were left for 30min. After staining, the gels were transferred to the de-staining solution at room temperature. De-staining was done till the bands appear and background becomes clear.

4. Results

The electrophoretic pattern of proteins separated from skim milk (1:40 diluted), casein (0.6 mg/mL) and whey (1 mg/mL) are presented in Figure 20.

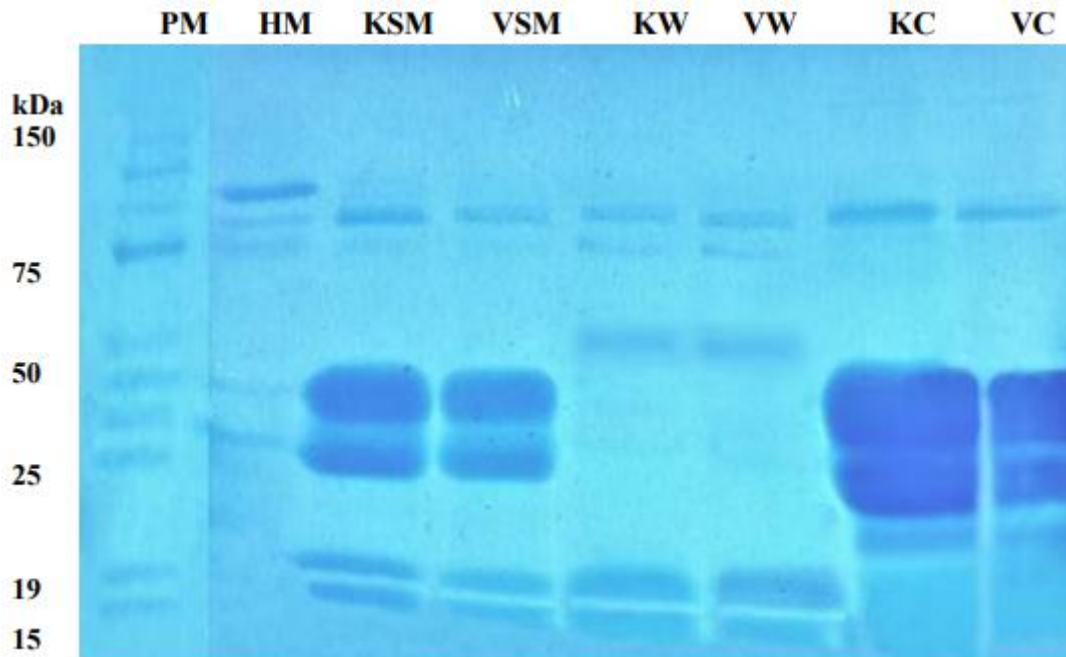


Figure 1: Electrophoretic pattern of milk proteins

PM: Protein marker HM: Human milk KSM: Kasargod Dwarf Skim milk VSM: Vechur skim milk KW: Kasargod Dwarf Whey VW: Vechur whey KC: Kasargod Dwarf casein VC: Vechur Milk casein

Figure 1 depicts the presence of approximately seven protein bands in Kasargod and Vechur dwarf breeds' skim milk. Lactoferrin (Lf) (76 KDa), immunoglobulin (Ig) (66 KDa), α -S1caseins (α -S1-CN) (23KDa), α -S2caseins (α -S2-CN) (21KDa), β -casein(β -CN) (24KDa), β -lactoglobulin (β -Lg) (18KDa) and α -lactalbumin (α -La) (14 KDa) were among the identified fragments. Human milk was also compared to Kasargod Dwarf and Vechur milks, revealing the existence of secretory component (SC)(78kDa) and serum albumin (B) (66kDa), as well as the absence of β -lactoglobulin (Lg) 18 kDa. Many other researchers have observed similar findings (Huma *et al.*, 2018; Kausar *et al.*, 2017).

Lactoferrin, one of the proteins present in milk is acclaimed for its therapeutic and antioxidant potential. Anisha *et al.* (2012) successfully characterized the full coding region of the Lf gene isolated from Vechur cow milk, by expressing the gene in a prokaryote system. The studies demonstrated that the isolated lactoferrin had enhanced antimicrobial and antioxidant activity compared to that from other cow milk. The recombinant lactoferrin expressed in the bacterial system showed remarkable suppression of bacterial growth similar to the level of ampicillin in the strains of *E.coli*, *Staphylococcus* and *Streptococcus* (Chinnamma *et al.*, 2015). Single nucleotide polymorphisms detected in the Lf gene revealed the presence of a higher amount of arginine, which illustrates the enhanced bioactivity of Lactoferrin in Vechur milk (Anisha *et al.*, 2012). These findings strengthen the scope of utilization of Vechur cow milk in therapeutic and medicinal preparations. Anu *et al.* (2018) performed the whole-exome sequencing of Kasargod Dwarf and the detailed genome study revealed the unique characteristics responsible for its rare phenotypes, such as short stature, heat tolerance, disease resistance and lower feed requirement. Studies revealed that lactoferrin isolated from colostrum of Kasargod Dwarf cattle possesses more effective antioxidant

potential when compared to commercially available bovine lactoferrin (Aswathy *et al.*, 2020).

5. Conclusion

Hence the present study revealed the protein fractions of milk from Vechur and Kasargod dwarf cattle. The defatted milk samples were compared for protein fragments using SDS-PAGE. The results unveiled the presence of 76kDa protein fragment in both the milk which seem to be Lactoferrin (Lf). Other fractions observed were immunoglobulin (Ig) (66 KDa), α -S1caseins (23KDa), α -S2caseins (21 KDa), β -casein(24 KDa), β -lactoglobulin (18 KDa) and α -lactalbumin (14 KDa). The milk from indigenous cattle breeds is claimed to possess nutritional and therapeutic properties and to prove the worth of consuming and asserting the beneficial health effects of milk from indigenous breeds, a comprehensive clinical or animal study is required.

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