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Research Article

COMPREHENSIVE ANALYSIS OF PHYSICOCHEMICAL PROPERTIES AND BACTERIOLOGICAL QUALITY OF YAK, DZOMO, AND HILL CATTLE MILK FROM THE NORTHERN HIMALAYAS

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ABSTRACT: The current investigation was aimed to assess the physicochemical and bacteriological quality of raw milk (N=223) obtained from Yaks, Yak hybrids (Dzomo) and Indigenous hill cattle (Pahari) reared in high-altitude regions of the Northern Himalayas. Results revealed significant variations in the milk composition among the three species. Hill cattle milk exhibited a pH of 6.46 ± 0.11, specific gravity of 1.028 ± 0.078, 4.40 ± 0.14% fat, 8.58 ± 0.30% SNF, 13.14 ± 0.58% TS, 4.54 ± 0.05% lactose, and 3.50 ± 0.10% protein. Dzomo milk had a pH value of 7.10 ± 0.05, specific gravity of 1.028 ± 0.013, 7.03 ± 1.80% fat, 6.13 ± 1.61% SNF, 12.75 ± 1.24% TS, 2.61 ± 0.81% lactose, and 2.53 ± 0.79% protein. Yak milk had a pH of 6.78 ± 0.13, specific gravity of 1.027± 0.011, 5.25 ± 0.69 % fat, 8.58 ± 0.21 % SNF, 12.72 ± 0.71 % TS, 4.46 ± 0.24 % lactose, and 3.31 ± 0.16 % protein. 9.4% of the samples had total viable counts more than the permissible limits set by the Food Safety and Standards Authority of India (FSSAI). Only 2 samples were found to contain *E. coli* indicating hygienic practices being followed in the study region. The overall results revealed good quality of milk, reflecting fairly good animal husbandry practices being adopted by farmers in high-altitude regions.

Keywords: Yak, Dzomo, Milk, Chemical composition, Bacteriological quality, *E. coli*, Public health, Himalayas.

INTRODUCTION

Among various sources of foods, animal-origin products such as milk, meat, eggs, etc. are important sources of biological proteins and are also considered high-value commodities to humans [1]. Therefore, together with nutrition, they are an integral component of major economies. Among these, milk is one of the most widely consumed foods and a sizable number of people consume milk and milk products daily in their diet.

Himachal Pradesh, a northern Himalayan state of India is bestowed with great biodiversity in flora and fauna. It is rich in domesticated species like yak, hill cattle (recently recognized as the "Pahari cow" breed by NBAGR), Gaddi sheep, and poultry (Himsamridhi). The hill cattle along with yak and their hybrid (dzomo) are a major source of milk for people living in high-altitude regions such as Kinnaur, Lahaul, and Spiti of Himachal Pradesh [2]. It is believed that the milk of hill cattle has better quality and has an edge over crossbreeds [3]. However, comprehensive studies on these parameters are very meager.

In Kinnaur, Lahaul, and Spiti regions, yak and dzomo are considered the lifeline of highlanders. Yak (*Bos grunniens*) is a prominent milk-producing animal inhabiting the high altitudes of the Himalayan region (2500 - 6000 meters above sea level). They are very resilient to harsh climatic conditions and can survive even at -40°C. They play multiple roles in rural animal husbandry practices. They are a source of milk, meat, fur, and hide. They are also being efficiently utilized for draught purposes in tough terrains. Although, yak milk output is not officially tracked globally but ballpark figure for their annual milk production ranges from 0.7 to 40 million tonnes [4]. Even though yak milk products are economically less significant, they

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are extremely important to the pastoral nomads living in the habitats on the Himalayan foothills. Their milk is an essential part of the herders' daily diet. Herders have used yak milk for centuries to make churpi (soft and hard varieties), ghee, butter, and other fermented products, which have been a significant source of essential nutrients for people, especially those living in high terrains [4]. Although, some studies have been conducted in China on yak milk quality very few studies have been reported from India [5, 6].

Keeping in view the availability of sparse information on various aspects of milk obtained from Yaks, Dzomo, and hill cattle in the literature, the present study was envisaged with the objective of evaluating physicochemical and bacteriological quality in their milk produced at high altitude regions of Himachal Pradesh.

MATERIALS AND METHODS

Sampling of raw milk samples

A total of 170 fresh raw bovine milk samples comprising 60 from hill cattle, 58 from Dzomo, and 52 from Yaks were collected randomly in sterile containers. The sample quantity collected was 50 mL for all the samples. The sampling was done from high-altitude regions and pastures of Kinnaur, Lahaul, and Spiti districts of Himachal Pradesh. The samples were immediately analyzed at the point of collection for their physical parameters (color and odor). Thereafter, all the samples were labeled and transported to the laboratory under ice-cold conditions and stored in the dark at -20 °C till processing to avoid matrix alteration (curdling and protein precipitation).

Physicochemical analyses

All the samples were examined within 48-72 hours in an ultrasonic milk analyzer for chemical parameters which included pH, specific gravity, Fat %, Solids Not Fat (SNF) %, Total Solids (TS) %, lactose %, and protein %, The physical parameters included color and odor examination. Prior to examination, the samples were allowed to thaw to room temperature. After each sample processing, the analyzer was given multiple washings to prevent false positive or false negative readings.

Bacteriological analyses

The bacteriological quality of raw milk samples was evaluated based on total viable count (TVC), Total coliform count (TCC), and occurrence of *E. coli* using standard bacteriological procedures prescribed in IS: 1479, Part III. All the culture media were procured from HiMedia (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Plate count agar (PCA) was utilized for the TVC of milk samples. To estimate the TCC, Violet Red Bile Agar (VRBA) was employed. Eosin Methylene Blue (EMB) agar was used as selective cum differential media for isolation of *E. coli*. Tryptone water, Methyl Red Vogus-Proskauer (MR-VP) broth medium, citrate agar, 10% hydrogen peroxide (H_2O_2), oxidase discs, gram-staining kit, triple-sugar iron (TSI) agar, and 4% urea agar were employed for the biochemical characterization of *E. coli*. Arabinose, dextrose, glucose, lactose, melibiose, maltose, mannitol, mannose, rhamnose, raffinose, sorbitol, sucrose, trehalose, and xylose sugars were used for the sugar fermentation tests.

Total viable count (TVC)

Within 24-48 hours of being collected, the raw milk samples were submitted to TVC analysis. Briefly, 5 sterilized 20 ml capacity glass tubes were filled with 9 ml of the sterilized normal saline solution (NSS) in each of the tubes. A milk sample (1 ml) was added to the first tube and mixed thoroughly making dilution 10-1. Then, 1 ml from the first dilution tube was transferred into the second (dilution 1:100) tube and so on in successive tubes (3^{rd} tube = 1:1000, 4^{th} tube = 1:10,000 and 5^{th} tube 1: 100,000) *i.e.* 10 fold serial dilution procedure was followed. Finally, 1 mL of a diluted aliquot from the 5^{th} tube (dilution factor 105) was poured into the sterile petri dish in duplicate and mixed with 15-20 ml of the molten PCA medium by gently rotating the plate clockwise and anti-clockwise on a leveled surface. Following a 24-48 hour incubation period at 37°C, the results were noted as

TVC (cfu/mL) = number of colonies × dilution factor (10^5)

Total coliform count (TCC)

TCC analysis was performed on milk samples 24-48 hours after they were collected. Samples were diluted with NSS using a 10-fold serial dilution method as followed in TVC. Then, using the pour plate procedure in duplicate, 1 mL of an aliquot from the tube containing the 10^{-2} dilution was placed on a standard Petri plate, and molten sterile VRBA was used as media. After that, the plates were incubated at 37°C for 24 to 48 hours, and the outcomes were reported as:

TCC (cfu/mL) = number of colonies × dilution factor.

Identification of *Escherichia coli*

E. coli was isolated and identified from the samples that tested positive for coliforms. After being inoculated into tryptone water, a single coliform colony was cultured for 24 to 48 hours at 44 °C. The presence of *E. coli* was revealed by the formation of a red color upon the addition of 2-3 drops of KOVAC's reagent. But in order to confirm the presence of *E. coli*, additional biochemical testing (oxidase test, indole test, and citrate test) was performed on the KOVAC's positive samples followed by sugar fermentation tests. For sugar fermentation tests, the sugars used were arabinose, dextrose, glucose, lactose, melibiose, maltose, mannitol, mannose, rhamnose, raffinose, sorbitol, sucrose, trehalose, and xylose as per CLSI guidelines [7].

Statistical analysis

The statistical analyses were performed using IBM® SPSS® statistical package (SPSS Inc., Chicago, IL), version 22 for Windows and Microsoft Excel data analysis tool. The results of the physicochemical study were reported as mean ± SD/SE and were shown in tables and graphs using Excel spreadsheets. The univariate analysis of variance (ANOVA) was utilized in conjunction with Tukey's post hoc test to ascertain the significance of difference ($p<0.05$) among the results.

RESULTS AND DISCUSSION

All the samples had a color and smell that were acceptable. The milk had a white color without any objectionable and unpleasant smell. All the collected samples were deemed appropriate for further quality evaluation. The results of chemical analyses are presented in Table 1. The pH values obtained for all the milk samples were within acceptable range, *i.e.,* 6.4 to 7.1 indicating proper storage condition without any spoilage. The average values of specific gravity for all the milk samples ranged between 1.027 and 1.028 indicating the absence of added water.

The mean fat % was found to be 4.40 ± 0.14, 7.03 ± 1.80, and 5.25 ± 0.69 in hill cattle, dzomo, and yak milk, respectively with an overall mean of 5.55 ± 0.84 %. On the basis of Tukey's post-hoc analysis, the chemical composition of hill cattle and dzomo milk samples differed significantly ($p<0.05$). The highest fat % was observed in dzomo milk. However, no study has been previously conducted on dzomo milk. So the results were compared with chemical analysis of milk obtained from cows. Mahmood and Usman [8] reported fat % in the range of 3.44 to 4.96 % in cow milk from Pakistan. However, the fat % obtained in the present study is higher than those reported by them. Similar work was done by Eisa [9] in Sudan, where the fat % was reported to be 3.38 % in bovine milk.

The mean SNF content in hill cattle, dzomo, and yak milk was found to be 8.58 ± 0.30 %, 6.13 ± 1.61 %, and 8.58 ± 0.21 %, respectively. The overall SNF content was 7.76 ± 0.7 %. There was no significant difference in the SNF content of all the milk samples ($p>0.05$). The results of the present study are comparable with the findings of Patange *et al.* [10] from Parbhani, Maharashtra, and Gangwar *et al.* [11] from Pantnagar, Uttrakhand, wherein they investigated SNF content in indigenous cattle breeds of India (Sahiwal and Deoni). A SNF content higher than hill cattle was reported in those breeds with 9.2 ± 0.05 % in Sahiwal and 8.55 ± 0.01 % in Deoni cattle.

In hill cattle, dzomo, and yak, the mean TS content found was 13.14 ± 0.58 %, 12.75 ± 1.24 %, and 12.72 ± 0.71 % respectively with an overall mean of 12.87 ± 0.8 %. Similar findings were reported by Ahmed *et al.* [12] on dairy cattle milk from Sudan in which they found 12.77 % TS content. There was a statistically significant difference between the TS content of hill cattle and dzomo milk ($p<0.05$). The highest TS content was observed in indigenous hill cattle, similar results have also been reported by Gangwar *et al.* [11] with higher TS content (14.3 %) in the milk of Sahiwal cow.

The mean lactose quantity was found to be 4.54 ± 0.05 %, 2.61 ± 0.81 %, and 4.46 ± 0.24 % in hill cattle, dzomo, and yak milk, respectively with an overall mean of 3.87 ± 0.37 %. Ostan *et al.* [13] reported a lactose content of 5.30 ± 0.29 % in cow milk. Therefore, the values obtained in the current study are less than those reported earlier. Hill cattle milk usually had higher lactose levels, which could affect its digestibility for lactose-intolerant consumers. In contrast, yak and dzomo milk may be more easily digestible for these populations due to relatively lower lactose content.

The mean protein content in hill cattle, dzomo, and yak milk were 3.5 ± 0.1, 2.53 ± 0.79, and 3.31 ± 0.16, respectively with an overall mean of 3.5 ± 0.1. Ozrenk and Inci [14] reported a protein content of 2.83 ± 0.88 % in cow milk from Turkey. A similar study was conducted by Ruska and Jonkus [15] from Latvia, where protein content in dairy cows was found to be 3.43 ± 0.04 % which was comparable to the present study. Franzoi *et al.* [16] also reported the protein content to be 3.46 % in cow milk from Italy. All three

Table 1. Chemical composition of milk samples.

Sample Type	No. of Samples	pH	Specific gravity	Fat (%)	SNF (%)	TS (%)	Lactose (%)	Protein (%)
Hill cattle milk	60	6.46 ± 0.11	1.028 ± 0.078[a]	4.40 ± 0.14[a]	8.58 ± 0.30[a]	13.14 ± 0.58[a]	4.54 ± 0.05[a]	3.50 ± 0.10[a]
Dzomo milk	58	7.10 ± 0.05	1.028 ± 0.013[a]	7.03 ± 1.80[b]	6.13 ± 1.61[b]	12.75 ± 1.24[b]	2.61 ± 0.81[a]	2.53 ± 0.79[a]
Yak Milk	52	6.78 ± 0.13	1.027± 0.011[a]	5.25 ± 0.69[ab]	8.58 ± 0.21[a]	12.72 ± 0.71[ab]	4.46 ± 0.24[a]	3.31 ± 0.16[a]
Overall	170			5.55 ± 0.84	7.76 ± 0.70	12.87 ± 0.80	3.87 ± 0.37	3.11 ± 0.34

*Results are expressed as Mean ± SD; Different superscripts in a column denote significant difference ($p < 0.05$), Tukey's post hoc test).

Table 2. Bacteriological counts for screened milk samples.

Bacteriological quality parameter	No. of samples analysed	Samples exceeding FSSAI limits	Sample source
TVC	170	16 (9.4 %)	Dzomo milk
TCC	170	5 (2.9 %)	Dzomo milk
E. coli	5*	2 (1.18%)	Dzomo milk

*Samples found positive for coliform count were tested for presence of *E. coli*. #FSSAI limit for TVC = 2×10^5 cfu/mL; TCC = 10 cfu/mL.

species tend to be richer in protein which can be beneficial for muscle maintenance, growth, and overall health of consumers. This makes their milk more attractive for consumers looking for protein-rich diets, potentially boosting their market in areas focusing on high-protein consumption.

A detailed review of the literature revealed that Wu *et al*. [17] reported chemical composition with respect to pH, fat, protein, and lactose content in fresh yak milk as: 6.48 ± 0.05, 7.15 ± 0.30 %, 5.04 ± 0.27 %, and 4.99 ± 0.26 %, respectively. The values of yak milk obtained in the present study w.r.t. pH, fat, protein, and lactose content are 6.78 ± 0.13, 5.25 ± 0.69 %, 3.31 ± 0.16 % and 4.46 ± 0.24 %. Therefore, the results obtained in the present study are in agreement with Wu *et al.* [17], who reported similar values for milk obtained from yaks reared in the Tibetan region of the Himalayas. This could be attributed to the similarity in vegetation or fauna on which the animals graze in these regions of the Himalayas. However, the result of the present study is not in agreement with those reported from China, wherein they reported slightly higher values of 6.12 %, 4.95 %, 5.03 %, and 16.88 % for fat, protein, lactose, and total solid content, respectively in yak milk [18].

The variations in milk composition among yak, dzomo (a hybrid of yak and cattle), and hill cattle can have notable implications for nutritional value and consumer preferences. Dzomo having higher fat content compared to hill cattle milk can provide more energy. This is especially important in cold regions where these species are typically found. Yak milk has intermediate fat levels between dzomo and hill cattle, offering a balance between richness and caloric density. Higher fat content can also mean a greater presence of fat-soluble vitamins (A, D, E, K), which enhances the nutritional profile of the milk obtained from yaks and dzomo. Some consumers may choose yak or dzomo milk due to perceived health benefits, such as higher nutrient content, particularly in regions with cold climates where yak milk is traditional. Additionally, the lower lactose content in yak and dzomo milk may make them more appealing for those with mild lactose intolerance. In some regions, the choice of milk can be tied to cultural or economic factors, with certain populations favoring one type of milk over another due to historical preferences or local availability. Hence, exploring these variations more deeply in future studies can help target marketing strategies for dairy products based on consumer preferences and nutritional demands.

For bacteriological quality evaluation, all 170 raw milk samples were processed using the standard protocol established for total viable count (TVC) and total coliform counts (TCC). The mean viable microbial load on the raw milk sample ranged between 5.38 $\log_{10}$ - 7.38 $\log_{10}$ cfu/mL and the total coliform count between 2.74 $\log_{10}$ - 2.90 $\log_{10}$ cfu/ml. Of the samples

examined, 16/170 (9.4 %) were found to exceed limits established for TVC as per microbiological requirements set by FSSAI (Table 2). On VRBA, 5 samples *i.e.* 2.9 % were found to contain coliforms. Two out of five coliform-positive samples tested positive for *E. coli* on the basis of cultural, morphological, and biochemical characteristics.

However, in chemical analysis, the samples were found to be within an acceptable range of pH. However, the presence of a total viable count above permissible limits in 9.4 % of samples could be attributed to poor hygienic practices adopted by a few farmers owing to a lack of awareness/education. However, most of the samples (90.6%) were found to be acceptable in the context of the microbiological requirement of milk. Therefore, the results indicate that most of the animal handlers in the study region have adequate knowledge of clean milk production. The absence of coliforms in 165 samples (97.1%) indicates good collection and storage practices adopted by the farmers. Since coliforms are indicator organisms for fecal contamination, therefore their absence from the majority of raw samples indicates good hygienic practices adopted by the rearers. Since, only 1.17 % of samples tested positive for *E. coli*, therefore the results reflect the good husbandry practices of the people in high altitude regions of Himachal Pradesh. The results are consistent with the observations made during reconnaissance surveys and field visits to the study area that people involved in animal husbandry practices in Kinnaur, Lahaul, and Spiti regions follow hygienic measures, especially during milking of their animals. They wash the utensils and dry them up under sunlight before using them for milk collection. Further, hand washing is usually followed for milking animals. Moreover, the animal shed is routinely cleaned because the dung of animals is being used as manure promoting natural farming practices. Together with these facts, other obvious reasons for the low bacterial count in raw milk could be attributed to low temperatures in the study regions throughout the year thereby allowing fewer microorganisms to multiply during storage. Therefore, the overall bacteriological examination results indicate that farmers in the study region have a fair knowledge of clean milk production.

However, even though the study reports low contamination rates, the potential health risks associated with *E. coli* contamination in milk are serious and warrant further attention. The potential sources of contamination could be improper handling or storage of milk, cross-contamination from animal feces during milking or processing, or through use of unclean water for cleaning utensils. Despite low contamination rates, *E. coli* in milk, particularly pathogenic strains can pose serious health risks, including gastrointestinal infections, diarrhea, and in severe cases, hemolytic uremic syndrome (HUS), which can lead to kidney failure. Contamination usually occurs due to poor hygiene during milking, improper handling, or raw milk consumption. Therefore, milk should always be pasteurized milk to avoid *E. coli* risks. It should be handled and stored safely below 4°C. Producers should always ensure hygienic milking practices and regular testing for contaminants.

The results of the present study can be compared with an earlier conducted study by Bandyopadhyay *et al*. [19] on yak milk and churpi (a fermented yak milk product). 77.7% of the churpi samples and 48.6 % samples of yak milk were found positive for *E. coli*. Similarly, in Egypt to find out how common it was and how dangerous it could be for raw milk and its derivatives to contain *E. coli*. Out of 187 dairy products analyzed, *E. coli* was isolated from 55 (76.4%) raw milk samples [20]. However, in the present study, the prevalence of *E. coli* was found to be only 1.17%, which is much lower than the prevalence reported in these previous studies. Therefore, it can be concluded that the quality of milk is impacted by management practices. However, there is always a scope to educate the farmers on various aspects of hygienic milk production to make animal rearing a sustainable system with the potential for the export of hill cattle, dzomo, and yak milk.

This study opens new avenues for future research such as exploring the impact of seasonal variations on milk quality and impact of different feeding practices influence the physicochemical properties of milk and exploring the microbial diversity in milk. There is also a scope to evaluate how environmental factors, like temperature and humidity, affect milk's physicochemical characteristics and bacterial counts in study regions.

CONCLUSION

Information on the physicochemical and bacteriological quality of milk produced in high-altitude regions of the Himalayas is very meager. No comprehensive study was conducted on this aspect in the past. The overall results of the current study affirmed that the physicochemical and bacteriological properties of milk obtained from hill cattle, dzomo, and yak had acceptable values and therefore conformed to the quality and established standards. However,

detection of coliform especially *E. coli* in some of the samples can be a significant health concern especially when the information on such aspect is missing from published literature. Therefore, routine monitoring of animal-origin foods together with extension activities directed towards education and awareness of farmers on food safety and quality is very essential and recommended.

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