# Recent Advances in Processing of Non-bovine Milk and Milk by-products

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## Editors

Yogesh Kumar
Shahaji Phand
Swagatika Priyadarsini
Sushrirekha Das

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**Editors:** Yogesh Kumar, Shahaji Phand, Swagatika Priyadarsini and Sushrirekha Das

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# PREFACE

The consumption of non-bovine milk and milk by-products has gained significant attention in recent years, owing to their nutritional, medicinal, and economic potential. With diverse varieties across regions—such as camel, sheep, goat, and donkey milk—the processing and utilization of these milk products have become a crucial area of research and innovation. Despite their immense potential, the technologies and methods associated with the processing of non-bovine milk have not been as widely explored or standardized as those for bovine milk.

In response to this growing need, the ICAR-National Research Centre on Camel, Bikaner, in collaboration with MANAGE, Hyderabad, organized an online training program on "Recent Advances in Processing of Non-Bovine Milk and Milk By-products." This training program aimed to address critical knowledge gaps, share research findings, and foster collaboration among scientists, industry professionals, and academics working in the field of dairy science and technology.

The e-book you are holding is an outcome of this comprehensive and collaborative effort. It brings together contributions from experts who have shared their insights on various topics such as innovative processing techniques, challenges, and opportunities in the utilization of non-bovine milk and by-products. From the microbiological and chemical properties of non-bovine milks to novel processing technologies and product development, this compilation seeks to provide a holistic view of the advances in the field.

The e-book serves as a valuable resource for researchers, practitioners, and students interested in dairy science, food technology, and animal husbandry. It is our hope that this compilation will not only further scientific inquiry in this important area but also encourage greater application of research findings for the benefit of farmers, dairy producers, and consumers alike.

We extend our gratitude to all the contributors, participants, and collaborators who have made this endeavor possible. Without their commitment to knowledge-sharing and passion for advancing this field, this publication would not have come to fruition.

**Yogesh Kumar**
**Shahaji Phand**
**Swagatika Priyadarsini**
**Sushrirekha Das**

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**CHAPTER- 1**

# Sustainability in non-bovine milk based dairy industries: Opportunities, challenges and way forward

A. Sahoo*

ICAR- National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka

*Correspondence: directornianp@gmail.com

## Introduction

The non-bovine population, viz. sheep, goat, camel and equines has got significance bearing with respect to their contribution to rearing communities as a source of meat, milk and fiber from sheep and goats, milk, draught power and fiber from camel and principally, transportation in case of equines. The milk from donkeys has recently been emphasized due to its nutraceutical value. Camel and equines also serve in defense operations (war and watch). Invariably, milk from these non-bovine species has gained its importance due to nutraceutical and therapeutic usage in various human ailments. In particular, the social-bondage, environmental-linkage, and dependence of pastoral communities with these livestock species has multiferous benefit to mother 'Earth' besides their contribution in the form of produce and byproducts like meat, milk, fiber, manure, skin and bones. The conventional management practices of these non-bovine species do not require specialized skill and manpower, but considering the challenges of animal sectors to fulfil the requirement of food from animals without increasing their population, the existing low-input system needs to be upgraded to high input-high output production system to maximize return on investment and to make it environmentally sustainable.

## Contribution of non-bovine milk

Non-bovine milk is the milk produced from species other than cows and buffaloes. Especially, the milk from minor species such as goat, sheep, camel, donkey, mithun and yak are considered as non-bovine milk. Around 81% of the global milk production is contributed by cows, and 15% by buffaloes. Likewise, 2% of the global milk production is contributed by goats, 1% by sheep, and 0.5% by camels. The remaining 0.5% is shared by equines and yaks. In developing countries, one third of the milk production is contributed by buffaloes, goats, camel and sheep, indicating that most of the non-bovine milk is produced in developing countries. In developed countries, most of the milk is produced from cows. Hence, developing countries have opportunities to capture the global market for non-bovine milk. Food regulatory body of country, FSSAI has also laid standards for goat, sheep and camel milk apart from cow and buffalo milk (FSSAI, 2023). Above all, the non-bovine population comprising sheep, goat and camel has only 3.5% contribution to total milk production in the world. Presently, donkey milk is also gaining importance, but no data available with regards to its milk production. This little contribution has greater significance with respect to sustaining livelihood of the rearers and providing them nutrition, manufacturing unique milk products and usage as a source of nutraceutical adjuvants and in various therapeutic applications (Sahoo and Rahman, 2023). It provides the dairy industry with an exceptional opportunity to exploit its characteristics for human benefits, such as use of camel milk as a source for producing medicinal food, and use of donkey milk for producing milk with hypo-allergic qualities for infant nutrition. As against bovine milk, milk from minor dairy species have also been proven to be healthier from infancy to older age owing to their higher digestibility and other nutritive components. Increasing interest in bioactive peptides among researchers has lately augmented the exploration of minor dairy species such as sheep, goat, camel, mare, donkey, mithun and yak.

India is behind the world average, if we compare the small ruminant population vis-à-vis contribution to milk production (Table 6). There is lot of scope particularly in sheep, which is not generally considered as a dairy animal in India. So, the question, "Can sheep promise to be a triple-purpose animal?" and provide meat, milk and fiber seems a definite possibility, if we look at the promising dairy-type sheep breeds in India, viz. Patanwadi, Malpura, Chokla, Muzaffarnagri, Jalauni, Bharat Merino, Avishaan, etc. Selection of dairy traits in these native breeds holds promise that can multiply the return from the sale of sheep milk.

**Table 1.** Small ruminant population and milk production

| Items | World | | India | | % of world population | |
|---|---|---|---|---|---|---|
| | Sheep | Goat | Sheep | Goat | Sheep | Goat |
| Population (in millions) | 1284.85 | 1111.28 | 74.29 | 148.75 | 5.78 | 13.38 |
| Milk animals (in millions) | 259.18 | 219.79 | 9.365 | 32.75 | 3.61 | 14.90 |
| Milk yield (Thousand tonnes) | 10504.5 | 20725.3 | 223.96 | 6070.25 | 2.13 | 29.29 |

## Non-bovine milk as nutraceutical adjuvant

Milk from non-bovine species can be used as successful probiotic carriers as these substrates could facilitate satisfactory probiotic viability during storage and promised to have potential in enhancing the functional properties of probiotics such as gastrointestinal tolerance and adhesion to intestinal epithelium. In general, probiotics isolated from non-bovine dairy sources seem to be harmless and safe to consume after proper preparation and the non-bovine milk and milk products possess tremendous potentials as successful probiotic carriers to humans. Non-bovine milk also has potential application in developing various infant milk formula (IMF). The non-bovine milk from caprine, equine and yak contain higher β-casein: αs-casein ratios to bovine milk and could serve as β-casein or casein concentrate sources for IMF production. More specifically, equine milk (and donkey milk) has several notable similarities to human milk, including total protein, lactose, and ash contents, whey protein: casein ratio and α-lactalbumin: β-lactoglobulin ratio. Similarly, camel milk lacks β-lactoglobulin and can therefore be used to manufacture whey protein ingredients for use in IMF to provide hypoallergenic effects. Caprine milk has also been demonstrated to be an excellent source of prebiotics (e.g. oligosaccharides) and its whey could be a useful source of ingredients (protein concentrates, enriched oligosaccharide fractions) for the formulation of caprine-based IMF. Legally, goat milk can be used as a natural source of nucleic acids monomers and is a suitable and valuable component for the development of tailor-made food, e.g. designer IMF.

Milks from non-bovine animal species are recently gaining momentum mainly due to the fact that they are considered suitable to supplement the needs of special population groups (i.e., infants, the elderly) with inherent and unique functional properties, namely, antimicrobial activity, immunomodulation, and hypoallergenicity, especially highlighting their effect on human health (Aspri et al., 2017). There is strong evidence that domestic non-bovine species only carry A2 beta-casein and A2 milk is an easily digestible product. It has been proposed that the effect of diet on human health and well-being is a “food-matrix effect” rather than effects of various individual food components. Further, characterization of nutraceutical properties in non-bovine milk and dairy products should accounts for following analysis, observations and interpretations.

- Composition and analysis: identification, purification, and characterization of bioactive molecules
- Dairy products: milk processability and carbon footprint, and improving the functionality of non-bovine milk proteins
- Health benefits: impact of processing on biomolecular activities (bioactive evaluation *in vivo* and *in vitro*), nutritional properties, and allergenicity.

## Salient health benefits of non-bovine milk

Researchers have reviewed the chemical composition of non-bovine milk and the role of various bioactive components in human health (Sahoo and Rahman, 2023). Some of the salient health benefits are elaborated here for elucidating its importance, possible opportunities, challenges and way forward for establishing non-bovine milk based dairy industries in India and other camel-rearing countries like Saudi Arabia, Africa, Pakistan, Mongolia etc.

- Goat milk has been documented to show anti-viral, anti-diabetic, anti-hypertensive, anti- carcinogenic, anti-allergic, anti-inflammatory, angiotensin converting enzyme (ACE) inhibitor activity, antimicrobial, hypocholesterolemic, cell proliferation, prebiotic activity, increasing mineral uptake, and anti-appetizing effect (Nayik et al., 2021).
- Sheep milk and its products have been shown to have anti-inflammatory, anti-diabetic, anti- cancerous, anti-viral and ant-bacterial effects. In addition, because of its CLA and Orotic acid content, it was reported to prevent type-2 diabetes, Alzheimer's disease, and cancer (Flis and Malik, 2021).
- Camel milk is popularly known for its anti-diabetic properties because of its antioxidative effect to restore insulin and HbAlc levels (Meena et al., 2016). The antioxidative effect of camel milk could be because of its selenium and high vitamin C content (Barłowska et al., 2011). Peptides derived from camel milk proteins have shown significant antioxidant, anti-diabetic, anti-obesity, antihypertensive, antibacterial, antibiofilm, anticancer, anti-inflammatory, anti-haemolytic and anti-hyperpigmentation activities (Redha et al., 2022).
- Donkey milk appears to be closer to human milk, especially in terms of lactose and protein content. Historically, it is considered as therapeutic food and cosmetic product. Several studies confirmed that donkey milk has high digestibility, antiviral, antimicrobial, antioxidant, antitumor, anti-inflammatory, anti-allergic, anti-atherogenic, anti-aging, and hypocholesterolemic effects (Balos et al., 2023).
- Yak milk also has potential to show antihypertensive, anti-inflammatory and antioxidant properties. The antihypertensive properties might be because of its unique peptides and the anti-inflammatory properties, promotion of heart health and brain function could be due to higher levels of beneficial fatty acids, viz. conjugated linoleic acids, omega-3 fatty acids, etc. (Kalwar et al., 2023).
- Mithun milk is a nutrient rich food that contributes to nutritional security of the pastoral communities. It is also rich in vitamins A, D, and E, calcium and polyunsaturated fatty acids (PUFA), linoleic and linolenic acids (Devi et al., 2023).

## Technological properties of non-bovine milk

One of the major problems associated with non-bovine milks is the development of dairy products that can compete with bovine dairy products. Among the milk of non-bovine species, sheep milk is better for cheese making because of its high protein, fat and calcium per casein unit. The milk of other non-bovine species is good for direct consumption; hence some technological interventions are needed to develop dairy products conventionally made from bovine milk (Barlowska et al., 2011). It has been stated that a considerable diversity exists in the analyzed parameters and traits of milk, which results in various directions of milk utilization. Sheep milk with high protein, including casein, and fat, make it a very good raw material for processing, especially cheesemaking. The composition of goat milk allows using it as the raw material for dairy processing and also to some extent as a therapeutical product (low content or lack of αs1-casein). Milk from equine species (donkeys) have the most comparable protein composition to human milk (low content of casein, lack of αs1-casein fraction and β-lactoglobulin, and high content of lysozyme) and additionally, characterized by a fatty acid profile distinctive from milk of other analyzed animal species. Camel milk also has valuable nutritional properties as it contains a high proportion of antibacterial substances and 30 times higher concentration of vitamin C in comparison to cow milk. Therefore, identifying the technological properties for processing in to various marketable products is always challenging because cow milk accounts for more than 80% of world milk production and supplies the most universal raw material for processing as reflected in the broadest spectrum of manufactured products. However, the e-commerce platforms depict promising market for non-bovine milk products, such as milk powders, milk soaps etc. In European countries, milk of goat and sheep as well as cheeses prepared from such milks are sold at premium rates and methods have been prescribed for admixing of cow milk into such milks as well as cheeses prepared from milk of minor species (EU, 2018).

Since the composition of non-bovine milk may affect its processability, it would be important to further investigate the relationship between the composition of non-bovine milk, milk biomolecules, processing, and physicochemical properties. Such links will help to better understand the impact of processing (e.g., fermentation, thermal and non-thermal processing, new and advanced processing technologies, etc.) on the nutritional properties and allergenicity of such milks. These studies will help to provide more suitable process conditions to develop valuable dairy products without compromising their health benefits for consumers.

## Socio-economic constraints and marketing challenges

Non-bovine milk is an important food category due to their versatility, their health benefits, and their availability to people living in climatic conditions unsuitable for the traditional dairy cow (e.g., harsh, arid conditions). It is noteworthy to say that milk from non-bovine species has unique properties that can resolve as nutraceutical adjuvant for various human ailments. The constraints in separate collection, processing and packaging, pricing and distribution of milk from the non-bovine species seem to be primary reasons of low adoption and utilization by the needy people. The other factors can be categorized into nutrition and feeding, breeding and reproduction, diseases, parasites, climate change, and underdeveloped dairy products market. In this scenario, there is the need to expand the markets by further sensitization on the nutritional and medicinal advantages of non-bovine milk and milk products. Traditionally non-bovine milk that is drunk in raw or naturally fermented form has now undergone unprecedented transformations such as pasteurization, directed fermentation, cheese or yoghurt processing, and manufacture of milk powder for the export market. Such emergence will pave the way for the stakeholders in the sector to offer diversified products corresponding to the tastes of increasingly urbanized consumers, more sensitive to "modern" products. It is noteworthy to emphasize that the non-bovine livestock sector linked to pastoralists and their life-cycle, which is imperatives upkeep of the environment and socio-economic sustainability of the native and/or tribal communities. According to Kratli and Koehler-Rollefson (2021), pastoralism can be important for conserving biodiversity, maintaining soil fertility, cooling the climate, ethical food production, and healthy diets. The animal produce and products are of organic nature and business perspectives in marketing and upkeep of their bioactive properties seems essential. Some of the important insights on development of non-bovine milk collection, processing and marketing to augment this environmental-friendly and climate-resilient livestock sector are outlined here.

- Expert insights on nutrition for specific population groups: Both sheep and goat milk are increasingly being used to manufacture products with specific health benefits for adults and children;
- Contribution of non-cow milks to health: Learn about milk composition from sheep, goats, camels and other animals and what can these types of milk add to overall health, gut microbiota and immune system;
- Stay ahead of consumer trends: A chance to find out about new consumer trends and the development of products to meet individual consumer needs and wishes. Higher and rising growth rates for buffalo and goat milk indicate a growing appetite for these types of milk.
- Highlighting the important role of milk on global food security and a sustainable food system.

In addition to providing a renewed vision of socio-economic sustainability, policies and social aspects of non-cow milk production, we should explore in line with current market trends and policies, and chances to network with other producers, advisors, develop business contacts, as well as engaging with scientists.

## Conclusion

The problems in production and processing of non-bovine dairy products have been attributed to poorly understood behavior of non-bovine dairy proteins and other variabilities in chemical attributes. There is still a gap in knowledge about these milks' processability and their adaptability to mass production. study of the relationship between the physicochemical characteristics of non-bovine milk and their processability, with particular attention to the bioactive molecules of these milks and their activities (evaluation in vivo and in vitro). Advanced research should aim at elucidating the health-benefiting and techno-functional properties of non-bovine proteins as well as their utilization for the development of new processed products. Therefore, the production of derived dairy products with high nutritional value from such milks requires more in-depth investigation. Carbon footprint studies should be further developed and implemented to develop a sustainable strategy for non-bovine milk production and processing with respect to the health benefits of milk components. It is also advocated for rethinking livestock as partners in food production, rather than machines that input and output.

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CHAPTER- 2

# Regulatory compliances and standards in non-bovine milk and milk products

Rajesh Kumar Sawal

ICAR-National Research Centre on Camel, Bikaner

Correspondence: rksawal01@gmail.com

## Introduction

Milk of different mammalian species differ in chemical composition which is attributed to the nutritional requirements of off springs. However, among them (Table 1), milk of few species is utilized by the mankind Majorly from Cattle, Buffalo, Goat, Sheep, Camel and to small extent from Equines. Looking the trade prospects compositional attributes of domesticated ruminant species have been categorized and recognized by FSSAI/ Institutions recognizing Food safety aspects in different countries as milk and milk products are consumed by mankind.

**Table 1.** Chemical composition of milk of different species

| Species | Water | Fat | Protein | Lactose | Ash |
|---|---|---|---|---|---|
| Human | 87.7 | 3.6 | 1.8 | 6.8 | 0.1 |
| Sow | 89.6 | 4.8 | 1.3 | 3.4 | 0.9 |
| **Non Bovine** | | | | | |
| Donkey | 90.0 | 1.3 | 1.7 | 6.5 | 0.5 |
| Mare | 89.1 | 1.6 | 2.7 | 6.1 | 0.5 |
| Camel | 86.5 | 3.1 | 4.0 | 5.6 | 0.8 |
| Goat | 86.5 | 4.5 | 3.5 | 4.7 | 0.8 |
| Sheep | 79.4 | 8.6 | 6.7 | 4.3 | 1.0 |
| **Bovine** | | | | | |
| Buffalo | 84.2 | 6.6 | 3.9 | 5.2 | 0.8 |
| Cow | 86.6 | 4.6 | 3.1 | 4.9 | 0.7 |
| **Pet animals** | | | | | |
| Dog | 75.4 | 9.6 | 11.2 | 3.1 | 0.7 |
| Cat | 84.6 | 3.8 | 9.1 | 4.9 | 0.6 |
| **Wild animals** | | | | | |
| Reindeer | 68.2 | 17.1 | 10.4 | 2.3 | 1.5 |
| Elephant | 67.8 | 19.6 | 3.1 | 8.8 | 0.7 |
| **Others** | | | | | |
| Guinea pig | 82.2 | 5.5 | 8.5 | 2.9 | 0.9 |

| | | | | | |
|---|---|---|---|---|---|
| Llama | 86.5 | 3.2 | 3.9 | 5.6 | 0.8 |
| Porpoise | 41.1 | 45.8 | 11.2 | 1.3 | 0.6 |
| Whale | 70.1 | 19.6 | 9.5 | - | 1.0 |

**Regulatory compliances**

Differences in the compositional variability are observed during the entire lactation length of the species however, basal standards have been devised, so that customer gets milk with the required minimal composition for which two basic parameters have been optimized for the milk packaged by dairies in general so that the customer gets similar product regularly for consumption. It has also helped to maintain standards even among dairy entrepreneurs' else differences in quality affect pricing of milk in general as milk collected by the vendors/ farers is usually mixture of cattle/ buffalo/ sheep/ goat/ camel milk. Similar to standards for fluid milk, norms have also been formulated for preparation of other dairy products which need to be followed by the traders to maintain quality.

**Table 2.** Natural range of essential components as in milk

| **Bovine milk** | **Milk Fat %** | **SNF%** |
|---|---|---|
| Cow milk | ≤ 3.5 | ≤ 8.5 |
| Buffalo milk | ≤ 6.0 | ≤ 9.0 |
| Goat/ Sheep milk | ≤ 3.0 | ≤ 9.0 |
| Mixed milk | ≤ 4.5 | ≤ 8.5 |
| **Non-Bovine milk** | | |
| Camel milk | ≤ 2.0 | ≤ 6.5 |
| Donkey milk | ≤ 2.0 | ≤ 6.0 |

***Note***: Protocol has to be prepared using large sample data

Milk is normal mammary secretion derived from animals. This fluid is used by mankind in different forms. Experimentation and entrepreneurship in the field of Dairy Technology/ Engineering; different type of products could be developed to meet consumer requirements. Standard for each product has been developed for milk-based products so that if same product is prepared elsewhere, similar nutritional components are expressed in the product and is prepared with similar processes. Many such products and process have been outlined

- Food products & standards
- General standards/ procedures
- Boiling –refers to process of heating under atmospheric pressure
- Analogue product-not derived from milk
- Composite milk product- milk is essential component of product

- Srikhand-contains fruits
- Ice cream containing fruits
- Flavored fermented milks
- Drinks based on fermented milks
- Heat treatment-pasteurization, sterilization, boiling, UHT milk
- Milk product- refers to product made from milk
- Cheese, Milk powder, Cream, Sweetened milk, Ghee, Lassi, Khoa

## Different processes

- Pasteurized milk- heating milk to at least 72$^{O}$C and holding at such temperature continuously for at least fifteen seconds
- Reconstituted milk-adding water to milk powder appropriate milk product composition as per the standards for that product
- Sterilized milk- heating milk to at least 115$^{O}$C for 15 minutes
- Labeling of product
- Adding of essential nutrients to make composite milk products

Different regulations govern establishment of dairy units so that inhouse facilities are maintained for testing of milk/ milk product during different stages of its manufacture by the Dairy processing units. FSSAI being legal requirement for trade, thus every unit need to get the FSSAI license. Apart from its registration, concerned unit is also bound by local/ state industrial norms as registration under MSME, clearance by Pollution Control Board, registration for GST, Trademark etc as outlined below.

## Regulations governing food product registration

- FSSAI legal requirement for any business involved in production,
- distribution, sale of dairy products
- FSSAI ensures the products your sale need to meet quality
- Standards set by the government
- HACCP implementation in Dairy industry
- Trade license
- SSI or MSME registration
- Trade mark registration
- GST registration
- FSSAI Regulatory and supervisory body

Opening up of a Dairy farm is major concern in present day scenario as most of the dairy units have been shifted to the sub-urban areas of the municipal corporations, thus needful permission need to be taken from the district authorities as animal waste disposal is one of the major concern of human and animal health. Other requirements for registration have also been spelled below.

- The prerequisites to run a dairy farm include registration in the local veterinary and dairy development department. For the license to start a dairy farm, one needs to contact Municipal Corporation Department or a local panchayat based on the place where the dairy farm will be setup.
- FSSAI Dairy Registration
- FSSAI Registration is a legal requirement for any business involved in the production, distribution, or sale of dairy products. Here's why it's essential:
- Ensures Food Safety: FSSAI registration ensures that the dairy products you produce or distribute meet the safety and quality standards set by the government.
- Consumer Trust: Having an FSSAI registration instills trust in consumers, as it signifies compliance with food safety norms.
- Legal Requirement: It's a legal mandate. Operating without a valid FSSAI registration can lead to severe penalties, including fines and product seizures.
- Business Expansion: FSSAI registration is often a prerequisite for partnering with retailers, supermarkets, and other businesses.

***To complete your FSSAI dairy registration, you'll need the following documents:***

1. **Identity proof**: Your identity proof, such as a PAN card, Aadhar card, or passport.
2. **Address proof**: Proof of your business address, which can include utility bills, rental agreements, or property documents.
3. **FSSAI declaration form**: This form (Form A or Form B) varies based on the scale of your dairy business.
4. **List of dairy products**: Specify the dairy products you intend to produce or distribute.

**MSME** stands for **Micro, Small and Medium Enterprises.** Registration of the dairy unit under MSME helps the entrepreneur to get financial aid and get recognition among the other industries to improve upon its trade aspect.

1. It provides legal recognition to businesses that are classified as micro, small, or medium enterprises (MSMEs).

2. The certificate helps businesses to access various government incentives and schemes. In India, MSMEs constitute a significant portion of the Indian economy.

## Benefits of having MSME Certificate

1. Enhanced market presence

2. Competitive advantage

3. Access to priority lending

4. Subsidy on trademarks and patents

5. Government incentives and schemes

Thus, regulatory compliances for use of non-bovine milk would help establishment of industrial units, develop products for human health use. This would definitely improve potential of non-bovine milk which is presently in an unorganized sector but is aspiring for its recognition. Apart from trade units it would pave way for saving the germplasm and monetary benefit for the farming community raising livestock as well.

**References will be shared on demand.**

CHAPTER- 3

# Nutraceutical Properties of Sheep Milk: Health Benefits and Recent Developments

Neha Thakur[1*], Raghavendar Singh[2], Laita Garg[1], and Monika Rani[1]

[1]Department of Livestock Products Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana

[2]Division of Biochemistry, ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh

*Correspondence: nehathakur@luvas.edu.in

## Introduction

Sheep milk is a nutrient-dense, creamy dairy alternative known for its high levels of essential vitamins, minerals, and proteins. It offers more calcium, phosphorus, and zinc than cow's milk, supporting bone health and immune function. Its natural sweetness and smooth texture make it ideal for cheese and yogurt production. Although less common than cow or goat milk, it is gaining popularity due to its digestibility, making it suitable for those with lactose intolerance.

Sheep milk's value extends beyond nutrition. It plays an important role in many cultures, particularly in Mediterranean countries, where it is used to produce specialty cheeses like Roquefort, Pecorino, and Manchego. Economically, it supports rural livelihoods and agricultural communities, especially in regions where sheep farming is sustainable due to adaptability to harsh climates and limited resources. The growing focus on sustainability and environmental impact also positions sheep milk as an eco-friendly dairy option.

In India, sheep milk holds significant importance, especially in states like Rajasthan, Gujarat, and parts of South India, where sheep farming is a common practice. While not as widely consumed as cow or buffalo milk, it is recognized for its nutritional value, particularly in predominantly vegetarian regions. Sheep farming in India supports rural incomes and offers a sustainable alternative to traditional livestock practices, helping alleviate poverty and diversify agricultural incomes.

Additionally, sheep milk is used in traditional Indian dairy products like paneer and curd, with potential for expanded use in high-value products like cheese and yogurt. As India moves towards sustainable agriculture, sheep milk production can enhance food security, promote rural development, and contribute to biodiversity conservation. Sheep milk's rich nutrient profile, digestibility, and versatility make it a promising dairy alternative, suitable for health-conscious consumers and those seeking sustainable food options. With its potential for growth in both global and Indian markets, sheep milk represents a valuable opportunity for innovation and expansion in the dairy sector.

## Important Statistics and Market Trends

Globally, sheep milk production has been on the rise, reflecting increased awareness of its benefits. In 2023, global sheep milk production is estimated at around 2.1 million metric tons. Major producers include China, Greece, Turkey, and Spain, where sheep milk is a key component of traditional diets and culinary practices. It is not only a staple but also a sought-after commodity in the dairy market, with products like cheese and yogurt becoming more popular. The market, valued at approximately USD 9.5 billion in 2023, is expected to grow at a compound annual growth rate (CAGR) of 5.2% from 2024 to 2028. This growth is driven by increasing consumer awareness of sheep milk's health benefits and the rising demand for nutritious dairy alternatives. Innovations in dairy processing and product development are also supporting this expansion, catering to health-conscious consumers looking for nutrient-rich options.

In India, sheep milk is gaining popularity, though it is still less commonly consumed compared to cow and buffalo milk. As of 2023, India’s sheep milk production is estimated at about 250,000 metric tons. Key sheep-rearing states include Rajasthan, Gujarat, and Himachal Pradesh, where traditional practices and growing consumer interest are driving increased production. The Indian dairy market is slowly shifting as consumers become more aware of the nutritional benefits of sheep milk.

Several factors are contributing to the rise of sheep milk in India. There is a growing demand for alternative dairy products with unique health benefits. Sheep milk’s higher protein content, richer fat profile, and beneficial nutrients such as calcium, vitamin D, and CLA make it appealing to health-conscious consumers. Additionally, advancements in dairy product development, particularly those by the Central Sheep and Wool Research Institute (CSWRI) in Avikanagar, are improving the availability and attractiveness of sheep milk products. CSWRI’s innovations in product development and production techniques are enhancing the popularity of sheep milk in the Indian market.

## Nutritional Profile of Sheep Milk

Sheep milk is distinguished by its rich nutritional profile, which surpasses that of cow and goat milk in several aspects (Table 1). Understanding these nutritional components is essential for appreciating its health benefits.

***Protein Content***

Dairy products are a reliable source of high quality proteins, which are well balanced in amino acids. Sheep milk contains approximately 5.8 grams of protein per 100 grams, significantly higher than cow milk's 3.3 grams and goat milk's 4.1 grams. This higher protein content is attributed to the unique composition of casein and whey proteins in sheep milk. Making up about 80% of the protein content, casein in sheep milk is predominantly beta-casein, which is easier to digest and less likely to cause allergies compared to the alpha-casein dominant in cow milk. Beta-casein has been shown to improve gut health and support immune function. The remaining 20% of sheep milk protein consists of whey proteins such as lactalbumin and lactoglobulin. These proteins are rich in essential amino acids and contribute to muscle repair and growth.

Bioactive peptides may be produced from goat or sheep milk proteins since their primary structures are close to those observed for bovine proteins.

Nucleosides and nucleotides, which are non-protein nitrogen compounds, are found in high amounts in colostrum but decrease in mature milk. However, mature milk from small ruminants like sheep and goats still contains high levels of nucleotides and ribonucleosides. Ruminant milk mainly has uridine monophosphate (UMP), adenosine monophosphate (AMP), and cytidine monophosphate (CMP), while sheep and goat milk also contain uridine diphosphate (UDP). Goat and sheep milk have lower levels of orotic acid compared to cow milk, but this varies in cow milk depending on the breed, diet, climate, and lactation stage. The differences in nucleotide levels may be due to unique secretion processes in each species, and these compounds, which are part of RNA and DNA, may help with cell renewal, especially in the intestines. There are potential uses, such as adding them to infant formula to boost the immune response to viruses. Pasteurization may reduce CMP and AMP but does not affect orotic acid levels.

***Fat Composition***

Sheep milk has a unique fat composition that makes it nutritionally valuable and distinct from other types of milk, like cow or goat milk. It is particularly rich in fat, with an average fat content of 6-8%, which is higher than cow (3-4%) and goat milk (4-5%). The fat in sheep milk contains a high proportion of medium-chain fatty acids (MCFAs), such as caproic, caprylic, and capric acids, which are easier to digest and have potential health benefits like antimicrobial properties. This makes sheep milk easier to tolerate for some individuals with digestive issues.

In addition to its high total fat content, sheep milk is a rich source of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), which are essential for heart health and have anti-inflammatory effects. The milk also contains higher levels of conjugated linoleic acid (CLA), a fatty acid known for its potential role in weight management, boosting immune function, and reducing the risk of certain cancers.

The fat globules in sheep milk are smaller than in cow milk, which contributes to a creamier texture and better distribution of fat, making it ideal for dairy products like cheese and yogurt. The high-fat content and composition of sheep milk also contribute to the richness and flavor of its dairy products. This unique fat profile, combined with its high content of essential nutrients, makes sheep milk an excellent choice for high-quality dairy production, especially in cheeses like Roquefort and Pecorino. Recent studies have shown that the fat composition of sheep milk can be influenced by factors such as diet, breed, and environmental conditions, which can modify the levels of healthy fatty acids like CLA and omega-3. This makes it a subject of interest for both nutrition and agriculture experts, as selective breeding and dietary adjustments in sheep could enhance the milk's health benefits.

***Mineral profile***

The mineral content in sheep milk is notably higher than in cow and goat milk:

i. Calcium: Sheep milk provides approximately 250 mg of calcium per 100 grams, surpassing cow milk and goat milk. Calcium is essential for bone health, muscle function, and cardiovascular health.
ii. Phosphorus: With about 220 mg per 100 grams, phosphorus in sheep milk supports bone mineralization and energy metabolism. Adequate phosphorus levels are crucial for maintaining a healthy bone structure.
iii. Magnesium: Sheep milk contains higher levels of magnesium (1.7-2.5 mg/L) compared to other types of milk. Magnesium is vital for muscle function, nerve transmission, and regulating blood pressure. It also works alongside calcium to support bone health.
iv. Zinc: Present at about 1.1 mg per 100 grams, zinc is vital for immune function, wound healing, and DNA synthesis. Zinc deficiency can lead to impaired immune responses and increased susceptibility to infections.
v. Iron: Although present in smaller amounts, sheep milk contains more iron (0.01-0.02mg/L) than cow and goat milk, contributing to the production of hemoglobin and the prevention of iron deficiency anemia.

vi. Others: In addition, it contains 15-20 mg/L of potassium, which helps regulate fluid balance, nerve function, and muscle contractions, along with 4-5 mg/L of sodium, necessary for proper nerve and muscle function. Trace elements like copper (0.003-0.005 mg/L) and selenium (0.2-0.4 µg/L) are also present, contributing to antioxidant defense and supporting iron metabolism.

These values may vary based on sheep breed, diet, and environmental conditions but demonstrate the high nutritional value of sheep milk.

***Vitamin profile***

Sheep milk is a rich source of essential vitamins, contributing to its high nutritional value. It contains 50-70 µg of vitamin A per 100 mL, which is important for vision, immune function, and skin health, along with 0.15-0.30 µg of vitamin D, crucial for calcium absorption and bone health. The milk also provides 0.1-0.5 mg of vitamin E, an antioxidant that supports skin health and immunity, and 1.5-4 mg of vitamin C, which is vital for immune function and collagen formation. Additionally, sheep milk contains B vitamins, including 0.05-0.10 mg of vitamin B1 (thiamine) for energy metabolism, 0.3-0.5 mg of vitamin B2 (riboflavin) for energy production and skin health, and 0.5-1 mg of vitamin B3 (niacin) for maintaining healthy skin and nerves.

Vitamin B6, present in amounts of 0.05-0.10 mg, plays a key role in brain development and immune function, while vitamin B12, at 0.5-1 µg, is essential for red blood cell formation and nerve health. Sheep milk also contains 1.5-2 µg of folate (vitamin B9), which is important for DNA production and cell division, particularly during pregnancy. These vitamins make sheep milk highly beneficial for various bodily functions, including immunity, metabolism, and overall health. The vitamin content may vary slightly depending on factors such as the sheep's diet, breed, and environmental conditions.

(Note: All vitamin values are per 100ml of sheep milk.)

**Table 1:** Nutritional Comparison of Sheep, Cow, Goat, and Human Milk

| **Nutrient** | **Sheep Milk (per 100g)** | **Cow Milk (per 100g)** | **Goat Milk (per 100g)** | **Human Milk (per 100g)** |
|---|---|---|---|---|
| **Protein** | 5.8 g | 3.3 g | 4.1 g | 1.0-1.2 g |
| **Fat** | 6.7 g | 3.7 g | 4.5 g | 4.0-5.0 g |
| **Calcium** | 250 mg | 120 mg | 134 mg | 32 mg |

| | | | | |
|---|---|---|---|---|
| **Phosphorus** | 220 mg | 95 mg | 110 mg | 15 mg |
| **Zinc** | 1.1 mg | 0.9 mg | 0.8 mg | 0.4 mg |
| **Vitamin B12** | 0.8 µg | 0.9 µg | 0.2 µg | 0.4-0.5 µg |
| **Vitamin D** | 1.2 µg | 1.0 µg | 0.5 µg | 1.0-2.0 µg |
| **Vitamin A** | 0.4 µg | 0.3 µg | 0.5 µg | 0.6-0.8 µg |
| **CLA** | High | Low | Low | N/A |
| **Omega-3** | Present | Low | Present | High |

## Health Benefits and Nutraceutical Properties

Sheep milk is gaining attention for its remarkable nutritional profile and numerous health benefits. Its unique composition, rich in essential nutrients and bioactive compounds, makes it an ideal candidate for inclusion in a health-conscious diet. Sheep milk's components not only meet daily nutritional requirements but also exhibit several biofunctional properties, making it a potent nutraceutical. This note delves into the health benefits and nutraceutical properties of sheep milk, highlighting its potential as a functional food.

### *Nutrient-Rich Composition*

Sheep milk is nutrient-dense, containing higher levels of essential nutrients than cow or goat milk (as previously discussed in section 3). It is rich in high-quality proteins, including all essential amino acids, and fats like conjugated linoleic acid (CLA), medium-chain triglycerides (MCTs), and omega-3 fatty acids, which support muscle growth, heart health, and weight management. Additionally, sheep milk provides significant amounts of vitamins A, D, E, and B vitamins, as well as minerals like calcium, phosphorus, magnesium, and zinc, which are crucial for bone health, immune function, and overall wellness.

### *Bioactive Compounds and Functional Properties*

Sheep milk is not only a rich source of nutrients but also contains several bioactive compounds with significant health benefits:

i. Bioactive Peptides: These peptides are released during the digestion of milk proteins and have a range of health-promoting properties. They exhibit antioxidant activity, which helps in neutralizing harmful free radicals, thereby reducing oxidative stress and lowering the risk of chronic diseases such as cardiovascular disease and cancer. Additionally, bioactive peptides in sheep milk have antimicrobial properties that help inhibit the growth of pathogens in the gut, and immunomodulatory effects that enhance the body’s immune response.

ii. Oligosaccharides: Present in small quantities, oligosaccharides in sheep milk play a crucial role in promoting gut health. They act as prebiotics, substances that stimulate the growth of beneficial bacteria like Bifidobacteria and Lactobacilli in the gut. These bacteria help maintain a healthy digestive tract, enhance immune function, and protect against infections and inflammatory conditions.

iii. Nucleosides, Nucleotides, and Polyamines: These minor components of sheep milk contribute to various physiological processes. Nucleosides and nucleotides are involved in cellular energy transfer, DNA and RNA synthesis, and the overall repair and regeneration of tissues. Polyamines, such as spermine and spermidine, play a role in cell growth, differentiation, and the modulation of immune functions. They are also involved in protecting cells from oxidative damage and regulating the gut microbiota.

***Health Benefits***

Sheep milk offers a multitude of health benefits due to its unique composition:

i. Cardiovascular Health: The presence of beneficial lipids, such as CLA and omega-3 fatty acids, contributes to improved cardiovascular health by reducing the levels of low-density lipoprotein (LDL) cholesterol (often referred to as "bad" cholesterol) and enhancing high-density lipoprotein (HDL) cholesterol levels. These changes can lead to a lower risk of atherosclerosis and coronary heart disease. Furthermore, the antioxidant properties of bioactive peptides in sheep milk can help in preventing the oxidation of LDL cholesterol, which is a critical factor in the development of heart disease.

ii. Digestive Health: Sheep milk is easier to digest compared to cow milk, largely due to its smaller fat globule size and the higher proportion of short- and medium-chain fatty acids. These properties make sheep milk particularly suitable for individuals with lactose intolerance or those who have difficulty digesting cow milk. The presence of oligosaccharides also supports digestive health by promoting the growth of beneficial gut bacteria, which aids in digestion and helps maintain the integrity of the intestinal lining.

iii. Bone Health: With its high calcium and phosphorus content, sheep milk supports bone density and strength, making it particularly beneficial for older adults and post-menopausal women who are at increased risk of osteoporosis. Regular consumption of sheep milk can help in maintaining bone mass, reducing the risk of fractures, and supporting overall skeletal health. Vitamin D, present in sheep milk, also plays a crucial role in calcium absorption and bone mineralization.

iv. Immune Support and Anti-Inflammatory Effects: Sheep milk contains immunoglobulins and bioactive peptides that possess immunomodulatory properties. These components can enhance the body's natural defense mechanisms, making it more resistant to infections. The anti-inflammatory properties of certain fatty acids, such as CLA, can help in managing inflammatory conditions and reducing the risk of chronic diseases related to inflammation, such as rheumatoid arthritis and inflammatory bowel disease.

## Developments at ICAR-Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan

Sheep milk is used to produce a variety of dairy products such as cheese, yogurt, and butter. These products retain much of the beneficial components of sheep milk, making them ideal for health-conscious consumers. For instance, sheep milk cheese is rich in calcium and phosphorous and contains bioactive peptides that may have antihypertensive effects. The processing of sheep milk also yields by-products that are rich in biofunctional components, offering additional opportunities for developing functional foods and nutraceuticals.

The Central Sheep and Wool Research Institute (CSWRI) in Avikanagar has been instrumental in advancing sheep milk research and development in India. CSWRI has developed a range of sheep milk-based products, including cheese, peda, kulfi, gulab jamun, flavoured milk and paneer. These products are designed to meet consumer demand for nutritious and health-enhancing dairy options. The institute's efforts in product development aim to increase the availability and consumption of sheep milk in India. Research at CSWRI focuses on optimizing the nutritional content of sheep milk through selective breeding and improved feeding practices. This includes efforts to increase levels of beneficial nutrients such as CLA and omega-3 fatty acids, which contribute to the health-promoting properties of sheep milk. CSWRI is also working on enhancing the sustainability of sheep farming practices. This includes developing efficient feeding systems, improving animal health, and reducing the environmental impact of sheep milk production. These efforts aim to make sheep milk a more viable and sustainable option for consumers and producers alike.

## Current trends in sheep milk research

Recent developments in sheep milk research highlight significant advancements in its nutritional benefits, health impacts, and industrial applications. Recent studies have emphasized the superior nutritional profile of sheep milk compared to other dairy sources. Studies have shown that sheep milk may enhance gut health due to its probiotic properties and prebiotic fibers. Regular consumption of sheep milk has been linked to improved gut microbiota composition and reduced markers of inflammation. Moreover, sheep milk's bioactive compounds, including antimicrobial peptides and antioxidants, have been associated with better immune function. Research indicates that the antioxidant activity of sheep milk is notably higher compared to cow's milk, suggesting potential benefits for managing oxidative stress and chronic diseases.

In the realm of dairy technology, innovations are enhancing sheep milk processing and product development. Advances such as microfiltration and high-pressure processing have been shown to preserve the nutritional quality of sheep milk while extending product shelf life. These methods effectively maintain the integrity of essential nutrients and flavors in sheep milk-based cheeses, leading to higher-quality products.

Sustainability and efficiency in sheep milk production are also receiving attention. Research into breeding and management practices aims to improve milk yield and quality while minimizing environmental impact. Genetic improvements in sheep breeds have been shown to enhance milk production and disease resistance, contributing to more sustainable production systems.

Market dynamics and consumer trends are increasingly supporting the growth of sheep milk. There has been a notable rise in consumer demand for alternative dairy products, with sheep milk and its derivatives gaining popularity due to their health benefits. This trend is driving further research into market behavior and product innovation. Consumer preferences for sheep milk-based yogurts and cheeses are on the rise, reflecting a growing acceptance of these products for their unique taste and nutritional advantages.

Recent research has illuminated the impressive nutritional benefits and health advantages of sheep milk, along with advancements in processing technology and sustainable production practices. Studies demonstrating the higher nutrient content, positive health effects, and innovations in dairy processing underscore the growing potential of sheep milk as a valuable component in the global dairy market. Continued research in these areas will be crucial in expanding the understanding and applications of sheep milk, ultimately enhancing its role in promoting health and sustainability.

## Future Directions and conclusion

Sheep milk, with its rich nutritional and bioactive properties, holds great promise in the development of nutraceuticals and functional foods. Its components—proteins, beneficial fats, vitamins, minerals, and bioactive compounds—offer health benefits such as cardiovascular protection, immune support, and improved digestive health. As consumer interest grows in nutrient-dense dairy alternatives, sheep milk is increasingly seen as a valuable addition to health-conscious diets.

The potential applications of sheep milk extend beyond traditional uses, with significant opportunities in global and Indian markets. The rising focus on health and wellness is driving consumer demand for dairy products that offer enhanced nutritional value. Future trends indicate a continued expansion of sheep milk's role in the dairy sector, propelled by advancements in production technology, innovative product development, and greater awareness of its benefits.

Sheep milk also aligns with the growing consumer preference for sustainability and ethical sourcing. Compared to cattle farming, sheep farming typically has a lower environmental impact, which supports the shift towards sustainable agricultural practices. The relatively smaller carbon footprint of sheep milk production makes it a more sustainable option for environmentally conscious consumers.

Furthermore, sheep milk's digestibility and bioactive compounds, including its anti-inflammatory and immune-boosting properties, underscore its potential as a functional food. Recent advancements, particularly in India, highlight increasing research and development in sheep milk production and utilization. Continued innovation and exploration of its benefits will further solidify its role in sustainable, health-focused food systems.

In conclusion, sheep milk presents significant growth potential within the dairy industry. Its unique nutritional profile and associated health benefits position it as a viable alternative to conventional dairy products. As awareness of its benefits grows, sheep milk is poised for greater innovation and expansion, offering both health-conscious consumers and the dairy market new opportunities for growth and development.

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## CHAPTER- 4

# Innovations in Development and Characterization of Probiotics from Milk Sources

Surya Tushir*

ICAR- Central Institute of Post-Harvest Engineering & Technology, Ludhiana, Punjab

*Correspondence: surya.kadian@gmail.com

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## Introduction

The human gastrointestinal tract is home to a vast microbial community, including beneficial, harmful, and conditionally pathogenic microorganisms. These microbes, which include *enterobacteria*, *lactobacilli, lactic acid cocci,* and *bifidobacteria*, interact with each other and the host and further play a role in influencing health. The microbial balance is affected by diet; plant-based foods promote fermenting species, while meat-based foods support putrefactive microorganisms. Beneficial microbes like *lactobacilli* and *bifidobacteria* produce metabolites that suppress harmful bacteria, maintaining gut health. In contrast, pathogenic microbes such as *Clostridium* and *Bacteroides* produce toxins that can cause disease. Factors influencing this balance include diet, water quality, stress, hygiene, health status, drug use, and age. In order to support and maintain healthy gut flora high concentrations of beneficial microbes used are known as probiotics.

Probiotics are live microorganisms that provide numerous health benefits when consumed. They are often referred to as "good" bacteria as they are naturally found in the human gut and play a crucial role in maintaining a healthy digestive system and immune function. Probiotics help balance the gut microbiota, which can enhance overall health and prevent diseases. They are essential for maintaining a well-balanced gut microbiome, promoting overall well-being. Probiotics improve digestion by aiding in the breakdown of food and enhancing nutrient absorption. They also boost the immune system by strengthening the body's natural defenses. Additionally, probiotics have anti-inflammatory properties, which can help alleviate conditions like bloating and irritable bowel syndrome (IBS). Regular consumption of probiotics supports a healthy gut microbiome, leading to improved nutrient absorption, better gut function, and a stronger defense against harmful pathogens. They are particularly important in treating various gastrointestinal and functional disorders. Probiotics come in various strains, each offering unique health benefits.

## Types of probiotics

Probiotics encompass a wide range of microorganisms, primarily bacteria and some yeasts. Each type of probiotic offers specific health benefits, and the effectiveness can depend on the strain, the individual's health condition, and the environment in the gut. Probiotics are available in various forms, including fermented foods, dietary supplements, and fortified foods. Here are some of the most common types of probiotic microorganisms:

1. *Lactobacillus*: This is one of the most common types of probiotics. Different species of Lactobacillus are found in various parts of the human body, especially in the digestive and urinary systems.

   - *Lactobacillus acidophilus*: They are commonly found in yogurt and other fermented foods.
   - *Lactobacillus rhamnosus*: These are known for its benefits in treating diarrhea and supporting gut health.
   - *Lactobacillus reuteri*: These are found in the gut of many animals and humans, beneficial for oral health and reducing colic in infants.

2. *Bifidobacterium*: These bacteria are primarily found in the large intestines and are essential for digestive health.

   - *Bifidobacterium bifidum:* These help with the digestion of dairy products.
   - *Bifidobacterium longum*: They are known for its role in reducing gastrointestinal discomfort and boosting the immune system.
   - *Bifidobacterium breve*: They are found in the intestines and breast milk, it helps break down plant fibers and aids in overall gut health.

3. *Saccharomyces boulardii*: This is a type of yeast used as a probiotic. It is effective in preventing and treating various types of diarrhea, including those caused by antibiotics.

4. *Streptococcus thermophilus*: These strains are commonly used in the production of yogurt, this bacterium helps in the digestion of lactose, making it beneficial for people with lactose intolerance.

5. *Enterococcus faecium*: They are also found in the intestines, this probiotic can help prevent the growth of harmful bacteria and support gut health.

6. *Leuconostoc:* These bacteria are involved in the fermentation process of foods like sauerkraut and kimchi. They help in preserving food and contributing to gut health.

7. *Escherichia coli Nissle 1917*: It is a non-pathogenic strain of E. coli used as a probiotic, known for its benefits in treating inflammatory bowel diseases like ulcerative colitis.

8. *Lactococcus lactis*: It is used in the production of cheese and buttermilk, this bacterium helps in maintaining a healthy gut microbiota.

9. *Propionibacterium freudenreichii*: It is used in the production of Swiss cheese, this bacterium produces propionic acid, which has preservative properties and contributes to gut health.

## Sources of probiotics

Probiotics can be found in a variety of fermented foods and supplements. Probiotic-rich foods can be broadly categorized into dairy and non-dairy sources.

### *Dairy Sources of Probiotics*

Dairy products are some of the most popular and widely consumed sources of probiotics. The fermentation process involved in producing these foods promotes the growth of beneficial bacteria. Milk is the most common source of probiotic bacteria. The diverse microbial communities found in raw milk offer a rich pool of beneficial bacteria with significant potential for probiotic development. Key dairy sources include:

1. **Yogurt:** One of the most well-known probiotic foods, yogurt is made by fermenting milk with bacterial cultures, primarily *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Many commercial yogurts also include other probiotic strains like *Lactobacillus acidophilus* and *Bifidobacterium.*
2. **Kefir:** Kefir is a fermented milk drink that contains a diverse range of probiotic bacteria and yeasts. It is known for its high probiotic content, which can include strains such as *Lactobacillus*, *Leuconostoc*, and *Acetobacter.*
3. **Cheese:** Certain types of cheese, especially aged cheeses like Gouda, cheddar, and Swiss, can contain probiotics. The strains present depend on the type of cheese and its fermentation process.
4. **Buttermilk:** Traditional buttermilk, a by-product of butter making, is fermented and contains probiotics. However, cultured buttermilk, which is commonly found in stores, is specifically fermented with probiotic cultures.

***Non-Dairy Sources of Probiotics***

Non-dairy probiotic sources are essential for those who are lactose intolerant, vegan, or simply prefer non-dairy options. These foods offer a variety of probiotic strains and are often used in traditional diets around the world:

1. **Kimchi: It is a** Korean fermented vegetable dish which is rich in probiotics, particularly *Lactobacillus* species. It is made by fermenting cabbage and other vegetables with a blend of spices and seasonings.
2. **Sauerkraut:** Sauerkraut is fermented cabbage, similar to kimchi but typically without the spices. It is a good source of *Lactobacillus* and other beneficial bacteria.
3. **Kombucha:** This is a fermented tea drink made using a symbiotic culture of bacteria and yeast (SCOBY). Kombucha is known for its probiotic content, including various *Lactobacillus* and *Acetobacter* species.
4. **Tempeh: It is a** fermented soybean product originating from Indonesia and is a good source of probiotics, particularly *Bifidobacterium* and *Lactobacillus*. It is also rich in protein and a popular meat substitute.
5. **Miso:** Miso is a traditional Japanese fermented soybean paste used in soups, dressings, and marinades. It contains beneficial bacteria, such as *Tetragenococcus halophilus*, and is rich in nutrients.
6. **Pickles:** Fermented pickles, not those preserved in vinegar, contain probiotics. The natural fermentation process involves lactic acid bacteria, contributing to their probiotic content.

## Probiotic strain selection

Probiotics can be made from a variety of yeast and bacterial types. The most often utilized are from the genera Saccharomyces, Bifidobacterium, and Lactobacillus. While these strains have many advantages, choosing probiotics with the right capacity has proven to be a difficult procedure that requires consideration of security as well as effectiveness requirements. In order to determine whether industrial scale production is feasible, strain selection involves screening, identifying, and evaluating biomass growth conditions, such as substrates, pH, temperature, and growth kinetics. This allows for the determination of appropriate kinetic parameters for strain comparisons. In order to guarantee the genetic and metabolic stability of particular strains, it is also important to take into account the conditions under which microorganisms are maintained and preserved in stock collections. In this way, the selection of strains can be divided into three stages:

1. Selection and characterization of strains

2. Capacity assessment *in vitro* probiotic

3. Capacity assessment *in vivo* probiotic

***Characterization of strains***

Strain characterization is a comprehensive process used to identify and differentiate microorganisms, particularly bacteria, at the strain level. A combination of phenotypic and genotypic methods is typically employed for accurate strain characterization.

1. Phenotypic methods:

These methods explore the genetic makeup of probiotics to understand their potential functional capabilities. Often serving as the initial step in strain characterization, these analyses can be relatively simple and cost-effective. They include **physiological and biochemical tests**, which evaluate the organism's metabolic capabilities, such as sugar fermentation, enzyme production, and growth conditions. Other methods like **antibiotic susceptibility testing** determines the organism's sensitivity to various antibiotics, while **phage typing** uses specific bacteriophages (viruses that infect bacteria) to differentiate strains. **Serotyping** identifies specific antigens on the bacterial surface, further aiding in distinguishing different strains.

2. Genotypic Methods:

These methods provide a detailed understanding of the genetic diversity and relationships between different bacterial strains. 1**6S rRNA gene sequencing** is a commonly used method for identifying bacteria at the species level. While it provides a general overview of the bacterial species present, it may not offer sufficient resolution for distinguishing between closely related strains. To achieve more precise differentiation, **whole-genome sequencing (WGS)** can be employed. WGS provides a comprehensive genetic blueprint of the organism, allowing for in-depth comparisons between strains, including the identification of specific genetic variations that may impact functionality or pathogenicity. **Pulsed-field gel electrophoresis (PFGE)** further aids in strain differentiation by separating large DNA fragments, creating unique banding patterns for each strain. This technique is often used in outbreak investigations to compare the genetic fingerprints of bacterial isolates. R**ibotyping** uses restriction enzymes to digest ribosomal RNA genes, producing distinct patterns that can differentiate strains. This method is effective for typing and classifying bacteria based on the genetic variation in their ribosomal RNA genes.

***Isolation of Probiotic Strains***

The journey begins with finding potential probiotic candidates. Researchers isolate bacteria from diverse sources like fermented foods (yogurt, kimchi), the human gut itself, or even environmental samples

1. **Sample Collection**

Probiotic strains are usually isolated from natural sources such as human or animal intestines, fermented foods, and dairy products. Common sources include yogurt, kefir, sauerkraut, and infant feces.

2. **Enrichment**

The sample is incubated in selective media that promotes the growth of probiotic bacteria while inhibiting unwanted microorganisms. For example, MRS broth is often used for Lactobacillus enrichment.

3. **Culturing**

The enriched sample is spread on selective agar plates and incubated under suitable conditions (e.g., anaerobic environment, 37°C) to promote the growth of colonies.

4. **Isolation**

Individual colonies are picked and sub cultured on fresh agar plates to obtain pure strains. This step may be repeated to ensure the purity of the isolates.

***In-vitro tests to evaluate probiotic capacity***

Series of tests should be conducted in the laboratory setting to assess the characteristics and potential benefits of isolated strains, particularly those intended for probiotic applications.

1. Growth and survival assays

These assays measure a strain's ability to grow and survive under various conditions that mimic the human digestive tract. This may involve testing tolerance to acidic environments, Bile Salts, Simulated Intestinal Fluid (SIF) and Simulated Intestinal Mucosal Fluid (SIM)

2. Antimicrobial activity assays

It is the strain's ability to inhibit the growth of pathogenic bacteria. It can help compete with and reduce harmful gut microbes. Different assays may be used to assess activity against various pathogens.

3. Enzyme activity assays

Certain enzymes like lactase aids in lactose digestion, amylase and lipase helps in breaking down carbohydrates and fats, respectively and Bile Salt Hydrolase (BSH) breaks down bile salts, potentially aiding digestion and cholesterol reduction.

4. Adhesion assays

It measures a strain's ability to adhere to intestinal epithelial cells. This is crucial for probiotic functionality, as adherent strains can better colonize the gut and exert their beneficial effects.

5. Stress tolerance assays

The human gut environment can be stressful for bacteria, with factors like temperature fluctuations and oxidative stress. It assesses a strain's ability to withstand such stresses, indicating its potential robustness in the gut.

***In vivo tests to evaluate probiotic capacity***

*In-vivo* analysis is crucial for evaluating the efficacy and safety of probiotics in living organisms. It complements in-vitro studies by providing a more realistic assessment of probiotic function within the complex environment of the host. This stage plays a critical role in assessing the safety and efficacy of probiotic strains identified as promising candidates. **A wide range of *in vivo* models, from simple invertebrates to complex human trials, have been employed to investigate probiotic potential.**

1. Animal models:

Animal models are essential tools in preclinical probiotic research. They offer a controlled environment to study probiotic effects before human trials. However, it's crucial to select appropriate models to maximize their predictive value. Rodents (rats and mice) are commonly used due to their affordability, rapid breeding, and well-established genetic backgrounds. They are valuable for exploring basic probiotic mechanisms and preliminary safety assessments. However, significant physiological differences compared to humans limit their translational potential. Rodent models are well-suited for studying immune responses, such as in inflammatory bowel disease and allergies, and exploring the complex interplay between the gut and brain.

2. Larger Animal Models (Pigs)

Pigs have emerged as superior models for probiotic research due to their striking physiological similarities to humans, particularly in terms of digestive system and metabolism. This close resemblance enhances the predictive value of findings. Pigs can be used to model human gastrointestinal diseases, allowing for a comprehensive evaluation of probiotic efficacy and safety. Moreover, their ability to be raised under controlled conditions and the availability of advanced research tools make them ideal for studying probiotic effects on the gut microbiome and immune system.

**3.** Human trials

These trials involve administering probiotics to human participants under controlled conditions to assess their impact on health and well-being. These trials are conducted in 3 phases:

- Phase I: Focuses on safety and tolerability in a small group of healthy volunteers.
- Phase II: Investigates the efficacy of the probiotic in treating a specific condition in a larger group of patients.
- Phase III: Confirms the benefits and risks of the probiotic in a large-scale study involving multiple centres.

## Strain Conservation

There are several ways to preserve probiotic strains for long-term storage. The choice of method depends on factors like desired storage duration and ease of revival. Where immediate access is less important, but maintenance of the characteristics of the species and the strains is the primary objective, long term storage is adopted. Short term storage is mainly done for daily or weekly use. Rich undefined media such as MRS broth LAPTg broth, M17 broth , Elliker broth for LAB and media such as YPG, YGC are used for yeasts. Freeze drying is commonly used for the long term preservation and storage of microorganisms in stock collections as well as for the production of starter cultures for the food industry. Cellular damage may happen during freezing or freeze drying, producing a mixed population of dead and mildly wounded cells with unharmed ones. Damage may not always result in death because wounded cells have the ability to heal and resume normal functions in the right conditions. By using Lyophillization technique strains can be stored for years. This allows for the creation of strain banks for future use, transportation, and incorporation into various applications. Storage under liquid nitrogen, which is also known as cryopreservation can also be used for long term storage. Its ability to maintain near-original viability for extended durations makes it a good option of strain banking and germplasm conservation efforts across various fields.

## Novel Probiotic based products

The most widely used probiotics are strains of lactic acid bacteria like *Lactobacillus*, *Bifidobacterium*, and *Streptococcus (S. thermophilus)* are known to withstand pancreatic enzymes, bile salts, and gastric acid, as well as to stick to colonic mucosa and easily colonize the intestinal tract. However, not all of these criteria have been taken into consideration when choosing probiotics. Many functional foods include lactobacilli in their composition. Milk also naturally contains a diverse microbiome of bacteria, including lactic acid bacteria (LAB) like *Lactobacillus* and *Bifidobacterium.* These are some of the most common probiotic strains. Fermented milk products like yogurt and kefir have been used for centuries and are known sources of probiotics. The fermentation process promotes the growth of beneficial bacteria. *Lactobacilli* are particularly important in the manufacture of probiotic foods. Several species of the genus *Lactobacillus* are used as starters in the manufacture of yoghurt, cheese and other fermented liquid products

### *Yoghurt with probiotic strain*

The foods high in lactic acid make up a significant portion of our contemporary diets. Yogurt is regularly consumed by about 80% of people either directly or as a dietary supplement. The incorporation of pure culture starters for *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* to facilitate lactic acid fermentation is a distinctive feature of this product. The body benefits from these conventional lactic acid bacteria because of the metabolites they produce, which suppress pathogenic and putrefactive flora and enhance lactose utilization. *Lactobacillus delbrueckii subsp. bulgaricus NBIMCC 3607* probiotic starter culture satisfies all the standards for probiotic cultures and has a high reproductive capacity. The yogurt made using this technique has a one-month shelf life; during that time, the acidity is kept within the acceptable range, and by the end of the extended storage period, the number of viable *L. bulgaricus NBIMCC 3607* cells per gram of the product surpasses one billion. It is possible to attain a comparable outcome with any of the existing technologies. Yogurt with high lactobacilli concentrations has more restorative and preventative qualities. Probiotics become the most widely used product as a result.

***Bio-yoghurt***

Most strains of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* do not persist in the intestinal tract, there are restrictions on the use of yogurt in medical settings, including antibiotic therapy. Thus, in addition to the conventional microorganisms *L. bulgaricus* and *Str. thermophilus*, probiotic bacteria are added to the composition of starter cultures for lactic acid products to produce products with medicinal qualities that are referred to as bio-yoghurt (yogurt, dry mixes, ice cream, soft and hard cheeses, products for infant feeding). *L.acidophilus, L.paracasei ssp.paracasei, L.paracasei biovar shirota, L.rhamnosus, L.reuteri, L.gasseri, Bifidobacterium infantis, Bif.breve, Bif.longum, Bif. bifidum, Bif.adolescentis, and Bif.lactis* make up the majority of the microbiota found in bio-yogurt. In addition to the traditional yoghurt organisms, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, certain yoghurt products have undergone modified in recent years to incorporate live cells of strains of *Lactobacillus acidophilus* and species of *Bifidobacterium* (known as AB-cultures). Thus, bio-yoghurt is defined as yoghurt that has living probiotic microorganisms in it, which are said to have positive health effects. For the probiotic effect to be felt, bio-yogurt needs to include more than one million viable probiotic bacteria cells (108–109 cfu/g).

***Probiotic bacteria in fermented sausages***

For the meat industry, biological preservation of ground beef is a significant and current concern. Finding the right strains of microorganisms to provide the final products a pleasing flavor and protective qualities is the key to finding a solution.

Adding probiotic strains to the starting cultures' composition is a recent development in the manu facturing of dry meat products. They supply substantial levels of microflora that are good for human health as well as appropriate conduction of the fermentation process in meat dishes. Non-thermally treated meat products are good sources of probiotic microorganisms. *Lactobacillus gasseri JCM 1131T* has been determined to be a good candidate for meat fermentation in investigations on members of the Lactobacillus genus. Furthermore, *Lactobacillus gasseri JCM 1131T and Lactobacillus acidophilus* are the two most common species in the human digestive tract, and *Lactobacillus gasseri JCM 1131T* has the capacity to stick to the gastrointestinal mucosa. The strains like *Pediococcus pentosaceus NBIMCC 1441* and *Lactobacillus plantarum NBIMCC 2415* exhibit strong antimicrobial activity, well-expressed fermentative activity without gas release, resistance to low pH, moderate proteolytic and lipolytic activity, and antioxidant activity, which is linked to the synthesis of free amino acids, volatile fatty acids, carbonyl compounds, and other compounds that affect the flavor and taste of meat products. The probiotic strain *Lactobacillus plantarum NIBMCC 2415* has a high concentration of viable cells (8x108 - 3,5x1010cfu/g) in the product, making it probiotic and beneficial. These indicators also lengthen the product's shelf life.

***Probiotic bacteria in sourdough bread***

One of the staples of the modern person's diet is bread. Bread is regarded as a perishable food since it frequently deteriorates due to microbes. Using lactic acid bacteria strains that are imported in the form of sourdough is one of the natural methods for preserving bread. This method offers quick and dependable stability of the predominant microflora in the manufacturing cycle. Selected strains of homo- and heterofermentative lactic acid bacteria are added as components of the starter cultures. By using substrates that cause lactic and acetic acid to develop, the latter cause the medium to become more acidic in terms of pH and total titratable acidity (TTK). The synthesis of acetate through heterofermentative metabolism is crucial for the development of flavor. The ability of the strains *Lactobacillus casei C, Lactobacillus brevis I, Lactobacillus plantarum NBIMCC 2415, and Lactobacillus fermentum J* to grow in a flour and water mixture, attain high cell viability levels, and accumulate acid is defined by their natural fermentation of sourdough. The sourdough starter culture that was developed for wheat bread enhances its organoleptic properties and technological capabilities. Furthermore, it has been discovered to prevent mold spores and "wild" yeasts from growing in flour.

***Soy probiotic foods***

Foods high in soy are thought to be beneficial in reducing chronic illnesses like cancer, osteoporosis, atherosclerosis, and menopausal problems. Whole, fully grown, ripened soybeans are used to make soy milk. The lactose in soy milk is absent. With the right strains of lactic acid bacteria (*Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum, Leuconostoc mesenteroides, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus casei,* etc.), it can be used to carry out lactic acid fermentation and produce a variety of fermented soy foods. The setting is ideal for the creation of novel probiotic supplements. Numerous studies have been conducted on soy milk yogurt. Because fermented soy milk products can be prepared at higher protein levels at a comparable or lower cost than ordinary fermented milk products, they may offer nutritional and economic benefits. With the exception of methionine, soy proteins have a good amino acid balance and satisfy all essential amino acid requirements. Selecting strains of *lactobacilli (Lactobacillus acidophilus A)* and *bifidobacteria (Bifidobacterium bifidum L1)* alone and in combination with *streptococci (Streptococcus thermophilus T3)* results in soy probiotic milk and beverages, which are characterized by a high concentration of active cells of *lactobacilli and bifidobacteria* (1011 - 1014cfu/g) and moderate titratable acidity, allowing 20 days of storage under refrigeration.

***Probiotic bacteria in fruits and vegetables***

Due to the presence of various lactic acid bacteria, almost all fruits and vegetables have the ability to naturally ferment. Probiotic strains and lactic acid bacteria form part of the starting cultures for fermented foods. Probiotic fruits and vegetables can grow within the fruit matrix, and the viability of their cells depends on the strain, the kind of substrate, and the final acidity of the product. They can also withstand high salt concentrations in the medium, grow quickly, and produce an acidic environment that inhibits the growth of extraneous microflora. The majorities of these are members of the genera Lactobacillus (*Lactobacillus brevis, Lactobacillus plantarum, and Lactobacillus casei) and Leuconostoc (Leuconostoc mesenteroides) and Pediococcus (Pediococcus pentosaceus*) and can be used as monocultures and as combinations. Leuconostoc synthesizes dextranase, which aids in the growth of other lactobacilli and bifidobacteria during its growth in vegetable juice. An alginate gel structure containing a probiotic banana product fermented with Lactobacillus acidophilus is produced. The cells in alginate gel and carrageenan matrices are shielded from the harm caused by freezing and freeze-drying by the presence of bacteria.

## Future of probiotics

Probiotics, however, have a bright future apart from the dairy probiotic products and presents significant opportunities for targeted delivery and improved functionality. Non-dairy beverages, including fruit juices and plant-based milks, are emerging as promising new carriers for these beneficial bacteria. This opens doors for individuals with lactose intolerance or those following vegan diets to reap the rewards of probiotics. Microencapsulation technologies play a pivotal role in this transition. By creating a protective barrier around the probiotic cells, these technologies ensure their survival during processing and storage, paving the way for their incorporation into a wider variety of food and beverage products. One of the major challenges associated with probiotics is their vulnerability to harsh stomach acids and processing conditions. Microencapsulation techniques, using materials like alginate, offer a compelling solution. By creating a protective shell around the probiotic cells, microencapsulation enhances their viability throughout the product's shelf life and during processing steps like mild heat treatment. This ensures that a sufficient number of live probiotic bacteria reach the gut, maximizing the potential health benefits for consumers.

Nanotechnology offers a revolutionary approach to probiotic delivery. By using nanoparticles as carriers, scientists can create targeted probiotic formulations. These nanocapsules can shield probiotics from the harsh environment of the stomach and intestines, ensuring their safe passage to specific areas of the gut where they can exert their health benefits most effectively. This targeted delivery has the potential to significantly enhance the efficacy of probiotics for various health conditions. Furthermore, new probiotic strains with specific, targeted health benefits can be designed to address conditions like irritable bowel syndrome or allergies – the possibilities are truly remarkable. Probiotics may be able to contribute even more to the preservation and promotion of human health as studies on the gut microbiome continue to yield new insights.

## Conclusion

The development of novel probiotics from milk sources presents a promising frontier in advancing gut health and overall well-being. Probiotics, encompassing various strains such as Lactobacillus, Bifidobacterium, and Saccharomyces, play a critical role in maintaining and restoring microbial balance within the gastrointestinal tract. The exploration of dairy and non-dairy sources has expanded the potential applications of probiotics, from traditional fermented dairy products to innovative non-dairy and functional foods. Advances in strain selection, characterization, and evaluation methodologies, both in vitro and in vivo, underscore the importance of rigorous scientific processes in ensuring the efficacy and safety of probiotic strains. Emerging technologies such as microencapsulation and nanotechnology offer exciting opportunities for enhancing probiotic stability, delivery, and functionality. As consumer awareness of the gut microbiome and its influence on overall health continues to grow, the demand for innovative probiotic products is expected to rise. Consequently, continued research and development in this area are essential to meet the evolving needs of the market and to harness the full potential of milk as a probiotic delivery system.

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CHAPTER- 5

# Donkey milk products: processing and value addition

Anuradha Bhardwaj*, Varij Nayan, Yash Pal, Ashish Kumar Singh, Hema Tripathi, Bhupendra Nath Tripathi

ICAR- National Research Centre on Equines, Hissar, Haryana
*Correspondence: anuradha.bhardwaj@icar.gov.in

## Introduction

Donkey (*Equus asinus*), considered as an animal with versatile customs and is mainly known as a pack animal but also provides milk, meat and also uses in breeding as well as in transportation too. The total Livestock population is 536.76 million in the country showing an increase of 4.8% over Livestock Census-2012. Contribution of livestock in total agriculture and allied sector GVA (at Constant Prices) has increased from 24.32 per cent (2014-15) to 28.63 per cent (2018-19). Total population of Horses, ponies, Mules & Donkeys is 0.55 million during 2019. Total population of Horses, Ponies, Mules & Donkeys has decreased by 51.7% over previous livestock census (2012) (20th Livestock Census-2019) (Table 1-2; Figure 1-2).

**Table 1.** Indian Equine Population

| Indian Equines | Population (Million) (2007) | Population (Million) (2012) | Population (Million) (2019) | % Decrease (2012-2019) |
|---|---|---|---|---|
| **Horse & Ponies** | 0.61 | 0.62 | **0.34** | **45.2%** |
| **Mules** | 0.14 | 0.20 | **0.08** | **57.1%** |
| **Donkeys** | 0.44 | 0.32 | **0.12** | **61.2%** |

**Table 2.** The Major States of India with Donkey Population (in Lakhs)

| States | Population in 2012 (in lakhs) | Population in 2019 (in lakhs) | % Change |
|---|---|---|---|
| **Rajasthan** | 0.81 | 0.23 | -71.31 |
| **Maharashtra** | 0.29 | 0.18 | -39.69 |
| **Uttar Pradesh** | 0.57 | 0.16 | -71.72 |

| | | | |
|---|---|---|---|
| **Gujarat** | 0.39 | 0.11 | -70.94 |
| **Bihar** | 0.21 | 0.11 | -47.31 |
| **Jammu & Kashmir** | 0.17 | 0.10 | -44.55 |
| **Karnataka** | 0.16 | 0.09 | -46.11 |
| **Madhya Pradesh** | 0.15 | 0.08 | -45.46 |
| **Himachal Pradesh** | 0.07 | 0.05 | -34.73 |
| **Andhra Pradesh** | 0.13 | 0.05 | -65.16 |

The traditional dairy-cattle-based industry is becoming increasingly diversified with milk and milk products from non-cattle dairy species. The interest in non-cattle kinds of milk has increased because there have been several anecdotal reports about the nutritional benefits of these kinds of milk and reports of individuals tolerating and digesting some non-cattle kinds of milk better than cattle milk and of certain characteristics that non-cattle milk is thought to share in common with human milk. Thus, non-cattle kinds of milk are considered to have potential applications in infant, children, and elderly nutrition for the development of specialized products with better nutritional profiles. However, there is very little scientific information and understanding about the physicochemical and digestion behavior of non-cattle kinds of milk. Donkey milk is still undiscovered and unexplored research area in India and is the need of the hour. Milk is a nutritive drink obtained from various animals and consumed by humans. Generally, milk is obtained from dairy cows and buffaloes; although milk from Goats, Camel and Donkey is also used in various parts of the world. It is well known that the best nutritional option for newborns is their mother's milk: however, some infants may not be exclusively breast fed during the first months of life. Therefore, there is a need for another substitute of close composition and properties as human milk. In such cases, when breast feeding is not possible, a cow's milk free diet often resolves symptoms, although some infants can present intolerance to the foods used as alternatives. The nutritive content of donkey's milk is almost similar to breast's milk hence beneficial for babies that could not feed on mother milk due to diseases or improper milk formation etc.

The milk of cow, goat and sheep contributes the majority of the global milk production and buffalo milk production is in second place worldwide (Tozzi *et al.*, 2016). Bovine milk represents over 80% of the world's milk production and is a major source essential nutrient for human health (Wochner *et al.*, 2018). Medhammar *et al.*, (2012) reported that donkeys, Bactrian camel, reindeer, musk ox, llama, moose, yak and alpaca have been underutilized milk producing animals and are defined as "species with underexploited potential for contributing to food security, health and nutrition". **Donkey milk** is being used as substitute of breast milk in several countries. Now-a-days, the consumers' interest in donkey milk is escalating in

European countries due to its compositional similarity to human milk, with good tolerability and palatability. Hence, in western countries, donkey dairies are emerging to produce an alternative milk source for human infants. Also, there is enhanced interest of consumers for donkey milk in southern states of India, while there are no official reports or published data with reference to its production, quality and hygiene. Consumer acceptance for donkey milk will further increase in India after its approval as a food item by FSSAI.

Equine (donkey) milk production differs greatly from that of conventional dairy species, especially in terms of milk supply which is much more limited. The equid mammary gland has the low capacity (maximum 2.5L) and a part of the milk production should be left to foal and that milking may be carried out two or three hours after separation from the foal (Doreau 1991). Donkeys produce milk from 20 to 90 d after foaling for three times a day. A female gives between 0.5 and 1.3 liters of milk a day for about 6-7 months. Among the functional proteins detected in donkey milk, there are molecules active in antimicrobial protection such as lysozyme and lactoferrin. The lactoferrin content of donkey milk is intermediate between the lower values of cow milk and the higher values of human milk. Lysozyme in donkey milk is present in large amounts, indeed ranges from 1.0mg/ml milk to 4 mg/ml, depending on the analytical method used (chemical or microbiological): this substance is present also in human (012 mg/ml) but only in trace amounts in cow and goat protease and may play a significant role in the intestinal immune response (Tidona *et al.*, 2011). Donkey mammary secretions contain human-like leptin at levels close to human milk (3.35 e 5.32 ng/ml). The bioactive peptides insulin like growth factor 1, ghrelin and triiodothyronine were also found in frozen donkey milk. These molecules, and many others present in human milk, are increasingly receiving attention from a nutraceutical point of view because of their potential direct role in regulating food intake, metabolism, and infant body condition.

Donkey milk is used as natural hypo-allergenic milk, because it is tolerated by about 90% of infants with food allergies, e.g., breast milk protein allergy, a common food allergy in childhood with a prevalence of approximately 3% during the first 3 years of life (Salimei and fantuz, 2012). Donkey’s milk is similar to human milk for its lactose, proteins, minerals and omega-6, 9 fatty amino acid content. In terms of energy despite the high lactose content of donkey milk the average fat content is lower.

The Donkey is a domesticated version of the wild ass. The donkey was domesticated about 5,000 years ago in Egypt, Iraq and Iran and was important, along with horses and camels in the development of trade and mobility. They were an important pack animal for the Roman legions who introduced the donkey to Britain. Genetic fingerprints indicated that wild African asses were the ancestors of domestic donkeys, making donkeys the only important domestic animal known to come from Africa. Donkey DNA analysis showed that there were 2 distinct populations of domestic donkey (Science Vol 304, p 1781): one descended from the Nubian wild ass (*Equus asinus africanus)* and the other being similar to the Somalian wild ass

(*Equus asinus somaliensis*) (http://www.arthurgrosset. com/mammals/donkey.html, retrieved August 21, 2010). The Indian donkeys are not much studied animals using molecular genetics tools as well donkey milk was not recognized in eligible milk list also. However, based on the phenotype, the Indian donkeys have been grouped under "Large White" or "Small Grey" categories. There are only three breeds assigned to donkeys (http://www.nbagr.res.in/regdnky.html) in India i.e. Halari donkeys which are large white donkeys of Gujarat, Spiti donkeys and recently Kachchhi donkeys have been registered. The total donkey population in the country has decreased by 61.0 % over the previous census and the total donkeys in the country are 0.12 million numbers in 2019. India is bestowed with precious equine germplasm and equines have contributed significantly in the development of human race from the time immemorial. India possesses about 0.54 million equines as per 2019 livestock census. Major population of equids comprising of donkey, mule, horse and ponies provide livelihood to the rural and semi-urban societies. There are seven registered horse breeds i.e. Marwari, Kathiawari, Kachchhi-Sindhi, Spiti, Zanskari, Bhutia and Manipuri and three registered breeds of donkeys, namely Spiti, Halari and Kachchhi. The management of genetic diversity is a major factor of any program of preserving species and breeds to protect genetic resources of animals. Therefore, it is important that breeds of horse and donkey are preserved and conserved both in-situ and ex-situ especially in view of their reducing populations in India (Pal *et al.*, 2020). The donkey milk profiling in India needs to be done and it has been observed that equine milk has tremendous medicinal value, curative agent for metabolic and allergic diseases, use in cosmetics and anti-aging property. In some part of the country, donkey milk is sold for medicinal uses. The donkey milk fetches a good amount of money for donkey milk providers and is in fair demand. Donkey milk-based cosmetics and health mixtures can be an attractive agribusiness for equine framers in India. This new possibility of increasing the income of the equine owners is very widespread among general public. ICAR-NRCE is establishing a Jenny Dairy Unit at Hisar campus with Jennies of Halari breed. It will serve as a demonstration farm for developing donkey milk-based research and also for entrepreneurship accordingly.

**Table 3.** Donkey breeds of India

| Sl. No. | Breed name | State | Database |
|---|---|---|---|
| 1 | Spiti | Himachal Pradesh | INDIA_DONKEY_0600_SPITI_05001 |
| 2 | Halari | Gujarat | INDIA_DONKEY_0400_HALARI_05002 |
| 3 | Kachchhi | Gujarat | INDIA_DONKEY_0400_KACHCHHI_05003 |

## Milk yield in donkey

In terms of milk supply, donkey milk production differs greatly from that of conventional dairy species. Recent studies on donkey lactation curves showed that individual milk yield ranged between 1.54

and 1.73 kg/day on specialized farms (Bordonaro *et al.,* 2013), which generally raise animals in semi extensive conditions and care about their wellness. Equid mammary gland has a low average capacity (maximum 2.5 L); therefore, to increase milk supply, dairy equids may need to be milked many times a day (Salimei and Fantuz 2012).Alabiso *et al.,* (2009) showed that the highest milk yield can be obtained with three milkings per day compared to two per day, with an increase in milk fat content, too. The donkeys that gave birth in an autumn–winter period yielded more milk than donkeys foaling in spring–summer period since seasonal thermal stress can have detrimental effect on the quantity and quality of milk.

## Composition of donkey milk

The composition of donkey's milk differs considerably from the milk of cow, buffalo, goat, camel and sheep. In comparison with bovine milk, donkey's milk contains less fat, protein and inorganic salts but more lactose, with a concentration close to that human milk. Lactose is a source of fast energy. It makes this milk sweet, palatable and well accepted by children. On the other hand, the majority of adults and certain ethnic groups exhibit intolerance to milk sugar (Guo *et al*., 2007). The high lactose content also stimulates the intestinal absorption of calcium, which is essential for bone mineralization and for nervous system development in infants (Schaafsma 2003). Moreover, the high lactose content suggests use of donkey milk for probiotic purpose (Coppola *et al.,* 2002) because it is an ideal substrate for a correct development of intestinal lactobacilli and makes donkey's milk an ideal matrix for the preparation of probiotic drinks following the incubation with *Lactobacillus rhamnosus* strains (Coppola *et al.,* 2001).

The gross composition of milk is affected by genetic and environmental factors, including the breed, individuality of animals, stage of lactation, frequency and completeness of milking, maternal age, health and type of feed. Many times, fat content in donkey milk was estimated negligible in our studies (Pal *et al.,* 2016) and hence, donkey milk is termed as natural defatted milk. Hence forth, it is encouraging for studies on the possible use of donkey milk in dietotherapy.

The pH of donkey milk, as well as human milk, is neutral or slightly alkaline, likely due to low content of caseins and phosphates, in comparison to cow milk (Pal *et al*., 2018). The average size of donkey milk fat globules may be important for milk digestibility. Although there are no explanatory results in the literature, it seems that the diameter of the milk fat globules has a different effect on the way in which fat is digested and metabolized (Michalski *et al*., 2005a). In fact, some authors have speculated that smaller native milk fat globules may have the best digestive parameters due to the larger surface available for the lipase action (Raynal-Ljutovac *et al*., 2008). Differences between donkey and other dairy species have also been found regarding the number of milk fat globules/mL of milk. In fact, the number of donkey milk fat globules has been found to be lower than that found in cows, goats and sheep (Martini *et al*., 2013a). Donkey milk fat globules showed an average diameter of 2 μm for 70% of total globules, resulting similar to the horse ones but smaller than both human (4 μm) and cow (2.8–4.6 μm) fat globules (Claeys *et al*., 2014, Martini *et al.*, 2013). Moreover, equine milk does not cream due to the lack of cryoglobulin, a protein that adsorbs onto the fat globules as the temperature is reduced, and thus the agglutination of fat globules occurs very slowly (O'Mahony and Fox 2014).

Milk contains numerous nutrients. The content of most minerals is higher in donkey milk than in human milk, but significantly lower than in ruminant milk (Fantuz *et al.*, 2012). The vitamin content of donkey milk is generally comparable or slightly lower than human milk, and, on average, lower than the vitamin content of ruminant milk, except for the vitamin C level (Claeys *et al*., 2014).

The principal salts in donkey milk are calcium, phosphorus, potassium, sodium, and magnesium. The concentrations of these elements, except potassium, are higher in donkey milk than in human milk but all are considerably lower compared with species such as cows, buffaloes, goats or sheep. The calcium, phosphorus concentrations were observed to be about 2-3 times higher than in human milk (Salimei and Fantuz 2012).Casein micelles are primarily a source of amino acids, calcium, phosphate and bioactive peptides for neonates (Shekar *et al*., 2006). Although the mechanism of tolerance of donkey's milk has not yet been fully clarified, it is reasonable to hypothesize that the reduced allergenic properties of this milk can be related to structural differences of its protein component with respect to cow's milk. The lower casein concentration in donkey's milk (about 50% of total protein) compared with bovine milk (80% of total protein) (Zicker & Lonnerdal 1994) and the relevant percentage of essential amino-acids make this milk a new dietetic food and a promising breast milk substitute (Guo *et al.*, 2007). A more recent study on DM has revealed the percentage of four caseins (αs1, αs2, β and κ-CN) in decreasing order: β (54.28) > αs1 (35.59) > αs2 (7.19) > κ-CN (2.79) as evaluated by Urea-PAGE analysis at pH 8.6, followed by immuno-detection with polyclonal antibodies, coupled to densitometric analysis (Cosenza *et al*., 2019).

**Table 4:** Chemical composition of milk from different species (Guo et al, 2007)

| **Components g/100g** | **Donkey** | **Bovine** | **Human** |
|---|---|---|---|
| Total solids | 8.8-11.7 | 12.5-13.0 | 11.7-12.9 |
| Fat | 0.3-1.8 | 3.5-3.9 | 3.5-4.0 |
| Lactose | 5.8-7.4 | 4.4-4.9 | 6.3-7.0 |
| Ash | 0.3-0.5 | 0.7-0.8 | 0.2-0.3 |
| Total protein | 1.5-1.8 | 3.1-3.8 | 0.9-1.7 |
| Casein | 0.64-1.03 | 2.46-2.80 | 0.32-0.42 |
| Whey protein | 0.49-0.80 | 0.55.0.70 | 0.68-0.83 |
| pH | 7.0-7.2 | 6.6-6.8 | 7.0-7.5 |

Milk proteins have been studied in depth for well over 50 years and lot of studies were performed in order to analyse the various milk protein components, in various milk from different mammals. However, many questions concerning milk protein expression, structure and protein modifications remain still not completely covered such as some details of protein modifications due to disease and processing. The milk proteome is extremely complex because of the presence of post-translational modifications, alternative splicing and different genetic variants. The post-translational modifications are: glycosylation, phosphorylation, disulphide bonds formation and proteolysis, they create a large number of different protein variants from a single gene product. In milk the molecular composition of proteins is very important since it influences the functional properties of milk proteins such as solubility, clotting aptitude, thermal denaturation and the nutritional properties of the milk. Usually, the analytical procedures used for structural analysis of milk proteins are based on chromatographic techniques (ionic exchange, reversed phase chromatography, size-exclusion chromatography) followed by one-dimensional electrophoretic techniques (PAGE, Urea-PAGE and SDS-PAGE), or more efficiently on bi-dimensional electrophoresis technique (2-DE). Recent clinical evidence has renewed the interest in donkey milk because of high tolerability in infants with cows' milk protein allergy. To be successful as a substitute for human milk in infant nutrition, donkey milk must be capable of performing many biological functions associated with human milk. The specific protein fraction in donkey milk can be good indicators of its potential role. From the proteomic map the presence of two isoforms of α-lactalbumin, three isoforms of β-lactoglobulin, lysozyme, albumin and lactoferrin was revealed. The high lysozyme and α-lactalbumin content found in donkey milk may be responsible for the low bacterial count reported in literature. Lysozyme, lactoperoxidase and lactoferrin have been recognized as antimicrobial and bacteriostatic agents. Their action may extend the conservation of fresh donkey milk and the relative potential commercial supply. In the past it has been widely used to replace human milk because its chemical composition and particularly protein content are close to that of human. Great attention must be obviously given to the hygienic characteristics of donkey milk production.

## Therapeutic properties of donkey milk

Cows are the primary dairy animal species throughout the world because of the abundance of their lacteal secretion, needed to satisfy the demand of milk and dairy products for human nutrition. However, cow milk is not suitable for the infants affected by cow milk protein allergies (CMPA), the prevalence for which was estimated between 5% and 15%, including infants who show symptoms related to adverse reactions to cow milk protein (Vandenplas *et al*. 2007). Donkey milk has been successfully used in clinical studies, in children with cow's milk protein allergy (CMPA), and has good palatability (Monti *et al*., 2007; DelloIacono and Limongelli, 2010). Its composition is more similar to human milk than ruminant milk, however it is poor in lipids, and adequate lipid integration is needed for a toddler's diet (D'Auria *et al*., 2011). Recently, the potential role of donkey milk has also been of increasing interest in the prevention of atherosclerosis and cardiovascular diseases (Tafaro *et al*., 2007).

The good tolerance of donkey milk in children suffering from CMPA (Monti *et al*., 2007) could thus be due to the levels of its major allergenic milk components, in fact its low casein content and casein:whey protein ratio play an important role in the sensitization capacity of the milk (Lara-Villoslada *et al*., 2005). Other factors may also help to explain the good tolerability of donkey milk, for example the number of casein fractions, the primary structure of the milk proteins, and the differences in digestibility of potential milk allergens, factors which have not yet been analyzed in depth (Salimei and Fantuz 2012). In addition, the lactose content represents a substrate for the development of intestinal microbiota with health-promoting properties (Coppola *et al.*, 2002).

In fact, milk contains a variety of bioactive compounds with special properties associated with the development, growth and survival of infants beyond those provided by nutrition alone (Schanbacher *et al.*, 1998). The major antimicrobial proteins in milk are immunoglobulins, lactoferrin, lactoperoxidase and lysozyme (Tanaka 2007). The protein, α-lactalbumin has a property of antiviral, antitumor and anti-stress. Cow’s milk represents the most common feeding during the infant weaning and widely used as a substitute for human milk, but the cow’s milk is one of the most common food allergies in children, it can lead to an abnormal immunological response (Criscione *et al.*, 2009). Cow milk protein allergy (CMPA) is the most common food allergy in early child hood; affecting 2 to 5% of the child population with less than three years of age (Huang and Kim 2012).

## Cosmetic properties of donkey milk

The use of donkey milk as a moisturizer in cosmetics is more fabulous. In cosmetics, donkey’s milk is used for its cleaning and hydrating action combined with an antioxidant action that prevents the aging. In fact, the fat content in donkey’s milk nourishes the skin and gives it softness. Cleopatra and other privileged ladies of ancient times were taking their bath in donkey milk in order to maintain their skin fresh, lenitive and shiny. Nowadays, so-called therapeutic and cosmetic properties of donkey milk seem to be validated by many trials (Brumini *et al.*, 2016). Donkey milk is rich in vitamins and polyunsaturated fatty acids (Aspri *et al*., 2016) and contains anti-ageing, anti-oxidant and regenerating compounds, which are described as naturally active in skin hydration and skin ageing prevention. The lipid fraction is characterized by high levels of essential fatty acids and low saturated fatty acids (Gastaldi *et al*., 2010). Compared with ruminants' milk, the considerable presence of unsaturated fatty acids found in donkey milk makes it very useful in the prevention of the cardiovascular, auto-immune and inflammatory diseases (Martemucci and D'Alessandro 2012, Martini *et al.*, 2014). In addition, donkey milk could act therapeutically in numerous cases, such as liver problems, infectious diseases, fevers, asthma, etc. (Hippocrates 1843, Pliny the Elder 1893).

## Value Added Donkey Milk Based Products

Traditionally donkey milk is utilized for the preparation of cosmetics, drinking purpose and sporadically for the manufacture of certain artisanal cheeses. Raw (unprocessed) and heat-treated (pasteurized and ultrahigh temperature (UHT)) donkey milk is currently available. Donkey milk has been frozen, freeze-dried (lyophilized), and powdered, among other methods of processing.

There is increasing attention towards donkey milk because of its nutritional, nutraceutical, and functional properties so that it is considered as a functional food and raising interest in the development of donkey milk products. Cheese, ice cream, milk powder, fermented milk products, and other dairy products have all been made from donkey milk. The thermal stability of donkey milk is low. Even at low temperatures, such as 75°C for 10 minutes, sedimentation was observed. Donkey casein micelles are more vulnerable to heat treatment than whey protein due to their low colloidal stability and presence of a high calcium environment.

### *Cheese*

Since donkey milk has low rennet clotting activity, cheese manufacturing using donkey milk is difficult. Moreover, low total solid content and lower proportions of caseins, especially α-S1-casein, resulted in a very weak gel and prevent the formation of a firm curd after renneting. The rennet curd has been reported to fragile. Donkey milk with a low-fat content may affect the texture and flavour of the cheese produced. Recent study carried out by using pure camel chymosin as a clotting agent for donkey milk cheese production resulted in 3.32 percent yield of fresh cheese. After 12 hours, the fresh cheese had a total solids content of 35.65%. Niro*et al*. (2017) investigated the efficacy of donkey milk as a dietary supplement. In Italian Grana cheeses, a replacement for hen's egg white lysozyme in preventing cheese blowing. The microbiological and sensory quality of cow-donkey mixed milk was fine. The authors proposed using donkey's milk in hard cheese production as a new technical solution to prevent late-blowing defects.

### *Ice cream*

Donkey milk was used to make strawberry flavoured ice cream, which was then mixed with two strains of lactic acid bacteria that were found to have probiotic properties. *Bifidobacterium adolescentis*ATCC15703 and *Lactobacillus plantarum* 998 with a fat and overrun of about 24.7 and 1.2 percent, the ice cream had a very low-fat content. There was a reduction in vitamin C content after 4 months of storage at -20°C. The total phenols and antioxidant potential of the ice cream, however, did not alter. Throughout the storage time, the viable probiotic bacteria count was over 9 log CFU/g.

***Donkey milk powder***

Donkey milk and donkey milk powder are good substitutes for cow milk and infant milk powder since they contain lower levels of fat and cholesterol, a good calcium-phosphorus ratio, and are resistant to oxidation. Among the milk of two breeds of donkeys, i.e. dwarf grey and largely white, it was noticed that dwarf grey breed donkey milk was better for powder processing. It was related to the higher particle size, protein and better nutrient composition i.e., lysozyme, Ca, $B_3$, cysteine, omega-3. Heat treatment of donkey milk at $85^0$C resulted in a greater loss of lysozyme and β-lactoglobulin. The levels of lysozyme and beta-lactoglobulin drop by 60% and 87 %, respectively. Heat treatments above 65°C resulted in a 100 percent loss of lactoferrin.

At temperatures less than 85°C, lysozyme was found in raw and heat-treated foods in equivalent amounts. The spray drying procedure considerably reduced lysozyme activity (58 percent residual activity) and lactoglobulin content (6.43 mg/mL in fresh milk vs 5.51 mg/mL in spray-dried milk). The high temperature used to process donkey milk reduced lysozyme activity and beta-lactoglobulin levels.

## *Fermented milk products*

Carminati *et al*. (2014) isolated and characterized culturable and cultivable acidifying and thermoduric LAB microbiota in donkey milk. *Lactobacillus plantarum* strains have been suggested as possible candidates for producing novel fermented milk from donkey milk, not only because of their probiotic potential but also because of their ability to improve the growth of *Streptococcus thermophilus*. The pH values of the fermented donkey kinds of milk were 2.91± 0.16 and 1.78 ±0.66. Lysozyme resistance was strong in both *S. thermophilus* and *L.plantarum*. Coppola *et al*. (2002), studied donkey milk as an excellent option for making probiotic beverages. They looked at how heat treatment affected the microbiological and biochemical consistency of ass's milk (Martina Franca breed) as well as the growth of four distinct *Lactobacillus rhamnosus* strains. Lactobacillus bacteria are commonly utilized to make probiotic beverages from equid milk, such as donkey and mare milk. *Koumiss* is fermented equid milk that has been utilized in Central Asia since antiquity. The lower protein content mainly casein and lack of adequate nutrient from microbial proliferation could be the reason for poor fermentation characteristics of donkey milk. Further experimentation are required in terms of increasing the total solids in donkey milk by supplementing goat or bovine milk, ultrafiltration, use of hydrocolloids and utilization of cross-lining enzymes.

## Agri-entrepreneurship through donkey dairy farming

It has been observed that donkey milk has tremendous medicinal value as curative agent for metabolic and allergic diseases along with its use in cosmetics. In some part of the country especially the southern regions, donkey milk is sold for medicinal uses. The donkey milk fetches a good amount of money for donkey milk providers and is in fair demand. Donkey milk-based cosmetics and health mixtures can be an attractive agribusiness for equine framers in India. This new possibility of increasing the income of the equine owners is very widespread among general public.

Since time immemorial the donkey milk was known for its unique therapeutic values. However, as cow's milk became a more popular choice for the masses due to high production and breeding policies, the values of donkey's milk was forgotten with time. But, economic gains from donkey milk are still on air, it is the costliest milk around the world fetching around 2000 to 5000/- per liter according to the demand. Not only this now- a-days the cosmetic properties of donkey milk are gaining momentum. One Agri-entrepreneur from Kerala, Mr Aby Baby decided to embark on a journey to bring back the lost glory of donkey's milk. Mr Aby Baby is now a man with a donkey farm. Aby Baby's one-of- its-kind donkey farm at Ramamangalam is the source of donkey milk used in a range of natural wellness and skincare products manufactured by him and getting favorable economic gain. Mr Aby Baby is finally finding joy in his venture after receiving positive feedback from consumers. Similary, Ms Pooja Kaul a young entrepreneur and founder of "Organiko" is leading her firm Organiko for making Donkey milk Soaps in India.

## Future perspectives

While the donkey was shunned aside for its 'asinine' values, its milk was venerated as the elixir of life. With increasing numbers of research reports worldwide, donkey milk is now being taken as "gold mine" for future. In contrast to the affluent Western societies in India donkey serves for the livelihood of the landless, small and marginal farmers. Maximum donkeys are found with nomads and labor at brick kilns who don't know how to gain economic benefit from the animal except as pack and load carrying animal. If these donkey owners and marginal donkey farmers made aware of the benefits and the products of donkey milk, this can be a great financial boon to them. The future prospects are really high by proper breeding, milking and product marketing.

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## CHAPTER- 6

# Functional and therapeutic properties of camel milk

Artabandhu Sahoo and Swagatika Priyadarsini*

ICAR-National Research Centre on Camel, Bikaner

*Correspondence: s.priyadarsini@icar.com

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## Introduction

The chemical composition of camel milk (CM) is influenced by various factors including the breed, age, and geographical habitat of the camel, along with the timing and frequency of milking, as well as the season and stage of lactation. Beyond its nutritional elements encompassing both macro and micro nutrients, CM also harbours non-nutritional components such as bioactive peptides, probiotics, exosomes, hormones, growth factors, and immunoglobulins, contributing to its holistic properties.

## Characteristics of camel milk:

Appears as an opaque white colour with a density of 1.029g/cm$^3$, viscosity of 1.72 mPa and a 6.2 - 6.5 pH (mostly acidic). The flavour profile remains subtle saltiness (may vary based on the types of fodder consumed by camels)

**Table 1.** Nutritional and non-nutritional components of camel milk

| **Sl. No.** | **Components of camel milk** | | |
|---|---|---|---|
| | **A. Nutritional components** | | **B. Non-nutritional components** |
| 1. | Water (83-90%) | 1. | Exosomes |
| 2. | Total solid (9.0-11.5%) | 2. | Probiotics |
| 3. | Protein (2.1-3.4%) | 3. | Bioactive Peptides |
| 4. | Lactose (2.9-4.9%) | | |
| 5. | Fat (2.7-4.3%) | | |
| 6. | Vitamins | | |
| 7. | Mineral | | |

Moreover, the content of these components varies due to geographical location, age, breed, feeding condition, method of estimation, managemental practices etc. It is proven that camel milk contains more nutrients than cow's milk, including 4 times more vitamin C and 10 times more iron than cow's milk. Studies have found that camel milk contains a large amount of unsaturated fatty acids that are needed by humans, and 1 mL of camel milk contains 52 units of insulin compared to 16 units of cow milk. For people who are allergic to cow's milk products, camel milk is a kind of dairy product that is safe to consume.

**Table 2.** Unique characteristics of camel milk proteins pertaining to its nutraceutical properties.

| Sl. No. | Uniqueness in camel milk proteins | Remarks |
|---|---|---|
| 01. | Lacks β- lactoglobulin | Non allergenic and equivalent to human milk proteins |
| 02. | Lacks new β-casein | Non allergenic |
| 03. | β-casein constitutes 65% of total caseins | Equivalent to human milk proteins |
| 04. | 18% of whey proteins are immunoglobulins | Equivalent to human milk proteins |
| 05. | Presence of Peptidoglycan Recognition Protein (PGRP-S) | Antibacterial property |
| 06. | Casein micelles have a relatively larger diameter; hence the surface area is decreased | Poor aggregation and gel formation leading to improper coagulum formation. Hampers the ability to form firm curd |
| 07. | Presence of IgG2 and IgG3 as extra two types of immunoglobulins | Additional immunomodulatory property |
| 08. | Presence of insulin like peptides | Hypoglycemic effect |
| 09. | Enzymatic digestion of lactoferrin produces antimicrobial peptide | Acts a preservative |
| 10. | Higher concentration of Glycosylation dependent cell adhesion molecule (PP3) | Anti-inflammatory for gastrointestinal and respiratory tract infections |

## Nutraceutical properties of camel milk

Camel milk has been studied to have multiple nutraceutical properties. It has been proved to be effective in managing several human diseases, whose salient mechanisms are mentioned below:

1. The antidiabetic property of camel milk involves multiple mechanisms:

   i. Insulin-like activity: Camel milk contains insulin-like peptides that may mimic the action of insulin in the body. These peptides could potentially help regulate blood sugar levels by facilitating glucose uptake into cells, similar to insulin.

   ii. Beta-cell regeneration: Studies suggest that camel milk may have a protective effect on pancreatic beta cells, which are responsible for producing insulin. By promoting the regeneration or preservation of these cells, camel milk could enhance insulin secretion and improve glucose metabolism.

   iii. Anti-inflammatory properties: Chronic low-grade inflammation is associated with insulin resistance and type 2 diabetes. Camel milk contains bioactive compounds with anti-inflammatory properties, which may help reduce inflammation in insulin-sensitive tissues and improve insulin sensitivity.

   iv. Antioxidant activity: Oxidative stress plays a role in the development of diabetes complications. Camel milk is rich in antioxidants, such as vitamins C and E, selenium, and superoxide dismutase, which can neutralize harmful free radicals and protect pancreatic beta cells from oxidative damage.

   v. Gut microbiota modulation: Emerging research suggests that camel milk may influence the composition and activity of the gut microbiota, which plays a crucial role in metabolic health and insulin sensitivity. By promoting a favorable gut microbiota profile, camel milk could indirectly improve glucose homeostasis.

   vi. Enhanced glucose utilization: Some studies have suggested that camel milk may improve glucose utilization in peripheral tissues, such as skeletal muscle and adipose tissue, thereby reducing blood sugar levels and improving insulin sensitivity.

2. The mechanism underlying the ameliorative effect of camel milk on autism is still a subject of ongoing research. However, several potential mechanisms have been proposed based on experimental and clinical studies:

   i. Immunomodulatory Properties: Autism is believed to involve immune dysregulation and neuroinflammation. Thus, the immunomodulatory effects of camel milk due to the presence of lactoferrin, lysozymes, immunoglobulins, etc. may help mitigate inflammation in the brain, potentially alleviating some symptoms of autism.

ii. Gastrointestinal Function: Many individuals with autism spectrum disorder (ASD) experience gastrointestinal issues. Camel milk may improve gut health due to its unique composition, including bioactive peptides and probiotics, which could positively impact gut microbiota composition and function. This, in turn, may contribute to improvements in behavior and cognitive function associated with autism.

iii. Nutritional Composition: Camel milk contains various nutrients and bioactive compounds, including vitamins, minerals, and immunoglobulins, which could support overall health and potentially address nutritional deficiencies commonly observed in individuals with autism. These nutrients may play a role in neurological development and function.

iv. Neuroprotective Effects: Some studies suggest that camel milk may have neuroprotective properties arising from its antioxidant activity, protecting brain cells from damage and promoting neuronal growth and repair. These neuroprotective effects could potentially mitigate the neurological abnormalities associated with autism.

v. Social and Behavioral Effects: Although less understood, some researchers speculate that certain components of camel milk may influence neurotransmitter levels or pathways involved in social behavior and communication, which are impaired in individuals with autism. This could contribute to the observed improvements in social interaction and communication skills reported in some studies.

3. Benefits of camel milk colostrum:

Camel colostrum is an “early” milk produced within the initial five days post-parturition by she-camels, undergoes transformation into mature milk during the subsequent two days. This transition involves noteworthy alterations in chemical and functional components, setting camel colostrum apart from both mature camel milk and colostrum from other animals like cows, goats, and sheep. Chemical analyses conducted by various researchers highlight distinct disparities in the composition of camel colostrum compared to mature milk. Notably, camel colostrum exhibits elevated levels of protein, ash, oligosaccharides, vitamins, and minerals, while non-protein nitrogen and lactose concentrations in both camel milk and colostrum remain closely aligned. The protein composition of camel colostrum differs significantly from conventional milk due to its heightened concentration of whey proteins, particularly IgG, a crucial element conferring immunity to the new born. Camel colostrum demonstrates a unique protein profile, characterized by a deficiency of Lactoglobulin, a primary whey bovine protein responsible for allergies in children, and an abundance of lactoferrin (LF), an antimicrobial protein. This richness in LF, averaging 2.3 g/L compared to 0.5 g/L in bovine colostrum, mirrors similarities between camel and human colostrums. The post-partum period sees significant reductions in fat and protein contents of camel colostrum. Seven days post-calving, total fat decreases to an eighth of its initial value, while total protein undergoes a fourfold decrease from. Camel colostrum, distinguished by its lower lactose and fat levels, encompasses high concentrations of total protein, IgG, lactoferrin, long-chain polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), vitamin C, and other oligosaccharides. Beyond its nutritional richness, camel colostrum's high immunoglobulin content, including IgG, IgA, and IgM, facilitates passive immunity transfer from mother to calf. In summary, camel colostrum stands out for its distinctive

| Camel colostrum | |
|---|---|
| | **Relatively higher in vitamins- A, E and B1** |
| | **Three subclasses of IgG** |
| | **Less fat and casein** |
| | **High antioxidant activities of colostrum proteins** |
| | **Colostrum isolates have probiotic activities** |
| | **Higher level of lysophosphatidylethanolamine, phosphatidylserine and phosphatidylinositol** |
| | **Low level of phospholipids** |
| | **Most similar to human colostrum** |

composition, supporting neonatal health and growth. Harvested within the initial hours after birth, camel colostrum serves as a valuable nutritional supplement with potential therapeutic applications. Its unique characteristics, particularly the unaltered functionality in foods for human use and clinical trials, underscore the superiority of camel colostrum, presenting promising avenues for combating specific human health ailments.

4. Camel milk exosomes represent a novel and promising therapeutic agent with its proven anticancerous, antiproliferative, antimicrobial and antidiabetic efficacies.

**Conclusion**

Overall, the ameliorative effect of camel milk on human diseases is likely multifactorial, involving a combination of immunomodulatory, gastrointestinal, nutritional, and neuroprotective mechanism. Further research is needed to elucidate the specific pathways involved and to determine the efficacy and safety of camel milk as a complementary therapy for human diseases.

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CHAPTER- 7

# Functional and Therapeutic Properties of Non-Bovine Milk Other than Camel

Aruna Kuniyal*, Rakesh Ranjan, Shyam Sundar Chaudhary

ICAR-National Research Centre on Camel, Bikaner

*Correspondence: aruna.kuniyal1995@gmail.com

## Introduction

Milk is considered as a valuable source of nutrients since the Neolithic period when hominids made the gathering societies. The first domesticated animals were goats and sheep that were utilized for both milk and meat. Consequently, with domestication of bovines that have higher milk production as compared to small non-ruminants, the value of milk from such species declined. With the bovines milk yield, dairy sector has witnessed tremendous growth in milk production in the past five decades. Generally, cow milk is the most commonly consumed milk, followed by buffalo contributing almost 95% of total milk production. Eighty-five percent of the world milk production is derived from cattle, 11% from buffalo, 2.3% from goat, 1.4% from sheep and 0.2% from camel. But now with the increased knowledge on the functional and therapeutic properties of milk from non-bovine species which is considered good for human health, milk from non-bovine species such as sheep, goats, camels, mares, donkey, yak, and reindeer is gaining attention. This milk which provides therapeutic effect or health benefits beyond providing basic nutrition is termed as Functional Milk.

## Goat Milk

Goat milk is richer in vitamin A, vitamin B1, vitamin B2, vitamin B5, vitamin C, niacin, Calcium, Phosphorus, Zinc, Potassium, and Selenium than cow milk. Goats convert all carotene into Vitamin A in the milk, hence is higher in content compared to cow milk and comparable to human milk, and therefore the milk from goat is whitish as compared to the cow milk which is yellowish. The high concentration of Calcium and Phosphorus in milk along with its highly bioavailability favors their deposition in the organic matrix of bone, leading to an improvement in bone formation parameters. Further the Zinc and Selenium are essential micronutrients contributing to the antioxidant defense and for the prevention of neuro-degenerative diseases.

The goat milk contains 13% more calcium, 25% more vitamin A, 134% more potassium, 3 times more niacin, and 4 times more copper. The mineral content of goat milk is 3 to 4 times that of human milk, which can be a considerable burden on kidneys in infants. Therefore, it is recommended that goat milk be given to infants (preferably over 6 months of age) after dilution and supplementation of missing components. Goat milk has higher Mono Unsaturated Fatty Acids (MUFA) and Polyunsaturated Fatty Acids (PUFA), and medium-chain triglycerides (MCT) than cow milk that are beneficial to humans suffering from cardiovascular diseases, gastrointestinal disorders. The MCT is 36% in goat milk versus 21% in cow milk. The major MCT are caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids which are partly responsible for its characteristic "goaty" odour. This further imparts medicinal value to the goat milk.

Another characteristic of goat milk is the presence of a complex array of nucleotides. These nucleotides facilitate maturity in the immune system of milk-fed offspring and therefore goat milk can be an effective adjuvant for infant formulae. Goat milk nucleotides are also involved in lipoprotein metabolism, by increasing High Density Lipoprotein (HDL) and synthesizing apolipoprotein A1 and Apo A1 V in pre-term infants as well as long-chain PUFA in neonates. The colostrums and milk from goats are rich source of polyamines. The polyamines content in goat milk is higher than in milk of other mammals. These polyamines are important for optimal growth, gastrointestinal tract (GIT) cell function, and maturation of GIT enzymes, and are found to reduce food allergy in infants. The average amino acid composition of goat and cow milk shows higher levels of 6 out of the 10 essential amino acids (threonine, isoleucine, lysine, cystine, tyrosine, and valine) in goat milk as compared to the cow milk.

The oligosaccharides content of goat milk is higher than in cow milk (4-5 times) and sheep milk (10 times), but much lesser than in human milk. These oligosaccharides are complex and have similarities in profile with human milk, and possess prebiotic effect, and stimulate gut Bifidobacterium and Lactobacillus spp. There are many functional properties provided by oligoasaccharides that are anti-adhesive, anti-microbial, immune modulators, intestinal epithelial cell response modulation, and nutrient provider for neonatal brain development and also for the growth of desired gut microflora. Thus, goat milk can be an attractive natural source of human-like oligosaccharides for infants.

The digestibility of goat milk proteins is better than of the bovine milk proteins, as during acidification it forms softer clots in the stomach that are efficiently digested by proteases. The absence of agglutinin combined with higher short and medium chain fatty acids prevents clustering of fat globules, thus making the milk easier to be digested.The significance of goat milk in infant diet is on account of its easier digestibility and less allergy causing than cow milk. Further, goat milk has anti-diabetic, anti-allergenic, anti-cancerous, anti-inflammatory, anti-diarrheal, and malabsorption syndrome properties. Small amounts of the αS1 casein fraction and the small size of casein micelles cause goat milk to be rapidly digested by proteolytic enzymes of the stomach, as a result of which goat milk is also recommended for people with gastrointestinal problems, especially ulcerations.

Unlike cow milk, which is slight acidic, goat milk is alkaline in nature, which is very useful for people with acidity problems.This alkalinity is due to the higher protein content and different arrangement of phosphates. In studies with rats, which had malabsorption syndromes, it was found that goat milk improved the intestinal absorption of copper, which was attributed to the higher contents of cysteine in goat milk than in cow milk.

About 60% of allergic reactions in humans are caused by the casein and lactoglobulin. The most allergenic is the casein fraction αS1, the content of which in goat milk is very small unlike in cow milk, in which it forms about 33% of all caseins. It has been reported that 40–100% of allergic patients, sensitive to cow's milk proteins, are able to tolerate goat's milk proteins. One of the functional components of goat milk is alpha-lactalbumin (α-La) which is about 21% of all proteins. It serves different functions in the body such as it is a calcium carrier and it can bind other metals such as magnesium, cobalt and zinc. It acts as an immune factor (which is particularly important in infant feeding) and also has an anti-cancer effect. Because of a high methionine content, α- LG provides protection against cancer development. Beta-lactoglobulin (β-Lg) forms the largest part of all whey proteins in milk (54%). It is a carrier of retinol, which is essential for the normal development of infants and for the vision process. It also has the ability to bind fatty acids, as well as antioxidant properties (by binding Cu and Fe ions, it inhibits the oxidation of milk fat).

Goat milk contains Conjugated linoleic acid (CLA), which is also known as rumenic acid. The CLA inhibit the incidence and growth of cancer in animals and humans, by preventing the proliferation of malignant melanoma. It has also shown to prevent osteoporosis and atherosclerosis and also increase immunity and reduces fatty tissue. Regular consumption of goat milk by anemic patients improves their recovery because it enhances the nutritional use of iron and enhances the regeneration of haemoglobin. The goat milk has been found to contribute towards increasing platelet counts of patients suffering from viral diseases like chikungunya and dengue fever and selenium deficiency. The goat milk and sweet goat whey is also the highest sources of bioorganic sodium which is a Naturopathic medicine to treat arthritis that comes with the advancing age.

## Sheep Milk

Sheep milk contains almost twice the amount of protein as compared to goat and cow milk. These proteins have different molecular forms with different amino acid sequences and have a positive impact on digestibility and thermostability. Sheep milk contains higher medium-chain triglycerides (MCT) and PUFA content than cow milk. The sheep milk is found superior to cow milk in all 10 essential amino acids.

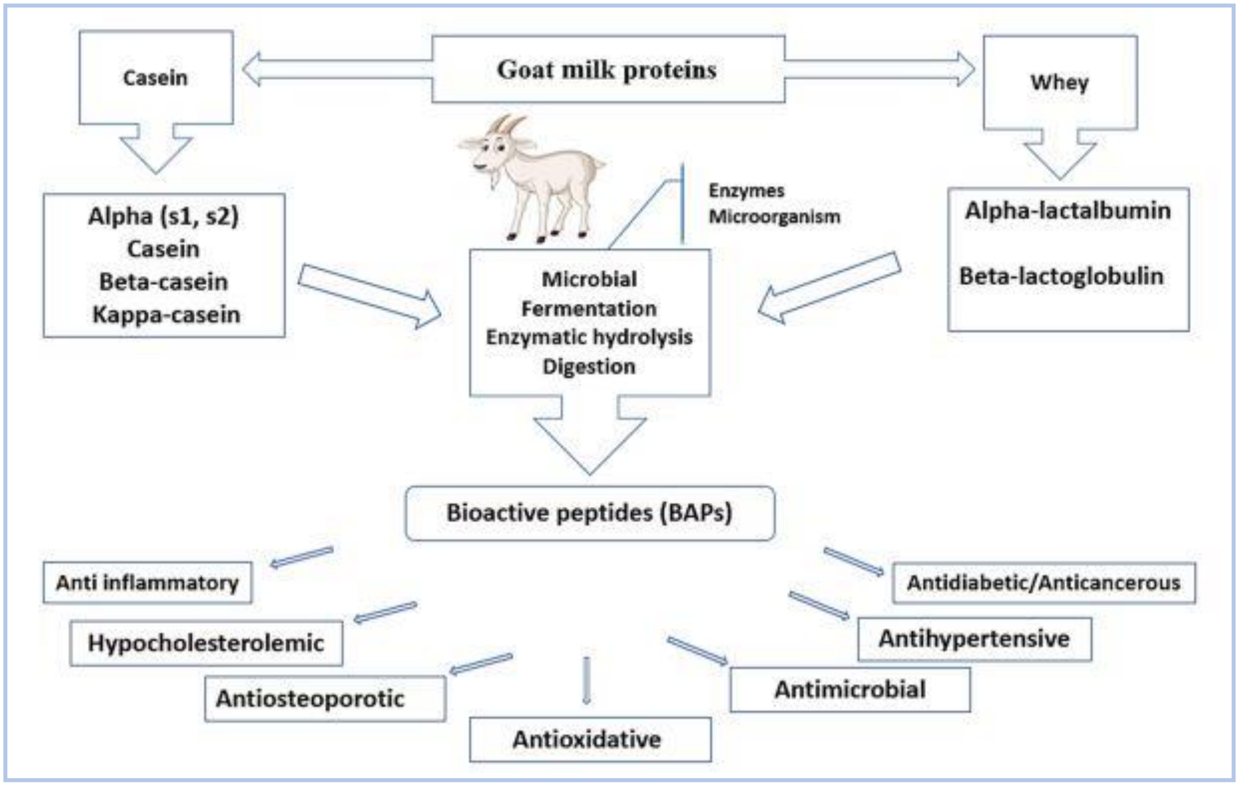


**Fig 1.** Benefits from goat milk protein derived bioactive peptides (ALKaisy *et al*., 2023)

Sheep milk has a sweet and soft flavour and aroma and a creamy texture due to small fat globules, thus making it easily digestible. The milk proteins of sheep have a smaller size of micelles than cow milk (260 nm) and are almost similar to those of goat milk. Similar to goat milk, sheep milk is characterized by higher vitamin A concentration in comparison with cow milk as all of the β-carotene in milk from sheep is converted into retinol, resulting in the white color of that milk. Being the richest source of butyric acid (C4:0), omega 3 fatty acid, conjugated linoleic acid, and α-linolenic acid, sheep milk has been proven beneficial against cancer, coronary heart disease, and osteoporosis; and it also stimulates the immune system.

The concentrations of both water-soluble and fat soluble vitamins are more in sheep's milk compared to goat's and cow's milk. Sheep milk is also considered a good source of minerals as it is highly rich in Calcium, Phosphorus, Magnesium, Zinc, Manganese and Copper. The milk being rich in vitamins and minerals is used in anti-aging formulations and therapeutic use in psoriasis and skin eczema. Further, the milk has positive effects on bone structural integrity and bone health due to more availability of calcium and phosphorus. Additionally, consumption of sheep milk or its derivatives can boost intake of protein, carbohydrates, beneficial fats, essential minerals, and important vitamins.

The orotic acid (vitamin B13), an important precursor in the biosynthetic pathway of pyrimidine nucleotides is highest in sheep milk than bovine, goat and human milk. It helps in the conversion of folic acid and vitamin B12, reduces cholesterol concentration in the blood, has detoxifying and antiarthritic action (reduces the concentration of uric acid in the blood). It can also prevent over-fatness of tissues, increases body weight gains through muscle mass gain and growth of connective tissue. Further, it can protect parenchymatous organs (liver, kidneys) and the heart from degeneration, cirrhosis and steatosis.

Sheep milk as like goat milk is an excellent source of bioactive peptides. The Angiotensin Converting Enzyme (ACE) inhibitor peptide has potential beneficial effects in the treatment of hypertension as it promotes the activation of vasodepressor bradykinin. Further, several peptides with anti-oxidative, antihypertensive and antimicrobial properties have also been isolated from sheep milk. Lactoferrin as a sheep's milk protein has antibacterial, antiviral, antioxidant, anticancer and antiinflammatory effects. Lysozyme and α-lactalbumin show reactive oxygen species (ROS)-dependent cytotoxicity in many tumor cells. Addittionaly, Lactoferrin, α-lactalbumin and β-lactoglobulin exhibit neuro-protective effects.

The ω6/ω3 ratio is 4.4 in sheep milk and is lower as compared to goat milk. A lower ratio of ω6/ω3 fatty acids is more desirable in reducing the risk of many heart diseases. So, sheep milk is considered beneficial for heart disease patients. Moreover, sheep milk is a better and more effective substrate for probiotic bacteria due to high protein and fat content in milk with good buffering capacity and growth factors including peptides etc.

Both proline and hydroxyproline are found in the highest amounts in sheep's milk proteins. They have role in collagen synthesis. Their demand increases in the prenatal period and just after birth and are crucial for the proper growth and development of young organisms. The dairy products which are rich in proline protect against Alzheimer's disease and other amyloidogenic diseases. High polar lipid fractions in sheep milk improve human health by reducing the level of atherogenic lipoprotein cholesterol, modulating the intestinal microflora and reducing inflammation in blood serum and liver.

Of all the ruminants, sheep's milk turns out to be the richest (1.1%) in CLA, but the concentration of this component in milk depends on the season. The CLA has a special role in the regeneration of the nervous system. It also inhibits both benign and malignant tumors by inhibiting cell growth and development. Administration of LF-CLA complex to rats with Alzheimer's disease resulted in a 2-fold decrease in Aβ in the hippocampus because of the ability of CLA to destroy existing Aβ and inhibit the formation of new oligomers of this protein. In the group treated with the LF–CLA complex, there was a significant decrease in the levels of reactive oxygen species (ROS), nitrite (NO) and malondialdehyde (MDA), which indicates the antioxidant effect of CLA and the ability to scavenge free radicals.

Antibodies in people allergic to cow milk (IgE) weakly recognize the protein fractions casein and whey proteins present in sheep milk. Therefore, sheep milk is an ideal substitute for cow milk allergy sufferers and can provide high levels of nutrients with lower sensitization in allergic people. Different sheep milk cosmeceuticals such as soaps, lotion and creams are available in market due to its rich combination of minerals, nutrients and vitamins such as vitamin A and E. Both vitamins A and E act as antioxidants within the skin, seeking for free radicals and eliminating them from the system.

## Donkey Milk

Donkey milk has a long history of use – within both Western and Eastern cultures, it has been recognized as "therapeutic food" as well as highly appreciated cosmetic product. Donkey milk has remarkable similarity with the human milk. Therefore, donkey milk can be used as new dietetic food or potential substitute for human milk for infants and also can be used as a substitute for infants who suffer from cow milk protein allergy (CMPA).

The donkey milk contains less fat, cholesterol, total proteins, casein, and inorganic salts. The milk is highly digestible and as milk is low in fat it is considered good for cholesterol management. Fat content in donkey milk has been found negligible therefore; the donkey milk is termed as natural defatted milk. Equine milk doesn't cream due to the small amount of cryoglobulin (agglutinins), a protein that adsorbs onto fat globules because of reduced temperature; and hence agglutination of fat globules occurs very slowly

The donkey milk is rich in lactose (with a concentration closer to human milk), whey proteins, calcium, selenium and vitamin D3. Lactose is considered as a source of readily available energy and it makes the milk sweet, palatable, and acceptable to children. The high lactose content also stimulates intestinal absorption of Calcium, Magnesium and Phosphorus and the utilization of Vit D which is important for bone mineralization and development of the nervous system in infants. High lactose content further suggests the use of donkey milk for prebiotic purposes which are a perfect substrate for the accurate development of intestinal lactobacilli.

The main salts - calcium, phosphorus, potassium, sodium, and magnesium, and their concentration, except that of potassium, is higher in the donkey milk than in the human milk, but considerably lower than in the milk of cows, buffaloes, goats, and sheep. The pH of donkey milk, as well as human milk, is neutral or slightly alkaline, probably due to low caseins and phosphates.

Donkey milk is rich in various protective proteins (α-lactalbumin, lysozyme, lactoferrin, lactoperoxidase, and immunoglobulins), and shows strong antioxidant, anti-bacterial, anti-viral, anti-fungal, hypoglycemic, anti-parasitic, anti-inflammatory and anti-tumor activity. The lactoferrin content of donkey milk is less than cow milk but high than human milk. The lysozyme is highly thermostable and resistant to acid and protease and plays a significant role in intestinal immune response. Moreover, donkey mammary secretions contain human-like leptin at levels close to human milk. The bioactive peptides- insulin-like growth factor 1, ghrelin, and tri-iodothyronine are also found in donkey milk which has direct role in regulating food intake, metabolism, and infant body condition.

The donkey milk is considered as natural hypo-allergenic milk as it is well tolerated by about 90% of infants with varied food allergies and breast' milk protein allergy. Further, clinical studies confirmed that diet containing donkey milk is safe and valid therapy for most complicated cases of food allergy. Therefore, donkey milk can be considered functional food, having in mind that its fatty-acid profile and content of essential fatty acids are beneficial for cardiac health. Beneficial effects of donkey milk have also been proved in the process of osteogenesis, in the treatment of patients with atherosclerosis, coronary disease or premature ageing as well as in hypocholesterolemic diets. In Serbia, donkey milk has traditionally been used as a natural remedy for treating asthma and bronchitis.

Fat globules have been found lesser in size in donkey milk than in cows, goats, and sheep milk. Smaller native milk fat globules are of utmost digestive parameters due to the larger surface available for lipase action. Several in vitro and in vivo studies showed that donkey's milk has nutraceutical and functional properties that can support immunity, alter metabolism and beneficially modify gut microbiota.

The use of donkey milk as a moisturizer in cosmetics is more fabulous. In cosmetics, donkey's milk is used for its cleaning and hydrating action combined with an antioxidant action that prevents the aging.

## Yak Milk

Milk of yak is creamy white, thick, sweetish, fragrant, and rich in proteins, fats, lactose, minerals, and totals solids. In general, yak milk has a higher nutrient density and is loaded with omega 3 fatty acids, amino acids, and antioxidants, besides vitamins and minerals. The varied products are a rich source of conjugated linoleic acid and vaccenic acid. Several studies have also reported on anti-inflammatory, antioxidative, anti-hypoxia, and anti-fatigue properties of yak milk. Compared with other animal milk fats, yak milk fat may include certain distinctive fatty acids because of its particular grazing habitat. These fatty acids have been linked to potential health advantages, including an anti-carcinogenic and anti-diabetic effect and a positive influence on the brain, heart, and eyes.

Despite having more protein than bovine milk, the amino acid profile of yak milk is comparable with bovine milk. Yak milk caseins, which are both a source of antihypertensive peptides and a typical dietary protein, might be used as a multipurpose component in various value-added functional meals. Several probiotic strains have also been identified in yak milk and its products that provide numerous healthier benefits.

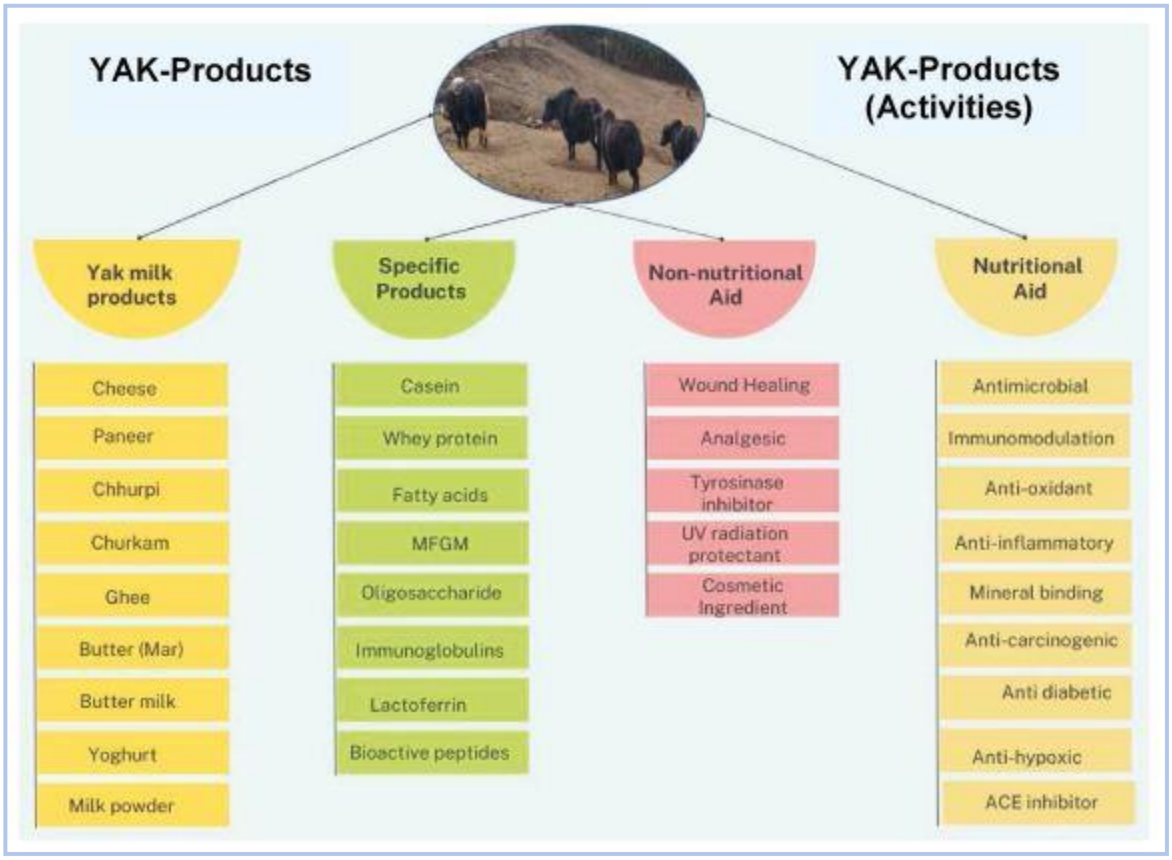


**Fig 2.** Yak milk products and their benefits (Singh *et al*., 2023)

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CHAPTER- 8

# Yak milk products: Processing and value addition

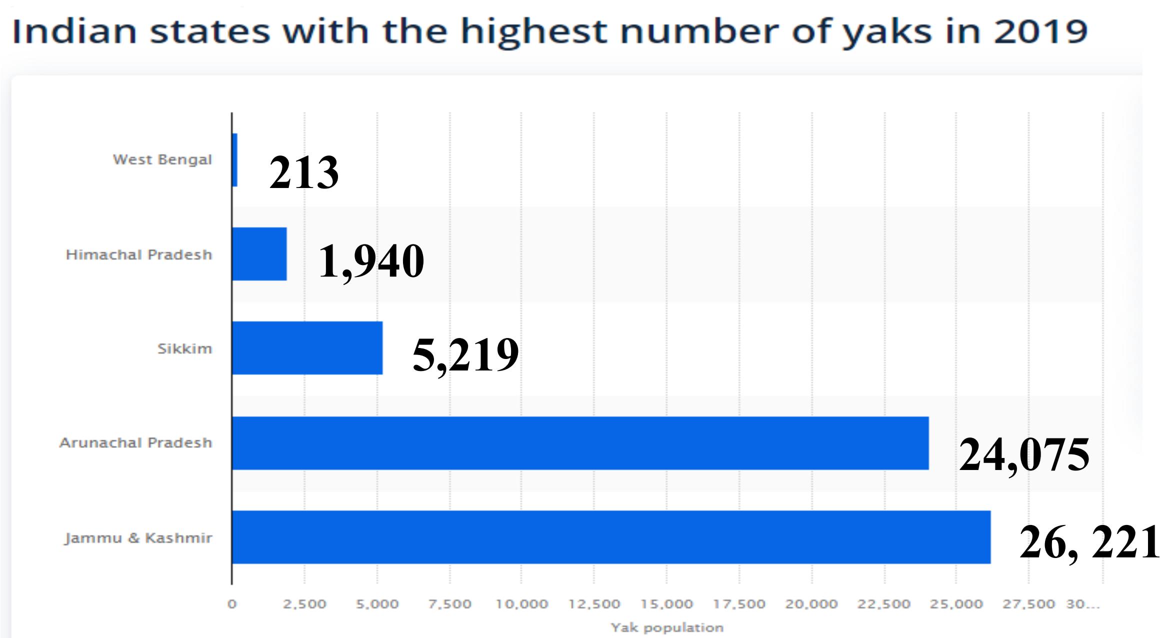
Shubham Loat*, Khriengunuo Mepfhuo, Martina Pukhrambam, Dinamani Medhi, Vijay Paul, Mokhtar Hussain, Mansukh Singh Jatana, Ninad Bhatt and Mihir Sarkar

ICAR-National Research Centre on Yak, Dirang-790101, West Kameng district, Arunachal Pradesh

*Correspondence: loatshubham25@gmail.com

## Introduction

The yak is a remarkable animal because of its distinct physical and structural characteristics that allow it to adapt to rugged mountains with unfavourable climates. Yaks are regarded as the "almighty livestock" by the herding communities since they are deeply ingrained in the customs, socioeconomic processes and culture. Yak (*Peophagus grunniens L.*) is a unique bovine species living in the difficult terrains, which appeared some two million years ago and provides the indigenous people with meat, milk, butter, cheese, wool, fiber, leather, fuel and travel. Yaks are well adapted to the harsh climate and hypoxia occurring under high altitude grazing conditions of the Himalayan Mountains. The population of yaks in India as per the 19th Livestock census is 0.077 million (19th Livestock Census All India Report 2012).



## Yak-rearing tracts of India

Indian yaks are classified into Arunachali yak, Himachali yak, Ladakhi yak and Sikkimi yak based upon the geographical area inhabitation and so far, only Arunachali yak has been described as a breed in India. Yaks are reared under transhumance on high altitude pastures under cold & hypoxic climate by various ethnic pastoral nomads for their livelihood and nutritional security. These pastoral nomads are known as Changpas in Ladakh, Brokpas in Arunachal Pradesh & Dokpas in Sikkim. Yaks play an important role in human survival in high-altitude areas, as they provide essential items such as yak milk, meat, hide, and fuel (dried yak dung) for the local people (Wang et al., 2014). Moreover, yaks are frequently used as a source of power for transportation in high-altitude areas.

## Yak-milk and its Characteristics

- Yak milk is creamy white, sweetish, fragrant & thick in consistency.
- It contains 84.37-80.37% water, 15.63-19.63% total solids, 3.45-4.27% protein, 5.29-8.73% fat & 0.6-0.8% ash.
- The protein composition of yak milk differs from that of bovine milk in terms higher contents of κ-casein, β-casein and β-lactoglobulin (Li *et al*. 2010).
- Milk fat of yak at very high altitudes is richer in PUFA and CLA (Nikkhah *et al*., 2011).
- High contents of colloidal and soluble calcium and phosphorus are other advantages of the Yak milk (Nikkhah *et al*., 2011).

## Importance of Yak milk and milk-products

Recently, Yak milk has attracted much attention due to its unique characteristics and therefore, it is an urgent need to expand its production capacity. Milk and its by-products are one of the most important protein sources produced by the mammary glands of mammals, providing complete dietary nutrition, bio-available amino acid sources, and essential micronutrients, as well as readily digestible food for the human diet. Yaks offer significant economic benefits to the pastorals living in the high altitudes and it has been used for farming and threshing, fur, meat, and high quality milk (Guo et al., 2014). Yak milk and dairy products such butter (Mar), ghee, curd, wet cheese (*chhurpi*) and hard cheese (*Churkam*) gives the pastoral nomads an essential source of vitamins and nutrients (Li et al., 2011).

**Figure 1: (Left to right)** Butter (Mar), ghee, curd, wet cheese (*chhurpi*) and hard cheese (*Churkam*)

Yak milk also plays a vital role in health protection. In recent years, there has been increasing research attention on yak milk. Yak milk not only has a high nutritional value but also holds potential benefits for human health. Yak milk can be used directly or processed into various traditional foods such as butter, cheese, yogurt, and Tibetan tea by local herdsmen.

### Processing and value-addition of Yak milk products:

- By adding value to the yak milk through better processing and packaging, these products can become more profitable and appealing to the consumers.
- Product development innovations like cheese with nutritious benefits and flavoured drinks broaden the potential customer reach beyond regional markets.
- After consumption, the herders sell the remaining yak milk products.
- Yak milk products give them vital nutrients during their migration time when other sources are sparse and help them survive the harsh winters in the high mountains.
- Yak milk value-addition has always been accomplished through the use of traditional technology.
- Curd inoculums are used to ferment the skimmed yak milk.
- Churpi is the soft, moist cheese that remains after the butter is churned out and is meant to be eaten right away.
- The longer-lasting dried chhurpi, known as churkham, is kept in yak skin bags.
- To make ghee, butter is purified and then similarly stored. Before being sold in the market, the cheese and ghee kept inside yak skin bags must be cleaned of their strong odor.
- The most sought-after yak milk products on the market are ghee and chhurpi, with demand always exceeding supply.
- Furthermore, there is only a meagre supply of the product outside of the West Kameng and Tawang districts' marketplaces.
- Brokpas charge between Rs. 600 and Rs. 650 per kilogram for chhurpi and Rs. 400 for ghee.

- Shopkeepers in retail typically add a profit margin of Rs 50 to the sales of chhurpi; bite-sized portions are sold for Rs 10 each.
- The retail price of ghee is Rs 600 per kilogram.
- The conventional production process requires a lot of labour. Also, outsiders are frequently hesitant to consume yak milk products because of the unhygienic processing and packaging of the milk and its products.
- The ICAR-National Research Centre on Yak has developed a number of initiatives to help the community meet the demand for milk and its by-products in an effort to grow the market.
- A female yak yields 0.98 to 1.04 kg of milk on average every day over the course of 180-day lactation period, or roughly 185 kg of milk in total.
- Yak milk is more nutrient-dense than that of other cows, despite its low production.
- The Indian Council of Agricultural Research-NRC-Yak in Dirang conducted an evaluation of the physicochemical characteristics and nutrient composition of Arunachali yak milk that is raised on farms.
- The results showed that this milk is more nutritious than other types of bovine milk.

**Preparation and processing of some of the Yak milk products:**

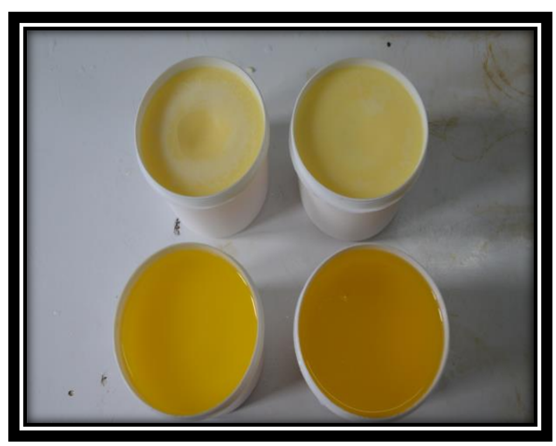
***Yak Ghee***

- Ghee is used for frying variety of dishes.
- The standard process of preparing ghee is as follows:

1) Heat the milk cream or butter in an iron kettle at 700-800ºC till complete liquid form is obtained.

2) The scum at the top is removed and the product is allowed to settle for one hour.

3) The ghee is then packed and stored in a cooler place for proper crystallization and grain formation.



***Yak Paneer:***

- Paneer is a dairy product prepared by acid coagulation of hot milk.
- Traditionally, yak paneer is prepared by using juice from a citrus fruit called thungi or whey obtained from the preparation of churpi (wet cheese).
- The standard process of preparation is as follows:

1) Heat the milk to 90ºC for 15 seconds and cooling it to 76ºC.
2) Then, 1 % citric acid boiled and kept at 70ºC is added to it (about 1-1.5 litres of 1% citric acid per 5 litres of milk).
3) The content is allowed to cool to 70ºC. The coagulum settles within 10 minutes.
4) The coagulum is transferred to a muslin cloth or strainer. The whey is allowed to drain and the coagulated mass is pressed with weight (about 5 times weight of coagulum) for 10 minutes.
5) The blocks are immersed in chilled water at 4ºC for 3 hours.
6) Excess water is drained and the product is packed and stored at 4ºC.

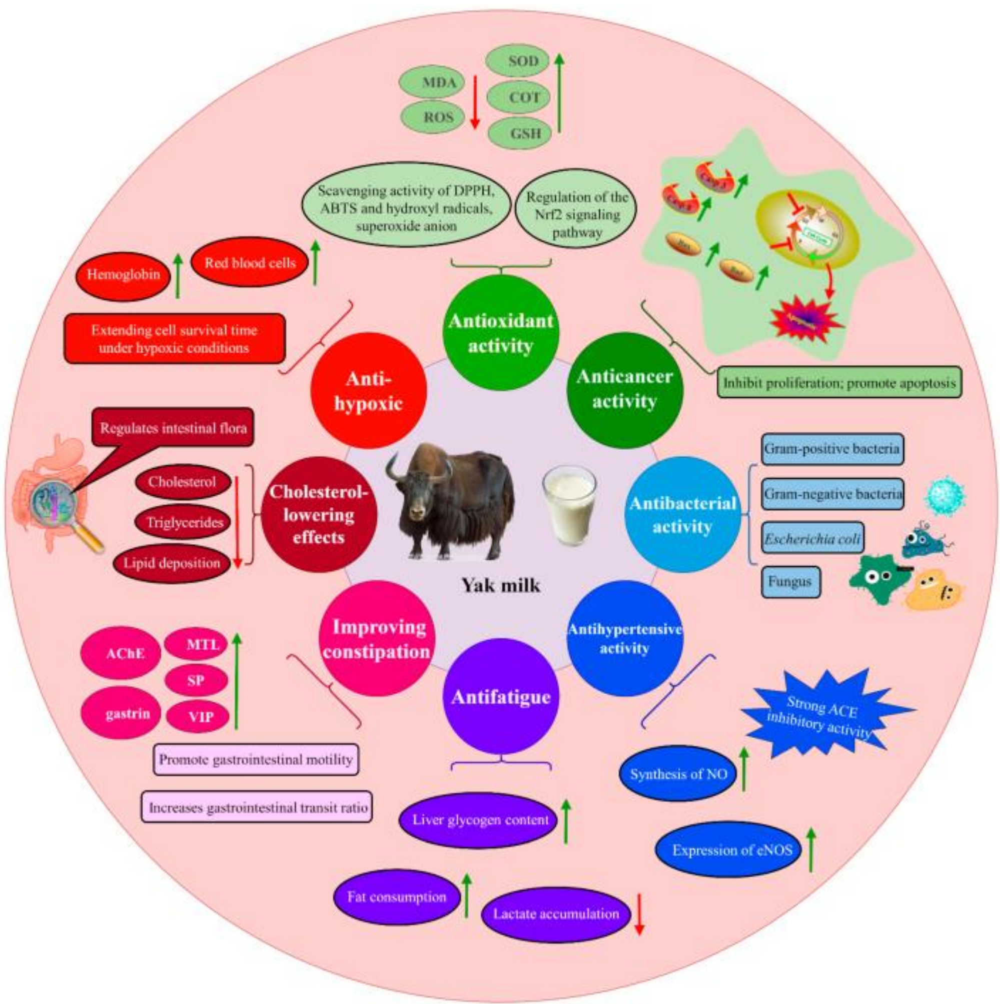
***Yak Churkam (Candy):***

- Churkam is a hard candy relished both by the local communities and the tourists. It is a good source of energy.
- The standard process of preparation is as follows:

1) The raw milk is heated to 50ºC for 5 minutes for cream separation.
2) A very hard pressed paneer is obtained which is then cut into small cubes of uniform size. Whey collected is concentrated by heating and coagulated milk cubes are added into it.
3) The whole content is stirred well to get concentrated mass. The cubes layered with the concentrated whey is taken out and placed in a tray with parchment paper.
4) The tray is placed in hot air oven at 45ºC for 16 hours to get a completely hard mass.

5) The final product is packed and stored at 4ºC.

## Additional benefits of Yak milk and its products:



Yak milk is generally regarded as naturally concentrated milk that has been enhanced with a higher nutrient density, rich in antioxidants, omega-3 fatty acids, amino acids, and minerals. Richer than cow's milk in terms of protein, fat, lactose, minerals, and total solids is thick and delicious yak milk. Additionally, a Geographical Indication (GI) tag was recently applied to the Arunachali Yak chhurpi. Compared to other types of milk, yak milk appears to contain a richer and more diverse range of nutrients. During its primary lactation period, yak milk has high levels of fat (5.5–7.5%), protein (4.0–5.9%), and lactose (4.0–5.9%). The unique amino acids, fatty acids, high levels of vitamins, specific enzymes, and beneficial microorganisms found in yak milk may have a positive impact on the health of nomadic populations in the high-altitude regions (Guo et al., 2014). Studies have found that the bioactive components in yak milk possess various bioactive functionalities, including antioxidant, anticancer, antimicrobial, blood pressure-lowering, anti-fatigue and constipation treatment properties.

## Conclusion:

The practice of herding nomadic yaks is essential to the maintenance of both inherited traditional knowledge and ecological consciousness. However, as time changes, its subsistence economy is contributing to its slow downfall. Therefore, adding value to yak milk offers a unique chance for the financial revival of yak pastoralism. Arunachal Pradesh Yak Churpi is the first among the yak milk products to get the coveted Geographical Indication (GI) tag. Other products will follow the same suit. ICAR-NRCY is actively involved in forming yak herders group based on commodity (yak milk, yak fibre or both) of interest to give yak herders a sense of cohesion and working as a collective. So, far there are 05 yak herders group and more are in the pipeline. The first yak herders cooperative, 'Arunachal Pradesh Yak Herders and Products Development Cooperative Society Ltd' bearing Registration No. Coop (ORG) 1301/2024 has been formed on 18th January 2024. This will provide a platform to popularise and eventually market yak products at profitable prices, thereby, contributing to the upliftment of the herders and yak husbandry. In the Himalayan region, the Brokpas and their yak milk products represent a singular junction of culture, commerce and ecology. Initiatives to maintain their traditional way of life in the facet of contemporary challenges are crucial for the future of their community and the preservation of the cultural legacy of the area.

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CHAPTER- 9

# Non bovine milk powder: Thermal drying vs freeze drying

Swagatika Priyadarsini*, Yogesh Kumar, Mitul Bumbadiya and Vishwa Ranjan Upadhyay

ICAR-National Research Centre on Camel, Bikaner, Rajasthan, India

*Correspondence: drswagatika.vet@gmail.com

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## Introduction

Non-bovine animal milk refers to milk sourced from animals other than bovine group (cow, buffalo, yak, Mithun etc.) of animals such as goats, sheep, camels, mare, donkey and other mammals. While cow's milk is the most commonly consumed and produced type of milk globally, non-bovine milk has gained increasing attention for its unique nutritional properties, potential health benefits, and cultural significance in various regions. These alternative milk sources offer diverse flavor profiles and compositional differences that cater to specific dietary needs and preferences. Non-bovine milk often has distinct nutritional profiles compared to cow's milk. For instance, goat's milk is known for its higher levels of certain minerals like calcium and magnesium, as well as easier digestibility due to its smaller fat globules and different protein structure. Sheep's milk is rich in protein and fat, making it highly nutritious and beneficial for cheese production. Camel's milk, traditionally consumed in many desert regions, is valued for its high vitamin C content and potential benefits for individuals with lactose intolerance or allergies to cow's milk. Buffalo milk, with its higher fat content, is ideal for making rich, creamy dairy products like mozzarella cheese.

The consumption of non-bovine milk is deeply rooted in the cultural practices and traditions of many societies. In Mediterranean and Middle Eastern countries, goat and sheep milk products like feta and yogurt are dietary staples. Camel milk has a long history in Middle Eastern, African, and Asian cultures, often considered a superfood due to its nutrient density and medicinal properties.

Drying of milk is a critical process in the dairy industry that involves the removal of water from liquid milk to produce milk powder. This transformation extends the shelf life of milk, reduces transportation costs, and enhances convenience for storage and use. Additionally, dried milk powders also have culinary values. The process of drying milk encompasses various techniques, each tailored to preserve the nutritional quality and functional properties of milk while achieving the desired dryness. The principles of drying milk include thermal and freeze drying. The selection of a drying method depends on factors such as the desired end-product characteristics, production scale, and economic considerations. Each method impacts the physical and chemical properties of the resulting milk powder, influencing its solubility, flavor, and nutritional value. Understanding these processes and their implications is essential for optimizing milk powder production to meet diverse consumer needs and industrial applications.

In this chapter we will learn about the processes involved in preparing milk powder, there advantages and limitations. In the end, we will also discuss the reasons for the preference of freeze drying over thermal drying of non-bovine milk.

## Thermal Drying

Thermal drying of milk is a widely used process in the dairy industry, involving the application of heat to remove water from liquid milk. Thermal drying can be achieved by either drum drying or spray drying.

### *Spray Drying*

Spray drying is the most prevalent method for drying milk due to its efficiency and ability to produce high-quality milk powder. Uniform particles are obtained in this procedure which provides a superior instantizing product, reduced product losses, less over- and under-drying and more efficient drying.

The process involves several steps:

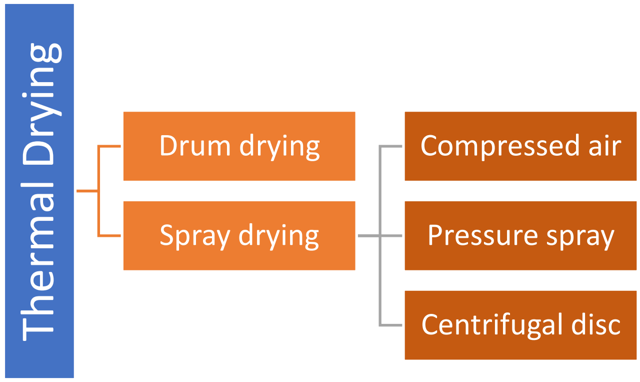


**Figure 1.** Methods of thermal drying of milk.

1. Atomization: Liquid milk is pumped into an atomizer that disperses it into fine droplets (50 to 150 microns in diameter).
2. Drying Chamber: These droplets are introduced into a chamber with hot air (typically between 150-260°C). The large surface area of the droplets allows for rapid evaporation of water.
3. Separation: The dried particles are separated from the moist air. The resulting powder is collected at the bottom of the chamber or in cyclones.

4. Cooling and Packaging: The milk powder is cooled and then packed for storage and distribution.

Spray drying is favored for its ability to produce powders with good solubility, controlled particle size, and minimal heat damage to the nutrients.

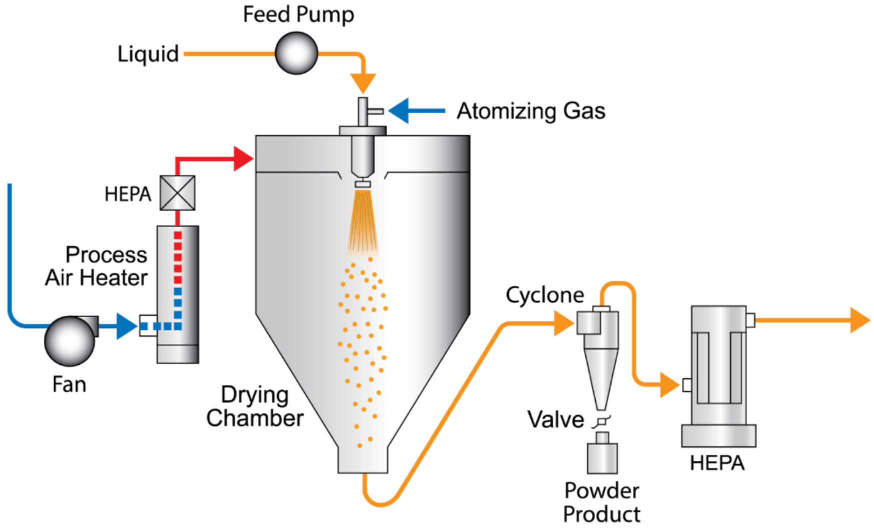


**Figure 2.** Principle of Spray drying.

***Drum Drying***

Drum drying, though less common than spray drying, is another method used for drying milk. It may have single drum, twin drum or double drum, and the drum material can be of Steel, Alloy steel, stainless steel, cast iron or chrome or nickel-plated steel. Usually, steel drums are used in this case. Drum drying involves the following steps:

1. Milk application: Liquid milk is applied as a thin layer onto the surface of heated drums (often set between 120-170°C).
2. Drying: The drums rotate, and the milk film dries almost instantaneously due to the direct contact with the heated surface.
3. Scraping: Dried milk is then scraped off the drums using a blade.
4. Cooling and Packaging: Similar to spray drying, the dried product is cooled and packaged.

Drum drying is efficient and cost-effective but tends to produce powders with a denser structure and sometimes a cooked flavor due to the higher temperatures and direct contact with the heat source.

***Comparative Analysis***

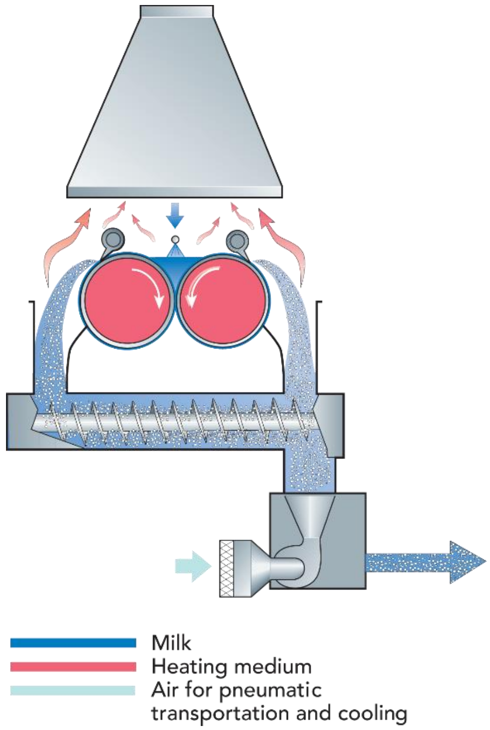


**Figure 3.** Principle of drum drying.

Both spray drying and drum drying have their unique advantages and limitations:

- Spray Drying:
  - Advantages: Produces fine, uniformly sized particles; preserves nutrients better due to rapid drying; excellent solubility; most economical when large quantities of milk are handled
  - Limitations: Higher energy consumption; more complex equipment and maintenance; involves large capital investment in plant and buildings; loss of nutrient quality such as the destruction of water soluble vitamins.
- Drum Drying:
  - Advantages: Lower energy costs; simpler equipment; suitable for producing flakes and powders with a high bulk density.

- Limitations: Can impart a cooked flavor to the product; less control over particle size; potential nutrient degradation due to higher temperatures and longer exposure.

***Other thermal drying methods***

While spray drying and drum drying are the primary methods, other techniques like roller drying and vacuum drying are sometimes used for specialized applications.

- Roller Drying: Similar to drum drying but typically involves a continuous belt or multiple rollers, offering greater control over film thickness and drying conditions.
- Vacuum Drying: Involves reducing the pressure to lower the boiling point of water, allowing drying at lower temperatures. This method is less common but useful for heat-sensitive products.

***Effect of thermal drying on milk:***

Effect on Salt:

- Calcium phosphate, present in milk, is less soluble at high temperature. Thus, the concentration of soluble calcium and phosphate is decreased during heating.
- Caseinate-phosphate micelles aggregation may occur (reversibly or irreversibly).

Effect on protein:

- Denaturation of whey protein (The order of denaturation of whey proteins are immunoglobulin, blood serum albumin, beta-lactoglobulin then alpha-lactalbumin)
- Cooked flavor: –SH groups are released from whey protein → results in the liberation of volatile sulphides including $H_2S$.

Other effects:

- Browning of milk occurs during thermal processing due to either Maillard reaction or Caramelization of sugar (lactose)
- Compound such as furfuryl alcohol, furfuryl aldehyde, maltol, acetol, acetaldehyde, acetic, formic and pyruvic acid, NH3, H2S and CO2 may also form during thermal processing of milk.

## Freeze drying

Freeze drying, also known as **lyophilization**, is a dehydration process that preserves milk by removing water through sublimation. Lowering the pressure and temperature in a controlled manner below the triple point of water achieves sublimation. This technique is highly effective for retaining the nutritional quality, flavor, and structure of milk, making it a preferred method for producing high-quality milk powder, especially for specialized applications such as infant formula, pharmaceuticals, and premium food products.

The freeze-drying process involves three main stages: freezing, primary drying (sublimation), and secondary drying (desorption).

1. Freezing:

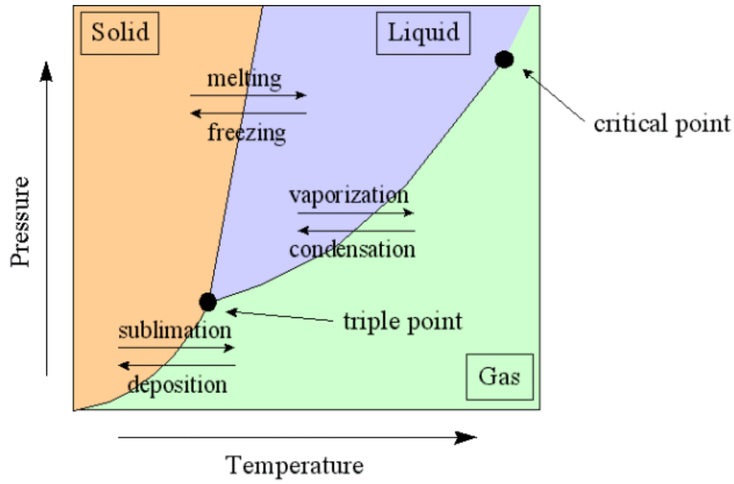


**Figure 4.** Triple point in freeze drying.

- The milk is first cooled to temperatures well below its freezing point (typically around -40°C).
- This causes the water in the milk to form ice crystals. Rapid freezing is essential to create small ice crystals, which helps in preserving the structure of the milk solids.

2. Primary Drying (Sublimation):

- The frozen milk is placed in a vacuum chamber. The pressure is reduced to below the triple point of water, allowing ice to sublimate directly into water vapor without passing through the liquid phase.
- Heat is applied gently to provide the latent heat of sublimation, which facilitates the removal of ice. This step is carried out at low temperatures to avoid melting the ice and damaging the milk's structure.
- This stage removes the majority of the water content, typically reducing the moisture content to around 5-10%.

3. Secondary Drying (Desorption):

- The remaining water, which is primarily bound water, is removed by gradually increasing the temperature while maintaining the vacuum.
- This step ensures the final moisture content is reduced to a very low level (typically less than 2%), making the milk powder stable and suitable for long-term storage.

- The entire freeze-drying process can take several hours to days, depending on the quantity and composition of the milk.

***Effect of freeze drying on milk:***

- Nutritional Preservation: Freeze drying operates at low temperatures, which helps in preserving sensitive nutrients, such as proteins, vitamins, and bioactive compounds, that might be degraded by heat in other drying processes.
- Retention of Flavor and Aroma: The gentle drying process maintains the original flavor and aroma of the milk.
- Structure and Texture: The process preserves the physical structure of the milk solids, resulting in a product that reconstitutes well when mixed with water.
- Extended Shelf Life: The low moisture content in freeze-dried milk powder prevents microbial growth and reduces oxidative spoilage, enhancing shelf life.
- There is an almost complete absence of air throughout the drying cycle (operated under vacuum)
- Moisture content of the finished product can be reduced to extremely low values
- Higher retention of calcium and iron

***Applications of Freeze-Dried Milk***

- Infant Formula: Due to its high nutritional retention, freeze-dried milk is ideal for infant formula.
- Medical and Specialty Diets: The preservation of bioactive compounds makes it suitable for medical nutrition products and specialty diets.
- Premium Food Products: High-end food products use freeze-dried milk for its superior quality and taste.
- Space Missions and Military Rations: Freeze-dried milk is lightweight and has a long shelf life, making it ideal for space missions and military rations.

***Challenges and Considerations in freeze drying***

While freeze drying offers numerous benefits, it also presents some challenges:

a. Cost: Freeze drying is more expensive than other drying methods due to the high energy consumption and longer processing times.
b. Complexity: The equipment and process control required for freeze drying are more complex and require specialized knowledge and maintenance.
c. Scale: Freeze drying is often less suitable for large-scale operations due to its slower throughput compared to spray drying.

***Emerging Trends and Market Potential***

The growing interest in non-bovine milk is also driven by dietary trends, such as the increasing demand for lactose-free and alternative dairy products. Additionally, concerns about sustainability and animal welfare have prompted consumers to explore diverse milk sources. The market for non-bovine milk and related products is expanding, offering opportunities for innovation in dairy alternatives and functional foods.

## Conclusion

Non bovine milk varieties are considered as superfoods. They have high medicinal values. Understanding the unique qualities and benefits of these milk sources can help in promoting their consumption and integrating them into modern dietary practices. In contrast to thermal drying, freeze drying retains nutraceutical values of the milk. The benefits of freeze drying extend beyond nutritional retention to include extended shelf life and improved product stability, making it particularly valuable for specialized applications such as infant formula, medical nutrition, and premium food products. Despite the higher costs and complexity associated with freeze drying, its advantages in preserving the integrity and quality of non-bovine milk make it a worthwhile investment for producers aiming to meet the growing demand for alternative dairy products. As consumer preferences shift towards more diverse and health-conscious dietary options, the role of freeze-dried non-bovine milk in the market is set to expand. By leveraging this technology, producers can offer innovative and high-quality products that cater to a wide range of nutritional needs and cultural preferences, thus enhancing the appeal and accessibility of non-bovine milk in the global dairy industry.

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CHAPTER- 10

# The Potential Implications of Pesticide Residues in Non-Bovine Milk

Mranalini Prerna* and Samar Kumar Ghorui

ICAR- National Research Centre on Camel

*Correspondence: mranaliniprerna89@gmail.com

## Introduction

India is indeed one of the largest milk producers globally and also holds a prominent position in global agriculture, with a diverse range of crops and significant agricultural output. Pesticides are crucial in ensuring high yields and protecting crops and livestock from pests and diseases. Non-bovine milk is gaining popularity as an alternative of bovine milk owing to its therapeutic benefits but the unregulated or excessive use of pesticides, often due to a lack of awareness or enforcement of regulations, leads to residues contaminating various parts of the food chain, especially milk and dairy products. Pesticides contain not only the active ingredients that kill pests but also other chemicals that help the pesticide work or spread. These additional chemicals, along with the breakdown products of the pesticide itself, potentially pollute the environment. Various classes of pesticides, encompassing Organochlorines, Organophosphates, Synthetic Pyrethroids, and Triazines, have been identified in diverse dairy products. Depending on the type and properties of the pesticide, residues can be excreted through urine, adsorbed in adipose tissue, or excreted into the milk. Pesticides and their degradation products can impair the use of surface waters and groundwater, which can indirectly affect milk production. Pesticides are known to cause chronic or acute health hazards to humans, causing both immediate poisoning and chronic diseases. These diseases can attack the nervous system, hormones, fertility, and contribute to cancer. Pesticide residues detected in milk and dairy products represent a global public health concern with significant implications for international trade. Dairy farm owners need to be aware of the potential presence of pesticide residues in milk and milk products, as these residues can pose health risks to consumers. They should be educated about its sources in milk, such as the transfer of pesticides from feed, fodder, and drinking water to animal bodies and awareness should be raised about the importance of implementing pesticide residue monitoring programs in dairy farms to ensure food safety and decrease exposure risks for consumers.

Pesticides are chemical substances or mixtures used to control, repel, or eliminate pests such as insects, weeds, fungi, rodents, and pathogens that can harm crops, livestock, or human health. They play a vital role in modern agriculture by protecting plants from pests and diseases, increasing crop yields, and ensuring food security. Pesticides come in various forms, including insecticides, herbicides, fungicides, rodenticides, and bactericides, each designed to target specific types of pests. While pesticides offer significant benefits in pest management and crop protection, their use raises environmental and health concerns due to potential residues, ecological impact, and risks associated with exposure. Sustainable pesticide use, integrated pest management (IPM) practices, and regulatory oversight are essential for balancing the benefits and risks of pesticides in agriculture and ensuring safe food production systems.

**Table 1.** Some Commonly used Pesticides

| **Pesticide** | **Examples** | **Properties** | **Toxicity** |
|---|---|---|---|
| Organochlorine | Lindane, DDT, Endosulfan | Low solubility in water, high solubility in oils, fats, lipids etc.,<br>Not prone to environmental degradation. | High acute toxicity as well as chronic toxicity |
| Organophosphates | Malathion, Chlorpyrifos | liquids or semi solids and volatile. Some compounds have slight solubility in water. | Exhibits acute extreme toxicity to slight toxicity |
| Carbamates | Carbaryl, Propoxur, Methomyl | Non-volatile solids and prone to environmental degradation | Chronic toxicity is insignificant |
| Pyrethroids | Cypermethrin, Deltamethrin | volatile and Non-volatile solids or semisolids which are insoluble in water | Chronic toxicity |

## Potential sources of pesticide contamination in non-bovine milk production

***Feed Contamination*:** Crops or forage used as animal feed are sprayed with pesticides. Residues of these pesticides can then be transferred to the milk produced by the animals consuming the contaminated feed.

***Water Contamination*****:** Water sources are contaminated with runoff from nearby fields where pesticides are applied and carry residues into water bodies which may then be used in milk production.

***Environmental Exposure***: Animals graze in fields treated with pesticides or come into contact with contaminated soil or vegetation.

***Veterinary Medications***: Sometimes not directly pesticides, certain veterinary medications for the treatment skin infections, flies or ticks are used which can act as contaminants and may leave residues in milk if not used according to regulations.

***Pesticide Drift***: Pesticide drift occurs when pesticides sprayed on crops drift away from the target area due to factors like wind or improper application. Non-target crops or vegetation near the spraying area can absorb these drifting pesticides, potentially leading to contamination in milk-producing animals if they consume these plants.

***Equipment and Facilities***: Contamination can also occur during the handling and storage of milk, if equipment or facilities are not properly cleaned or if they come into contact with surfaces contaminated with pesticides.

***Misuse of Pesticides***: Improper use or overuse of pesticides can increase the risk of contamination. This includes using pesticides not approved for specific crops or livestock, exceeding recommended application rates, or ignoring pre-harvest intervals.

***Residue Persistence***: Some pesticides have a longer persistence in the environment or in animal tissues, which increases the likelihood of residues being present in milk even after the initial application.

***Global Trade***: In regions where non-bovine milk products are imported, there is a risk of contamination during transportation or storage if proper regulations and monitoring are not followed.

## Pesticide Impact on Human Health

Various pesticides majorly target the nervous and endocrine systems, causing potential toxicity to humans and leading to significant direct or indirect harmful health concerns. Human exposure to pesticides can occur directly due to direct handling of pesticides agriculture, public health, livestock, or indirectly through consuming contaminated food and water or inhaling pesticide droplets from the contaminated environment. Children are mainly more prone to its toxicity due to their physical characteristics, behaviour, and physiology; exposure to even low levels of pesticides during early development can result in severe health impacts.

Research has also linked pesticide exposure to increased rates of various human diseases, like cancers, Alzheimer's, Parkinson's, asthma, bronchitis, infertility, birth defects, hyperactivity disorder, autism, organ dysfunction, and system failures. Pesticides can also induce genetic and epigenetic changes through cellular-level processes, potentially disrupting and mimicking endocrine functions which leads oxidative stress and damaging cellular functions.

## Pesticide Impact on Environment

***Water Contamination*****:** Pesticides leach into soil and groundwater or run off into surface water bodies like rivers, lakes, and streams and bioaccumulate in aquatic organisms, potentially leading to indirect contamination through the food chain.

***Soil Degradation*****:** Continuous use of pesticides degrades soil quality by disrupting microbial communities, reducing soil fertility, and impairing nutrient cycling.

***Impact on Biodiversity***: Pesticides can harm non-target organisms such as beneficial insects, pollinators, birds, and soil organisms disturbing ecological balance in agricultural ecosystems.

***Persistence****:* Some pesticides have long persistence in the environment, remaining in soil, water, and vegetation for extended periods which disrupts ecosystem balance.

***Health Risks for Livestock***: Direct exposure of livestock to pesticides, either through contaminated feed, water, or environmental exposure, can pose health risks. Pesticide residues may accumulate in animal tissues, including milk-producing glands, leading to milk contamination.

## Testing Pesticides residues in milk

***Chromatographic Techniques***: High-performance liquid chromatography (HPLC) and gas chromatography (GC) are commonly used chromatographic techniques for analyzing pesticide residues in milk. These techniques separate and identify pesticides based on their chemical properties.

***Mass Spectrometry (MS)****:* Coupling chromatographic techniques with mass spectrometry enhances the detection and quantification of pesticide residues. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are used for identifying specific pesticides and measuring their concentrations accurately.

***Immunoassays****:* Enzyme-linked immunosorbent assays (ELISA) and other immunoassay methods are used as a rapid screening techniques detect the presence of pesticide residues in milk. While less specific than chromatographic methods, but they provide quick results and can be used for preliminary screening purposes.

***Multi-Residue Methods***: Multi-residue methods (MRMs) are complete approaches that allow simultaneous analysis of multiple pesticide residues in a single sample. These methods are efficient for screening a wide range of pesticides and are commonly used in monitoring programs.

***Advanced Techniques*****:** Advancements in analytical technology has led to the development of advanced techniques such as high-resolution mass spectrometry (HRMS), tandem mass spectrometry (MS/MS), and ultra-performance liquid chromatography (UPLC). These techniques offer enhanced sensitivity, specificity, and efficiency in detecting and quantifying trace levels of pesticide residues in milk.

## Strategies to reduce pesticide contamination in milk

### *Monitoring food safety and protecting Human health*

National regulatory bodies, FSSAI, Food and Drug Administration (FDA) in the United States, or the European Food Safety Authority (EFSA) in the European Union, establish legal limits or maximum residue levels (MRLs) for pesticides in milk. These MRLs are based on scientific risk assessments that consider factors like toxicity, exposure levels, and consumption patterns.

Organizations like the Codex Alimentarius Commission, jointly established by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations, develop international food standards, including MRLs for pesticides in milk. Codex standards serve as benchmarks for harmonizing regulations globally.

### *Risk Assessment and Scientific Evaluation*

Regulatory agencies implement monitoring programs to routinely test milk samples for pesticide residues. These programs involve sampling milk at different stages of production, processing, and distribution to assess compliance with MRLs and detect any potential contamination issues**.** International monitoring programs, such as the Global Environment Monitoring System for Food and Agriculture, support coordinated efforts to monitor pesticide residues in food, including milk. These programs enhance data sharing and collaboration among countries to address global food safety challenges.

### *Public Awareness*

Agencies educate industry stakeholders, healthcare professionals and consumers about safe food handling practices and the importance of purchasing products that meet established safety standards. These organizations contribute in communication of efforts by sharing research information, guidelines, and best practices related to pesticide residues in milk. They promote public awareness campaigns initiatives to enhance food safety awareness globally.

***Integrated Pest Management***

According to European union it means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment.

***Biopesticides***

They also known as biological pesticides, use pathogenic microorganisms that target specific pests, providing a sustainable and efficient approach to pest management. They present a reduced risk to the environment and human health compared to conventional pesticides. The main types of biopesticides include biofungicides (such as *Trichoderma*), bioherbicides (like *Phytopthora*), and bioinsecticides (including *Bacillus thuringiensis*). Incorporating biopesticides into agricultural practices and public health programs offers significant potential benefits.

***Insect growth regulators***

Insecticides containing growth-regulating properties (IGR) damage insects by interfering with biochemical pathways necessary for their growth and development. Exposure to these compounds may lead to insect mortality through irregular regulation of juvenile hormones, impairing cuticle formation or ecdysis.

***Crop Rotation***

The manipulation of crop sequence to break the life cycle of pests through rotation with crop species belonging to different families is a major lever to strengthen robustness of cropping and farming systems.

***Proper Disposal of remains of insecticides and empty packaging***

Never pour the remaining insecticide into rivers, pools or drinking-water sources. Decontaminate containers where possible. For glass, plastic or metal containers this can be achieved by triple rinsing, i.e. part-filling the empty container with water three times and emptying into a bucket or sprayer for the next application. The used packages shall not be left outside to prevent their re-use. The packages shall be broken and buried away from habitation.

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CHAPTER- 11

# Synergizing camel dairying with arid agriculture: A scientific assessment of climatic stress, adaptation and milk production

Vishwa Ranjan Upadhyay[1]*, Swagatika Priyadarsini[1], Mitul Bumbadiya[1] and Manisha[2]

[1]ICAR-National Research Centre on Camel, Bikaner, Rajasthan (India)

[2]ICAR-National Dairy Research Institute, Karnal, Haryana (India)

*Correspondence: vishwaranjanhzb@gmail.com

## Introduction

The dromedary camel (*Camelus dromedarius*), a large ungulate, is highly adapted to desert environments, exhibiting remarkable resilience to heat and dehydration. Camels are uniquely suited to arid climates, with the ability to survive up to a week without water, making them one of the most drought resilient livestock species. This multipurpose species is essential to the livelihoods of pastoralists in arid and semi-arid regions, being well-adapted to extreme temperatures, desertification, and scarce natural resources, while offering a reliable source of food, transport, and traction. One of the camel's most distinctive heat adaptation strategies is the efficient water conservation and resistance to osmotic stress. Additionally, their ability to regulate body temperature, minimizing heat gain during the day and promoting heat dissipation at night, while reducing evaporative cooling to conserve water, demarcate them from other livestock species. These physiological adaptations collectively enable camels to thrive in arid environments and efficiently manage water and temperature stress. Despite their remarkable thermotolerance, increasing drought and shrinking grazing lands due to climate change are becoming major concerns, leading to malnutrition and other health challenges for camel herds. Additionally, climate change is contributing to the emergence and spread of vector-borne diseases, such as camel pox, peste des petits ruminants, babesiosis, theileriosis, trypanosomosis etc. Dairy camels, in particular, are notable for their ability to continue lactation under harsh environmental conditions. However, lactating camels are more vulnerable to environmental changes, as they experience elevated metabolic heat production and heat storage, making them more susceptible than non-lactating camels. Climate models suggest that heat stress will intensify under future climate scenarios, posing a significant threat to the health and production of even climate resilient species like small ruminants and camel.

## Physiological, anatomical and behavioural resilience characteristics in camel

The camel exhibits exceptional adaptability to prolonged periods of water and food scarcity for thriving in harsh environments through a combination of physiological, morphological and behavioural adaptations. Physiologically, camels minimize water loss by maintaining high urine osmolarity and reducing glomerular filtration rates. They tolerate water deprivation by absorbing water in the lower intestine, excreting minimal water through faeces and can drink water equivalent to up to one-third of their body weight when rehydrating. The unique water sac structure in the stomach and specialized adaptations in kidneys are critical in conserving water within the body. Camel kidneys excrete highly concentrated urine, allowing them to tolerate elevated salt levels. Camel erythrocytes are anucleate and elliptical in shape, which likely aids their circulation in dehydrated conditions by enhancing blood flow. These cells are highly adapted to dehydration, maintaining fluidity and withstanding osmotic stress. Notably, their membrane phospholipid composition increases fluidity, enabling the cells to withstand significant osmotic fluctuations without rupturing, even during rapid rehydration. Their flexible membranes, rich in saturated and short-chain fatty acids, provide greater stretchability and resilience under osmotic pressure. Camel erythrocytes have an extended lifespan, further contributing to water conservation. Camel platelets also exhibit remarkable thermal resistance, tolerating temperatures of 43-45°C without significant structural or functional damage, unlike human platelets. Even at extreme temperatures of 50°C, which severely affect human platelets, camel platelets remain largely functional. Additionally, camels have the capacity to drink large volumes of water up to 200 liters at once to quickly replenish fluid loss. Camels employs adaptive heterothermy, having a wide range of body temperature variation, storing heat during the day and releasing it at night. By this mechanism, camel can fluctuate its body temperature between 34 and 42 °C, thus minimizing perspiration and avoiding water losses through evaporation. The rete mirabile, a specialized vascular structure, facilitates selective brain cooling through counter-current blood flow. These arrangement or distribution of camel arteries and veins help in mitigating the high blood temperature of the body reaching the brain, thus protecting the animal from potential brain damage. These specialised mechanisms of adaptive heterothermy and selective brain cooling uphold the homeostasis of vital organs and regulate heat loss without incurring extra energy. Morphologically, camels possess specialized features such as body hair, chest pads, third eyelids, and humps, aiding in thermoregulation and survival in desert environments, including sandstorms. At the anatomical level, several adaptations have been identified: the one humped camel is provided with a single hump filled with fat rather than the common belief of being filled with water. The high fat content in camel humps serves as an energy store which is used in periods of food limitation. Camel nostrils have a muscular nature which allows camel to fully control its opening and closure, thus avoiding sand inhalation in case of sandstorm events. The feet of camel are thick and characterized by leathery pads which spread widely on hitting the ground, consequently preventing the animal from sinking into the warm sand. Camel legs are long compared to other desert animals and during walking each two legs move on one side, rocking side-to-side, therefore giving another reason for being nicknamed the ship of desert. Moreover, in the recumbent position, the camel sternum takes a “plate like” conformation permitting more air circulation.

Daily oscillations in physiological variables have been documented for numerous parameters, including locomotor activity, body temperature, heart rate, blood pressure, hormonal secretion, and urinary excretion. While the rhythmic patterns of individual variables have been extensively studied, research on the temporal relationships between the rhythms of different physiological variables is limited. In camels, only a few studies have explored daily rhythmicity, focusing on variables such as body temperature, sweat rate, plasma aldosterone, plasma melatonin and other general physiological indicators. These variables display varying degrees of rhythmicity, each peaking at different times of the day. For instance, plasma cholesterol reaches its peak 24 minutes after midnight, while plasma calcium peaks three hours before midnight. Notably, the core body temperature rhythm persisted even in the absence of environmental cues, indicating its endogenous regulation. Therefore there is need for further research into their molecular adaptation mechanisms to fully understand their behavioural needs, thermoregulation and adaptability.

## Biotic and abiotic stressors

Under field conditions, animals frequently encounter multiple concurrent stressors, including heat stress, nutritional deficiencies, water scarcity and housing challenges, which collectively lead to a range of health and production issues. Microclimatic factors such as temperature and humidity significantly affect animal behaviour and physiology. Climate change exacerbates ecological pressures, including reduced food and water availability, temperature fluctuations, and increased frequency of extreme weather events. These shifting environmental dynamics influence species diversity and natural selection processes within ecosystems. Disruptions in environmental variables and weather patterns alter ecosystem balance, resulting in shifts in biodiversity and species interactions. Additionally, these changes increase exposure to new pathogens, potentially leading to the emergence or resurgence of infectious diseases and affecting disease transmission by altering the distribution and abundance of vectors.

In desert regions, camels face numerous biotic and abiotic stressors impacting their survival and productivity. Biotic stressors include parasitic infections from ticks, mites, and gastrointestinal nematodes, as well as vector-borne diseases like trypanosomosis, babesiosis, and theileriosis, all exacerbated by climate change. Other challenges include bacterial and viral infections such as camel pox, PPR, brucellosis, and tuberculosis, which impact health, reproduction, and milk production. Abiotic stressors involve extreme heat (>42°C), water scarcity, nutritional deficiencies due to sparse vegetation, respiratory issues from sandstorms, and damage from dust. Temperature fluctuations and high salinity in water sources further complicate thermoregulation and hydration. Overall, climate change, and in particular rising temperature significantly influences body dynamics, both directly and indirectly, exacerbating disease pressures and production losses.

## Thermal stress

As ambient temperature and relative humidity rise, maintaining stable body temperature through heat dissipation mechanisms can become challenging, leading animals to experience heat stress. This stress manifests in decreased dry matter intake, reduced milk yield, and impaired reproductive performance, affecting overall production. Housing factors such as ventilation, cooling, flooring, animal density, and regrouping influence the severity of stress, with deficiencies exacerbating seasonal stress. These multiple stressors can reduce growth, production, and reproductive potential by redistributing nutrients to vital functions. Deviations from the thermoneutral zone trigger adaptive responses to restore thermal balance, altering welfare and growth. Animals employ physiological, molecular, endocrine, biochemical, and behavioural adaptations to maintain homeostasis, regulating variables like respiration rate, pulse rate, and body temperature. Climatic stressors affect metabolism, altering enzymatic reactions, biochemical profiles, and blood metabolites. Elevated temperatures impact the hypothalamic appetite centre, intestinal function, and metabolic pathways, leading to reduced growth. Heat stress also adversely affects reproductive organs, including the anterior pituitary, pre-ovulatory follicles, corpus luteum, and embryo development, resulting in decreased fertility and increased foetal loss.

Scientific research highlights the significant impact of environmental stress, particularly heat stress, on the physiological parameters of camels, affecting their energy balance across seasons. Camels utilize adaptive mechanisms such as adaptive heterothermy and selective brain cooling to regulate body temperature, but they can still experience heat stress, particularly when dehydrated. Although camels have a wider thermoneutral range and various adaptive traits, they suffer from heat stress when ambient temperatures exceed the upper critical threshold (varies by breed), especially when coupled with dehydration. Dehydration thus challenges the camel's ability to manage heat stress effectively. Unlike many livestock species that use panting for heat dissipation, camels primarily rely on perspiration. Heat stress notably increases packed cell volume (PCV), hemoglobin (Hb), total protein, albumin, serum glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), tri-iodothyronine (T3), and thyroxine (T4) levels in camels. Lactating camels exhibit greater thermal sensitivity compared to non-lactating ones, with higher body temperatures indicating a heightened vulnerability to environmental changes.

Consequently, lactating camels experience significant deviations in thermophysiological parameters such as respiratory rate (RR), heart rate (HR), and various temperature measures compared to dry camels. Therefore, lactating camels must manage both environmental heat and metabolic heat to maintain a stable body temperature. It is decisive, therefore, that lactating camels must dissipate both the heat that gained from the environment together with its own metabolic heat to maintain a constant body temperature.

## Camel milk

The rise in non-bovine milk consumption over the past fifty years underscores the nutritional benefits and potential health advantages of camel milk, making it a viable alternative to traditional bovine milk. Camelids, particularly dromedaries and Bactrian camels, have a genetic predisposition for high milk production, but their unique udder morphology and physiological traits can complicate mechanical milking. Camel milk production averages around 3.1 million tons annually, ranking fifth worldwide behind cows, buffalo, goats, and sheep. Bactrian camels produce 1.5 - 5 kg of milk per day over a lactation period of 10 to 12 months, while dromedaries yield 3 - 10 kg daily, influenced by breed, forage, lactation stage, and management. The global camel milk market, valued at USD 10.2 billion in 2019, is projected to grow due to higher digestibility and therapeutic properties of camel milk. Camel milk is rich in essential nutrients and bioactive components, including higher levels of copper, iron, zinc, potassium, magnesium, and vitamins A, B, and C. It also contains beneficial probiotics and protective proteins such as lysozyme, lactoferrin, lactoperoxidase, and immunoglobulins, enhancing systemic immunity and offering health benefits beyond those of cow's milk.

The actual amount of milk produced during the lactation period is affected by several factors viz. breed, parity, season of calving, geographic region and management factors (nutrition, frequency of milking). Additionally, milk quantity and quality are significantly influenced by the extent of mammary tissue available for milk production, the efficiency of secretory cells in synthesizing milk components, and the availability of appropriate nutrients required for milk synthesis. Previous research on camel milk traits has predominantly focused on physiological changes and biochemical markers in milk and blood, with limited investigation into gene expression related to milk characteristics. Future strategies for camelids should address this gap to optimize performance and validate the costs of domestication, especially often given the inadequate feeding and housing management for this species. Transitioning camel farming from traditional nomadic practices to technologically advanced management systems is crucial for optimizing milk production. Comprehensive strategies should include improved shelter management, nutritional interventions, genetic enhancement, and healthcare practices. Additional management practices involve adjusting herd composition, cost-effective stall feeding, managing rangelands, providing training in camel husbandry, implementing intensive shelter management, and refining feeding practices.

## Effect of thermal stress on camel production traits

Heat stress significantly affects milk yield and composition in dairy animals. Although most research has concentrated on cattle, the impact of heat stress on dairy camels has received less attention. Camels are noted for their ability to maintain milk production during drought conditions due to unique physiological adaptations that support continued milk yield under prolonged inclement conditions. They produce more milk for longer periods compared to other arid-adapted domestic animals, which is crucial for pastoral and agro-pastoral communities. However, prolonged drought can eventually lead to decreased milk production under thermal stress conditions. Studies indicate that high ambient temperatures and severe water deprivation reduce camel milk yield and fat content, with reductions generally proportional to the degree of dehydration. Seasonal changes also significantly influence camel milk composition, with variations in total solids and other milk components linked to heat stress, feed quality, and water availability. Camel milk yield peaks within a specific temperature-humidity index (THI) range. During dry and hot seasons, increased diurnal body temperature and high THI values suggest that local weather conditions, including temperature and relative humidity, significantly impact camel thermoregulation. In contrast, the wet season is associated with higher concentrations of milk protein, casein, total solids, solid-not-fat, and lactose, likely due to better forage quality. Specifically, milk fat is significantly lower in summer, while protein and lactose levels peak in February and are minimal in October and September, respectively. Thus, fat and protein concentration in camel milk decreases significantly during dry, hot periods, highlighting the effects of seasonal and environmental variables on milk composition. Additionally, variations in milk composition across locations are attributed to differences in management practices and the quality and quantity of available feed which are the indirect repercussions of thermal stress.

## Conclusion and way forward

Camels, known for their adaptability to extreme conditions compared to ruminants, offer significant potential for supporting impoverished farmers in a changing climate. Apart from drought purpose, these animals provide essential resources such as meat and milk, playing a vital role in sustaining pastoral communities, particularly in harsh ecological environments where other domestic animals struggle. However continuous environmental variability present major challenges to ecosystems, including arid regions where camels represent a promising avenue to bolster the socioeconomic resilience of underserved camel keepers by offering an alternative income source. Stressors such as drought, temperature extremes, and elevated $CO_2$ levels, combined with biotic stressors are reducing productivity and causing economic losses. Abiotic stressors are particularly impacting their welfare, affecting growth, reproduction, and immune function. This dynamic climate, exposes camels to various stressors, prompting them to prioritize nutrients for vital functions over production pathways. This results in decreased growth, milk and meat production, immune function, and overall health. Effective management of these stressors requires optimal energy and resource availability. Despite global efforts, camel milk production lags behind that of cattle and buffalo, highlighting the need for improved strategies and management practices to enhance camel productivity and counter their adaptation cost in extreme climates.

While adaptation strategies like tree planting for shade and provision for drinking water can alleviate some effects of rising temperatures on camel health, but additional measures are needed for comprehensive mitigation. Sustainable practices, technological advancements, and proactive management are required for maintaining camel dairying in such challenged agro-ecosystem. Research indicates that shaded areas improve camel welfare by facilitating natural behaviours, while insufficient shade and overcrowding can negatively impact welfare. Lactating camels are more thermally sensitive than dry camels, as evidenced by increased thermophysiological and thermographic parameters. Effective management, including providing shade and cooling during peak heat, can improve body heat regulation, subsequently enhancing milk production and reproductive performance. Integrating genetic improvements with management practices such as shelter, health management, enhanced nutrition, environmental modifications, and stress reduction can mitigate the impacts of abiotic stressors on their health and productivity. Revising breeding policies and identifying biomarkers related to dairy traits are promising strategies to optimize productivity.

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## CHAPTER- 12

# Fortification of Non-Bovine Milk for Infant Formula: A Perspective

Sagar Ashok Khulape*, Swagatika Priyadarsini and Basanti Jyotsana

ICAR- National Research Centre on Camel, Bikaner

*Correspondence: sagar.khulape@icar.gov.in

## Introduction

The nutritional needs of infants, especially those who are preterm or have specific health challenges, are critically important for healthy growth and development. Traditionally, infant formulas have been based on bovine (cow) milk due to its availability and nutritional profile. However, some infants experience issues with cow milk-based formulas, including allergies, intolerance, and gastrointestinal disturbances. This has led to a growing interest in non-bovine milk options, such as goat, camel, and donkey milk, as alternatives for infant formula fortification. The fortification of non-bovine milk with essential nutrients could offer promising benefits for infants, addressing specific dietary needs while potentially reducing the risks associated with cow milk.

## Overview of Non-Bovine Milk as an Alternative

Non-bovine milk varieties differ significantly in terms of composition, taste, digestibility, and allergenic potential. While they have unique characteristics that might make them suitable for infants with cow milk allergies or sensitivities, they also present challenges related to nutrient profiles and bioavailability of certain vitamins and minerals. Key non-bovine milks under investigation include:

**1. Goat Milk:** Known for its close resemblance to human milk in terms of fat composition, goat milk contains smaller fat globules and shorter fatty acid chains, making it easier to digest. Its protein structure also differs from bovine milk, which might reduce allergenic potential, though it's not entirely hypoallergenic.

**2. Camel Milk:** Camel milk is rich in certain vitamins, minerals, and bioactive compounds, such as immunoglobulins, which may support immune function. It has a unique protein composition, including lower levels of beta-lactoglobulin, a major allergen in cow milk, potentially making it less allergenic. Camel milk is also high in vitamin C and contains protective enzymes.

**3. Donkey Milk:** Often regarded as the closest match to human milk in terms of lactose, protein, and fat content, donkey milk has low allergenic properties and has been historically used as a substitute for infants with cow milk intolerance. However, it is low in certain essential nutrients, such as vitamin D, necessitating fortification.

## Why Fortification is Necessary for Non-Bovine Milk-Based Formulas

Non-bovine milks offer unique benefits but are generally insufficient on their own to meet all the dietary requirements for infant growth and development. Fortification involves adding essential nutrients—such as vitamins, minerals, and fatty acids—to bridge any nutritional gaps and ensure that these milks provide adequate nourishment for infants. Important considerations include:

**1. Protein Content and Quality:** Non-bovine milks may vary widely in protein content. For instance, goat milk has a higher protein content than human milk but lacks certain whey proteins found in human milk that are crucial for infant digestion and growth. Fortification can enhance protein quality by adding these whey proteins or essential amino acids.

**2. Fatty Acids:** Omega-3 and omega-6 fatty acids, particularly docosahexaenoic acid (DHA) and arachidonic acid (ARA), are essential for brain and visual development in infants. Non-bovine milks are often lower in these fatty acids, so fortification with DHA and ARA is necessary to meet infant dietary requirements.

**3. Vitamins and Minerals:** Many non-bovine milks lack sufficient quantities of certain vitamins and minerals, such as vitamin D, vitamin B12, iron, and calcium. For instance, camel milk is rich in vitamin C but generally low in vitamin D and folate, while donkey milk lacks sufficient vitamin D, calcium, and phosphorus. Fortification ensures these essential nutrients are present in adequate amounts to support bone health, immune function, and other developmental needs.

**4. Digestibility and Bioavailability:** Non-bovine milks are typically easier to digest than cow milk due to their different protein structures. However, the bioavailability of certain nutrients may be lower, necessitating fortification with highly bioavailable forms of vitamins and minerals to maximize absorption.

## Potential Benefits of Non-Bovine Milk Fortification for Infant Nutrition

Fortifying non-bovine milk offers several potential benefits for infant nutrition, particularly for infants who cannot tolerate bovine-based formulas. Some of these benefits include:

**1. Enhanced Digestibility and Reduced Allergy Risk:** Non-bovine milks, such as goat and camel milk, are often less allergenic and easier to digest. They contain proteins that are more similar to those in human milk, and their different casein and whey ratios result in a softer curd formation in the stomach. This may alleviate digestive discomfort and reduce the risk of allergic reactions in infants with cow milk protein allergy (CMPA).

**2. Immune Support:** Camel milk, in particular, is rich in immunoglobulins, which may provide additional immune protection, a benefit especially relevant for infants with immature or compromised immune systems. These immunoglobulins and other bioactive compounds in camel milk may support the immune system by helping protect against infections and inflammation.

**3. Support for Neurological and Cognitive Development:** When fortified with DHA and ARA, non-bovine milk formulas can support cognitive and neurological development. These fatty acids are critical components of neural and retinal tissue, and their inclusion in fortified formulas can help infants achieve developmental milestones.

**4. Bone Health and Mineralization:** Preterm infants and those with high nutrient demands require calcium and phosphorus for bone growth and mineralization. Fortifying non-bovine milk formulas with these minerals helps address the calcium and phosphorus gaps often present in non-bovine milks, supporting healthy bone development.

## Challenges of Non-Bovine Milk Fortification

Despite the promising benefits, there are challenges associated with the fortification of non-bovine milks for infant formulas:

**1. Nutrient Imbalances and Optimization:** Each non-bovine milk type has unique nutrient strengths and weaknesses. Camel milk, for example, may be high in certain vitamins but low in others like vitamin D and folate. Careful formulation and fortification are needed to balance nutrient profiles and ensure infants receive a well-rounded diet. This includes precise adjustments for macro- and micronutrient content based on each type of non-bovine milk.

**2. Cost and Availability:** Non-bovine milks, particularly camel and donkey milk, can be expensive and less accessible. This presents a challenge for scaling production of fortified formulas, especially in regions where cost constraints are significant. Additionally, sourcing these milks reliably can be difficult due to limited production volumes, particularly for niche markets like infant nutrition.

**3. Regulatory Approvals and Standardization:** Infant formulas are among the most regulated foods, and new formulations must meet strict nutritional and safety guidelines. Non-bovine milk formulas may face additional scrutiny, particularly due to limited large-scale research on their effects in infants. Regulatory bodies may require extensive evidence before approving these formulas, delaying their availability in some regions.

**4. Potential Allergenicity:** Although goat and camel milk are generally less allergenic than cow milk, they are not entirely hypoallergenic. Some infants may still react to these milk proteins, so careful monitoring is needed when introducing non-bovine milk-based formulas to infants with known milk allergies.

## Current Research and Emerging Insights

Emerging research highlights the potential of non-bovine milk-based formulas, particularly in improving outcomes for infants with CMPA, preterm infants, and those with digestive challenges. Studies have shown that goat milk formulas, when fortified appropriately, can support adequate growth and weight gain in infants with milk allergies, with improved digestibility and tolerability. Research on camel milk has focused on its immune-supporting properties, demonstrating that its unique composition may have added benefits for infants with specific immune needs.

There is also growing interest in the use of prebiotics and probiotics with fortified non-bovine formulas. Adding prebiotics (non-digestible fibers that support gut bacteria) and probiotics (beneficial bacteria) can enhance gut health, improve digestion, and potentially reduce the incidence of gastrointestinal issues like colic and constipation in formula-fed infants. This approach aligns with research on the gut-brain axis, highlighting how improved gut health can have positive effects on neurological and immune function.

## Future Directions and Potential Developments

The fortification of non-bovine milk for infant formulas has the potential to grow as more research supports its benefits and addresses existing challenges. Future directions for non-bovine milk formula fortification may include:

**1. Optimization of Fortification Techniques:** Advances in biotechnology and nutritional science may lead to more precise fortification techniques, ensuring that non-bovine milk formulas meet the specific dietary needs of different infant groups (e.g., preterm infants, those with CMPA). Innovations in nutrient encapsulation, for example, can improve the bioavailability and stability of added nutrients.

**2. Incorporating Functional Ingredients:** In addition to prebiotics and probiotics, research may explore the use of other bioactive compounds, such as nucleotides and specific amino acids, that support immune function and cognitive development. This could further enhance the health benefits of non-bovine formulas.

**3. Sustainability and Sourcing Improvements:** Efforts to sustainably source non-bovine milk, especially camel and donkey milk, could help address cost and availability challenges. Developing standardized production systems for these milks might make them more accessible and affordable.

**4. Long-Term Studies on Health Outcomes:** Conducting long-term studies on the health outcomes of infants fed non-bovine milk-based formulas would provide valuable data on the potential benefits and risks. This research would be instrumental in securing regulatory approvals and gaining widespread acceptance.

## Conclusion

The fortification of non-bovine milk for infant formulas represents an innovative approach to infant nutrition, especially for those who cannot tolerate cow milk-based formulas. By tailoring the nutrient profiles of goat, camel, and donkey milk through targeted fortification, these formulas offer a viable alternative that may improve growth, immunity, and developmental outcomes for infants with special nutritional needs. Challenges related to cost, availability, and regulatory approval remain, but continued research and technological advancements could pave the way for these fortified non-bovine milk formulas to become a mainstream option in infant nutrition.

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CHAPTER- 13

# Physicochemical and nutritional properties of camel milk and its dairy products

Yogesh Kumar*, Mitul Bumbadiya and Swagatika Priyadarsini

ICAR- National Research Centre on Camel, Bikaner

*Correspondence: ysomvanshi@gmail.com

## Introduction

Camel milk is an important part of the staple diet in many parts of the world, especially in arid and semi-arid regions. Camel milk is rich in substances beneficial to health, such as bioactive peptides, lactoferrin, zinc, and mono and polyunsaturated fatty acids. These substances may help treat some important human diseases such as tuberculosis, asthma, gastrointestinal diseases, and jaundice. The composition of camel milk is more variable than that of cow milk. The influence of feed, breed, age, and milking stage on milk composition in camels is more significant. Region and season significantly alter the proportions of compounds in camel milk. Camel whey proteins are not only composed of several soluble proteins, but also contain indigenous proteases such as chymotrypsin A and cathepsin D. In addition to their high nutritional value, these whey proteins have unique characteristics including physical, chemical, physiological, functional, and technological characteristics that are useful in food application. Hydrolysis of camel milk proteins results in the formation of bioactive peptides, which affect major organ systems of the body and provide physiological functions to these systems. Camel milk has antioxidant, antimicrobial, angiotensin-I-converting enzyme (ACE)-inhibiting peptides, antidiabetic as well as anticholesterol activities.

## 1. Introduction to Camel

Camels belong to the family Camelidae named Artiodactyla. There are two types of camels, the Bactrian two-humped camel (Camelus bactrianus) and the Arabian or dromedary one-humped camel (Camelus dromedarius). Camels play an important role in the lives of many societies, especially in the arid areas of the Middle East and the Arabian region. Camels can adapt to different climatic conditions. They are used in transportation, sport, source of meat and milk; therefore, they contribute to increasing the economy and food security for people.

Based on the latest Food and Agriculture Organization (FAO) data, the population of camels in the world is around 29 million, of which about 95% are dromedary (one humped) camels. The lactation period of camels can vary from 9 to 18 months. The amount of milk obtained depends on many factors such as breed, animal health, stage of lactation, living conditions. Camel milk yield is low and unstable compared to cow milk, however, improved feed, water, and veterinary practices can increase camel milk yield as camels have similar udder structure.

Millions of people around the world are consuming milk daily due to the tremendous nutritional benefits of milk such as bone growth and development in young children, as milk is a good source of calcium and vitamin D. It has also been proven beneficial for older people, especially in menopausal women where calcium deficiency is a high-risk factor for the development of osteoporosis.

Milk is not only a source of nutrition, but its production also contributes to food security and income for most people in developing countries. Around 150 million households worldwide are engaged in milk production (FAO, 2012). It is particularly beneficial for small-scale producers due to quick cash receipts. Camel milk provides essential nutrition to humans. In addition, it also provides therapeutic benefits.

The present review highlights the composition and health benefits of camel milk as a natural source of bioactive components.

## 2. Camel milk

Camel milk is white opaque, tastes slightly salty and has a pH between 6.2 and 6.5, which is lower than cow milk (6.5-6.7). It is very low in fat, and contains 96% triglycerides and about 30 mg/100 g dry cholesterol. Its fat contains fewer short chain fatty acids than cow milk. In addition, the average size of fat globules is smaller than fat globules of bovine, buffalo and goat milk. Because camel milk is highly digestible. This can cause problems in technological applications.

Camel milk is rich in vitamins B1, B2 and C. Vitamin C is three to five times higher than in cow milk, making it an important part of the diet in arid regions where access to green foods is limited. It has been found that camel milk has antidiabetic, antibacterial and hepatitis-resistant properties (Aggarwal et al., 2009). To varying degrees, it resists contamination with microorganisms due to its specific inhibitory structures such as lactoperoxidase/thiocyanate/hydrogen peroxide structures, lactoferrin, lysozyme, immunoglobulins and free fatty acids.

## 3. Camel milk compared to cow milk

There are some significant differences between camel and cow milk. For example, camel milk lacks β-lactoglobulin (β-LG), a major protein in cow milk that can trigger allergic reactions. In addition, the whey proteins in camel milk contain higher amounts of antimicrobial agents such as lactoferrin, lysozyme, immunoglobulins and lactoperoxidase compared to cow whey.

These differences in camel milk proteins can reveal variable biological activities such as mineral-binding properties and immunoglobulin formation upon hydrolysis. Camel milk is white in colour and slightly salty afterwards and has a slightly lower density than cow milk, with an average value of 1.029 g/cm3. The pH ranges from 6.4 to 6.7. The water content varies from 87 to 90%, and the freezing point ranges between −0.57 and −0.61 °C.

- Cow milk is opaque white to yellow in colour due to the presence of carotene and depends on the breed, type of feed and fat content (NPCS Board, 2012). The water content in cow's milk ranges from 79 to 90%. The pH of cow's milk ranges from 6.4 to 6.6. Its density is about 1.030 g/cm3 and freezing point is about -0.54 °C. Cow's milk contains an average of 3.6% fat, 3.0% protein and 4.6% lactose.
- The composition of camel milk is more variable than that of cow milk. Region and season significantly affect the proportion of compounds in camel milk. The amount of lactose in camel milk is stable, and ranges from 3.5 to 4.5%. It is the main carbohydrate of camel milk. In addition, it contains a small number of various oligosaccharides that protect infants from pathogens, promote the formation of a bifidobacterium environment and help develop the nervous system.
- The total protein in camel milk ranges from 2.15 to 4.90%. Camel and cow milk have similar casein (αs1, αs2, β, and κ-casein) content, but they differ in the content of whey proteins. Thus, the ratio of casein and whey proteins is higher in cow milk than in camel milk. This affects the firmness of the coagulum, and camel milk forms a softer gel than cow milk.
- Casein is the main protein in camel milk, and it represents about 52–87% of the total protein, while whey proteins contribute 20–25%. Camel milk contains four fractions of casein and accounts for; The ratio of αs1 to αs2 and β to κ-casein in camel milk is quite different, which is 22:9.5:65:3.5 (Park and Heinlein, 2013). Camel milk contains more β-casein than α-casein, accounting for 65 and 21% of total casein, respectively.
- Compared to camel milk, cow milk has almost similar β-casein and α-casein percentages (36 and 38%, respectively) and higher content of κ-casein (13%), which is almost four times less in camel milk (3.47%). β-casein is more digestible and less allergenic to people, as it is more sensitive to peptic hydrolysis in the intestine. The high β-casein percentage makes camel milk beneficial for human health.

- The diameter of casein micelles in camel milk ranges from 20 to 300 nm, while that in cow milk is 40–160 nm. Overall, the average diameter of casein micelles in camel milk is larger and its mineral charge is higher. α-lactalbumin is the main whey protein of camel milk. It is more digestible and has higher antioxidant activity than α-lactalbumin derived from cow milk, which encourages the use of camel milk in infant feeding.
- Camel milk lacks β-lactoglobulin, which reduces allergenicity, but other whey proteins such as lactoferrin and immunoglobulins are present (Devendra et al., 2016). Lactoferrin is a glycoprotein that binds two ferric ions. Its content in camel milk ranges from 0.02 to 2.1 g/liter. It has antimicrobial, anti-inflammatory, immunomodulatory and antitumor activities.
- Lysozyme, another milk antimicrobial agent, is present in camel milk with a concentration of approximately 150 µg/L which is higher than that in cow milk (70 µg/L). Immunoglobulins (IgG) are whey proteins that play a major role in the passive immunity of newborns. The major immunoglobulin in camel milk is IgG. It is secreted in colostrum at a concentration of approximately 100 g/L but rapidly decreases to less than 10 g/L during lactation, as reported by Park & Heinlein (2013).

It is worth noting that differences in protein profile may affect the composition of fermented camel and cow milk. Fermented camel milk contains more antioxidant peptides possibly due to the composition of β-casein. So, β-casein in camel milk is smaller and contains more proline. Its hydrolysis results in the formation of bioactive peptides and releases amino acids such as phenylalanine and tryptophan with antioxidant properties.

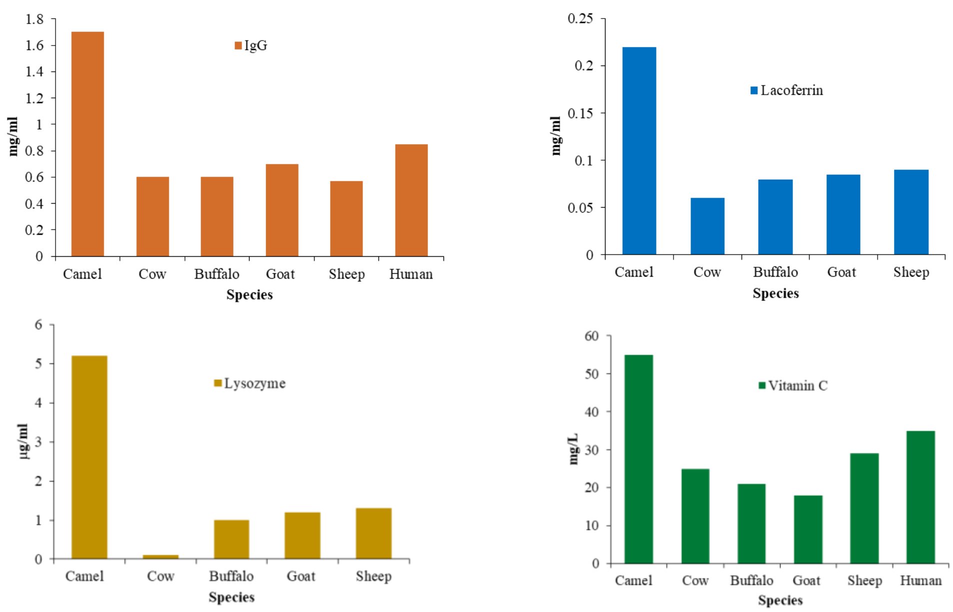


**Fig 1.** Concentration of IgG, lactoferrin, lysozyme and vitamin C in milk of different species.

The fat content of camel milk ranges from 1.2 to 4.5%. However, Park and Heinlein (2013) reported that the fat content of camel milk can reach 6.4%, and its profile is characterized by the presence of high amounts of unsaturated and long-chain fatty acids. This helps to reduce lipid levels in human serum. The content of long-chain fatty acids is 92–99%, and the percentage of unsaturated acids is 35–50%. These structural differences provide a "waxy texture" to camel milk fat. The low amount of carotene makes camel milk whiter in color than cow milk.

The mineral content of camel milk is particularly similar to that of cow milk; in Ca, P, Mg, Na, and K content. The main difference is in the content of Zn, Cu, Fe, and Mn, as camel milk contains higher concentrations of these minerals. The increased iron concentration in camel milk may be useful for the prevention of iron deficiency anemia. In addition, the lower concentration of citrate in camel milk compared to cow milk enhances lactoferrin antimicrobial activity, as smaller levels of citrate are needed to be beneficial. The total mineral content of camel milk fluctuates from 0.60 to 0.90%. The salty taste of camel milk can be explained by the increased amount of chloride derived from the fodder consumed by the animals.

In addition, camel milk contains a higher amount of ascorbic acid. Therefore, it can increase the shelf-life of its products and enhance its antioxidant and antiradical capabilities. The concentration of mineral salts and vitamins in camel milk depends on the breed, feed, water intake and lactation stage. Moreover, camel milk has higher content of vitamin C and niacin than cow milk. But it lacks vitamins B1, B2 and A, pantothenic acid and folic acid. The content of vitamin B6 and B12 is almost the same in both camel and cow milk.

On the other hand, camel milk has better heat stability than cow milk. Increasing the temperature of camel milk to 80°C breaks down 32–35% of whey proteins, while increasing to 90°C denatures 47–53% of whey proteins. Heat treatment of cow milk at 80°C denatures 70% of whey proteins, while 90°C denatures 81%.

Camel milk contains more inhibitory structures than cow milk, in particular, lysozyme and lactoferrin which are much higher than those in cow milk. Therefore, it can be stored for a longer period at room temperature. In addition, it contains peptides and proteins that have valuable effects on many biological processes such as assimilation, ingestion, development and immunity. Camel whey contains a diverse collection of proteins, such as immunoglobulins, serum egg white, α-lactalbumin, lactophorin, and peptidoglycan.

## 4. Health Benefits of Camel Milk

Milk is the most important source of nutrients for mammalian newborns. Milk contains biologically active substances and compounds that are essential for immunological protection and healthy growth. Camel milk has many beneficial nutritional and therapeutic properties, antibacterial, anticancer, antioxidant, antihypertensive and antidiabetic properties.

Peptides derived from dietary proteins have been extensively studied to investigate health effects in humans such as antioxidant activity, mineral binding, blood pressure reduction, immunomodulatory function and protective effect against various bacteria and viruses (Salami et al., 2010). Such peptides from milk proteins are widely accepted. Indigenous protease enzymes such as milk plasmin can hydrolyze proteins and release bioactive peptide fragments during storage or processing. Bioactive peptides can also be obtained through enzymatic hydrolysis with microbial and digestive enzymes. The activity of these peptides is based on their amino acid composition and sequence.

Health-related bioactive properties of camel milk protein hydrolysates have been reported recently. These hydrolysates were obtained through enzymatic hydrolysis of milk proteins, which are susceptible to proteolysis. Enzymatic hydrolysis is known to improve the functional properties of milk proteins, in addition to enhancing bioactive properties. However, camel milk proteins in their intact form have bioactive properties such as anti-hypertensive, hypo-allergic, anticancer and antidiabetic. Most of these properties have been demonstrated in vivo (human or rat models) for intact camel milk proteins. Recent research is focusing on generating bioactive hydrolysates from camel milk proteins and exploring their potential bioactive properties in in vitro and in vivo conditions.

Traditionally, camel milk is used to treat diseases such as tuberculosis, asthma, ascites and jaundice due to its content of natural bioactive components. Moreover, camel milk has better digestibility and nutritional value than cow milk, making it one of the alternative sources for human consumption. These bioactive components can be produced from milk proteins by probiotic bacteria during the fermentation process. Camel milk can also be used to cure gastrointestinal disorders. It has a good effect on stomach and intestinal diseases due to its high level of anti-inflammatory proteins, polyunsaturated fatty acids and vitamins that enhance carbohydrate metabolism.

Camel milk has antibacterial and antiviral properties due to the presence of lactoferrin, lysozyme, lactoperoxidase, hydrogen peroxide and immunoglobulins. These compounds can suppress both gram-positive and negative bacteria, such as Staphylococcus aureus, Listeria monocytogenes, and Escherichia coli. The amount of antibacterial components in camel milk is higher than that in cow milk. However, their beneficial properties are completely inactivated by storing the milk at 100°C for 30 minutes. In addition, whey protein of camel milk enhances anti-rotavirus functions to treat non-bacterial gastroenteritis.

Camel milk lactoferrin and IgG can inhibit hepatitis C and B viruses and prevent their replication in cells. IgG can recognize hepatitis C virus peptides at concentrations when human IgG does not detect the presence of the virus. In addition, camel milk can cure hepatitis B, as it enhances the immune response and prevents DNA replication of the virus. The abundance of antimicrobial components in camel milk gives it therapeutic effects against drug-resistant tuberculosis. Thus, camel milk can relieve symptoms such as cough, breathlessness, and fever.

Angiotensin-I-converting enzyme (ACE)-inhibiting peptides are present in the primary structure of many food proteins, including milk proteins. These peptides are also present in fermented camel milk. The probiotic bacteria used in fermentation break down the proteins into peptides and amino acids.

Bioactive peptides in fermented camel milk may have a positive effect on lowering cholesterol levels. Camel milk also contains orotic acid, which is known to reduce cholesterol levels in humans. Raw camel milk and fermented dairy products are a source of probiotic strains. Species of Lactobacillus, Bifidobacterium, Enterococcus and Streptococcus were isolated from camel milk and used in the dairy industry.

Due to the presence of insulin and insulin-like substances as well as immunoglobulins in small sizes, camel milk can be used to treat diabetes type 1 and type 2. The insulin level in camel milk is high and is around 52 units/liter (Ayub et al., 2018). In addition, these components affect the pancreas and liver, improving insulin secretion, therefore reducing the required dose of insulin (Kaskus, 2016). Along with the application for the treatment of diabetes, camel milk lowers blood sugar, reduces insulin resistance and improves lipid profile (Ayub et al., 2018).

Another potential health benefit of camel milk is the reduction of its ability to cause allergies, especially in children who are allergic to cow's milk. Such allergies are caused by the high content of α-casein and the low content of hypoallergenic β-casein, as well as the presence of β-lactoglobulin. In particular, cow milk allergy is a major concern in infants, as in the most severe cases, cow milk consumption can lead to anaphylaxis. Furthermore, camel milk immunoglobulins are similar to those of human milk, making it safe for children to consume. Furthermore, individuals with lactose intolerance can safely consume camel milk. Camel milk contains a higher amount of L-lactate compared to cow milk which is rich in D-lactate. L-lactate reduces milk allergenicity. The IgE of children allergic to cow milk does not react with camel milk; therefore, camel milk immunoglobulins reduce allergic symptoms.

Furthermore, camel milk has a potentially positive effect on people with autism. In the intestines of patients with this autoimmune disease, the breakdown of milk casein leads to the formation of casomorphin, a strong opioid responsible for brain damage. The high levels of β-casein content and β-lactoglobulin in cow's milk make it more likely to form opioids.

In addition, camel milk contains protective proteins (lactoferrin, lysozyme, and immunoglobulins) that can improve brain development. Treating blood, lung, liver, and breast cancer is another benefit of camel milk. It inhibits the proliferation of HepG2 and MCF7 cells as well as the stimulation of death receptors in cell lines and mechanisms caused by oxidative stress.

Camel milk improves the gut microbiota as its consumption helps to develop abundant amounts of Allobaculum, Akkermansia, and Bifidobacterium. The study by Wang et al. (2018) indicates that camel milk can increase the abundance of Allobaculum, which may have a positive effect on the physiological function of the organism. This genus produces short-chain fatty acids that improve colon health, prevent obesity, and reduce inflammation. Akkermansia, a mucin-degrading probiotic, is well-known for its beneficial effects on obesity, metabolic disorders, diabetes, and inflammation.

## Conclusion

Camel milk is rich in substances beneficial to health, for example, bioactive peptides, lactoferrin, zinc, and mono and polyunsaturated fatty acids. Whey proteins have nutritional importance because they provide energy and essential amino acids, and functional importance because they help improve texture, structure modification and overall appearance of food. Camel milk whey is a good source of nutrients and important bioactive peptides. Antioxidant, antimicrobial, angiotensin-converting enzyme (ACE) inhibitor, antidiabetic and anticholesterol activities are all representing major value for these bioactive peptides.

**Reference will be provided on demand**

# Recent Advances in Processing of Non-bovine Milk and Milk by-products

This e-book is the result of a collaborative online training program on *Recent Advances in Processing of Non-bovine Milk and Milk by-products*, conducted from 14th to 17th May, 2024. It will serve as an invaluable resource for dairy scientists, researchers, veterinary professionals, and extension workers across the country. The book covers a wide range of topics, including innovative methods in non-bovine milk processing, the health benefits of non-bovine milk, advances in milk by-product utilization, quality control measures, sustainable production practices, and emerging market trends for alternative dairy products. This comprehensive guide will be immensely helpful for professionals and farmers engaged in non-bovine dairy farming, as well as those involved in the development of innovative dairy products.

## Editors


**Dr. Yogesh Kumar**
Senior Scientist
ICAR- National Research Centre on Camel, Bikaner, Rajasthan

**Dr. Shahaji Phand**
Deputy Director
MANAGE, Hyderabad, Telangana

**Dr. Swagatika Priyadarsini**
Scientist
ICAR- National Research Centre on Camel, Bikaner, Rajasthan

**Dr. Sushrirekha Das**
MANAGE Fellow
MANAGE, Hyderabad, Telangana