Camel milk Research

Compositional, Molecular and Clinical studies

on

Milk from Livestock at Dayalbagh Dairy (Gaushala)

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Representation of global partners in the study

Camel milk research, an initiative at Dayalbagh, in the year 2024, being declared as 'Year of Camelids' by the UN, gives high significance to the series of studies focused on nutrinomics and axiomatic benefits of camel milk and underscores camel domestication and harmonious coexistence with cows, buffalos, and goats at Dayalbagh Gaushala Dairy (figure 1). The sustainable Agroecology and precision farming practices, organic farming and community participation as selfless service with supply of mixed milk to the Lactovegetarian community is a unique feature of Dayalbagh Way of Life. Dayalbagh Dairy (R.S.S Gaushala) established in 1926, was regarded as Asia's finest dairy[1]. Presently there are 1100 cattle comprising of high-yielding indigenous breed of cows and buffalos, 11 camels and 72 goats producing over 1,300 litters of milk daily, promoting lacto-vegetarianism. Having a blend of modern technology and ancient wisdom in a spiritually charged environment and working on scientific techniques related to breeding, feeding, health and facilities management, contributing immensely to the ecosystem, Dayalbagh Dairy is aligned to the Sigma Six Q-I-V-A Model of Dayalbagh Way of Life[2]. While milk, butter, and buttermilk and other milk products, serve as an important source of nutrition for human consumption, cow dung and urine help the cultivation of hundreds of acres of land at Dayalbagh without the use of any chemicals. The innovations at RSS Gaushala and availability of 'mixed milk' to the community, is showing positive impact on health, especially growth and development trends in children as observed on their longitudinal follow up with potential to prevent many diseases if supplemented from infancy. This observation is supported by the series of clinical studies and identification of specific metabolites in high abundance especially in Camel milk having effect on heart and brain functions. This research holds highly promising and cost - effective outcomes. Supporting community health initiatives at Dayalbagh, these findings contribute to a deeper understanding of milk's metabolic composition, with wider implications for dietary recommendations, functional food development, and healthcare applications. Moreover, this research underscores the importance of livestock biodiversity and its role in global food security and sustainability.

Figure 1: Harmonious coexistence of Camels, Buffaloes, Cows and Goats at Dayalbagh Dairy symbolising "Dayalbagh Way of Life"

List of abbreviations:

NMR- Nuclear Magnetic Resonance

LC-MS- Liquid chromatography mass spectrometry

PLS-DA- Partial Least Squares- Discriminant Analysis

sPLS-DA- Sparse Partial Least Squares- Discriminant Analysis

OPLS-DA -Orthogonal Partial Least Squares- Discriminant Analysis

VIP- Variable Importance in Projection

MSEA- Metabolite Set Enrichment Analysis

KEGG- Kyoto Encyclopedia of genes and Genomes

IMP- Inosine monophosphate

ADP- Adenosine diphosphate

UDP-Uridine diphosphate

TMAO- Trimethylamine N-oxide

Abstract Summary Milk is considered an elixir of life, a natural, richest and most inexpensive source of energy, proteins, micro and macronutrients as well as immune factors. Milk forms a complete nutrition specially for infants and children for their growth and development. After initial few months, breast feeding needs to be supplemented by other sources of milk to avoid malnutrition in young children. Milk also forms an important dietary component during adolescence, pregnancy, lactation and older age group. Milk from cow, buffalo, goat and camel available from livestock at Dayalbagh Dairy gaushala, forms an important part of lactovegetarian diet for the community. The present research is aimed to evaluate compositional and metabolomic analysis of each of above source of milk and its impact on health. A comprehensive mineral nutrient analysis of milk samples from cow, buffalo, goat and camel revealed that each of these sources have optimum levels of all the nutrients though each source has unique differential properties. Seasonal changes, grazing pattern, exercise, musical chanting, stage of lactation also affects the quality and quantity of milk. Camel milk was found enriched with highest content of most of the macro and micronutrients specially selenium and sodium and has higher protein content. Buffalo milk has highest fat and protein content, while cow milk has higher manganese content and notably highest iron content is found in goat milk. The analysis of physiochemical properties of milk is carried out by Kjeldahl method and Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES).

Global Metabolomic analysis by Liquid chromatography coupled Mass spectroscopy (LC- MS) studies on all the four milk samples identified 245 common metabolites. Multivariate statistical analysis using Partial least squares-discriminant analysis (PLS-DA) method identified 20 significantly differentiating metabolites with 13 metabolites up regulated in camel milk compared to other milks. Notably, among these 13 metabolites, “Pargyline” metabolite was found to possess Antidepressant and Antihypertensive properties attributing to the therapeutic properties of camel milk.

Nuclear magnetic resonance spectroscopy (NMR) analysis of all four milk samples revealed 48 metabolites, with 38 compounds showing significant changes based on ANOVA analysis. Principal Component Analysis (PCA) demonstrated clear discrimination among the four milk groups. Further, the PLS-DA model indicated the good classification performance. Using multivariate analysis and Variable Importance in Projection (VIP) scores, the study identified the top 15 compounds with VIP values > 1. Further comparison of the metabolite profile of

each milk source with cow milk, identified relatively higher concentrations of few compounds. Notably, camel milk contained higher nicotinamide ribotide, enhancing NAD+ synthesis. The higher levels of NR in camel milk suggest potential benefits for energy metabolism, antioxidant defences, and neuroprotection. While goat milk was rich in UDP-galactose and coenzyme A, supporting cell signalling and lipid metabolism. These findings emphasise that different milk sources have unique nutritional and medicinal metabolites, offering potential applications in targeted nutrition and therapeutics.

Clinical studies on camel milk supplementation, undertaken at Saran Ashram Hospital has revealed medicinal effects of camel milk on heart, brain, growth and development. With camel milk consumption, mean pulse rate declined by 4% (within normal limits) for the duration of experiment (2 hours) in about 54% of participants. The statistical analysis of before and after pulse oximetry observations upon milk consumption suggests that decline in pulse rate is statistically significant when camel milk is consumed while bovine and goat milk consumption did not show any conclusive impact on the oximetry readings. Above findings need further evaluation for potential implications in patients with heart failure, coronary artery disease and inappropriate sinus tachycardia.

Further, addition of camel milk as nutritional adjuvant, has improved the cognitive health and quality of life of children Sant Super Humane Evolutionary Scheme, especially neurodiverse /differently abled children. Evidenced by improvement in their cognitive scores, results show statistically significant change in scores of Childhood Autism Rating Scale (CARS) p =0.0019, Autism Treatment Evaluation Checklist (ATEC) p= 0.022, IQ p =0.0394 and EQ p =0.00561 after three months of camel milk supplementation. Improvement is seen in all 15 Domains in CARS score, more in verbal communication (26.39%), listening Response (23%), activity level (20.39%) and also in cognitive and sensory functions (17.25%) in ATEC score. The earliest change is observed after 15 days of consumption, peaking at 2 months and more appreciated in younger age group of less than 2 years.

Contrary to literature survey which shows high concentration of Vitamin D in camel milk, our study found it to be deficient in Vitamin D, as also observed in other sources of milk. Thus, fortification of milk or medicinal supplementation is recommended to prevent Vitamin D deficiency, which is prevalent in 79% of the local population.

The detailed analysis involving a series of studies above, highlights specific benefits associated with each type of milk, suggesting that a combination of different milk sources namely cow, buffalo, goat and camel as 'mixed milk' product could offer a more comprehensive range of nutritive and medicinal properties in a cost-effective way. Ongoing research on impact of mixed milk available from Dayalbagh Dairy during the year 2024, reveals positive change in trends on growth centile charts especially in height for age, during current year, as compared to previous years, as observed during 8 years of longitudinal tracking of the growth and development of children of Sant Super Humane Evolutionary Scheme of Dayalbagh. Future research prioritizes a careful proportioning of volumetric dosage using mixed milk for nutritional recommendations and for prevention and mitigation of diseases by its supplementation from infancy, through this unique cost-effective measure.

1. Milk nutrient profiling and seasonal changes in quality and quantity of milk production at Dayalbagh Dairy (RSS Gaushala)

Compositional analysis of milk produced at RSS Gaushala was carried out by several analytical techniques. The physiochemical properties of various milk sources was carried out by Kjeldahl method and Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES), The comparison of species-specific macro and micronutrient signatures of various milk samples are presented in Figure 2.1 and 2.2.

Macro nutrients	P	K	Ca	S (g/L)	Mg
Generic range	500-900 mg/L	50-349mg/L	1130mg/L	3.4-4.37mg/L	110mg/L

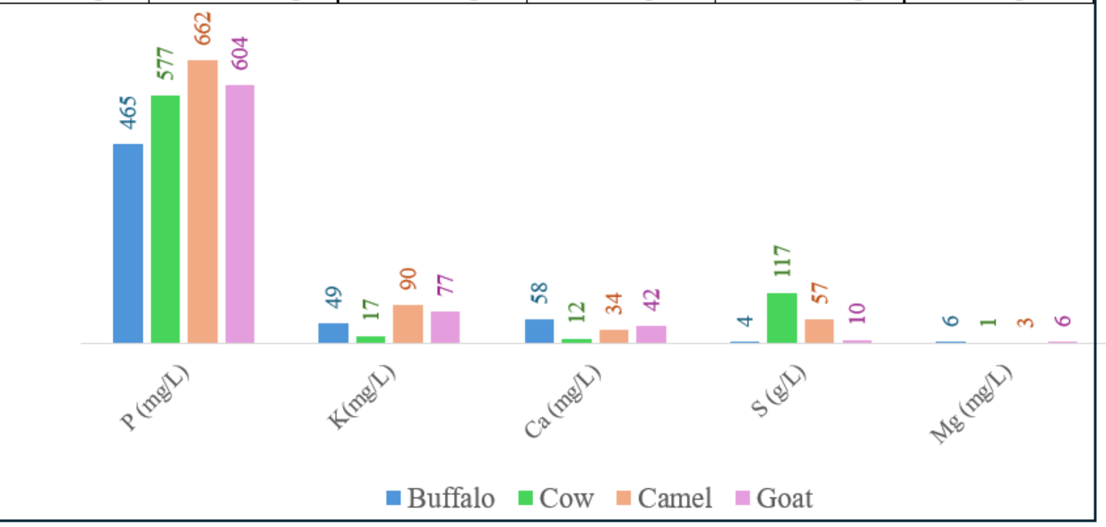

Figure 2.1 Comparative graph for species-specific macronutrient signatures of various milk samples

Micro nutrients	Na	Fe	Mo	Zn	Se	Mn	Cu
Generic range	0.98-400mg/L	0.3-0.5mg/L	0.05mg/L	0.888-4.0mg/L	0.002-0.06mg/L	0.03 mg/L	0.10-0.12mg/L

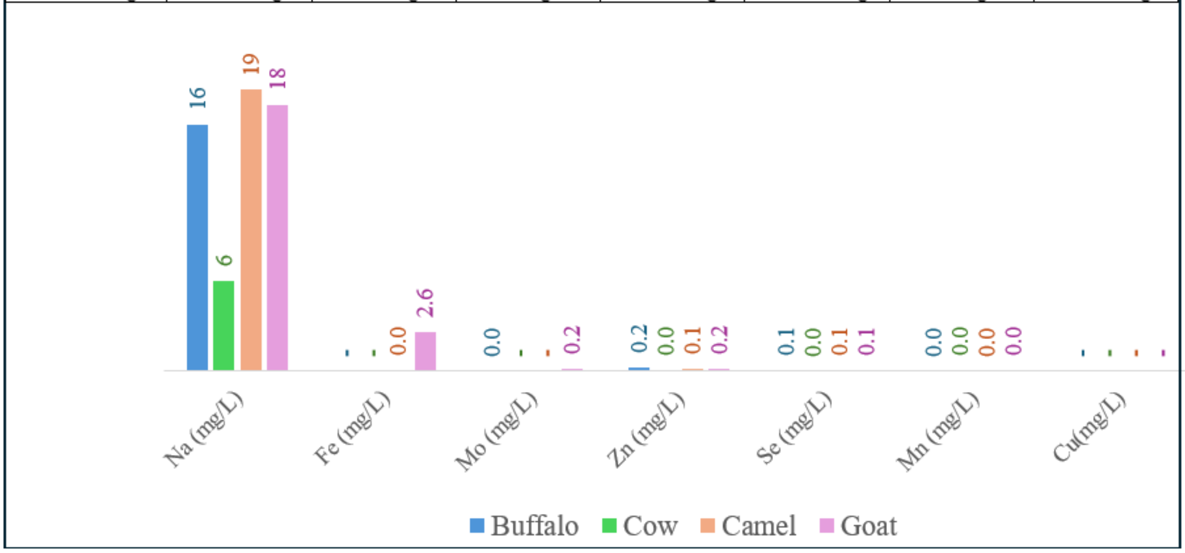

Figure 2.2 Comparative graph for species-specific micronutrient signatures of various milk samples

A comprehensive mineral nutrient analysis of these studies on milk samples from cow, buffalo, goat and camel revealed that camel milk is enriched with highest content of most of the macro and micronutrients including potassium, phosphorus and sodium that are required for normal body growth and development and energy metabolism, while camel milk has low iron content. The goat milk has the highest content of iron and optimum levels of calcium and magnesium. Goat milk also ranks second in potassium and phosphorus content next to camel milk, but goat milk has less protein content. Thus, mixing camel milk with goat milk is one of the options to make milk a wholesome diet. On the other hand, buffalo milk has the highest content of protein, calcium, magnesium and zinc. Milk protein is one of the essential body building proteins required for not only children but for all age groups thus buffalo milk when mixed with camel milk offers a wholesome dietary supplement. Cow milk has the highest content of Manganese, which has a direct role in balancing metabolic pathways linked to the production of compounds with therapeutic value, specifically for neural and brain development. Thus, mixing all the four milk sources is an appropriate option to provide balanced nutrients to the body for normal growth and development.

While, the levels of different toxic heavy metals are found to be below the detectable limit and also within the permissible limit prescribed by different International and national agencies including W.H.O, FSSAI (Table 1).

Heavy metals	Buffalo	Cow	Camel	Goat	Permissible limit
Al (mg/L)	0.143	0.521	0.458	0.222	10.0mg/L
As (mg/L)	0	0	0.003	0	0.02mg/L
Cd(mg/L)	0	0	0	0	0.08mg/L
Co(mg/L)	0	0	0	0	0.1mg/L
Cr (mg/L)	0	0	0	0	0.10mg/L
Ni(mg/L)	0	0	0	0	0.02mg/L
Pb (mg/L)	0.051	0.016	0.055	0.144	0.751mg/L

Table 1: Variation in heavy metal content of milk sourced from livestock of Dayalbagh Dairy

Milk samples from cow, buffalo, camel and goat collected from the gaushala in three seasons viz., winter (February), summer (May) and rainy (July) were tested for various sensorial characteristic (colour and appearance, body and texture, flavour along with overall acceptability) as well as physio-chemical analysis (acidity, pH, protein, fat, lactose, moisture and ash content) and compared to each other. Sensory parameters showed that the buffalo milk samples scored highest followed by cow, camel and goat milk in all the studied seasons. pH value of the samples followed the similar trend whereas acidity content was found highest in goat milk (0.16-0.17% LA) and lowest in buffalo milk (0.14-0.16% LA). Cow and camel milk also scored non-significant change in the acidity value. Highest fat as well as protein content along with lowest moisture percent was found in buffalo milk samples as compared to others whereas the maximum lactose was reported in camel milk (5.45-5.54%) in every tested season and noticed that the decrease was more in the month of May in all four species of milk. These studies offer valuable insights into the biochemical composition of milk from all the animal sources from gaushala and paves way for well-informed practices for food science, nutrition, and animal husbandry practices, potentially influencing dietary recommendations and the management of livestock to optimize the nutritional value of milk.

Camel milk production at Dayalbagh Dairy

Usually, the lactating camel can provide 4-5 Kg of milk per day during the first 3 months of lactation and the duration of camels' lactation periods can vary from 9 to 18 months, with milk production being influenced by factors including breed, animal health, lactation stage, and environmental conditions [3], emphasizing the importance of sustainable dairy practices.

At Dayalbagh gaushala, by employing sustainable dairy practices, camel milk production gradually increased from 277 liters /month in the month of January to about 827 liters /month in the month of May (Figure 3). In the months of January and February the concentrate from

Bikaner and Gwar *bhusa* were fed to camels in addition to its normal feeds. Also, camels were shifted to *Chandmari ka Tila* in *Anupam Upavan* Biodiversity Park, Dayalbagh. This systematic approach helped in rapid increase in milk production. Later, there was a slight decline in the milk production in the months of March and April. This was due to stopping of grazing on *berseem*. But later, with increase in grazing on leaves and intake of water due to heat, camel milk production again increased. As the rainy season started in July, a decline in milk production was observed with about 636 liters/month in the month of September. This decline in camel milk production is attributed to seasonal variation, as camels usually yield less milk during rainy season. Along with seasonal changes, one of the camels was pregnant.

	Monthly Camel Milk Production (in liters)			Growth vs last Month		
Month	**Morn**	**Eve**	**Total**	**Morn**	**Eve**	**Total**
Jan	170.0	107.0	277.0			
Feb	278.7	221.5	500.2	64%	107%	81%
Mar	308.6	234.3	542.7	11%	6%	8%
Apr	379.0	315.5	694.5	23%	35%	28%
May	420.5	406.4	826.9	11%	29%	19%
Jun	364.4	385.9	750.3	-13%	-5%	-9%
Jul	356.7	350.5	707.2	-2%	-9%	-6%
Aug	364.5	345.3	709.8	2%	-1%	0%
Sep	337.0	299.3	636.3	-8%	-13%	-10%
Oct	309.1	269.6	578.7	-8%	-10%	-9%
Nov	240.4	198.6	439	-22%	-26%	-24%
Dec	235.2	189.2	424.4	-2%	-5%	-3%

Figure 3.1 Monthly quantity of camel milk production at Dayalbagh Gaushala and its growth versus previous month

Fig. 3.2 Trend of "daily" camel milk production at Dayalbagh Gaushala, Jan- Dec. 2024

Avegrage Camel Milk Production per Day (Litres)					
	Duration	**Phases**	**Morning**	**Evening**	**Total**
1	1-18 Jan	Grazing and browsing on only leaves	4.4	2.0	6.4
2	19-29 Jan	Concentrate from Bikaner and Gwar Bhusa feeding started	6.6 +50%	5.4 +167%	12.0 +87%
3	30Jan-8 Feb	Two Camels and kids were shifted to Gaushala	8.0 +20%	6.4 +19%	14.3 +19%
4	9-15 Feb	Barseem Grazing started for Camels.	8.7 +10%	6.0 -6%	14.7 +3%
5	16-26 Feb	Three more Camels started to be milked	10.6 +22%	8.7 +46%	19.3 +31%
6	27 Feb-3 Mar	All Camels were shifted to Chandmari	12.4 +17%	9.7 +12%	22.1 +15%
7	4 Mar-10Apr	Barseem Grazing stopped and Browsing on leaves started	9.8 -21%	7.2 -25%	17.0 -23%
8	11-18 Apr	Camels started to go for approx 8-9 hrs in grazing. More water intake due to heat.	13.1 +34%	11.2 +55%	24.3 +43%
9	19 Apr-5Jul	Camels being taken to Sikanderpur and further areas	13.1 0%	13.0 +16%	26.1 +7%
10	6Jul-23Aug	Rains Started. It is stated that during rains the camel milk production decreases.	11.5 -12%	11.2 -14%	22.7 -13%
11	24Aug-2Sep	1 camel Kesar, Diagnosed Pregnant. Milk Production declined and stopped.	11.4 -1%	10.2 -9%	21.7 -5%
12	3Sep-11Dec	Camel Malini milk production decreased to average 1 ltr. End of lactaion length.	9.5 -17%	8.3 -19%	17.8 -18%
13	>12Dec	One camel stopped giving milk due to advanced pregnancy	7.4 -22%	5.5 -34%	12.9 -28%

Figure 3.3 Quantity and changes in camel milk production with changes in feed, water, seasons and lactation period at Dayalbagh Gaushala. %Growth is change in Average Camel Milk production (per day) from previous Phase

Factors affecting Quality and Quantity of Milk and Innovations at RSS Gaushala Dayalbagh

Nutrition has the capability to affect genetics and Milk is nature's complete food as it is the best lactovegetarian source of protein, carbohydrate, fats, vitamins and minerals. Thus, it is very pertinent to study the factors affecting quality and quantity of milk production in cattle and how certain innovations practiced at RSS gaushala Dayalbagh has optimized the same to bring white revolution

Challenges and solutions to balance quality and quantity of milk at Dayalbagh

Succulent and fodder of the milking stage is crucial for deriving maximum benefits from fodder in terms of protein, plant oestrogens and vitamins. But challenges are faced as tonnage of green fodder supplied supersedes quality. This is because most of the time mature fodder, which is devoid of nutrition, is received which is unable to have salutary effect on the milk yield and quality.

Optimization of different types of fodders - feed and their benefits

Over decades, the team consisting of animal husbandry, nutritionists, dieticians and animal doctors optimised regimen for the diet of milk providing cattle for optimum and healthy milk production. These measures include a nutritious diet consisting of balanced proteins, minerals and vitamins provided through GM (Genetic Modification free) and chemical free fodder. Two major aspects that are uniquely adapted RSS gaushala are the grazing in fodder- feed fields with physical activities and exposing cattle to spiritual hymns (RA DHA SVA AA MI) and mantras (Path). Post grazing, the cattle are bathed, cleaned and monitored for health parameters by a specialized dedicated team and then milked.

2. Metabolomic analysis of milk from camels at Dayalbagh using NMR and LC-MS analysis

Camel milk, a revered superfood known for its profound benefits to humanity, has been utilized for centuries, yet its critical constituents have remained largely unexplored. In this current study, we aimed to delve into the compositional profile of camel milk. Our preliminary clinical data revealed major health benefits for children. These observations prompted us to undertake compositional analysis of camel milk and compare with cow milk. A relatively rapid and accurate tool to identify major components of these milk samples is by Nuclear Magnetic Resonance Spectrometry. Samples were collected from cows and camels at RSS Gaushala, Dayalbagh, Agra, at 3 months (Feb-March 2024) and 5 to 6 months (May-June 2024) postpartum stage and 7 months postpartum stage (July 2024). The samples collected in July 2024 were deployed for NMR.

Utilizing complimentary tools such as Nuclear Magnetic Resonance (NMR) spectroscopy, researchers can obtain complete metabolomic fingerprints of milk(4). Although NMR is commonly used in milk metabolomics research for its reliability and quantitation capabilities, its sensitivity is limited to micromolar to millimolar concentrations. NMR spectroscopy offers several unique advantages over other metabolomic platforms. Compared with LC-MS and GC-MS (Gas chromatography-mass spectrometry), one of the advantages of NMR spectroscopy is the direct and quantitative relationship between molar concentration and the intensity of the NMR resonances, moreover being a non-destructive method it estimates actual structure of the metabolites (4). NMR has the potential to transform the field of metabolomics, yet its potential has barely been tapped. Few studies demonstrated its advantages, the Tian group (5) screened the differential metabolites between heat stress-free and heat stressed cows by LC-MS and ^{1}H NMR, and found that those metabolites could be regarded as potential biomarkers for monitoring the heat stress of lactating dairy cows Based on the milk metabolomic data from NMR, LC-MS and milk production traits, the Vervoort group (6)obtained models to estimate the energy balance of early lactation cows. Thus, NMR has specific advantages and offers reliable identification of metabolites.

By comparing cow milk with camel milk the NMR results revealed significant differences in 34 metabolites, with camel milk showing higher levels of 24 metabolites and cow milk with elevated levels of ten metabolites (Figure 4). Key metabolites, such as coenzyme A, orotic acid, and acetylcholine, hippurate, IMP, ADP, formate, fumarate, lactate, lipid, were more abundant in cow milk, while camel milk exhibited higher concentrations of nicotinamide ribotide, TMAO, guanosine, UDP-galactose, O-phosphocholine, phosphoenolpyruvic acid, carnosine, phosphocreatine, hydrochlorothiazide, S-adenosylhomocysteine and uridin. Nicotinamide ribotide (NR), essential for NAD+ synthesis, plays a critical role in metabolic processes. The higher levels of NR in camel milk suggest potential benefits for energy metabolism, antioxidant defenses, and neuroprotection, contributing to the medicinal properties associated with camel milk. Additionally, the presence of carnosine in camel milk further highlights its potential health-promoting properties. Carnosine, found in camel milk, is believed to possess antioxidant, anti-inflammatory, and neuroprotective properties that can support brain function and protect the central nervous system, highlighting the potential cognitive health benefits of consuming camel milk.

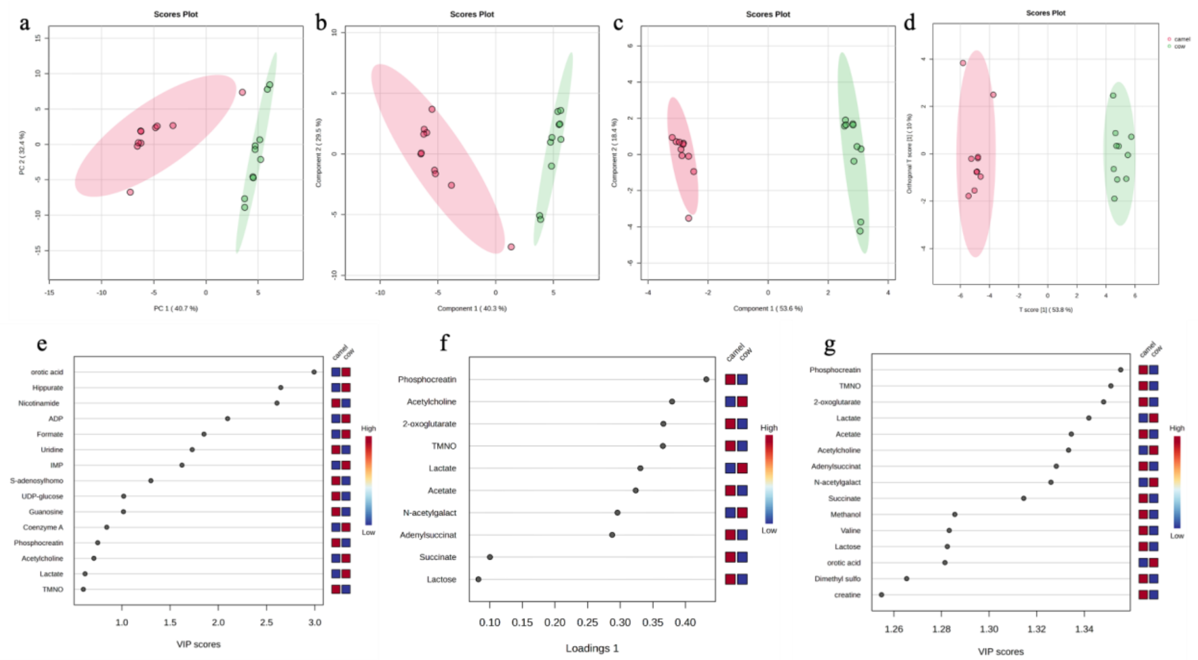

Figure 4 Multivariate analysis of metabolites from cow and camel milk samples obtained in NMR study. The Principle Component Analysis (PCA) plot (a) explains the variance. Supervised methods, including Partial Least Squares- Discriminant Analysis (PLS-DA) (b), Sparse Partial Least Squares-Discriminant Analysis [sPLS-DA] (c), and Orthogonal Partial Least Squares- Discriminant Analysis [OPLS-DA] (d), are presented. VIP score plots for PLS-DA (e), sPLS-DA (f), and OPLS-DA (g) are also shown.

To further identify other components present in cow and camel milk samples, we analysed milk samples by Liquid chromatography-Mass spectroscopy (LC-MS), a highly sensitive technique to identify molecules at nano/picomolar levels.

A comprehensive compositional profiling of camel and cow milk was carried out using non-targeted Liquid Chromatography-Mass spectrometry (LC-MS). Metabolomics, particularly through non-targeted Liquid Chromatography-Mass spectrometry (LC-MS), stands out as the most robust method and powerful platform for studying low molecular weight metabolites (MW < 1000) and several studies on milk metabolomics of various species demonstrated the successful application of LC-MS approach [(7).] In the present study, camel milk metabolomics identified and quantified metabolites. By leveraging bioinformatic software, we conducted pathway analysis and pathway enrichment for these identified metabolites. Metabolic profiling of both the LC-MS runs [using 3 months (Feb-March 2024) and 5 to 6 months (May-June 2024) postpartum stage], unveiled a total of 366 metabolites mapping to 99 KEGG pathways (Kyoto Encyclopaedia of genes and Genomes). Through pathway enrichment analysis utilizing the Over Representation Analysis approach, we identified enriched pathways such as Pantothenate and CoA biosynthesis, valine, leucine, and isoleucine biosynthesis, Sphingolipid metabolism, and tyrosine metabolism, with a p-value of <= 0.07 and FDR < 0.20 (False Discovery Rate) (Figure 5). Furthermore, our KEGG drug analysis unearthed approximately 40 drug-like substances with therapeutic potential, predominantly five nootropics (Choline Alfoscerate, Fluroxene, Fipexide, Tiagabine, Cabaser), four antihypertensive (Azelnidipine, Fosinoprilat, celiprolol, Inspra) and five antineoplastic agents (Safingol, Pixantrone, Cedefingol, Gemcitabine elaidate, and Iniparib) and two antidiabetic (Netoglitazone) and antiglaucoma (Latanoprost) drugs. But the accurate abundance levels of these drugs and other metabolites are not predicted in the study due to absence of replicates. Thus another LC-MS run using 7 months postpartum stage milk samples collected in July 2024 was performed in triplicates to identify the significant metabolites.

This LC-MS run (using milk samples collected in the month of July 2024) identified total of 245 common metabolites classified into 12 class categories. PLS-DA analysis of global metabolomics identified 13 metabolites out of top 20, namely, PG(18:1(11Z)/16:1(9Z)), 4-vinylbenzene-1,3-diol, 1-octadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1′-sn-glycerol), pargyline, Benzoic acid, 2-6-toluenediamine, 2-O-methylcytosine, Phenmetrazine, 3-methyleneindolenine, (S)-(+)-Methamphetamine, Gabapentin, Urocanic acid and (2S)-6-Amino-2-[(E)-(hydroxymethylene)amino]hexanimidic acid, identified as important metabolites by VIP score, were found to be present in higher abundance in camel's milk (Figure 6). Among them, "Pargyline" metabolite was found to possess Antidepressant and Antihypertensive properties attributing to the therapeutic properties of camel milk.

While PLS-DA analysis of variance of camel and cow milk showed 57.6% variance (component 1) and component 2 value was 14.5% (Figure 7). Pairwise analysis between camel and cow revealed 53 significant metabolites (FDR <= 0.01). Out of which, 13 were found to show higher concentration in Camel’s milk and 27 metabolites were found to be low (log2(FC) > + - 2 and adj. p-value < 0.05).

With respect to pathway analysis, we performed pairwise analysis of the samples taking cow data as reference and predicted significant metabolites. Further metabolite set enrichment analysis (MSEA) of these metabolites revealed significant pathways. Pairwise MSEA analysis of camel samples revealed 22 significant pathways. Six metabolites namely, Phenylalanine, Methionine, Taurocholic acid, Niacinamide, Quinoline and Cyclamic acid were the hits among these pathways. Methionine is essential for antioxidant function, liver health and protein synthesis. Taurocholic acids help with energy metabolism, DNA repair, immune system, fat digestion and gastrointestinal health. Phenylalanine plays a role in nervous system development, mood regulation and thyroid hormone synthesis. Although the role of Quinoline is not completely known, it may have neuroprotective and anti-inflammatory effects. Surprisingly, Cyclamic acid, not a natural metabolite, was also found in the camel milk. Interestingly, Niacinamide is also known as Nicotinamide or vitamin B3, is also identified in NMR and LC-MS analysis, thus emphasizing the importance of this metabolite role in camel milk medicinal properties.

Conclusion

This study provides valuable insights and a deeper understanding of camel milk's nutrient and chemical composition. Further the findings of this research underscore the remarkable versatility of camel milk composition, positioning it as a superfood with remarkable medicinal properties for addressing a myriad of diseases. Notably, the compounds and drugs identified in this study exist in forms that are readily absorbable, serving as precursor molecules with a notable safety profile devoid of the side effects often associated with chemically synthesized drugs. Furthermore, the integration of sustainable agroecology and precision farming practices at Dayalbagh has demonstrated tangible benefits by not only enhancing milk production but also preserving the milk's exceptional nutritional value. Camel milk emerges as a promising nutritional supplement suitable for individuals of all ages, holding the potential to significantly contribute to the management and prevention of various diseases. Ultimately, this research seeks to lay a foundational molecular framework for future investigations into the pharmacological impact of camel milk in the treatment of severe ailments, paving the way for further advancements in this promising field. Molecular studies comparing the metabolite profiles of all the milk sources are underway, which aids in characterization of unique and significant metabolites specific to each milk source.

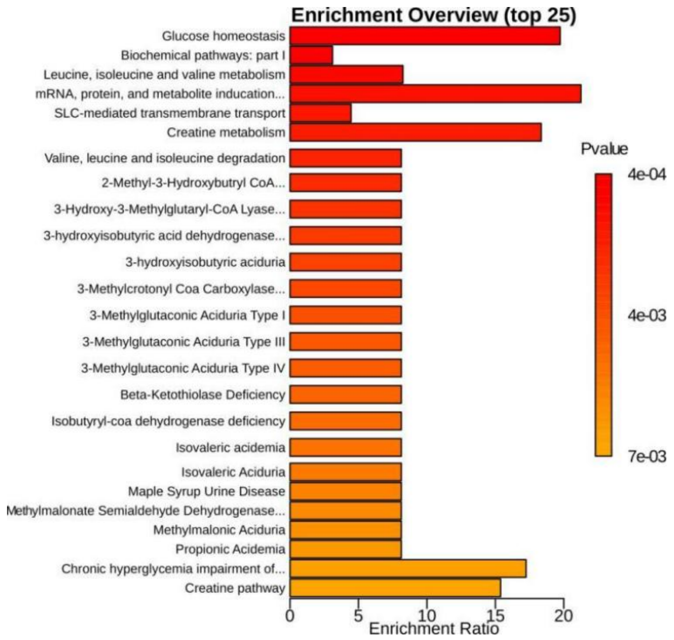

Figure 5 Representation of the significantly enriched pathways with respect to camel milk LC-MS analysis with p-value of <= 0.07.

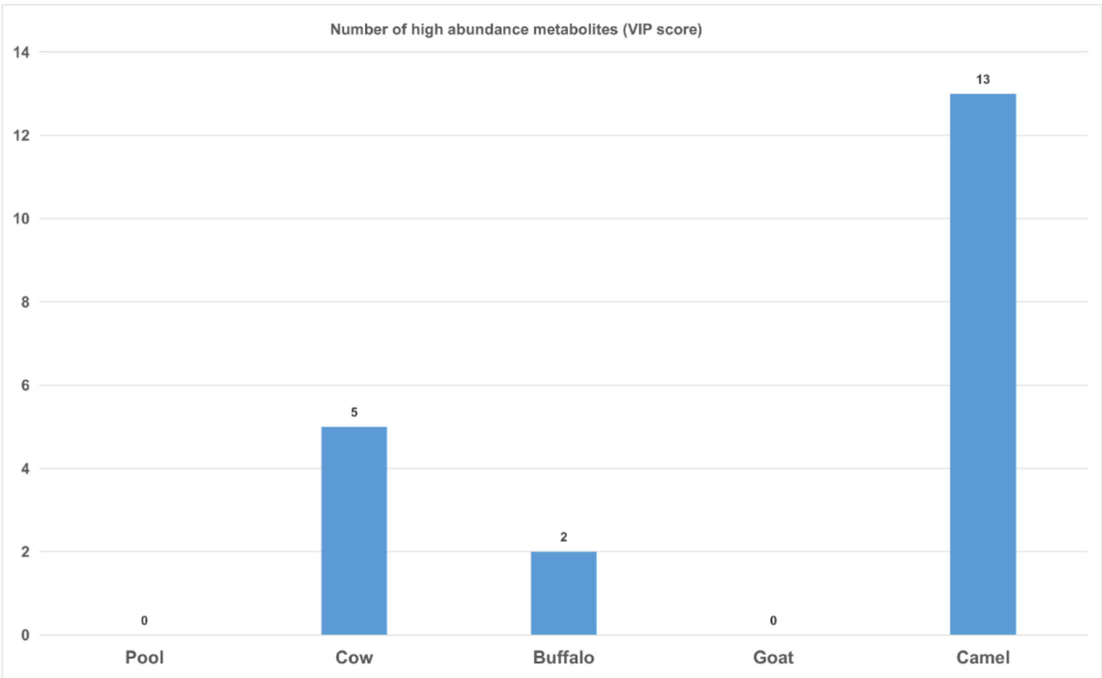

Figure 6 Global metabolite profiling of all milk samples using PLS-DA method identified important metabolites based on VIP (Variable Importance in Projection) scores in the LC-MS analysis. The highly abundant metabolites identified in all the milk sources based on VIP scores are represented in the above figure.

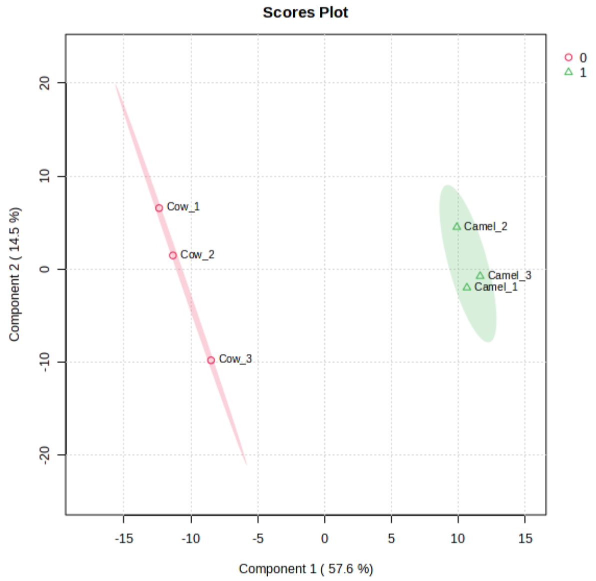

Figure 7 Partial Least Squares- Discriminant Analysis (PLS-DA) method based class separation for enhancing interpretability of Cow and Camel samples. PLS-DA analysis of variance of camel and cow milk showed highest variance of 57.6% for component 1 and component 2 value was 14.5%.

Note*: The part of above study is presented for oral presentation paper at 6th International Conference on Dayalbagh (Art) Science (& Engineering) of (Evolutionary/Re-Evolutionary) Consciousness (DSC) & the 47th (Inter) National Systems Conference (NSC), September 23-25, 2024, titled as “Compositional and metabolomic analysis of milk from Camels at Dayalbagh”*

3. Metabolomics of Milk Samples from Cow, Goat, Buffalo, and Camel from Dayalbagh Dairy using Nuclear Magnetic Resonance Spectroscopy

The insightful data obtained by NMR analysis of camel milk, encouraged us to include other milk samples from RSS gaushala. We carried out metabolomic analysis of all four milk sources by NMR spectroscopy. Milk being a highly nutritious and complex food, exhibits variability depending on its source. The characterization of milk metabolome from various animal species at Dayalbagh dairy offers a promising approach to assess its quality and variability.

Metabolite identification and Statistical analysis

Metabolite identification and spectral binning were performed in Chenomx v10.1 tool and imported HMDB 500 MHz metabolite library. The size of spectral binning was kept as 0.02 ppm bin, the bin file was uploaded in the metabonalayst 6.0 web server tool for further statistical analysis. The data was filtered, normalised by a median, log-transformed, and Pareto scaling methods were applied.

Notably, NMR analysis of all four milk samples revealed 48 metabolites, with 38 compounds showing significant changes based on ANOVA analysis. These metabolites were mapped to 32 pathways with a p-value of $<= 0.01$. Principal Component Analysis (PCA) demonstrated clear discrimination among the four milk groups, with PC1 explaining 38.5% of the variance and PC2 accounting for 29.7%. Conversely, Partial Least Squares Discriminant Analysis (PLS-DA) indicated that Component 1 explained 36.7% of the variance and Component 2 explained 10.2%. The PLS-DA model achieved an accuracy of 0.89, R^2 of 0.82, and Q^2 of 0.57 in cross-validation, indicating good classification performance. The model was validated using a 1,000-time permutation test, which yielded a p-value of 0.001. This indicates that the model's classification performance is statistically significant and not due to random chance.

From multivariate analysis, through Variable Importance in Projection (VIP) scores, the study identified the top 15 compounds with VIP values > 1, including UDP-galactose, IMP, S-adenosylhomocysteine, uridine, guanosine, ADP, hippurate, orotic acid, UDP-glucose, and methylcysteine (Figure 8, 9 and 10).

The comparison of metabolite profiles between cow milk and buffalo milk revealed distinct differences in metabolite levels. Using univariate t-test analysis, nine metabolites were found at higher levels in cow milk and five metabolites showed higher levels in buffalo milk. Further analysis using fold change and volcano plot techniques highlighted specific metabolites enriched in each type of milk, such as acetylcholine, hippurate, guanosine, orotic acid, IMP, formate, S-adenosylhomocysteine, uridine, and coenzyme A were cow milk, and nicotinamide ribotide, lactate, UDP-galactose and threonine in buffalo milk. Acetylcholine, a neurotransmitter crucial for muscle function and cognitive processes, was notably higher in cow milk, potentially offering therapeutic benefits. Orotic acid, known for its choleretic properties, and threonine, a nutritive supplement, were also found at elevated levels in cow milk, contributing to its potential health-promoting properties.

Comparing the metabolite profiles of cow milk and goat milk revealed significant differences in 26 metabolites, with goat milk showing elevated levels of 17 metabolites and cow milk with higher levels of nine metabolites. Through fold change and volcano plot analysis, cow milk exhibited increased levels of various metabolites such as ADP, IMP, and carnosine, while goat milk showcased higher levels of glycerophosphocholine, acetylcholine, and orotic acid. Glycerophosphocholine, a phospholipid metabolite, plays a crucial role in membrane synthesis and cell signalling, potentially supporting cognitive function and neuronal health. On the other hand, UDP-galactose, a nucleotide sugar found in goat milk, is essential for glycosylation reactions, influencing cell signalling, immune responses, and overall cellular function. The diverse range of metabolites present in different types of milk underscores their unique nutritional and potential health-promoting properties.

The detailed analysis highlights the specific benefits associated with each type of milk, suggesting that a combination of different milk sources in a mixed milk product could offer a more comprehensive range of nutritive and medicinal properties. By leveraging the distinct advantages of cow, buffalo, camel, and goat milk, a mixed milk blend may provide a synergistic effect, maximizing the health benefits derived from these diverse sources. Overall, these findings underscore the unique metabolite profiles of cow, buffalo, and camel milk, shedding light on their distinct nutritional compositions and potential health benefits. The present study provides preliminary differences among the milk metabolites of each source, further study is required to characterise the large number of metabolites present in these milk samples to find the unique potential signature of each milk sources, thus LC-MS analysis is undertaken which is more sensitive and yields higher number of significant metabolites.

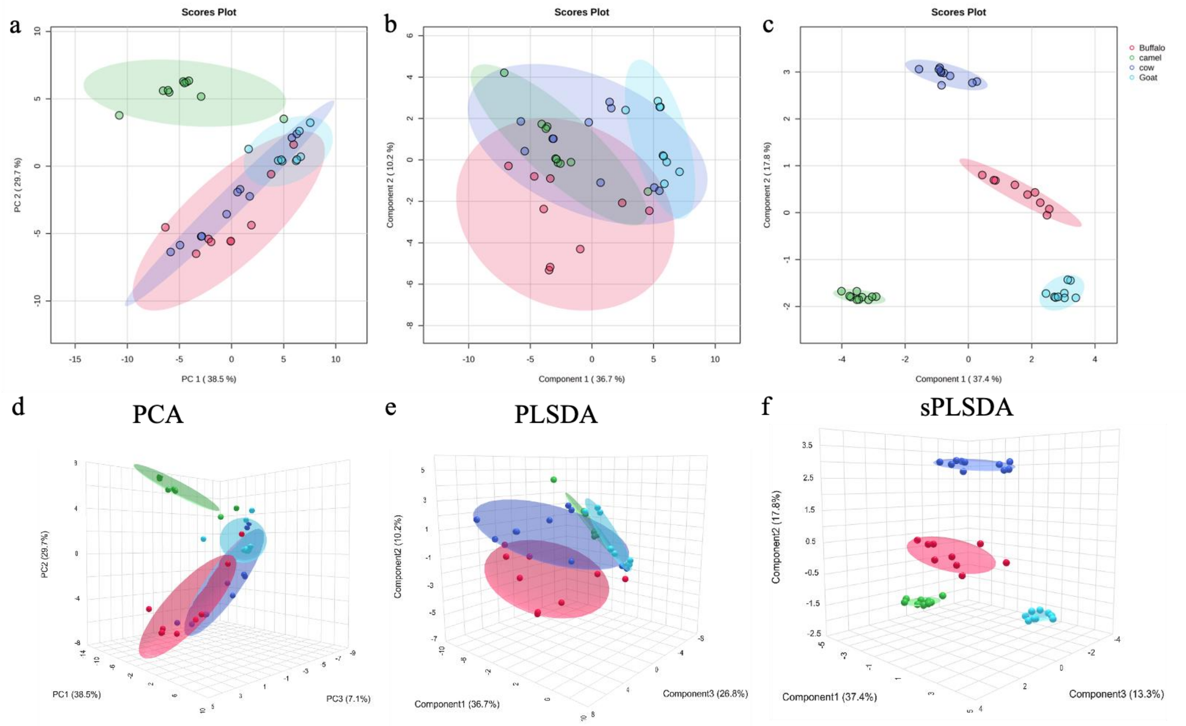

Figure 8 Multivariate analysis of the four milk sample groups (cow, goat, buffalo, and camel). The PCA plot (a) illustrates the explained variance. Supervised methods are represented by the PLS-DA (b) and sPLS-DA (c) models. Three-dimensional representations of these analyses are shown in (d), (e), and (f), respectively

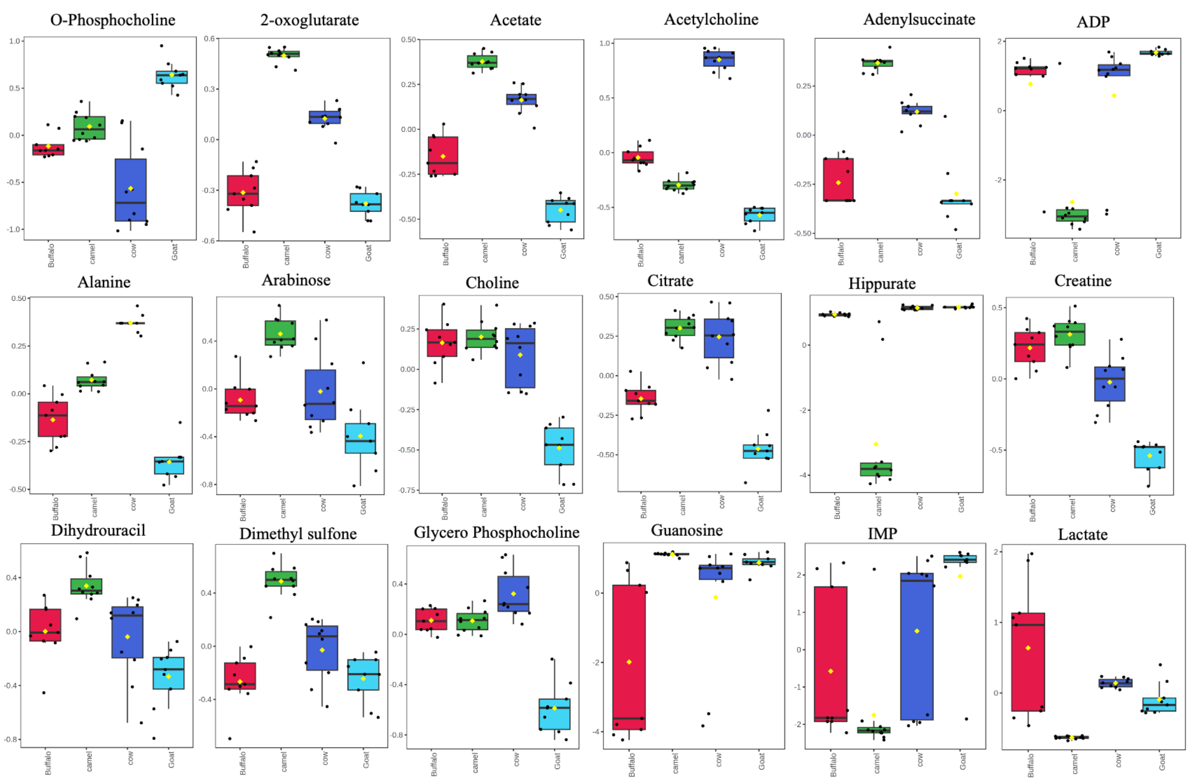

Figure 9 ANOVA analysis of cow, goat, buffalo, and camel milk samples. It represents a detailed view of significant metabolite differences among the samples.

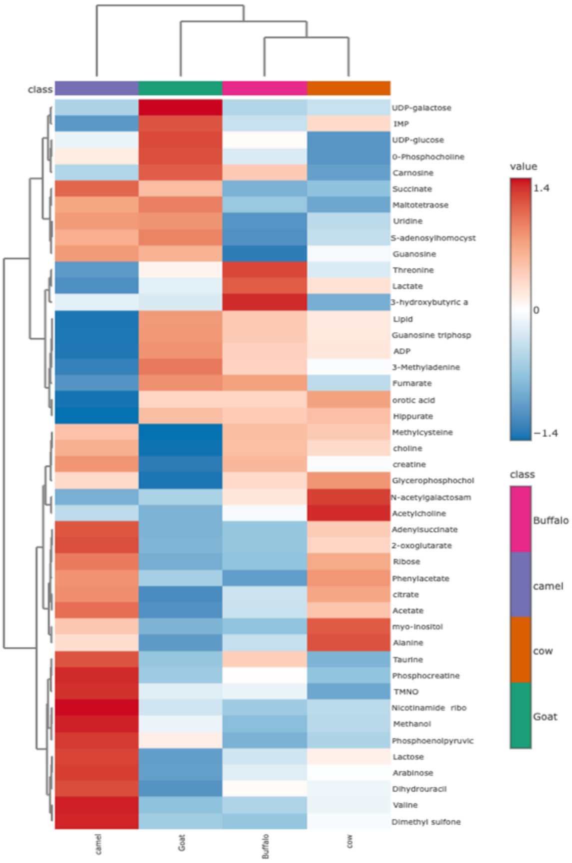

Figure 10 Heat map representation of significant metabolites in buffalo, camel, cow and goat milk from Dayalbagh Dairy

***Note**: The part of above study is presented for poster presentation paper at DSC 2025 winter session on 1st January 2025, titled as [“Mutual reciprocity and harmony at Dayalbagh Dairy promoting Lacto-vegetarianism and consciousness”]*

4. Comparative metabolomics of milk from Buffalo, Cow, Goat and Camel of the Dayalbagh dairy using LC-MS analysis

Milk is a vital source of nutrition with diverse health and wellness benefits. While the basic composition of milk is similar across different species, there exists a difference reflecting in milk properties indicating towards unique species-specific milk metabolites. The dairy at Dayalbagh accommodates diverse group of animal species including buffalo, cow, goat, and camels living in harmony which mirrors the "Dayalbagh way of life". Thus, characterisation of milk from these different sources from Dayalbagh dairy always remains a priority to understand its variability and comparative efficiency. Liquid Chromatography-Mass Spectrometry (LC-MS) is a more sensitive technology for global metabolic profiling of complex biological samples like milk samples [(7).] It can detect most of the metabolites, especially the low abundance compounds, which thus make it suitable for potential biomarker identification specific to each species. Applying metabolomic technology, the Yang group analyzed the differences of metabolites in the milk between Holstein and other minor dairy animals and found that different metabolic pathways were involved in the milk synthesis of diverse mammals [(8).] Yuan group studied the comparative metabolomics analysis of milk components between Italian Mediterranean buffaloes and Chinese Holstein cows based on LC-MS/MS technology [(9).] Using same LC-MS tool for comparative metabolomics of human milk with milk sourced 12 different kinds live stocks, Wang group [(10)] analysed for the milk source with more beneficial nutrients that is close to human milk composition Thus, metabolomics exhibited a better response to phenotypes, and provided extraordinary help for further study in physiology and pathology during lactation and other biochemical characteristics of milk [(7).]
In the present study, using non-targeted/label-free Liquid Chromatography-Mass Spectrometry (LC-MS), we conducted comprehensive metabolomic analysis of milk samples collected in the months of May 2024 and subsequently in July 2024 from buffalo, cow, goat, and camel of Dayalbagh dairy. In addition to these milk samples, pooled milk from all four milk sources representing the mixed milk sample was also analysed in the second LC-MS run.

Global metabolite profile analysis using initial LC-MS run (using milk samples collected in the month of May 2024), resulted in the identification of 643 metabolites with 56 found to be common among all the animal species. The pathway enrichment analysis using Over Representation Analysis (ORA) approach, identified Pantothenate and CoA biosynthesis and Ascorbate and aldarate metabolism as significantly enriched pathways specific to cow milk, whereas Sphingolipid metabolism and Arginine and proline metabolism were identified as enriched pathways specific to buffalo milk. Similarly, Pantothenate and CoA biosynthesis, valine, leucine, and isoleucine biosynthesis, Sphingolipid metabolism, and tyrosine metabolism were identified as significantly enriched pathways specific to camel milk and Pyrimidine metabolism, Pantothenate and CoA biosynthesis, beta-Alanine metabolism and Sphingolipid metabolism were identified as enriched pathways specific to goat milk. These all pathways were selected with a p-value of <= 0.07 and FDR < 0.20. Further comparative analysis couldn't identify differential metabolites, due to a smaller number of common metabolites (n=56) identified in this LC-MS run.

The second LC-MS run (using milk samples collected in the month of July 2024) identified total of 245 common metabolites classified into 12 class categories (Figure 11). Principal component analysis (PCA) suggested that the metabolite profiles of samples from the same species exhibit clustering, while separated patterns of metabolite profiles are observed across camel, goat, cow, and buffalo species. Using PLS-DA (Partial least squares-discriminant analysis) method for global metabolomics, 13 out of 20 metabolites were identified as important metabolites by VIP scores (Variable Importance in Projection) with respect to camel milk. With respect to Cow milk, 5 metabolites namely, D-glucosamine-6-phosphate, 2'-Deoxysepiapterin, 7,8-dihydro-8-oxoguanine, Celastrol and 2-(3,5-Dimethoxyphenyl)-7-hydroxy-4H-chromen-4-one were found in high abundance. In case of Buffalo, 2 metabolites, GLY-MET and L-a-Lysophosphatidylcholine, caproyl were in high abundance. Goat milk showed high level for 13 metabolites similar to Camel but the abundance was less than the camel metabolites. Mixed milk samples had 2 samples with high abundance similar to cow but having concentration less than cow metabolites.

About 140 potentially significant global metabolites were predicted using one way analysis of variance (ANOVA) with post-hoc analysis, among them two metabolites namely, "Gabapentin" and "Pregabalin" were identified as Neuropsychiatric agents and tranexamic acid was identified to possess anti-inflammatory property using KEGG drug analysis. Pair wise analysis between the camel milk and cow milk samples revealed 52 significant metabolites (FDR <= 0.01). Out of which, 13 were found to show higher concentration in Camel's milk and 27 metabolites were found to be low (log2(FC) > + - 2 and adj. p-value < 0.05). Similarly, analysis of Buffalo milk samples showed a total of 9 significant metabolites, including 4 metabolites each with log2(FC) > + - 2 and adj. p-value < 0.05. Likewise, 31 significant metabolites were identified in Goat milk samples. Out of the significant metabolites, 8 metabolites were up regulated and 15 were down significantly. We also performed pair-wise analysis of mixed milk sample verses cow milk sample. The analysis revealed 18 metabolites to be significantly regulated and out of these, 6 metabolites showed higher regulation and only one metabolite was found to be down regulated ($\log2(FC) > + - 2$ and adj. p-value < 0.05).

Metabolite set enrichment analysis (MSEA) of the significantly regulated metabolites revealed important pathways for the pair camel, goat and mixed milk samples. With respect to pathway analysis, we performed pairwise analysis of the samples taking Cow data as reference and predicted significant metabolites. The pathways with p value $<= 0.01$ were considered as significantly enriched pathway. Pairwise MSEA analysis of camel samples revealed 22 significant pathways. In MSEA analysis of Goat samples, 8 metabolites (L-Acetylcarnitine, Taurocholic acid, Creatine, Glutamine, Glycerophosphocholine, Niacinamide, Phenylalanine and Tranexamic acid) were found to be involved in the identified significant pathways (Table 2). L-Acetylcarnitine plays a role in maintaining cognitive function and in protecting neurons from oxidative damage. It also has a role in transport of long-chain fatty acids into mitochondria leading to the role in energy production. The creatine in milk help in enhancing muscle function and supporting cognitive development. Glutamine aids in gut health and supports immune function. Glycerophosphocholine is a naturally occurring phospholipid and a derivative of phosphatidylcholine which in-turn is a vital nutrient for brain development. It also helps in maintaining liver health as phosphatidylcholine is a critical component of liver cell membranes. Tranexamic acid was found to acts as antifibrinolytic agent but its role in milk is not known. Three metabolites namely Taurocholic acid, Phenylalanine and Tranexamic acid were found to be contributing to seven of the significant pathways identified for mixed milk samples. The roles of these metabolites are already discussed above. Further, MESA analysis also identified potential diseases targeting pathways specific to the unique metabolites of each milk sources, with p-value $<= 0.01$ (Figure 12). This approach has provided deeper insights into effective treatment of these selected diseases using specific metabolites constituting each milk sources. In conclusion, we can deduce that together, these metabolites play vital roles in supporting growth, development, metabolism and overall health, particularly in infants and young children who are primarily fed milk.

Also, it would be more effective to use mixed milk in the treatment of these diseases as it would be a perfect way to combine all the unique metabolites. Thus, understanding the global metabolite profiles and pathways provides species-specific milk metabolites with medical properties making milk from Dayalbagh gaushala as a wholesome and therapeutic food.

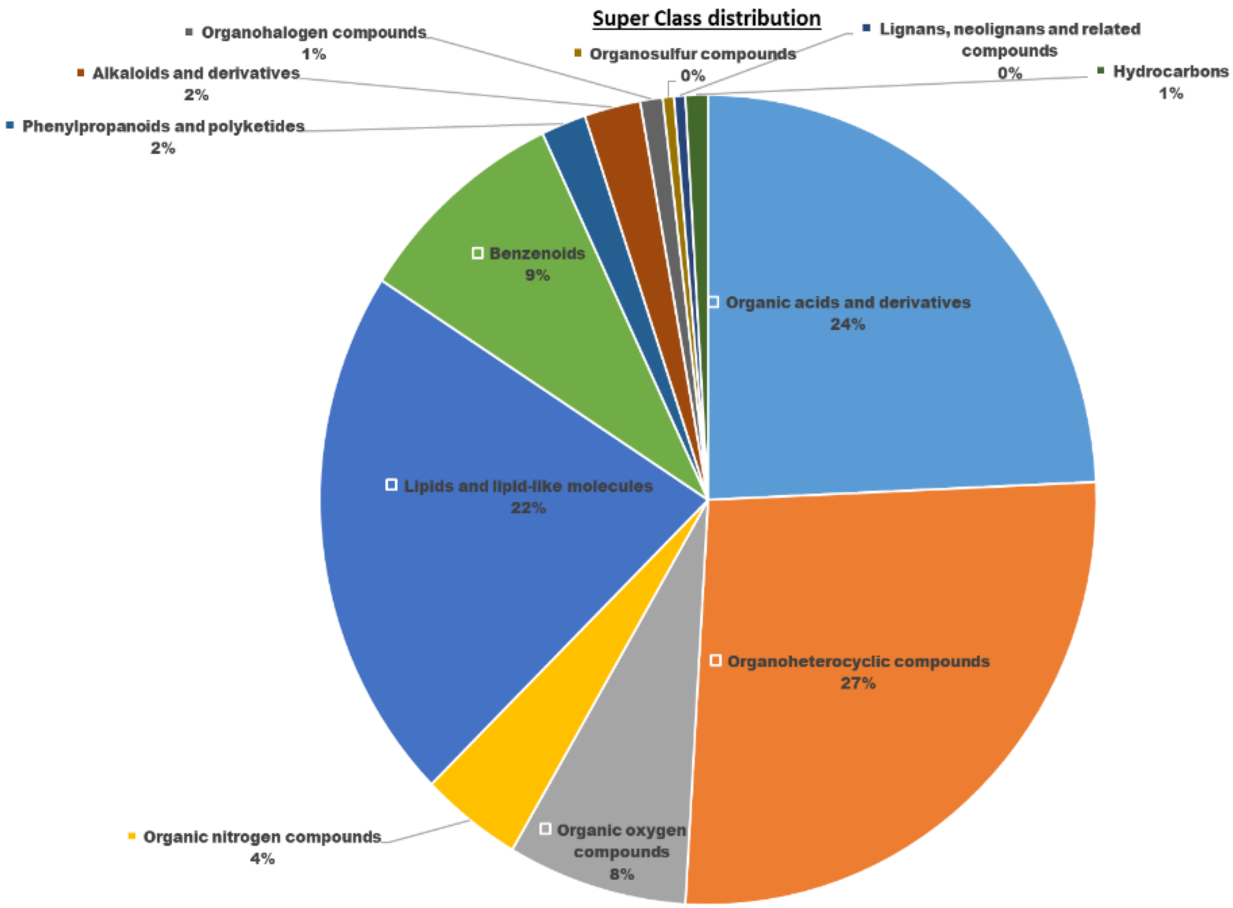

Figure 11 Classification of total metabolites into 12 different super class categories

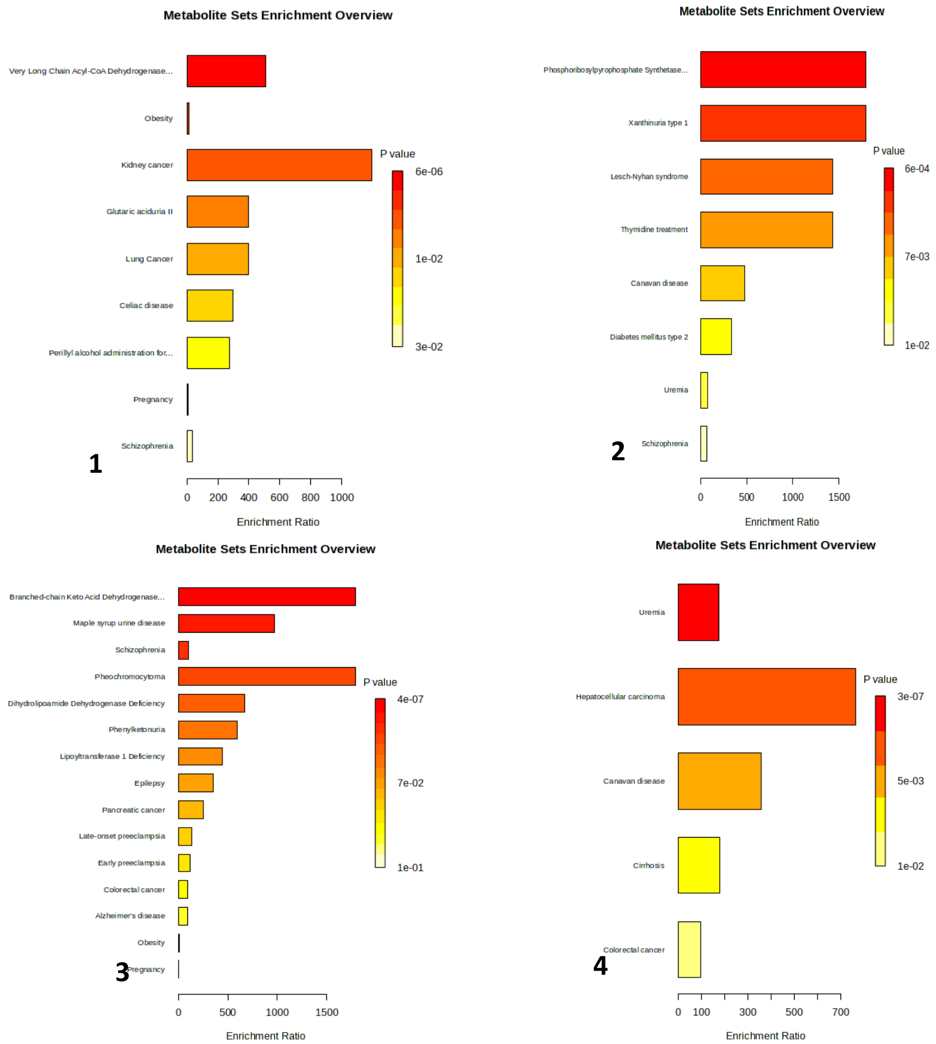

Figure 12 Representation of potential diseases targeting pathways specific to the unique metabolites of each milk sources. The numbers correspond to the following 1: Cow, 2: Buffalo, 3: Camel, 4: Goat

Table 2: List of enriched pathways analyzed using pairwise MSEA analysis

Milk Sample	Pathway	total	hits	Raw p
Camel/Cow	Defective SLC6A19 causes Hartnup disorder (HND)	13	2	0.000378
	Glucose homeostasis	21	2	0.00101
	Disorders of transmembrane transporters	98	3	0.00124
	Transcription/Translation	25	2	0.00143
	Amino acid transport defects (IEMs)	27	2	0.00167
	Amino acid transport across the plasma membrane	32	2	0.00235
	Na+/Cl- dependent neurotransmitter transporters	32	2	0.00235
	Tryptophan catabolism	33	2	0.0025
	SLC-mediated transmembrane transport	155	3	0.00463
	Degradation of AXIN	2	1	0.00467
	Transport of inorganic cations/anions and amino acids/oligopeptides	52	2	0.00612
	Uptake and function of diphtheria toxin	3	1	0.00699
	DNA Damage Recognition in GG-NER	3	1	0.00699
	Lac-Phe pathway	3	1	0.00699
	Trans-sulfuration, one-carbon metabolism and related pathways	58	2	0.00757
	Metabolism of vitamins and cofactors	189	3	0.00808
	Phenylketonuria	4	1	0.00931
	Oleoyl-phe metabolism	4	1	0.00931
	Cytosolic tRNA aminoacylation	66	2	0.00973
	tRNA Aminoacylation	66	2	0.00973
	Mitochondrial tRNA aminoacylation	66	2	0.00973
	Sensory Perception	203	3	0.00984
Goat/Cow	Defective SLC6A19 causes Hartnup disorder (HND)	13	2	0.000236
	Disorders of transmembrane transporters	98	3	0.000599
	Biomarkers for urea cycle disorders	22	2	0.000693
	Transcription/Translation	25	2	0.000897
	Amino acid transport defects (IEMs)	27	2	0.00105
	Metabolism of amino acids and derivatives	283	4	0.00106
	Phenylalanine and Tyrosine Metabolism	28	2	0.00113
	Nicotinate and nicotinamide metabolism	28	2	0.00113
	Amino acid transport across the plasma membrane	32	2	0.00147
	Na+/Cl- dependent neurotransmitter transporters	32	2	0.00147
	SLC-mediated transmembrane transport	155	3	0.00228
	Arginine and proline metabolism	49	2	0.00344
	Degradation of AXIN	2	1	0.00373
	Defective GFPT1 causes CMSTA1	2	1	0.00373
	Transport of inorganic cations/anions and amino acids/oligopeptides	52	2	0.00387
	Metabolism of vitamins and cofactors	189	3	0.00402
	Nicotinate metabolism	56	2	0.00447
	Generic Transcription Pathway	58	2	0.00479
	Transport of small molecules	208	3	0.00528

	Classical antibody-mediated complement activation	3	1	0.0056
	Uptake and function of diphtheria toxin	3	1	0.0056
	DNA Damage Recognition in GG-NER	3	1	0.0056
	Lac-Phe pathway	3	1	0.0056
	Biochemical pathways: part I	445	4	0.00573
	RNA Polymerase II Transcription	66	2	0.00617
	Cytosolic tRNA aminoacylation	66	2	0.00617
	tRNA Aminoacylation	66	2	0.00617
	Mitochondrial tRNA aminoacylation	66	2	0.00617
	Phenylketonuria	4	1	0.00746
	Oleoyl-phe metabolism	4	1	0.00746
	TCA cycle nutrient use and invasiveness of ovarian cancer	4	1	0.00746
	Transport of bile salts and organic acids, metal ions and amine compounds	76	2	0.00813
	Gene expression (Transcription)	79	2	0.00876
	SLC transporter disorders	80	2	0.00898
	Regulation of PTEN stability and activity	5	1	0.00931
	Escape of Mtb from the phagocyte	5	1	0.00931
	Phagocyte cell death caused by cytosolic Mtb	5	1	0.00931
	Tranexamic Acid Action Pathway	5	1	0.00931
	Sterol regulatory element-binding proteins (SREBP) signaling	5	1	0.00931
	Translation	83	2	0.00964
Mixed/Cow	Lac-Phe pathway	3	1	0.0028
	Disorders of transmembrane transporters	98	2	0.00302
	Phenylketonuria	4	1	0.00373
	Oleoyl-phe metabolism	4	1	0.00373
	Tranexamic Acid Action Pathway	5	1	0.00467
	Defective ABCB11 causes PFIC2 and BRIC2	6	1	0.0056
	SLC-mediated transmembrane transport	155	2	0.00745

Note : The above detailed studies on comparative analysis of all milk samples deploying NMR and LC-MS are under pre-publication status and awaited for communication with reputed international journals

5. A clinical trial of Camel milk consumption on Heart rate and Peripheral Oxygen Saturation

Introduction

A high heart rate and low peripheral oxygen (Spo2) are established markers for cardiopulmonary risk. The acute effect of camel milk consumption on heart rate and peripheral oxygen saturation (phase 1), in comparison with cow and goat milk (phase 2) and anecdotal interesting cases (phase 3) is presented in this proof-of-concept study.

Methods

This is an open label trial with three groups to evaluate the effects of camel milk, on heart rate and peripheral oxygen saturation and compare with goat milk and cow milk. For each trial, participants received boiled milk with quantity varying from 5ml to 150 ml for camel milk and 100 ml for both cow and goat milk, respectively. Total of 288 observations were collected for Camel, 31 for Cow and 36 for Goat milk oximetry. The heart rate and peripheral oxygen saturation were recorded with a heart oximeter once before milk consumption, and then at 30, 60-, 90- & 120-minute post-consumption. Paired t-test statistical method is used to analyse the data.

Results

Phase 1 *(Acute effects of camel milk consumption on heart rate and oxygen saturation)*

The study showed a significant decrease in heart rate at all time-intervals, with highest significance at 90 minutes (p-value < 0.05). The mean decrease in the heart rate was 4%. 54% of participants experienced a significant decline in the heart rate. Decrease in heart rate was observed with low quantities (5 - 30ml) as well as with high quantities (>30 ml) of camel milk. However, a decline of close to 7% was observed if more than 100ml of Camel milk is consumed. In addition, the study showed that females experience higher decline in mean heart rate (5%) than males (2%). No significant change in oxygen saturation levels before and after camel milk consumption was observed in this study.

Phase 2 *(Acute effect of camel milk consumption compared to cow and goat milk on heart rate and peripheral oxygen saturation)*

The camel milk group showed a significant decrease in heart rate at all time-intervals, with highest significance at 90 minutes (p-value < 0.05). The mean decrease in the heart rate was 4%. Close to 54% of participants experienced a significant decline in the heart rate.

The cow milk group also showed a significant decrease in heart rate at 30 min post consumption with a 90% confidence interval. However, the goat milk group showed an increase in heart rate with a significant increase at 30 min post consumption at 90% confidence interval and at 60 min at 95% confidence interval (Figure13)

Phase 3 *(Anecdotal interesting cases)*

CASE 1: A 9-year-old patient with TOF (Tetralogy of Fallot) with baseline oxygen saturation of less than 60% prior to consumption and chronically below 60s at home had an increase of oxygen saturation to 70-80 % at 30 min after consumption of camel milk.

CASE 2: A 10-year-old patient with Turner Syndrome with inappropriate Sinus tachycardia and on Anti-hypertensive medicines. Post Camel milk consumption over 3 months has significantly improved heart rates (now in normal range) and is now weaned off Anti-hypertensive medicines.

CASE 3: A 6-year-old patient with Down's syndrome, operated prior for cardiac conditions, had baseline oxygen saturation of 95% before consumption of camel milk. The oxygen saturation improved to 100% by 30 min after consumption.

CASE 4: A 4-year-old patient with Downs syndrome had SpO_2 of 88% before and improved to high 90s after 30 min of camel milk consumption.

Conclusions

In this open label, non-blinded trial

1. Camel milk consumption significantly reduces heart rate in healthy participants. The mean decrease in the heart rate was 4%. Female, and participants who consumed more than 100 ml had highest decline in heart rate.
2. Camel milk has significant benefit over cow and goat milk in reducing heart rate and could have beneficial effects in patients with heart failure, coronary artery disease and inappropriate sinus tachycardia.
3. Camel milk, cow and goat consumption has not significantly increased peripheral oxygen saturation. Since majority of participants already had baseline oxygen saturation close to 98 before intake of camel milk, the study was not adequately powered to detect a change.
4. Very interesting anecdotal cases have shown us directions to design future studies. A double blinded RCT with multicentre trial to compare acute and chronic effects of camel, cow, goat milk and mixed milk consumption on heart rate in healthy participants

is planned in future incorporating more parameters like mean blood pressures, HRV (heart rate variability) etc.

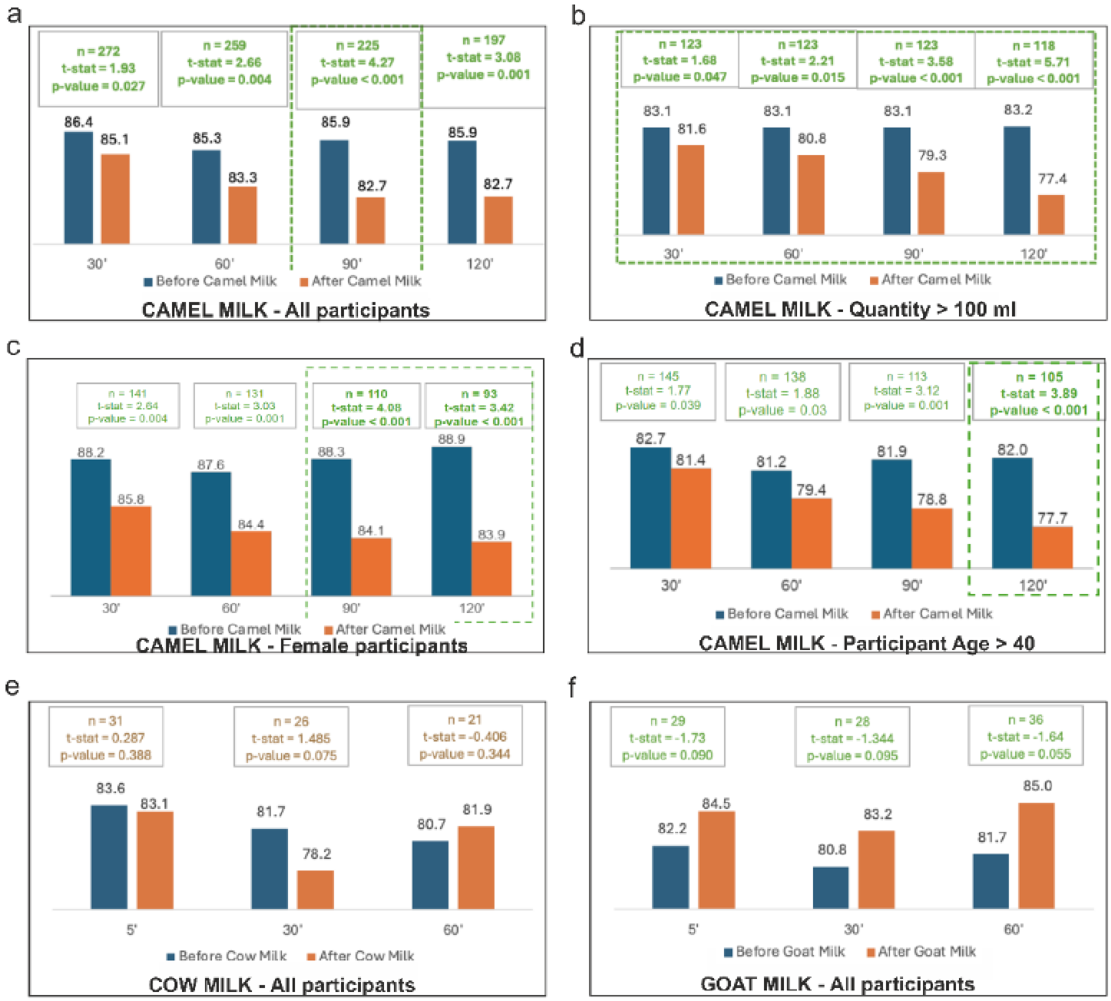

Figure 13 Average pulse rate readings of the subjects before and after milk consumption (30, 60, 90 and 120 minutes) with the sample size used for comparison, t-stat and p-value also indicated for each pair of mean comparison. (a) Camel milk related pulse rate changes for all the participants. (b) Camel milk related pulse rate changes participants who consumed more than 100 ml quantity milk. (c) Camel milk related pulse rate changes for female participants only. (d) Camel milk related pulse rate changes participants who are more than 40 years old. (e) Cow milk related pulse rate changes in participants. (f) Cow milk related pulse rate changes in participants.

Note: *The part of above study is presented as oral paper at 6th International Conference on Dayalbagh (Art) Science (& Engineering) of (Evolutionary/Re-Evolutionary) Consciousness (DSC) & the 47th (Inter) National Systems Conference (NSC), September 23-25, 2024, titled* *A clinical trial of camel milk consumption on Pulse Oximetry observations*

6. Impact of Camel Milk Consumption on Cognitive Health of Differently Abled Children of Sant Superman Evolutionary and Re-evolutionary Scheme of Dayalbagh

Introduction

Sant Superman Evolutionary Scheme of Dayalbagh is a bio-socio-cognitive scheme which is an all-inclusive model of Early Childhood Development (ECD). The scheme was launched by Most Revered. Prof. Prem Saran Satsangi Sahab in Jan 2017, since then all children including Differently Abled are given equal opportunity to participate in its activities like multicultural performances and healthcare exercises at Agroecological fields of Dayalbagh and connected E Cascade network. The longitudinal observational study of over 7 years, tracking the holistic growth and development of its participants, shows statistically significant difference as compared to controls. With addition of camels to its live stalk at Dayalbagh dairy, in Dec 2023 children started receiving camel milk as per the Gracious Guidance. Present study evaluates the impact of recent supplementation of camel milk on cognitive health of Neuro Diverse children. This study is being conducted at the Department of Paediatrics, Saran Ashram Hospital since January, 2024.

Methods

Camel Milk is already known to benefit autism spectrum disorder as per the review of literature. This self-controlled study includes 35 Differently Abled Neuro Diverse children from local as well as outstation participants, (Autism 14, Attention-Deficit/Hyperactivity Disorder 5, Motor Disorder 5, Congenital 4, Neuro behaviour disorder 2, and Communication Disorder 5). Assessment is done at regular interval of 3 months for pre and post score changes of cognitive markers after consuming boiled CAM (100-200ml/day). Diagnostic battery of tests included Childhood Autism Rating Scale (CARS), Autism Treatment Evaluation Checklist (ATEC), Diagnostic and Statistical Manual (DSM V) along with standard tools to assess Intelligence, Emotional and Developmental Quotient.

Results

The results show statistically significant (95% Confidence Interval) change in scores of Childhood Autism Rating Scale p value = 0.0019, Autism Treatment Evaluation Checklist p value = 0.022, Stanford Binet Intelligence Quotient p value = 0.0394 and Emotional Intelligence Quotient p value = 0.00561 after consuming camel milk for three months. Improvement that is the percentage decline in pre and post scores shows maximum change in Verbal Communication (26.39%), Listening Response (23%), Activity level (20.39%) in

Childhood Autism Rating Scale (Figure 14) and Cognitive and Sensory functions (17.25%) in Autism Treatment Evaluation Checklist (Figure 15). The earliest change is observed at 15 days peaking at 2 months of consumption more at younger age.

Conclusion The Superman Evolutionary scheme is a re-evolutionary scheme which keeps on adding new dimensions to its Systems model. This all-inclusive scheme encourages participation of Differently Abled children in all its activities to develop their full potential. Addition of Camel milk as nutritional adjuvant has further improved the cognitive functions and quality of life especially in Neurodiverse/Differently Abled children.

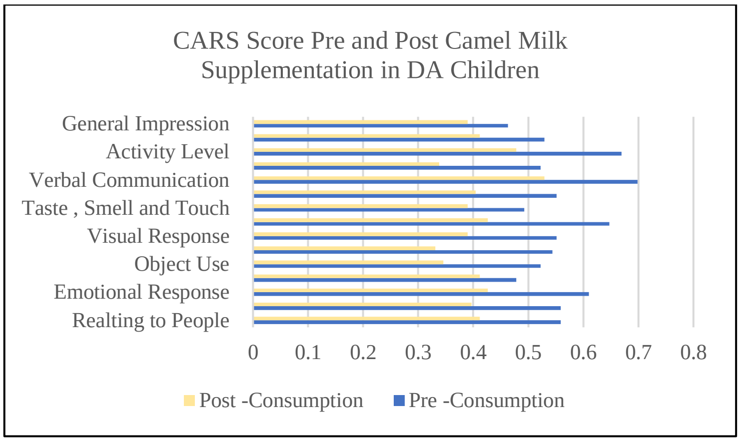

Figure 14 Childhood Autism Rating Scale n=17. Pre and post scores show a decline (improvement) in all 15 domains. The most significant improvement is seen in Listening Response, Adaptation to Change and Activity Level. P Value is 0.00338, hence significant at p<.05 at 95 % CI

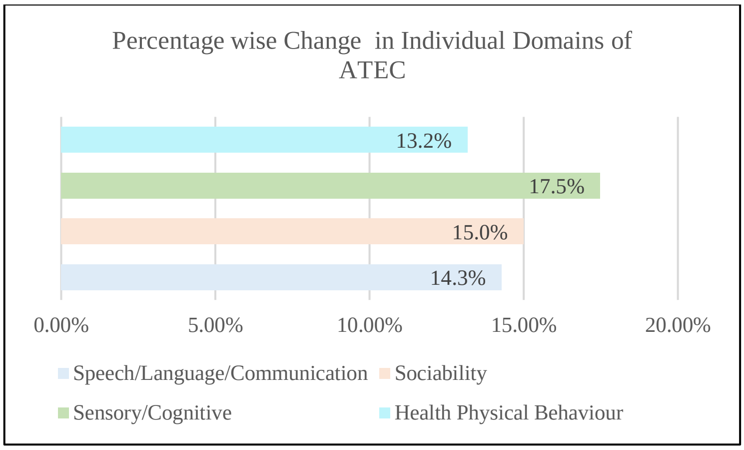

Figure 15 Autism Treatment Evaluation Checklist (ATEC) Cognitive and Sensory functions observed maximum change with p-Value of 0.01928, hence significant at p < .05 at 95 % CI. IQ Scores pre and post p-Value is 0.09, the result is not statistically significant at 95 % CI

***Note**: The part of above study is presented for Poster presentation paper at 6th International Conference on Dayalbagh (Art) Science (& Engineering) of (Evolutionary/Re-Evolutionary) Consciousness (DSC) & the 47th (Inter) National Systems Conference (NSC), September 23-25, 2024, titled as “Impact of Camel Milk Consumption on Cognitive Health of Differently Abled Children of Sant Superman Evolutionary and Re-evolutionary Scheme of Dayalbagh”*

7. Vitamin D deficiency: Fortification and Treatment at Saran Ashram Hospital Dayalbagh Educational Institute

Background The prevalence of vitamin D3 deficiency in Indian population in previous community-based studies is 51 to 94% and in hospital- based studies is 40 to 99.7%. Consequences of Vitamin D deficiency are rickets in children and osteomalacia in adults, skeletal diseases, metabolic disorders, cancer, cardiovascular disease, autoimmune diseases, infections, cognitive disorders, and/or mortality. Vitamin D is a group of fat-soluble vitamins, with vitamin D3 and vitamin D2 being most significant for humans. Studies confirm that, vitamin D3 is twice as efficacious as Vitamin D2, hence superior. While vitamin D3 can be sourced through animal food sources, vitamin D2 is derived from plants. This makes the lactovegetarian community deprived of vitamin D3 as a food item. However, external supplements of vitamin D3 are recommended to those who need it. Synthetic vitamin D3 is now available in India at reasonable cost, making it viable to be provided to the deserving communities, particularly growing children and osteoporotic females.

In present study, the incidence of vitamin D deficiency at Saran Ashram Hospital, Dayalbagh, Agra, which caters to a subsection of population essentially urban and semi urban type from neighbouring localities, is found to be about 79% in patients (Feb to May 2024), in a sample of 150 patients between the age 4 years to 79 years. Amongst these patients, about 50% were in the range of 'deficiency' (below 20ng/ml) and 11% were in the range of 'severe deficiency' (less than 10 ng/ml) (Figure 16).

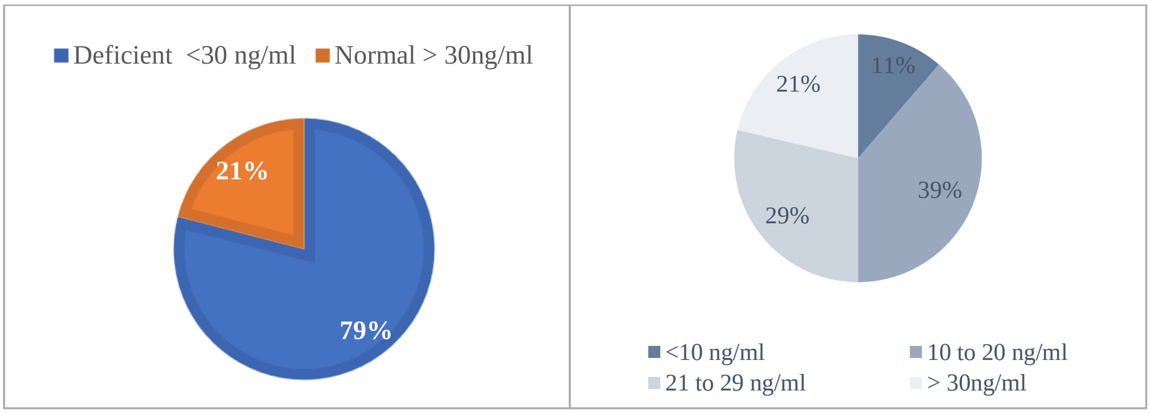

Figure 16 Prevalence of Vitamin D3 deficiency at SAH

Milk is generally deficient in vitamin D, however few studies have reported high vitamin D levels in camel. In present study we could not detect vitamin D in any of the milk samples from Dayalbagh Gaushala i.e. cow, buffalo, goat and camel done through DEI. Further no vitamin

D or its metabolites could be detected in our study through LC-MS and NMR studies done at ICGEB. This was implicated to probability of extraction and sample preparation methods. This arises the need to fortify milk and milk products with vitamin D and also provide treatment to those who are found to be deficient.

Fortification and Treatment To check bioavailability of vitamin D, we conducted a proof-of-concept study enrolling symptomatic children n=48, who were found to have deficiency of 25-hydroxy-vit D in blood sample and randomly assigned them one of the 4 treatment protocols with weekly therapeutic dose of vit D, while control group received 100 ml of Camel milk daily without any other supplementation of vitamin D.

1.Vitamin D sachet dry powder 60K UI

2. Chewable tablets 60K UI

3. Fortified chocolates 30K UI

4. Vit D liquid in nano form of 60 K UI

25-hydroxy-vitamin D levels were compared in pre and post samples with gap of around 2 months i.e. post completion of treatment. Results reveal that most significant effect on bioavailability was found by supplementing nano preparation of vitamin D, while no change could be detected in Camel milk group. This led to consider **Fortification of milk or milk products** like butter or chocolates to vulnerable sections of Dayalbagh community (growing children, pregnant or nursing mothers or elderly females with osteoporosis). Because butter has higher shelf life and is convenient to transport, fortification of butter was identified as best option for this fat-soluble vitamin. The dosage was adjusted to weekly or monthly schedules for reducing cost without reducing efficacy. Initially, patented product provided by students run university affiliated private ltd. company from Delhi University (FSSAI – Food Safety and Standards Authority of India), derived from vegetarian source D2, like Lichen/synthetic D3 (D2 and D3 sound as hanging, maybe these should be in brackets or renamed better) which is efficient and cost effective, was used for butter fortification. Against the market preparations which cost Rs 30 -70/ dose, our fortification with vitamin D2 or D3 costed approx. Rs 3 for prophylaxis (2800 IU/ week) and Rs 30 for therapeutic dose of 30K IU through butter supplied by Dayalbagh dairy per week. A proposal of running an incubation center at DEI to train students in a years' time, for enabling the activity as self-sustaining was also

8. Axiomatic benefits of milk consumption by mammals and Nutrionomics of mixed milk from divergent animals and its potential impact on health status of children

Axiomatic benefits of milk consumption by mammals have been documented since Vedic times. Mother's milk is considered the best source of nutrition for a new born baby [(11)] Exclusive breastfeeding is recommended by the WHO for the first 6 months of life because human milk protects against gastrointestinal infections and supplies balanced and adequate nutrient contents to the infant. It is advised that after exclusive breast feeding for 6 months, infants should receive complimentary feeding and weaning diet. Complementary feeding of milk from diverse domesticated cattle sources starts during infancy and beyond as milk being rich source of growth promoting nutrients including minerals, vitamins and immune boosting metabolites and essential growth sustaining macromolecules. Cow and buffalo milk is universally accepted milk type, which is easily digestible and liked by everyone in taste.

Optimal nutrition during early childhood is important for growth and development specially brain. Research suggests that young children during weaning phase are at risk of nutrient deficiencies e.g. iron deficiency as bovine milk and human milk are deficient in iron requiring either fortification or supplementation. There are also studies which state that human milk does not provide sufficient nutrition for the very low birth weight (VLBW) infants and lay emphasis on supplementation (or fortification) of human milk with the nutrients in short supply, particularly with protein, calcium, and phosphate to meet high requirements of babies, especially the preterm infants [(13,14)].

Relation between metabolomics and nutrigenomics and health is current area of research important for health policy decisions. Further, metabolomics to quantify chemical composition of milk is a gold standard to evaluate milk quality and help enhance its therapeutic applications. Microbiological and competitive protein-binding assays, inductively coupled plasma spectroscopy, and chromatographic analyses and nuclear magnetic resonance are among the methods that have been applied to milk nutrient analysis. We have identified and catalogued major macro and micromolecular components of four major milk sources from Dayalbagh in India. Micro-nutrient analysis observed high levels of Calcium and Iron in Goat milk and high protein in Buffalo milk. We have found that the highest level of Iron amongst the 4 milk types is in goat milk, with value of 2.609mg/L, much higher than the generic range of 0.3 – 0.5mg/L [(12),] thus can optimize iron requirements. Vitamin C is found in good quantity in Camel milk, thus can improve iron absorption. When we compare, it is the buffalo milk with highest content of Protein 3.368% and Calcium 57.601mg/L. Camel milk brings the highest level of Phosphorus 661.60mg/L. Further, we found that nicotinamide ribonucleotide (NR), a form of vitamin B3 (niacin), is uniquely present in camel milk, but present in less concentration in milk from cow, goat, and buffalo, supporting energy metabolism, antioxidant defences, and potential neuroprotective effects. Similarly in goat milk two unique molecules were identified: UDP-galactose and Coenzyme A (CoA). UDP-galactose (uridine diphosphate galactose) plays a vital role in synthesizing glycoproteins, glycolipids, and polysaccharides, influences cell signalling, immune responses, and overall cellular function, which can impact health and disease resistance in human.

‘Mixed milk’ a boon

Hence, a promising approach to improve infant and childhood nutrition can be consumption of “mixed milk” which has potential to prevent development of chronic diseases such as nutritional deficiencies, cardiovascular and neurological disorders and diabetes, also even cancer. These diseases are often associated with oxidative stress and Camel milk metabolomics indicate antioxidant along with many other medicinal properties.

Mixed milk from Dayalbagh Dairy has been supplied to all the residents of Dayalbagh colony since the start of this year 2024 and is valued as “Amrit paye” (Elixir of Life). The synergy of combining milk from all 4 animals termed as “mixed milk” is already working wonders, especially in differently abled children, under Gracious Guidance of Revered Prof. Prem Saran Satsangi Sahab. Thus, consumption of mixed milk offers combined benefits impacting overall growth and development by supporting neural, cardiac, immune and gastrointestinal functions in humans and is a practical and cost-effective solution as compared to fortification and has potential to prevent many diseases if supplemented from infancy. Based on this hypothesis a large multicentre community based longitudinal observational study comparing control vs experimental group of children is underway, tracking impact of mixed milk on holistic growth, development and wellbeing of children between age group of three weeks to 12 years at Saran Ashram Hospital of Integrative medicine, Dayalbagh. Outcome measures include anthropometry, cognitive and socioemotional scores and Hb concentration. Future research aims at careful proportioning of volumetric dosage using mixed milk for childhood nutritional recommendations for prevention and mitigation of diseases through this cost - effective measure.

Conclusions of the studies

The above series of studies undertaken during the "Year of Camelids" on intuitive guidance reveal highly significant outcomes, and highlight the essence of Lacto-vegetarianism. Clinical studies demonstrated certain novel medicinal benefits of camel milk on cardiovascular and nervous systems. This led to a quest to find the molecular basis of these clinical effects for identification of the specific metabolomics and enrichment pathways. Camel milk was found to have rich nutrient profile on deploying sensitive tools like Nuclear magnetic resonance (NMR) spectroscopy and Liquid Chromatography-Mass Spectrometry (LC- MS). Certain unique metabolites were highly abundant in camel milk like, nicotinamide ribotide (NR), which has neuroprotective properties and "Pargyline" with antidepressant and antihypertensive properties, being reported for the first time as per literature survey. On comparative study of specific physiochemical and therapeutic properties of other sources of milk, like cow, goat and buffalo from RSS Gaushala, certain common as well as unique metabolites were identified in each milk, using NMR and LC-MS. "Mixed milk" which has been supplied to the community at Dayalbagh, on Intuitive Guidance since Jan 2024 along with harmonious blend of camels, goats with cows and buffalos at Dayalbagh Dairy, is a novel example of mutual reciprocity having evident synergistic effect.

Preliminary results of longitudinal tracking of growth and developmental parameters in children along with incidence of diseases as compared to controls show benefits of mixed milk in height and weight gain, improved immunity, decreased incidence of allergic and gastrointestinal diseases and improved concentration, memory and sleep. Thus, Mixed milk is a pack of all necessary nutrients required for all the age groups with a potential to prevent many diseases, in a cost- effective manner if supplemented from infancy. This finding can have high socio- economic impact on global health if employed as a policy for childhood nutrition.

Acknowledgements

With immense gratitude and humble submission, we thank Revered Professor Prem Saran Satsangi, Chairman Advisory Committee on Education (ACE), a Non-Statutory Body, a think tank, Dayalbagh Educational Institution, Dayalbagh Agra 282005 U.P. India. As Managing Director, Foundation for Innovation and Technology Transfer (FITT), His Incessant Guidance and Mentorship at each and every step from the intuitive conceptual outline of the research to its health implications to the society at large, with special reference to children has shown us the path.

It is His Grace that the research has drawn selfless participation of experts from different fields to form a cohort which includes scientists, engineers, doctors, managers, IT professionals and agriculturalists from RSS Gaushala, Dairy Dayalbagh Educational Institute (Deemed to be University) and Saran Ashram hospital (SAH), DDU Veterinary Science University Mathura as well as Indian Institute of Agricultural Research (IARI), International Centre for Genetic Engineering and Biotechnology (ICGEB), and also international collaborators DARSANA and DARSAE as part of the Incorporated Charitable Organization (ICO). I am grateful to all the members of the team for their collaborative and interdisciplinary approach who readily responded and enriched the research experience and quality with their expertise and spared no effort in taking it forward.

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