

RESEARCH ARTICLE

Plasma mineral and biochemical profiles and milk composition alterations with parity in dairy cattle: a study from South Kashmir

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Abstract: Mineral deficiency is a region-specific problem with a significant impact on livestock productivity, and can be assessed by measuring blood mineral levels that depends upon the mineral content in available feedstuffs, different abiotic factors and biological conditions including parity. This study aimed to determine the mineral profile of feedstuffs and plasma along with the metabolic parameters and milk composition in dairy cows of different parities under farmers' field condition in district Shopian of Kashmir division. A total of 201 blood and 156 milk samples were collected randomly from dairy cattle in 6 tehsils of two veterinary blocks (Shopian and Keegam) of the district. These samples were divided into four groups based on parity of animals as: primiparous (Parity 1), multiparous (Parity 2), multiparous (Parity 3), and older multiparous (Parity 4 and above). The chemical composition of the feedstuffs were within prescribed normal ranges, while the available fodders were deficit in P, Cu and Zn but the concentrate feeds contained adequate levels of all minerals. All plasma macro-minerals and Fe were above the critical levels across all parities throughout the district; however, deficiency prevalence for Ca, P and Cl (macro-) as well as Cu and Zn (micro-minerals) in plasma were higher ($p < 0.01$) in multiparous compared to primiparous dairy cows, with deficiency rates increasing progressively with parity. Plasma biochemicals were within normal reference ranges, except for total protein, which remained below the standard range across all groups of dairy cattle. Most of the milk components varied among the parity groups with multiparous cows exhibiting higher levels of fat, SNF, and total solids, but lower protein and MUN contents

compared to primiparous cows. In conclusion, plasma mineral and metabolic profiles as well as milk composition varied with parity in dairy cows, with older multiparous cows exhibited higher deficiency prevalences, reflecting the need for targeted mineral supplementation to support efficient and cost-effective milk production.

Keywords: Blood metabolites, Dairy cows, Feedstuffs, Milk composition, Parity, Plasma minerals

Introduction

Among the nutrients required for normal physiological functioning in dairy animal, mineral elements are essential as they play crucial role in overall health, reproduction, and production of animal (Pal et al. 2024). However, consumption of feedstuffs deficit or excess in minerals for prolonged period can lead to biochemical, nutritional and physiological alterations that ultimately affects animal performances like milk production and composition, conception rate etc. in dairy animals (Fadlalla 2022). Assessment of blood and milk constituents serve as reliable indicators of the nutritional status and metabolic health of animals, and these constituents can be monitored through analysis of the blood mineral and biochemical profiles (Puppel and Kuczyńska 2016) and milk composition (Andjelic et al. 2022). As such, estimation of constituents of blood and milk are important to determine the prevalence and risk levels of specific metabolic disorders in the herd (Kuterovac et al. 2005; Calamari et al. 2016).

Various abiotic factors and biological conditions like lactation stages, milk yield, parity etc. affect the level of blood (Spears et al. 2022) and milk (Toghdory et al. 2022) contents, but the results are inconsistent due to differences in breed, production system, regional feeding practices, environment factors, and physiological conditions, etc. Parity causes shift of the nutrients as per physiological priorities, accordingly affects the metabolic demands of the animal and influence blood and milk constituent levels (Walter et al. 2022). These alterations make parity a key factor in understanding the metabolism and identifying potential deficiencies or imbalances in blood and/or milk.

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Despite the importance of metabolic profiling, limited data exist on the parity-wise dynamics of blood and milk constituents in dairy cattle under specific regional management and feeding systems. Thus, establishing further knowledge in this context may help to evaluate the physiological state of dairy cows for overcoming nutrient deficiencies or imbalances (if any) to improve the productivity. Being crucial for developing precision nutritional strategies to improve animal health and productivity through insights into parity-linked metabolic demands, the present study aimed to evaluate the changes in plasma mineral and biochemical profiles and milk composition across different parities in dairy cows of district Shopian of Kashmir, Jammu and Kashmir.

Materials and Methods

The study area

The study was conducted from April to August, 2021 in district Shopian of South Kashmir, with its unique agro-climatic conditions and traditional dairy farming practices, presenting a distinct context for this study. Being situated at latitude 33° 72' N and longitude 74° 53' E with an average elevation of 2057 meters above mean sea level, the district is mostly hills and its climate is primarily dry and temperate. Shopian and Keller are the two veterinary blocks that make up the district's administrative division.

Sampling

A total of 201 blood and 156 milk samples were collected from dairy cattle (preferably in mid- or late- lactation) selected randomly based on breedable population, wherein blood samples of 165 and 36 numbers, and milk samples of 126 and 30 numbers were taken in Shopian block and Keller block, respectively. Milk samples were collected from the same cattle from which blood samples were taken. The number of blood and milk samples collected from different tehsils in district Shopian are presented in Table 1. These samples were divided into four groups based on parity of the respective cattle from where samples were drawn: as primiparous (Parity 1), multiparous (Parity 2), multiparous (Parity 3), and older multiparous (Parity 4 and above) animals.

Moreover, samples of feeds and fodders commonly offered to cattle by dairy farmers were collected separately, pooled for different villages within each tehsil to get the uniform representative sample for each type of feed/fodder.

Sample processing and analysis

About 15 ml of blood was collected aseptically in capped collection vials containing anti-coagulant ethylene diamine tetra acetic acid (EDTA @1.5 mg/mL blood) from jugular vein of dairy animals, and thoroughly shaken to mix with the anti-coagulant. 10 ml of milk sample was also collected from the animal during morning milking into tubes on the same day blood was sampled. The samples were transported in vaccine carriers placed between ice packs to the laboratory for analysis.

Centrifugation of blood samples was done at 3000 rpm for 10 minutes to obtain plasma which was then stored in deep freezer at -20 °C in two sets for further analysis of mineral and biochemical constituents. The plasma samples with equal volume of concentrated nitric acid were kept for overnight in digestion tubes followed by low heat (70-80 °C) digestion with di-acid mixture (70% Perchloric acid : conc. Nitric acid in 1: 3 ratio). The final content was filtered through Whatman's filter paper No.1 (Kolmer et al. 1951). The concentration of calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), cobalt (Co) and manganese (Mn) in blood plasma were estimated using Atomic Absorption Spectrophotometer (GBC SensAA, Sr. no. A7156, GBC Scientific Equipment, Inc, Australia). Phosphorus was determined colorimetrically in blood plasma (Fiske and Subbarow 1925) using Autozyme Phosphorus kit (ACCUREX Biomedical Pvt. Ltd.). Sodium (Na), potassium (K) and chlorine (Cl) were estimated in blood plasma using flame photometer (Systronics, Mediflame 127). Also, the glucose level was estimated at the time of blood collection by using SD-Codefree Blood Glucose Meter (SD Biosensor Healthcare Pvt. Ltd., Gurgaon, Haryana, India). Another set of stored plasma samples were analysed for contents of biochemicals viz. cholesterol (Chol), triglycerides (TG), total proteins (TP), albumin (Alb), and urea-N (PUN) through standard methods using commercial diagnostic kits (Diasys Diagnostics Private Limited, India) on Photometer 5010V5⁺ semi-auto biochem

Table 1: Samples of blood and milk collected from dairy cattle in different tehsils of district Shopian, Kashmir

S. No.	Block	Total cattle population	Breedable cattle population	Surveyed tehsils	Number of blood samples collected	Number of milk samples collected
1.	Shopian	56000	36400	Shopian	28	21
				Imam Sahib	35	25
				Herman	41	34
				Zainapora	30	21
				Chitragam	31	25
				Total (A)	165	126
2.	Keller	15000	9750	Keegam (B)	36	30
				Total (A+B)	201	156

analyzer (Robert Riele INC, Berlin, Germany). Plasma globulin (Glb) content was calculated by difference of TP and Alb contents.

The milk samples collected were heated to 40 ± 2 °C for homogenization and then analysed for composition using automatic milk analyzer (Speedy Lab, Astori Technica s.r.l, Italy) based on Fourier-transform infrared spectroscopy. The collected and pooled feed and fodder samples were dried at 80°C, ground, labelled and stored in airtight polythene bags for laboratory analysis. These samples were evaluated for proximate composition including dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), non-structural carbohydrates (NSC) following standard procedures as per AOAC (2019). Additionally, the samples were subjected to wet digestion using di-acid mixture (Trolson 1969), followed by dilution with triple glass distilled water to prepare extractable aliquots, which were analysed for contents of macro- and micro-minerals using the same procedures employed for blood plasma analysis.

Statistical analysis

The data were subjected to statistical analysis using statistical software program SPSS (2011) for determination of means, standard error and test of significance by one-way ANOVA. Significant differences among treatment means was determined as per Duncan's multiple range test. These tests were one sided and were referenced for p value for their significance. Any p value less than 0.05 ($p < 0.05$) was taken to be statistically significant.

Result and Discussion

Feeding system and composition of available feeds/fodders

The livestock farmers take their dairy animals along with for grazing in nearby common property resources and orchards during the daytime in summer season, but completely stall fed them during winter season. Among roughages, mixed-grass as green fodder harvested from orchards were commonly offered fresh to dairy animals during summer months, and dry roughages mainly in the form of maize stovers and mixed grass hays collected and preserved in summers were offered during the lean winter seasons. In some lower altitude areas, paddy and oats straws were also being offered to the livestock during winters. Being busy in the horticultural activities, most of the dairy farmers preferred to offer the commercial compounded concentrate feed to their animals, while some of them were preparing home-made concentrate rations using the local feed ingredients.

The proximate and mineral composition (on %DM basis) of most common feeds and fodders fed to dairy cattle in district Shopian, Kashmir are presented in Table 2. The chemical composition of the feeds and fodders in all surveyed tehsils of the district were in normal ranges as prescribed for Indian feeds and fodders (ICAR

2013) with little variations which might be due to difference in plant species, soil fertility, harvesting stage, agro-climatic conditions, and storage practices (Belete et al. 2024). All the composite dry fodder samples analyzed contained low P, Cu, Zn and Mn, while the Ca, Mg, Cl, K, Na, Fe and Co concentrations were higher than the critical levels as prescribed by McDowell and Conrad (1977) and NRC (1984). The soils of hilly region are acidic in reaction due to leaching of the bases under the influence of high precipitation (Patgiri et al. 2024). Rainfall water leaches P resulting in dominance of Ca and Fe salts in the soils. High levels of Ca in soil is essential to reduce its acidity and may increase Ca concentration in fodders grown in it, but reduce the absorption of Cu by plant roots (Fan et al. 2012). In the present study, Cu deficiency in fodders may also be attributed to the typical soil condition that might be restricting its accumulation and availability in plants (Xu et al. 2024). The concentrate rations contained adequate levels of all the nutrients and minerals with low Zn content in home-made concentrate mixture probably due to less Zn content in constituent ingredients and little or no use of mineral mixture in its formulation by the dairy farmers of district Shopian. These results were in close agreement with the reports of feeds and fodders from other districts of Kashmir (Sheikh et al. 2019, Sheikh et al. 2022); however, the values for CP and NSC for maize stover analysed in the present study were less than those reported by Bhat et al. (2021).

Plasma macro-mineral and deficiency prevalence profile of dairy cattle

All the estimated plasma macro-mineral contents except Mg in dairy cattle were significantly influenced by parity (Table 3). The overall average of all the plasma macro-minerals were found above the critical levels throughout the district. The normal range for Ca, P, Mg, Na, K and Cl in dairy cattle has been reported to be 9 to 12, 4 to 7, 1.7 to 2.5 mg/dL, and 134 to 144, 4 to 5.9, 92 to 99 mEq/L, respectively (Radostits et al. 2000). The mean plasma Ca concentration in dairy cattle of all the tehsils of district Shopian was within the normal range with higher values ($p < 0.01$) in animals of early parity than higher parity animals. This lowering of plasma Ca content with increase of parity in dairy cattle could be attributed to high milk production in multiparous animals that consequently caused more Ca drainage. The overall prevalence of hypocalcaemia in the district was 41.56% with higher values ($p < 0.01$) in older multiparous animals than those in early parity. These results are in agreement to Reinhardt et al. (2011) who reported a rising likelihood of subclinical hypocalcemia with progression of age and number of lactations.

The mean plasma P concentration was higher ($p < 0.01$) in animals of 1st and 2nd parity compared to other multiparous animals, but the values were within the normal range for all groups of dairy animals in all tehsils of the district. Older cows have higher demand for P due to increased milk production, and a reduced ability to absorb P from intestines and to mobilize it from bones (Horst et

Table 2: Composition (on %DM basis) of most available feeds and fodders fed to dairy cattle in district Shopian, Kashmir

Attribute	Critical level	Home-made concentrate (n=17)	Commercial compound feed (n=95)	Paddy straw (n=11)	Oat straw (n=34)	Oats fodder (n=14)	Maize fodder (n=52)	Orchard grass
Proximate composition								
Dry matter	-	91.14±0.39	90.83±0.18	89.67±0.17	91.69±0.13	37.83±0.30	23.40±0.32	27.12±0.46
Organic matter	-	91.58±0.28	90.36±0.16	79.86±0.35	92.96±0.24	91.43±0.08	87.53±0.19	85.88±0.29
Crude protein	-	14.35±0.48	15.61±0.14	3.03±0.02	3.55±0.02	4.92±0.04	6.17±0.05	12.24±0.07
Ether extract	-	2.35±0.08	2.27±0.01	1.17±0.01	0.86±0.01	1.75±0.02	0.91±0.02	1.92±0.04
Non-structural carbohydrates	-	68.21±0.76	65.00±0.21	33.13±0.38	52.53±0.37	61.43±0.38	50.60±0.48	51.00±0.56
Mineral composition								
Calcium	<0.30 %	1.25±0.05	1.49±0.02	0.31±0.00	0.28±0.00	0.42±0.00	0.55±0.01	0.82±0.02
Phosphorous	<0.25 %	0.33±0.01	1.00±0.01	0.10±0.00	0.13±0.01	0.16±0.00	0.34±0.05	0.37±0.01
Magnesium	<0.20 %	0.35±0.01	0.59±0.01	0.26±0.01	0.24±0.01	0.23±0.00	0.34±0.01	0.44±0.01
Sodium	<0.08 %	0.31±0.01	0.60±0.01	0.62±0.03	0.52±0.01	0.34±0.02	0.04±0.00	0.15±0.00
Potassium	<0.25 %	0.99±0.03	1.07±0.03	2.27±0.12	2.41±0.09	2.51±0.13	3.26±0.07	3.28±0.09
Chlorine	<0.10 %	0.20±0.01	0.24±0.00	0.80±0.04	0.75±0.01	0.70±0.04	0.61±0.01	0.60±0.01
Copper	<8.00 ppm	9.63±0.62	14.76±0.75	4.67±0.25	7.72±0.23	10.07±0.43	9.14±0.56	7.27±0.56
Zinc	<30.0 ppm	26.40±1.59	76.62±1.79	19.06±0.73	21.14±1.00	8.45±0.74	25.78±1.09	28.07±1.43
Iron	<50.0 ppm	229.03±6.85	734.58±11.06	257±2.20	179.76±4.05	317.61±61.28	901.32±23.45	850.01±8.05
Cobalt	<0.10 ppm	0.11±0.00	1.01±0.02	0.15±0.00	0.12±0.00	0.20±0.01	0.50±0.01	1.09±0.05
Manganese	<40.0 ppm	46.73±1.97	59.19±1.88	34.03±1.59	27.55±1.53	37.11±1.67	51.83±2.50	49.17±1.42

Table 3: Plasma macro-minerals and deficiency prevalence profile in different parity groups of dairy cattle in district Shopian, Kashmir

Plasma macro-mineral	Critical level	1 st (n=57)	2 nd (n=55)	3 rd (n=48)	4 th and above (n=41)	Pooled district mean (n=201)	P value
Macro- mineral profile							
Calcium	< 8.00 mg/dL	9.17 ^B ±0.22	8.67 ^{AB} ±0.23	8.09 ^A ±0.18	7.93 ^A ±0.24	8.39±0.12	0.001
Phosphorous	< 3.50 mg/dL	5.78 ^C ±0.234	5.50 ^{BC} ±0.186	5.01 ^{AB} ±0.192	4.83 ^A ±0.194	5.28±0.105	0.004
Magnesium	< 1.20 mg/dL	4.26±0.30	4.08±0.32	3.69±0.27	3.32±0.21	3.80±0.14	0.079
Sodium	< 132 mEq/L	163.2 ^C ±0.48	162.7 ^{BC} ±0.46	161.4 ^{AB} ±0.416	160.6 ^A ±0.27	161.9±0.22	0.000
Potassium	< 3.00 meq/L	6.46 ^B ±0.29	6.34 ^{AB} ±0.26	5.82 ^{AB} ±0.22	5.65 ^A ±0.20	6.02±0.12	0.048
Chlorine	< 80.0 mEq/L	102.66 ^B ±0.89	100.64 ^{AB} ±0.94	99.15 ^{AB} ±0.96	96.75 ^A ±1.49	99.58±0.58	0.005
Macro- mineral deficiency prevalence profile							
Calcium		15.62 ^A ±0.53	38.23 ^B ±0.54	50.00 ^C ±0.95	54.35 ^C ±0.74	41.56 ^{BC} ±0.56	0.000
Phosphorous		3.85 ^{AB} ±3.33	0.00 ^A ±0.00	3.57 ^{AB} ±3.33	6.89 ^B ±4.36	3.51±1.57	0.000
Chlorine		0.00 ^A ±0.00	0.00 ^A ±0.00	0.00 ^A ±0.00	5.56 ^B ±5.56	1.48±1.38	0.001

The means across the rows with different upper case superscript differ significantly among the pa

al. 1997). Kume et al. (1998) reported higher (5.21 mg/dL) concentration of serum P in first parity animals, followed by third (4.87 mg/dL), second (4.82 mg/dL) and fourth or above parity animals (0.10 mg/dL). Overall prevalence of hypophosphataemia in dairy cattle was very less in the district (3.51%), with higher values in old multiparous animals. In contrast, Singh et al. (2016) reported widespread P deficiency among crossbred cattle of hilly areas of Jammu division.

Plasma Mg levels in dairy cattle across the district were above the critical threshold of 1.2 mg/dL, with no significant differences among the parity groups and no prevalence of hypomagnesaemia detected in any animal group of dairy cattle throughout the district. These results are in line to the findings of Fadlalla et al. (2020) who reported slight variation in serum Mg concentration among different parities in dairy cows. Mg deficiency is rare under normal conditions as it is generally present in appreciable quantity in fodders (Greene et al. 1983). In the present study also, Mg was in adequate amounts in all the feed and fodder provided to the dairy cattle which might be responsible for normal Mg levels in the animals under study. In contrast, Singh et al. (2016) reported 3.50% incidence of hypomagnesaemia in crossbred cows with higher prevalence in Kathua followed by Jammu district.

The plasma Na, K and Cl levels in all the parity groups of dairy cattle throughout the district Shopian were found to be higher than normal ranges with significant differences among the groups. The average plasma concentrations of these minerals were the highest in first parity animals which declined in animals of subsequent parities with the lowest values in older multiparous animals, and overall deficiency prevalence of 1.48% for Cl only. In contrast, Kume et al. (2003) reported no significant differences in serum Na and K levels among different parities in dairy cows. Higher content of these minerals in the feeds and fodders being offered, along with routine salt supplementation (100-150 g/cow/day) likely contributed to the elevated plasma levels of these minerals in the dairy cows across the region. Comparatively higher prevalence of Na and K deficiencies among cattle from various agro-climatic zones of North-West Himalayan region of Jammu division was reported by Singh et al. (2016).

Plasma micro-mineral and deficiency prevalence profile of dairy cattle

The results of plasma micro-mineral levels and prevalence of their deficiencies in dairy cattle of district Shopian, Kashmir are presented in Table 4. The mean plasma levels of all the micro-minerals except Fe were below the critical levels in animals of all the parity groups across the district. The average plasma Cu concentration was the highest ($p < 0.01$) in primiparous animals and declined in subsequent parities, and the reverse of this trend followed for plasma Fe ($p > 0.05$), Mn, Co and Zn ($p < 0.01$). Van Aken et al. (1991) also reported an increase in serum Cu concentration in animals with the advancement of age. The under

critical level of plasma Cu among animal of all the parity groups across the district could be attributed to lower Cu content in forage resources offered, limited availability of Cu from feeds due to high Fe content that interfere with Cu bioavailability (Spears 2003), and Cu loss through milk drainage. Prevalence of hypocupraemia was recorded in 80.0% of dairy cattle in the district with higher ($p < 0.01$) value in multiparous compared to primiparous cows. The finding corroborates with the reports of Kume and Tanabe (1993) who reported decline in plasma Cu levels in colostrum fed calves born to cows with higher parity.

The mean plasma Fe concentration in dairy cows was much above the critical level across all parities in the district, showing a numerical increase with each parity. This might be due to regular high Fe intake through feeds and fodders containing excessive level of Fe as soil of the hilly areas have higher Fe content (Hassan et al. 2017). As such, no prevalence of Fe deficiency was detected in any animal group of dairy cows across the district. These results are in agreement with the findings of other workers who also reported either negligible or no Fe deficiencies in adult cattle in different parts of the country (Singh et al. 2016).

The mean plasma Co level in dairy cows was below the critical level in all the parity groups across the district with higher ($p < 0.01$) values in multiparous compared to primiparous animals. No adequate literature is available to infer the effect of parity on plasma Co concentration in dairy animals. Shekher et al. (2017) reported that plasma Co concentration in crossbred cattle varied from 0.01 to 0.27 $\mu\text{g/dL}$ in different districts of Bihar against their critical levels of 0.05 to 0.07 ppm as suggested by McDowell (2003). Likewise, mean plasma Mn concentration was higher ($p < 0.01$) in multiparous dairy cows but below the critical level for all the parity groups throughout the district. This might be due to the antagonistic effect of other minerals like Ca, P and Fe on Mn concentration in animal body as reported by Furl et al. (2004). Overall prevalence of Mn deficiency in the district was 89.50% with the highest ($p < 0.01$) value in primiparous than multiparous cows.

Plasma Zn concentration was higher ($p < 0.01$) in multiparous animals with mean concentration below the critical level in all parity groups across the district which might be probably due to its lesser content in available feeds and fodders offered to the dairy animals and/or increased intake of Fe in diet which might have interfered in normal absorption of Zn (McDowell 2003). Overall prevalence of Zn deficiency in the district was 89.67% with the highest ($p < 0.01$) value in older multiparous than primiparous cows. As parity increases, there is greater mineral mobilization and potential depletion due to cumulative lactational and gestational stress (Wilde 2006). The results of the present study are in accordance with Kume et al. (1998) who reported higher (0.95 mg/dL) serum Zn levels in second as compared to (0.73 mg/dL) first parity.

Table 4: Plasma micro-minerals and deficiency prevalence profile in different parity groups of dairy cattle in district Shopian, Kashmir

Plasma mineral	Critical level	1 st	2 nd	3 rd	4 th and above	Pooled district mean	P value
Micro-minerals profile							
Copper	<0.65 µg/dL	0.64 ^C ±0.01	0.59 ^{BC} ±0.01	0.57 ^{AB} ±0.01	0.55 ^A ±0.01	0.59±0.00	0.000
Iron	<100 µg/dL	554.42±26.39	610.90±34.93	641.25±34.43	679.96±52.34	624.98±20.20	0.158
Cobalt	<0.10 µg/dL	0.01 ^A ±0.00	0.02 ^B ±0.00	0.02 ^B ±0.00	0.02 ^B ±0.00	0.02±0.00	0.021
Manganese	<2.00 µg/dL	0.13 ^A ±0.00	0.15 ^B ±0.00	0.16 ^B ±0.00	0.15 ^B ±0.01	0.15±0.00	0.001
Zinc	<0.50 µg/dL	0.40 ^A ±0.01	0.44 ^B ±0.01	0.42 ^{AB} ±0.01	0.40 ^A ±0.01	0.42±0.00	0.001
Micro-mineral deficiency prevalence profile							
Copper		58.33 ^A ±0.98	76.00 ^{AB} ±0.60	85.00 ^B ±0.71	95.91 ^C ±0.26	80.00±0.38	0.000
Manganese		96.42 ^C ±0.19	76.92 ^{AB} ±0.57	75.00 ^A ±0.72	80.48 ^B ±0.79	89.50±0.87	0.001
Zinc		93.75 ^B ±0.34	80.76 ^A ±0.75	90.91 ^{AB} ±0.33	95.00 ^B ±0.70	89.67±0.23	0.000

The means across the rows with different upper case superscript differ significantly among the parity groups

Table 5: Plasma metabolic profile of different parity groups of dairy cattle in district Shopian, Kashmir

Parameter	Reference value	1 st	2 nd	3 rd	4 th and above	Pooled district mean	P value
Energy profile							
Glucose	45-75 mg/dL	53.09 ^A ±1.45	55.59 ^{AB} ±1.27	57.24 ^{AB} ±1.05	59.06 ^B ±1.10	56.50±0.62	0.005
Cholesterol	65-220 mg/dL	124.39±2.64	122.41±2.96	120.75±2.01	116.15±3.32	121.59±1.41	0.223
Triglycerides	0-14 mg/dL	5.63±0.26	5.96±0.25	6.47±0.31	6.63±0.28	6.23±0.14	0.059
Protein profile							
Total proteins	5.7-8.1 mg/dL	5.34 ^A ±0.13	5.58 ^{AB} ±0.13	5.58 ^{AB} ±0.11	5.92 ^B ±0.15	5.64±0.70	0.031
Albumin	2.1-3.6 mg/dL	3.34 ^B ±0.09	3.16 ^{AB} ±0.09	3.06 ^{AB} ±0.08	2.96 ^A ±0.07	3.12 ^A ±0.04	0.010
Globulin	2.8-5.0 mg/dL	2.54±0.21	2.78±0.19	3.10±0.17	3.29±0.23	2.93±0.10	0.060
Plasma urea nitrogen	6-27 mg/dL	16.06±0.94	15.29±0.89	14.65±0.78	13.79±0.68	14.94±0.42	0.252

The means across the rows with different upper case superscript differ significantly among the parity groups

Table 6: Milk composition profile of different parity groups of dairy cattle in district Shopian, Kashmir

Parameter	Reference value*	1 st	2 nd	3 rd	4 th and above	Pooled district mean	P value
Fat (%)	3.5-4.5	4.36 ^a ±0.21	4.48 ^b ±0.20	4.52 ^b ±0.18	5.02 ^c ±0.24	4.60±0.25	0.034
Lactose (%)	4.6-4.9	4.53±0.06	4.63±0.04	4.67±0.05	4.71±0.07	4.63±0.05	0.086
Protein (%)	3.0-3.5	3.32 ^b ±0.02	3.21 ^b ±0.04	3.16 ^{ab} ±0.04	3.03 ^a ±0.01	3.18±0.02	0.011
Solid not-fat (%)	8.5-9.0	8.13 ^a ±0.17	8.48 ^b ±0.23	8.53 ^b ±0.20	8.72 ^c ±0.25	8.47±0.22	0.046
Total solids (%)	12.5-14.5	12.82 ^a ±0.42	13.25 ^{ab} ±0.26	13.43 ^{ab} ±0.30	14.24 ^b ±0.29	13.43±0.31	0.032
Urea N (mg/dL)	10-16	14.35 ^c ±0.18	13.81 ^{bc} ±0.20	13.02 ^b ±0.30	11.52 ^a ±0.28	13.17±0.21	0.009

The means across the rows with different upper case superscript differ significantly among the parity groups.

* Kalyankar et al. (2016)

Blood metabolic profile of dairy cattle

Blood biochemical parameters are crucial indicators of the metabolic activities in lactating animals (Giannuzzi et al. 2024). Levels of blood glucose, cholesterol, and triglycerides are key indicators of the energy status in ruminants (Pechova and Pavlata 2005). All energy parameters in dairy cattle of the district Shopian were within the normal ranges with statistical difference ($p < 0.01$) observed for blood glucose only, while plasma cholesterol and triglycerides were comparable ($p > 0.05$) among different parity groups (Table 5). The mean blood glucose level in dairy cows increased with parity which may be attributed to improved feed intake, metabolic adaptation, and enhanced gluconeogenic ability in older animals (Bell 1995). Ferreira et al. (2021) also reported an increase in blood glucose level with parity in Nellore cows under grazing. There was numerical decrease in plasma cholesterol and increase in plasma triglycerides levels with parity, although both remained within the normal reference ranges (Kaneko et al. 1999). These findings could be attributed to energy balance changes, metabolic stress, and physiological adaptations that worsen with each subsequent lactation (Nikkhah and Alimirzaei 2023). Wu et al. (2019) also reported higher serum cholesterol levels in animals of second parity than those of third or more parities.

All plasma protein profile parameters in dairy cows of different parity groups across the district Shopian were within the normal ranges except TP which was marginally below the reference range (Kaneko et al. 1999) indicating prevalence of nominal protein deficiency among dairy cattle throughout the district (Table 5). Livestock in the district were mainly offered fodder like maize stover that has a low protein value, which might be the reason for low plasma TP in dairy cattle of the district. Singh et al. (2016) also reported that the cattle from subtropical and intermediate zones of Jammu division were having significantly lower levels of plasma proteins. Mean plasma TP concentration in dairy cows increased ($p < 0.05$) while Alb decreased ($p = 0.01$) with each parity, and the values were recorded to be the at most for older multiparous animals compared to primiparous ones. This could be attributed to a number of physiological, metabolic, and hormonal changes brought about in dairy cows by each subsequent lactation (Pineyrua et al. 2018). Contradictorily, Chacha et al. (2018) reported significantly higher (3.07 ± 0.22 g/dL) levels of serum Alb in multiparous cows as compared to (2.29 ± 0.35 g/dl) primiparous cows. Mean plasma Glb and PUN levels varied non-significantly ($p > 0.05$) among the dairy cows of different parity groups with numerically higher values for plasma Glb and lower values for PUN in multiparous than primiparous animals. Ferreira et al. (2021) also reported lowering of serum urea N in multiparous grazing beef cows compared to primiparous ones. In contrast, Walter et al. (2022) reported higher serum urea concentrations in multiparous cows than their herd mates from two weeks postpartum until four weeks postpartum.

Milk composition profile of dairy cattle

The analysis of milk composition from cows of different parity groups revealed significant variations for several parameters (Table 6). Milk fat percentage increased ($p < 0.05$) progressively with parity from 4.36% in primiparous to 5.02% in older multiparous cows. This could be attributed to improved metabolic adaptation and nutrient partitioning in older cows. A similar trend was observed for total solids, which increased significantly ($p < 0.05$) across parities, consistent with the rise in fat content. Multiparous cows have a greater mobilization of nutrients from their body reserves to their mammary glands than primiparous cows (Pineyrua et al. 2018). Although lactose levels also showed a gradual increase with parity, the change was not statistically significant ($p = 0.086$), and values remained within the normal reference range. These results are in line with the findings of Ferreira et al. (2021) who also reported non-significant effect on milk lactose and a numerical increase in milk fat and total solids content with higher parity in Nellore cows under grazing. Milk protein content declined ($p < 0.05$) with increasing parity from 3.32% in primiparous to 3.03% in older multiparous cows. This decrease might be linked to higher milk yields diluting protein concentration or a decline in protein synthesis efficiency with age. Solid-not-fat (SNF) content in milk significantly ($p < 0.05$) increased with parity, reflecting higher levels of lactose and minerals in milk from older cows despite a drop in protein level. Milk urea nitrogen (MUN) levels declined ($p < 0.01$) with increasing parity from 14.35 mg/dL in first-parity cows to 11.52 mg/dL in older cows. Although values in all the parity groups were within the normal reference range, the reduction suggests improved nitrogen utilization or a possible decline in dietary protein intake or digestibility in older animals. These results are in agreement with the findings of Wu et al. (2019) who also reported significant ($p < 0.05$) effect of parity on milk protein and MUN contents in mid-lactation Holstein cows.

Conclusion

Parity significantly influences levels of plasma mineral and metabolite contents, and key milk components. Older multiparous cows showed a higher prevalence of deficiencies for Ca, P and Cl among macro-minerals, and Cu and Zn among micro-minerals. The plasma levels of Mg, Na, K, Fe and Co were adequate among dairy cows across all the parity groups throughout the district. All metabolic profile parameters fell within the normal physiological ranges for dairy cattle, except total protein which remained slightly below the standard range across the district. Milk of multiparous cows had higher fat, SNF and total solids contents, but lower protein and MUN levels. Dietary supplementations in different forms to dairy cattle could be a suitable approach, but framing and provision of region specific mineral mixture to overcome the specific deficiencies appears to be the best option for enhancing milk productivity economically.

References

- Andjelic B, Djoković R, Cincović M, Bogosavljević-Bošković S, Petrović M, Mladenović J and Cukić, A (2022) Relationships between milk and blood biochemical parameters and metabolic status in dairy cows during lactation. *Metabolites* 12:733. Doi: 10.3390/metabo12080733.
- AOAC (2019) Official methods of analysis. Association of Official Analytical Chemists, 21st edn. Gaithersburg, USA.
- Belete S, Tolera A, Betsha S and Dickhöfer U (2024) Feeding values of indigenous browse species and forage legumes for the feeding of ruminants in Ethiopia: a meta-analysis. *Agriculture* 14:1475. Doi: 10.3390/agriculture14091475.
- Bell AW (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J Anim Sci* 73(9):2804-2819. Doi: 10.2527/1995.7392804x.
- Bhat MA, Ganai AM, Sheikh GG, Beigh YA, Farooq J, Sheikh BA and Reshi PA (2021) Nutritional status of feeds and fodders fed to dairy cattle in South Kashmir. *Pharma Innov J* 10(3):225-228.
- Calamari L, Ferrari A, Minuti A and Trevisi E (2016) Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on fourier transform mid-infrared spectroscopy: preliminary results. *BMC Vet Res* 12:4. Doi: 10.1186/s12917-015-0621-4.
- Chacha F, Bouzebeda Z, Bouzebeda-Afri F, Gherissi DE, Lamraoui R and Mouffok CH (2018) Body condition score and biochemical indices change in Montbeliard dairy Cattle: influence of parity and lactation stage. *Glob Vet* 20(1):36-47.
- Fadlalla MT (2022) The Interactions of some minerals elements in health and reproductive performance of dairy cows. *New Advances in the Dairy Industry* (Ed. M.S. Qureshi), Intech Open.
- Fadlalla MT, Omer SA and Atta M (2020) Determination of some macro element mineral levels at different lactation stages of dairy cows and their correlations. *Sci Afr* 8:e00351. Doi: 10.1016/j.sciaf.2020.e00351.
- Fan J, He Z, Ma LQ, Nogueira TAR, Wang Y, Liang Z and Stoffella PJ (2012) Calcium water treatment residue reduces copper phytotoxicity in contaminated sandy soils. *J Hazard Mater* 199-200(15):375-382. Doi: 10.1016/j.jhazmat.2011.11.030.
- Ferreira M, Renno LN, Rodrigues II, Detmann E, Paulino MF, Filho SCV, Martins HC, Moreira SS and Lana DS (2021) Effects of parity order on performance, metabolic, and hormonal parameters of grazing beef cows during pre-calving and lactation periods. *BMC Vet Res* 17:311. Doi: 10.1186/s12917-021-03019-0
- Fiske CH and Subbarow Y (1925) The colorimetric method for the determination of phosphorus. *J Bio Chem* 66:375-378.
- Furll M, Sattler T and Anke M (2004) Secondary manganese deficiency as a herd problem in cattle-a case report. *Tieraerztl Prax G N* 32:126-132.
- Giannuzzi D, Piccioli-Cappelli F, Pegolo S, Bisutti V, Schiavon S, Gallo L, Toscano A, Ajmone Marsan PA, Cattaneo L, Trevisi E and Cecchinato A (2024) Observational study on the associations between milk yield, composition, and coagulation properties with blood biomarkers of health in Holstein cows. *J Dairy Sci* 107(3):1397-1412. Doi: 10.3168/jds.2023-23546.
- Greene LW, Fontenot JP and Webb KEJ (1983) Site of magnesium and other macro mineral absorption in steers fed high levels of potassium. *J Anim Sci* 57:503-510.
- Hassan M, Hassan R, Pia HI, Hassan MA, Ratna SJ and Aktar M (2017) Variation of soil fertility with diverse hill soils of Chittagong hill tracts, Bangladesh. *Int J Plant Soil Sci* 18(1):1-9. Doi: 10.9734/IJPSS/2017/34975
- Horst RL, Goff JP, Reinhardt TA and Buxton DR (1997) Strategies for preventing milk fever in dairy cattle. *J Dairy Sci* 80(7):1269-1280.
- ICAR (2013) Chemical composition of feeds and fodders. Indian Council of Agricultural Research, New Delhi- 110012.
- Kalyankar SD, Khedkar CD, Patil AM and Deosarkar SS (2016) Milk: sources and composition. In: *Encyclopedia of Food and Health* (Edited by: Caballero B, Finglas PM and Toldrá F), Academic Press, pp. 741-747. doi: 10.1016/B978-0-12-384947-2.00463-3.
- Kaneko JJ, Harvey JW and Bruss ML (1999) *Clinical Biochemistry of Domestic Animals*. Harcourt Brace and Co. Asia P/C Ltd., Singapore 238884.
- Kolmer JA, Spanbling EH and Robinson HW (1951) *Approved Laboratory Techniques*. Appleton Century Crafts, New York, USA.
- Kume S and Tanabe S (1993) Effect of parity on colostral mineral concentrations of Holstein cows and value of colostrum as a mineral source for newborn calves. *J Dairy Sci* 76: 1654-1660
- Kume S, Nonaka K and Oshita T (2003) Relationship between parity and mineral status in dairy cows during peri-parturient period. *Anim Sci J* 74:211-215.
- Kume S, Yanamoto E, Kudo T, Toharmat T and Nonaka I (1998) Effect of parity on mineral concentration in milk and plasma of Holstein cows during early lactation. *Asian-Australas J Anim Sci* 11(2): 133-138.
- Kuterovac K, Balas S, Gantner V, Jovanovac S and Dakic A (2005) Evaluation of nutritional status of dairy cows based on milk analysis results. *Ital J Anim Sci* 4(3):33-35. Doi: 10.4081/ijas.2005.3s.33
- McDowell LR (2003) *Minerals in Animal and Human Nutrition*. 2nd edn. Elsevier Science B. V. Amsterdam, The Netherlands. Pp. 33-92.
- McDowell LR and Conrad JH (1977). *Trace mineral nutrition in Latin America*. *Wld Anim Rev* 24: 24.
- Nikkhah A and Alimirzaei M (2023) Management updates on prepartal stress effects on transition cow and calf health. *Wld's Vet J* 13(2):250-257. Doi: 10.54203/scil.2023.vwj27.
- NRC (1984) *Nutrient requirements of domestic animals*. National Academy of Sciences-National Research Council. Washington, DC.
- Pal RP, Mani V, Tariq H, Sarkar S, Sharma A and Gupta D (2024) *Newer Trace Elements for Ruminants*. In: *Feed Additives and Supplements for Ruminants*, (eds: Mahesh MS, Yata VK). Springer, Singapore. Doi: 10.1007/978-981-97-0794-2_5
- Patgiri P, Swami S and Yumnam V (2024) Extracting insoluble inorganic phosphorus from organic farm soils in mountains: identifying effective organic acid extractants. *Grassroots J Nat Resour* 7(1): 66-80. Doi: 10.33002/nr2581.6853.070104
- Pechova A and Pavlata L (2005) Use of metabolic profiles of dairy cows in the control diet. In *Nutrition of cattle in terms of production and preventive medicine* (ISBN 80-86542-08-4), pp. 102- 111.
- Pineyrua JTM, Farina SR and Mendoza A (2018) Effects of parity on productive, reproductive, metabolic and hormonal responses of Holstein cows. *Anim Reprod Sci* 191: 9-21. Doi: 10.1016/j.anireprosci.2018.01.017.
- Puppel K and Kuczyńska B (2016) Metabolic profiles of cow's blood: a review. *J Sci Food Agric* 96:4321-4328.
- Radostits OM, Gay CC, Blood DC and Hinchcliff KW (2000) *Veterinary Medicine*. 9th edn. W B Saunders Harcourt Publishers Ltd.
- Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP and Horst RL (2011) Prevalence of subclinical hypocalcemia in dairy herds. *Vet J* 188(1):122-4. doi: 10.1016/j.tvjl.2010.03.025.
- Sheikh FA, Ganai AM, Mir NA, Sheikh GG, Ahmad HA and Beigh YA (2019) Chemical composition and nutritional profile of feed and fodders fed to cattle of Anantnag district of Kashmir valley during winter season. *Vet Res Int* 7(2):104-109.
- Sheikh GG, Ashraf A, Haq Z, Ganai AM and Reshi PA (2022) Socio-economic status of dairy farmers, management practices and nutritional status of dairy cattle in Pulwama District of Kashmir valley. *SKUAST J Res* 24(1):53-60. DOI: 10.5958/2349-297X.2022.00005.8.

- Shekhar P, Kumar P, Dimri U and Sharma M (2017) Micro mineral status of crossbred cattle under different physiological stages in Eastern India and their interrelation in soil and fodder. *Int J Livest Res* 7(4):158-165. Doi: 10.5455/ijlr.20170324031823.
- Singh R, Singh V and Baigh SA (2016) Haemato-biochemical and mineral status of crossbred cattle from various agro-climatic zones of North-West Himalayan region of Jammu division. *Indian J Anim Sci* 86(10):1125–1131.
- Spears JW (2003) Trace Mineral Bioavailability in Ruminants. *J Nutr* 133(5):1506S-1509S.
- Spears JW, Brandao VLN and Heldt J (2022) Invited review: assessing trace mineral status in ruminants, and factors that affect measurements of trace mineral status. *Appl Anim Sci* 38(3):252-267.
- SPSS (2011) Statistical Package for Social Sciences (Version 20.0), Software products, Marketing Department, IBM SPSS Inc., New York, USA
- Toghdory A, Ghoorchi T, Asadi M, Bokharaeian M, Najafi M and Nejad JG (2022) Effects of environmental temperature and humidity on milk composition, microbial load, and somatic cells in milk of Holstein dairy cows in the Northeast Regions of Iran. *Animals* 12:2484. Doi: 10.3390/ani12182484.
- Trolson JE (1969) Outline for in vitro Digestion of Forage Samples. Research Station Swist Current, Saskatchewan, Canada.
- Van Aken D, Bont JD, Van Holm LV and Ranawana SSE (1991) A study on mineral status of cattle in dairy farm in Sri Lanka. *Indian Vet J* 3(6):371-374.
- Walter LL, Gärtner T, Gernand E, Wehrend A and Donat K (2022) Effects of parity and stage of lactation on trend and variability of metabolic markers in dairy cows. *Animals* 12: 1008. Doi: 10.3390/ani12081008.
- Wilde D (2006) Influence of macro and micro minerals in the periparturient period on fertility in dairy cattle. *Anim Reprod Sci* 96(3-4):240-249.
- Wu X, Sun H, Xue M, Wang D, Guan L and Liu J (2019) Days-in-milk and parity affected serum biochemical parameters and hormone profiles in mid-lactation Holstein cows. *Animals* 9:230. Doi: 10.3390/ani9050230.
- Xu E, Liu Y, Gu D, Zhan X, Li J, Zhou K, Zhang P and Zou Y (2024) Molecular mechanisms of plant responses to copper: from deficiency to excess. *Int J Mol Sci* 25:6993. Doi: 10.3390/ijms25136993.