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# Effect of dietary inclusion of Chandrasoor (*Lepidium sativum*) seed powder on minerals and antioxidant profile of milk in lactating cattle

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ABSTRACT

The present study aimed to investigate the effect of dietary supplementation of Chandrasoor (*Lepidium sativum*) on the mineral and antioxidant profile of milk in lactating Tharparkar cattle. A total of 12 lactating Tharparkar cattle were selected from Shree Pinjrapole Gaushala, Sanganer, Jaipur, and randomly divided into two groups ($G_1$ and $G_2$). $G_1$ group was fed a basal diet formulated as per ICAR (2013) standards, consisting of wheat straw, green fodder, and a balanced readymade concentrate. In contrast, the $G_2$ group received an additional 150 g/day/animal of Chandrasoor seeds along with the basal diet. Minerals analysis of milk indicated a significant increase in calcium (Ca) and iron (Fe) in the Chandrasoor-supplemented group as compared to the control. Although phosphorus (P) and magnesium (Mg) levels showed a numerical increase, the differences were not statistically significant. Antioxidant assays, including DPPH, ABTS, total phenol, FRAP, and total flavonoids, exhibited a significant enhancement in the supplemented group, suggesting improved oxidative stability of milk. Based on these findings, it can be concluded that dietary supplementation of Chandrasoor at 150 g/day/animal significantly improves Ca, Fe, and antioxidant activity in milk, making it a promising feed additive for enhancing milk quality.

**Keywords**: Antioxidant, Chandrasoor, Feed additive, Tharparkar cattle

According to Animal Husbandry Statistics 2025, India is the leading milk producer globally, contributing 25% of total world milk production in 2024-25 (248 million tonnes), accounting for nearly one-fourth of global milk output. The country has consistently held the top rank in milk production, with the dairy sector contributing 4–5% to the national GDP. The National Dairy Development Board (NDDB) aims to increase India's global share to 30% (one-third of total milk production) by 2030, which can be achieved through enhanced nutritional efficiency and genetic improvement of dairy animals.

The antioxidant content in milk and its overall antioxidant potential can be influenced through the addition of natural feed additives. Dairy cow diets are frequently enriched with herbs, which contain high levels of biologically active compounds. These compounds have beneficial effects on the cow's health, ultimately enhancing milk quality (Paskudska *et al.* 2018). Chandrasoor contains bioactive compounds, including the alkaloids lepidiline A (1,3-dibenzyl-4,5-dimethylimidazolium chloride) and lepidiline B (1,3-dibenzyl-2,4,5-trimethylimidazolium chloride), which have been shown to enhance glutamic pyruvate Transaminase (GPT) activity (Valentová and Ulrichová, 2003). Increased GPT activity improves digestibility and nutrient assimilation, thereby promoting galactagogue properties in lactating animals when included in their diet. Furthermore, chandrasoor seeds are rich in phytochemicals with antioxidant properties, which contribute to enhanced immune function and reduced disease incidence in dairy cattle (Patel *et al.* 2016). Since nutrition plays a crucial role in the productive performance and quality of milk, dietary interventions such as Chandrasoor (*Lepidium sativum*) supplementation can contribute significantly to this goal and it is considered as an important medicinal crop in India (Tiwari *et al.* 2004).

Modern consumers are becoming more conscious about the significance of antioxidant compounds in enhancing the body's defense mechanisms, which play a crucial role in preventing lifestyle-related diseases such as cardiovascular conditions, cancer, diabetes, and obesity. Antioxidants obtained from natural sources are particularly beneficial in this regard. Likewise, milk serves as a source of antioxidants, primarily found in its protein content (such as β-lactoglobulin (β-Lg) and lactoferrin) as well as in its fat content, which includes vitamins E and A, along with β-carotene (Stobiecka *et al.* 2023). To meet market demands, efforts are being undertaken to develop nutritional strategies that optimize the production potential of dairy cows while improving the quality and composition

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of their milk. Therefore, the present study was planned to evaluate the impact of Chandrasoor (*Lepidium sativum*) supplementation on milk minerals and antioxidant profile in lactating Tharparkar cattle.

## MATERIALS AND METHODS

The present study was conducted at Shri Pinjrapole Gaushala, Jaipur, on healthy and lactating Tharparkar cows. Twelve lactating cows with average milk yield of 5 kg/day were randomly allocated to two groups: G1 (control) and G2 (supplemented with Chandrasoor seed powder). All the cows were restrained in a well-ventilated byre having concrete floor with individual feeding arrangement The cows were stall fed throughout the experimental period. Fresh and clean water was provided thrice a day i.e. morning, afternoon and evening throughout the experimentation. The animals were given prophylactic dose of anthelmintic 15 days prior to start of experiment. Proper hygienic conditions and healthy surrounding were maintained in the shed throughout the experimental feeding period. During the research period, health status of cows was monitored regularly.

The cows were fed as per ICAR (2013) feeding standards to meet the requirement of nutrients for 60 days. Wheat straw was offered daily *ad lib* in morning. The leftover was removed on the next day morning. Chandrasoor seeds were procured from the local market. The Chandrasoor seed powder was supplemented daily by mixing in the concentrate. An adaptation period of 15 days was allowed before the start of the experimental feeding trial. The cows were then placed on two dietary experimental feeds.

The cows in the G1 group were fed a basal diet comprising wheat straw, green fodder, and a commercial concentrate mixture, while those in G2 received Chandrasoor seed powder at 150 g/day/cow in addition to the basal diet. The feeding trial was carried out for 60 days. The milking of cows was done twice daily at 4:00 AM and 4:00 PM manually at the milking barn. Milk yields were recorded (in kg) using a digital weighing balance. The milk samples of crossbred cows were collected fortnightly on 0, 15, 30, 45 and 60 days in the morning and evening.

Milk mineral content and antioxidant profile were analyzed on day 0 and day 60. Calcium (Ca) and Magnesium (Mg) concentrations in milk were estimated using the EDTA titration method (Davis and White, 1962). Phosphorus (P) and Iron (Fe) contents were determined using the Fiske and Subbarow method and the 1,10-orthophenanthroline method, respectively, via UV-VIS spectrophotometric analysis. Antioxidant activities of milk were evaluated using different methods: DPPH radical scavenging activity (Williams *et al.* 1995), ABTS assay (Van den Berg *et al.* 1999), total phenol content (Barba et al. 2013), ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), and total flavonoid content (Ordon *et al.* 2006). Data collected during the present investigation were subjected to statistical analysis using the Student's t-test to compare means between groups, following the procedure described by Snedecor and Cochran (1994) using SPSS software version 24.0.

## RESULTS AND DISCUSSION

The results depicting the effect of chandrasoor seed supplementation on milk mineral composition and antioxidant profile are presented in Table 1.

*Minerals:* The mean values of milk calcium (mg/L) were found to be 944.7 ± 12.8 and 916.1 ± 9.5 at 0 day and 854.0 ± 4.7 and 1164 ± 12.00 at 60 days for $G_1$ and $G_2$, respectively. Statistical analysis using paired t-test revealed significant increase in calcium content of milk ($P \leq 0.01$) due to *L. sativum* treatment. Our findings were in agreement with Srivastav *et al.* (2024) who observed significantly higher milk calcium in Sahiwal cattle when supplemented with fenugreek seed. Chandrasoor seeds are regarded as a "superfood" due to their high nutritional composition (Azene *et al.* 2022). It is also a good source of calcium (377 mg/100 g) (Tufail *et al.* 2024). When included in the diet, they might enhance the overall calcium intake of animals, leading to increased availability of calcium in the bloodstream and subsequent secretion into milk. Contrarily, Sharma *et al.* (2019) reported no alterations in milk calcium due to dietary supplementation of fenugreek seeds to that of control. Similarly, Rahman *et al*. (2024) also reported no alterations in milk calcium due to dietary supplementation of Plantain and lemongrass as compared to control.

The mean values of milk phosphorus (mg/L) were found to be 970.64 ± 1.44 and 980.42 ± 9.62 at 0 days and 838.38 ± 22.60 and 1000.4 ± 6.00 at 60 days for $G_1$ and $G_2$, respectively which revealed no significant increase in phosphorus content of milk on Chandrasoor supplementation. our results were consistent with Rahman *et al*. (2024), who found no differences in milk phosphorus between the control and herbal dietary supplements. Srivastav *et al*. (2024) also recorded non-significant effect of dietary supplementation of fenugreek (*Trigonella foenum graecum*), giloy (*Tinospora cordifolia*) and Shatavari (*Asparagus racemosus*) on phosphorus content of milk Sahiwal cattle.

The mean values of milk magnesium (mg/L) were found to be 113.4 ± 16.0 and 119.9 ± 14.3 at 0 days and 103.7 ±

Table 1. Chemical composition (per cent DM basis) of the experimental feeds

| Attribute | Green fodder | Wheat Straw | Concentrate | Chandrasoor Seed |
|---|---|---|---|---|
| DM | 16.93 | 91.80 | 92.55 | 94.37 |
| OM | 87.47 | 89.06 | 87.22 | 94.79 |
| CP | 6.50 | 2.53 | 17.20 | 20.87 |
| EE | 1.27 | 0.86 | 2.62 | 20.27 |
| CF | 30.87 | 41.47 | 18.50 | 21.40 |
| NFE | 34.81 | 35.94 | 41.44 | 26.60 |
| TA | 12.50 | 11.00 | 12.78 | 5.21 |

Table. 2. Minerals and antioxidant profile in milk of experimental animals

| Parameters | Groups | | | |
|---|---|---|---|---|
| | $G_1$ (Control) | | $G_2$ (Treatment) | |
| | 0 Days | 60 days | 0 Days | 60 days |
| | | Minerals | | |
| Ca (mg/L) | 944.7 ± 12.8 | 854.0$^{a}$ ± 4.7 | 916.1 ± 9.5 | 1164.0$^{b}$ ± 12.0 |
| P (mg/L) | 970.64 ± 1.44 | 838.38 ± 22.60 | 980.42 ± 9.62 | 1000.4 ± 6.00 |
| Mg (mg/L) | 113.4 ±16.0 | 103.7 ± 13.4 | 119.9 ±14.3 | 128.0 ± 2.9 |
| Fe(µg/ml) | 0.92 ± 0.04 | 0.89$^{a}$ ± 0.03 | 0.90 ± 0.04 | 1.30$^{b}$ ± 0.01 |
| | | Antioxidants | | |
| DPPH % RSA | 15.21 ± 2.17 | 22.24$^{a}$ ± 2.45 | 19.00 ± 0.81 | 62.92$^{b}$ ± 2.00 |
| ABTS % RSA | 30.95 ± 0.70 | 37.38$^{a}$ ± 2.13 | 20.95 ± 1.41 | 53.66$^{b}$ ± 0.38 |
| FRAP (µmolFe (II)/L) | 809.66 ± 30.04 | 832.00 ± 25.60 | 833.83a ± 48.31 | 2075.8$^{b}$3 ± 88.81 |
| Total Phenol (mg GAE/L) | 660.56 ± 20.73 | 758.45$^{a}$ ±22.20 | 577.96 ±34.12 | 993.20$^{b}$ ± 9.29 |
| Total Flavonoids mg RE/L | 1.01 ± 0.07 | 0.89$^{a}$ ± 0.03 | 0.99 ± 0.17 | 2.86$^{b}$ ± 0.03 |

Mean value with different superscript differ significantly

13.4 and 128.0 ± 2.9 at 60 days for $G_1$ and $G_2$, respectively which revealed non-significant effect on magnesium in milk due to chandrasoor treatment. Our observations were in accordance with Srivastav *et al*. (2024) who found that supplementing Sahiwal cattle with herbal galactogogues had no discernible influence on milk magnesium.

The mean values of milk iron (µg/ml) were found to be 0.92 ± 0.04 and 0.90 ± 0.04 at 0 day and 0.89 ± 0.03 and 1.30 ± 0.01 at 60 days for $G_2$, respectively and revealed a significant increase in iron content of milk (P≤0.01) due to *L. sativum* treatment. Garden cress (*Lepidium sativum*) seeds were are a rich source of iron and Vitamin-C used to treat anemia or iron insufficiency without the need for additional supplements (Umesha and Naidu, 2015). They also have a high concentration of iron, which encourages the development of red blood cells (Falana *et al.* 2014). However, Sharma *et al*. (2019), who observed no significant changes in milk iron levels as a result of dietary supplementation with fenugreek seed powder (150g) in Jersey crossbred cows as compared to the control group.

*Antioxidant activity in milk*: The mean DPPH radical scavenging activity (RSA, %) of milk was recorded as 15.21 ± 2.17 and 19.00 ± 0.81 at day 0, which increased to 22.24 ± 2.45 and 62.92 ± 2.00 at day 60 in $G_1$ and $G_2$, respectively, indicating a highly significant (P≤0.01) improvement in antioxidant activity. The observed enhancement in DPPH scavenging capacity may be attributed to the presence of bioactive phytochemicals in chandrasoor (*L. sativum*) seeds, such as gallic acid, protocatechuic acid, p-coumaric acid, caffeic acid, and kaempferol derivatives, which possess strong antioxidant, anti-inflammatory, and anti-carcinogenic properties (Morya *et al.* 2022). These compounds are known to donate hydrogen atoms or electrons to neutralize free radicals, thereby improving the oxidative stability of milk. Furthermore, chandrasoor seeds have been reported to exhibit considerable DPPH radical scavenging activity, supporting their potential use in functional food and milk fortification (Al-Saad and Al-Saadi, 2021). In agreement with the present findings, Yadav *et al*. (2011) also reported high antioxidant activity in ethanolic extracts of *Lepidium sativum*, with an $IC_{50}$ value of 18.46 ± 0.27 µg/ml, indicating strong free radical scavenging potential.

*ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid):* The mean ABTS radical scavenging activity (RSA, %) of milk was recorded as 30.95 ± 0.70 and 20.95 ± 1.41 at day 0, which increased to 37.38 ± 2.13 and 53.66 ± 0.38 at day 60 for $G_1$ and $G_2$, respectively, indicating a highly significant (P≤0.01) effect of Chandrasoor supplementation on antioxidant activity. The enhancement in ABTS scavenging activity may be attributed to the presence of bioactive phytochemicals in Chandrasoor, which are known to improve the antioxidant potential of milk. Chen *et al*. (2003) reported that milk with higher fat content (3%) exhibited significantly greater total antioxidant capacity, as measured by the ABTS assay, compared to low-fat and skimmed milk, suggesting that fat-soluble antioxidant components contributed to the overall activity. Additionally, Malar *et al*. (2014) observed notable radical scavenging activity in different parts of garden cress, with the whole plant showing appreciable antioxidant potential. Further, Kadam *et al*. (2018) and Attia *et al*. (2019) demonstrated strong antioxidant activity of garden cress seed ethanolic extract, reporting values of 35.29 µg/mL in the ABTS assay, indicating its potent free radical scavenging capacity.

The increase in ABTS activity observed in the present study may therefore be due to the transfer of these antioxidant compounds or their metabolites into milk, enhancing its functional and nutritional quality.

*Total phenol*: The mean total phenolic content (mg GAE/L) of milk was recorded as 660.56 ± 20.73 and 577.96 ± 34.12 at day 0, which increased to 758.45 ± 22.20 and 993.20 ± 9.29 at day 60 for $G_1$ and $G_2$, respectively, indicating a highly significant (P≤0.01) effect of chandrasoor supplementation on phenolic content in milk. The observed increase may be attributed to the transfer of plant-derived phenolic compounds or their metabolites into milk. chandrasoor is known to contain bioactive constituents such as phenolics, phytosterols, and tocopherols, which contribute to its strong antioxidant potential (Tufail *et al.* 2024).

These findings are consistent with Oh *et al*. (2017), who reported that dietary supplementation with plant extracts enhanced endogenous antioxidant levels and free radical scavenging activity in animal products. The higher phenolic content observed in treated groups, particularly $G_2$, may therefore be responsible for the improved antioxidant status of milk, indicating its potential as a functional food with enhanced health benefits.

*FRAP* (*ferric reducing antioxidant power*)*:* The mean FRAP value (µmol/L) of milk was recorded as 809.66 ± 30.04 and 833.83 ± 48.31 at day 0, which increased to 832.00 ± 25.60 and 2075.83 ± 88.81 at day 60 for $G_1$ and $G_2$, respectively, indicating a highly significant (P≤0.01) effect of chandrasoor supplementation on reducing power. The marked increase in FRAP values, particularly in $G_2$, suggested enhanced electron-donating capacity and antioxidant potential of milk. This improvement may be attributed to the presence of bioactive compounds in chandrasoor, which contributed to ferric ion ($Fe^{3+}$) reduction and overall antioxidant activity.

These findings are in agreement with Stobiecka *et al*. (2023), who reported increased FRAP values in milk from herbal-supplemented groups due to improved metal chelating and reducing activities. Similarly, Paraskevakis *et al*. (2015) also observed a significant (P<0.01) increase in antioxidant capacity (expressed as FRAP) in the milk of goats supplemented with oregano. Kadam *et al*. (2018) also demonstrated strong reducing power in alcoholic extracts of garden cress seeds, supporting their potential as natural antioxidants.

*Total flavonoids:* The mean total flavonoid content (mg RE/L) of milk was recorded as 1.01 ± 0.07 and 0.99 ± 0.17 at day 0, which changed to 0.89 ± 0.03 and 2.86 ± 0.03 at day 60 for $G_1$ and $G_2$, respectively, indicating a highly significant (P≤0.01) effect of chandrasoor supplementation. The substantial increase in flavonoid content in $G_2$ may be attributed to the incorporation of plant-derived flavonoids or their metabolites into milk.

Supporting evidence has been reported by Aguiar *et al*. (2014), who observed significantly higher flavonoid content in milk of dairy cows supplemented with propolis extract. The enhancement in flavonoid levels and overall antioxidant activity in treated groups may be due to the presence of natural phenolic and flavonoid compounds in the herbal additive. Additionally, Oh *et al*. (2017) reported that dietary supplementation with plant extracts enhances endogenous antioxidant systems and free radical scavenging capacity.

The present study demonstrated that dietary supplementation of chandrasoor seed powder at 150 g/day/animal significantly improved mineral content and antioxidant properties of milk. The enhancement in antioxidant indices—including DPPH, ABTS, FRAP, total phenols, and flavonoids—indicated improved oxidative stability and functional quality of milk. Therefore, chandrasoor supplementation can be recommended as a natural feed additive to enhance milk quality through increased mineral content and antioxidant potential.

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