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# EFFECT OF TANNIFEROUS TREE LEAVES IN CAMEL'S DIET ON MILK YIELD AND QUALITY

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## ABSTRACT

Effect of tannin containing tree leaves in the diet on camel milk production and its quality was studied in 15 lactating camels (average body weight 554 kg) in mid-lactation stage. The animals were distributed following randomised-block design into 3 groups on the basis of similar live weight and milk production. The control group (GG) was fed with a standard basal diet of roughage and supplemental concentrate in the ratio of 70:30, where the roughage component included equal proportions (1:1) of groundnut (*Arachis hypogaea* L.) straw (GS), guar (*Cyamopsis tetragonoloba* L.) phalgati (GP). The treatment groups GGK received a similar ration with a varied roughage combinations, one having tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios and the other GGP received pala leaves (PL) at GS40:GP40:PL20 ratios. Milk yield was recorded daily and the milk samples were collected fortnightly for assessing the quality parameters. The milk yield (kg/d) of camel increased to 7.11±0.14 in GGK and 6.65±0.21 in GGP compared to 6.15±0.24 in GG. The physical parameters of camel milk like electrical conductivity and freezing point decreased in the tree-forage fed groups. Amongst the chemical constituents, milk protein content increased and fat:SNF ratio decreased in GGK and GGP compared to control. An improvement in antioxidant properties of camel milk was observed in the test groups due to consumption of tanniferous tree leaves. It may thus be concluded that incorporation of tanniferous tree forages of hot-arid climatic regions at 20% level by replacing the basal crop-residues in the diet of lactating camel had positive influence on milk quality and its antioxidant properties, which will have far-reaching economic impact in the trade of camel milk and milk products and thus uplifting the socio-economic status of camel rearers.

**Key words:** Arid zone roughage, leaves, milk production, tanniferous leaves

Camel milk is considered to be an important nutrition source for the inhabitants in arid and semiarid areas (Singh *et al,* 2017; Sahoo, 2021a). Unlike other milk-producing animals, camels can thrive under extreme hostile conditions of temperature, drought and lack of pasture and still produce milk (Sahoo, 2020).

The general composition of camel milk varies in various parts of the world, i.e. protein (3.5-4.5%), lactose (3.4-5.6%), fat (3.07-5.50%), ash (0.7-0.95%) and TS (12.1-15%) (Konuspayeva *et al,* 2009; Singh *et al,* 2017). This variation may be attributed to factors such as breed, age, the number of calving, nutrition, management, the stage of lactation and the sampling technique used. Thus, production of animals is directly affected by availability of feeds and feeding strategy adopted during the course of seasonal cycle, available in plenty after rain to continue end of winter and then a long summer scarcity period ranging from March to July (Sahoo, 2021b). An economic feeding schedule adheres to maximum and optimal utilisation of the locally available feed resources. The camel population of India is concentrated mostly in the 'Thar Desert' of India (AHSD, 2019) and the available forage biomass in this western arid Rajasthan comprises of seasonally grown pasture, salt bushes, browses and tree forages (Sharma and Sahoo, 2017). Therefore, suitable inclusion of alternate tree forages with conventional crop-residue based ration could be an economical way of sustaining lactation in camel. But, the tree leaves of this region are mostly tanniferous Khejri (*Prosopis cineraria*) and Pala (*Ziziphus nummularia*) leaves, which may exert antinutritional effect if not fed judiciously. Various approaches involved in the use of these unconventional feed resources have delineated newer concepts that implies inclusion at low levels to explore pro-nutritional effect for improving animal production (Singh and Sahoo, 2004). The phytochemical-rich tree leaves have bioactive properties (Makkar *et al,* 2007; Durmic

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and Blache, 2012; Xiao and Bai, 2019) that often alters the quality milk, meat and their products (Vasta and Bessa, 2012; Morales and Ungerfeld, 2015; Jerónimo *et al,* 2016; Sahoo, 2021a). There is limited information on modulation of antioxidant properties of camel milk. The present investigation was thus aimed at incorporating 20% of tree leaves in the basal roughage diet of lactating camel on milk yield and its composition and possible alteration in antioxidant constituents.

## Materials and Methods

Fifteen lactating she camels in mid lactation were randomly divided into 3 groups of 5 camels each based on comparable milk yield, body weight, number of lactations completed and days in lactation. The control group was fed with a standard basal diet of roughage and supplemental concentrate in the ratio of 70:30 (ICAR, 2013), where in the roughage component included equal proportions (1:1) of Groundnut (*Arachis hypogaea* L.) straw (GS), Guar (*Cyamopsis tetragonoloba* L.) *phalgati* (GP) and designated as GG. One treatment group received a similar ration with a varied roughage combinations having tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios and referred as GGK. Similarly, the other group (GGP) received Pala leaves (PL) at GS40:GP40:PL20 ratios. The experimental animals were housed in individual stalls and provided with uniform management practices during the course of this study, i.e. 12 fortnights.

### *Data recording and sample collection*

Samples of feed and forage samples were collected at fortnight intervals and pooled after drying for phytochemical analysis. The milk yield of individual camel was monitored daily and fortnightly average yield per animal was recorded during the whole period. Milk samples were collected at fortnight intervals in clean plastic sampling bottles and kept in an icebox during transportation and subjected to analysis directly.

### *Phytochemical analysis*

Representative samples of feed offered during the study were collected, dried in drafted hot-air oven at 55-60°C, ground to pass through 1 mm screen and stored in poly propylene air-tight containers for further analysis. Polyphenols were extracted in 70% acetone solution after treating with 2% acetic acid and petroleum ether with the help of sonicator (Sonirep 150, Ultrasonic disintegrator and process timer, MSE, UK), total phenols (TP) and total tannins (TT) were assessed by a modified Folin-Ciocalteu method using polyvinyl polypyrrolidone (Makkar, 2003) for separating non-tannin phenols (NTP) from tannin phenols. Condensed tannins (CT) were analysed by the butanol-HCl-iron method (Porter *et al,* 1986). Hydrolysable tannins (HT) were estimated as the difference between total tannins and condensed tannins. TP and TT were expressed as tannic acid equivalents, while CT as leucocyanidin equivalents.

### *Chemical assay of milk*

The physicochemical parameters including fat, protein, lactose, electrical conductivity (EC), density and freezing point determined by the infrared milk analyser (Lactoscan, MCC) previously standardised for camel's milk (Aple Industries services–LaRoche/Foron, France), as per the to manufacturer's instructions. The total solid was determined from its dry matter (DM) content by drying in a drafted hot-air oven and subsequently its mineral content i.e. inorganic matter (or total ash) was analysed as per AOAC (2000).

### *Antioxidant assay*

Total antioxidant capacity (TAC) was determined by ferric reducing antioxidant potential (FRAP) based on a redox reaction in which ferric tripyridal triazine complex (Sigma-Aldrich) reduced to its coloured ferrous form in the presence of antioxidant, which is measured after 40 min of incubation with an absorption maximum at 593 nm (Benzie and Strain, 1996) in a UV-VIS spectrophotometer (Uvikon XL; Bio-Tek Instruments, Winooski, VT, USA). The potential of the antioxidants in the milk serum to reduce $Fe^{3+}$ to $Fe^{2+}$ was expressed in µmol $Fe^{2+}$ in 1L milk serum, using a calibration curve of standard $FeSO_4.7H_2O$ (0–1000 mM; Sigma-Aldrich).

Free radical scavenging activity (FRSA) was measured by employing 2,2-diphenyl-1-picrylhydrazyl (DPPH; Fluka Chemicals) method that is based on the reduction of the stable free radical by antioxidants present in a methanol solution (Brand-Williams *et al,* 1995). The reduction of DPPH (105 mM) was followed by monitoring a decrease in its absorbance after 40 min (Uvikon XL). For each sample, six different volumes of milk serum were measured in a ratio of DPPH solution to milk serum varying between 3:1 and 1.5:2.5 (v/v), depending on the number of antioxidants present in the milk serum. Before measuring the absorption, the reaction mixture is centrifuged (5 min 1300 g; Sorvall RC 26 Plus) to obtain clear medium. For each volume of milk serum, the percentage of DPPH remaining at 40 min was calculated based on different standard curves of DPPH in methanol: water (ratio varying between 3:1 and 1.5:2.5, v/v). The antioxidative capacity is calculated as the amount of milk serum necessary to decrease the initial DPPH concentration by 50% ($EC_{50}$ value) and expressed as mL milk serum per 1mg DPPH and thus a lower $EC_{50}$ value is indicative of less milk serum needed to reduce the concentration of DPPH to 50%.

### *Statistical analysis*

Statistical analysis was performed using SPSS version 24 (SPSS Inc., Chicago IL). The effects of tree leaves feeding on milk quality parameters were analysed by ANOVA with the Tukey post hoc test. The milk yield data recorded fortnightly was subjected to repeated measure analysis. Any significant differences between the dietary groups was declared at $P<0.05$.

## Results and Discussion

### *Tanniferous constituents*

The conventional basal forages had least and/ or negligible phenolic constituents, GS accumulated more than GP (Table 1). On the other hand, all the phenolic and tannin constituents were high in Khejri leaves compared to Pala leaves, the most significant being TP (10.22 *vs* 5.41) and CT (4.47 *vs* 1.42). The phenolic composition of the test forages was in line with the values reported earlier (Sharma and Sahoo, 2017). The tree forages accumulate more phenolic constituents as a defence mechanism against browsing by herbivores (Salminen and Karonen, 2011) compared to cultivated crops (e.g. groundnut, guar) which are harvested for seed production. A similar difference in polyphenolics composition between Khejri and Pala leaves was also reported by Kanika *et al* (2022).

**Table 1.** Tanniferous constituents (% DM) of forages used in camel feeding.

| Attributes | Khejri leaves | Pala leaves | Groundnut straw | Guar phalgati |
|---|---|---|---|---|
| Total phenols | 10.22 | 5.41 | 1.21 | 0.55 |
| Non-tannin phenols | 1.51 | 1.20 | 0.39 | 0.28 |
| Total tannins | 8.71 | 4.21 | 0.82 | 0.27 |
| Hydrolysable tannins | 4.24 | 2.79 | 0.54 | 0.23 |
| Condensed tannins | 4.47 | 1.42 | 0.28 | 0.04 |

### *Milk yield and composition*

The milk yield of camels recorded highest in GGK followed by GGP and GG during the 12 fortnights observation (Table 2). The lactation pattern was more consistent in GGK compared to GGP and GG, where in the fall was sharp during 5 to 8 fortnights (Fig 1). Repeated measure analysis showed significantly higher milk yield in GGK compared to GGP and GG during $6^{th}$ and $7^{th}$ fortnights that ultimately influenced the average milk yield of GGK over that of the other two groups. The average milk yield of camel was in line with the observations made earlier (NRCC, 2020; Nagpal *et al,* 2003; Nagpal and Jabbar, 2005). An increase in milk yield in GGP and GGK compared to GG may be attributed to difference in nutrient uptake and utilisation of the polyphenolic-rich diet. More specifically, GGK recorded higher milk yield than GGP and this could be ascribed to difference in polyphenolic composition between Khejri and Pala leaves (Table 1). Bhatt *et al* (2020) reported a lower growth performance of lambs on a complete diet at higher levels of inclusion (50%) of tanniferous tree leaves. On the country, Aderao *et al* (2020) observed comparable lamb growth performance when the conventional cowpea hay was replaced with Pala leaves. Nagpal *et al* (2002a) observed higher feed intake and growth in camel calves fed on guar phalgati and Khejri leaves at 76:24 ratio. It may be inferred that the present levels of incorporation of Khejri and Pala leaves in the diet of camel could support higher milk yield in camels during the mid-lactation stage.

With regard to milk composition (Table 2), the physical attributes *vis* EC and freezing point were higher ($P<0.05$) in control compared to groups fed on tanniferous leaves. This was a typical observation and a possible role of plant polyphenols or tannins on electrolyte redistribution that affected these two attributes cannot be ruled out. Invariably, tree leaves have wider calcium: phosphorous ratio than the conventional crop residues (Nagpal *et al,* 2002b). Moreover, soil-plant-animal inter-relationship is one of the principal determinants in regulating the difference in mineral acquisition process in different agroecological regions (Kumar *et al,* 2016). According to Henno *et al* (2008), possible effects of season and diet and their combined effect should be considered for interpreting the milk freezing point data. The milk nutrient components except protein are relatively similar in all the dietary groups. The milk protein content in the tree leaves fed groups (GGK and GGP) was higher ($P<0.05$) than the control. This is most

**Table 2.** Milk yield and composition of lactating camels under different dietary groups.

| Item | GG | GGK | GGP |
|---|---|---|---|
| Milk yield/day | $6.15 \pm 0.24^a$ | $7.11 \pm 0.14^c$ | $6.65 \pm 0.21^b$ |
| **Physical parameters** | | | |
| pH | $6.48 \pm 0.009$ | $6.49 \pm 0.010$ | $6.50 \pm 0.008$ |
| Electrical conductivity (EC; mS/cm) | $6.48 \pm 0.072^a$ | $6.34 \pm 0.089^{ab}$ | $6.17 \pm 0.078^b$ |
| Density (kg/m$^3$) | $26.7 \pm 0.216$ | $26.08 \pm 0.223$ | $26.5 \pm 0.243$ |
| Freezing point ($^0$C) | $0.48 \pm 0.006^a$ | $0.45 \pm 0.004^b$ | $0.46 \pm 0.005^b$ |
| **Chemical constituents** | | | |
| Total solids | 10.50 + 0.105 | 10.64 + 0.105 | 10.56 + 0.091 |
| Fat (%) | $2.66 \pm 0.119$ | $2.51 \pm 0.111$ | $2.47 \pm 0.104$ |
| Protein (%) | $3.06 \pm 0.059^b$ | $3.29 \pm 0.044^a$ | $3.28 \pm 0.034^b$ |
| Lactose (%) | $4.00 \pm 0.050$ | $4.04 \pm 0.062$ | $4.02 \pm 0.058$ |
| Solid not fat (SNF; %) | $7.84 \pm 0.084$ | $8.13 \pm 0.098$ | $8.09 \pm 0.076$ |
| Minerals (%) | $0.78 \pm 0.010$ | $0.80 \pm 0.011$ | $0.79 \pm 0.010$ |
| Fat: SNF ratio | $0.339 \pm 0.0042^a$ | $0.309 \pm 0.0035^b$ | $0.305 \pm 0.0034^b$ |

GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)

GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios

GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio

abc Values within a row without a common superscript letter are significantly different (P < 0.05)

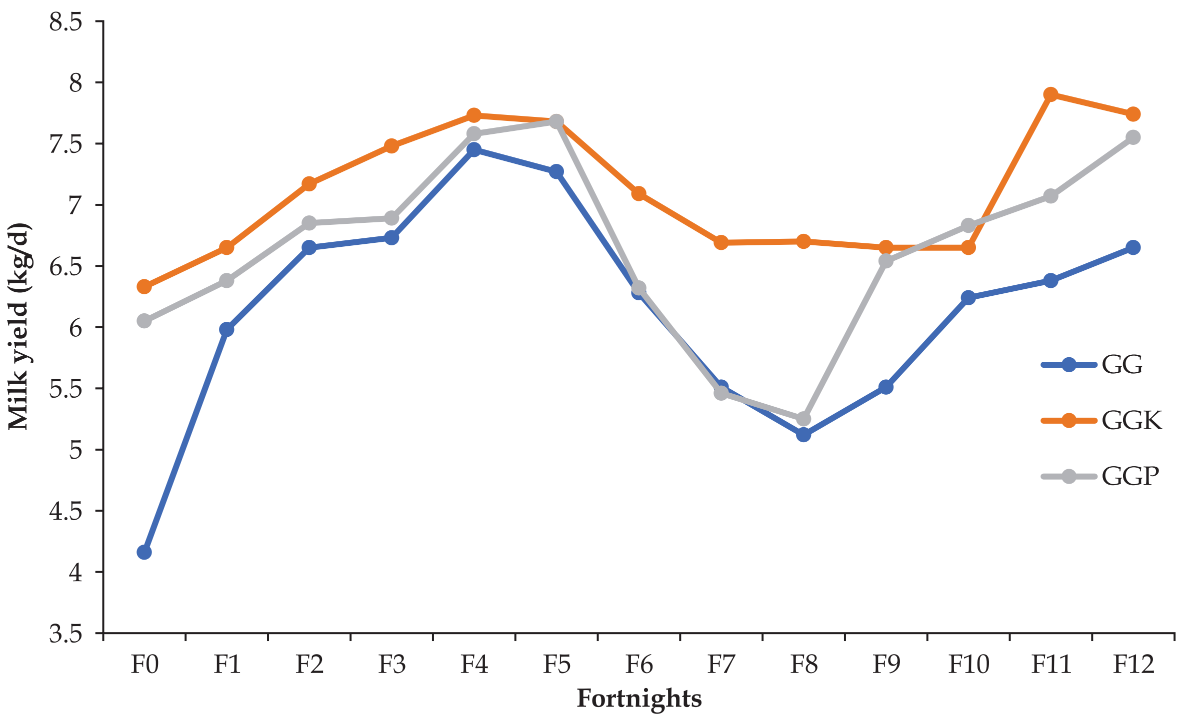


**Fig 1.** Fortnightly average milk yield of camels in different dietary groups.

GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)

GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios

GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio.

likely attributable to tanniferous forage intake in GGK and GGP that might have modulatory effect on N or protein utilisation. Plant tannins in moderate amounts reduces the degradability of proteins in the rumen, which induces greater absorption of amino acids by the gut and synergistic actions of

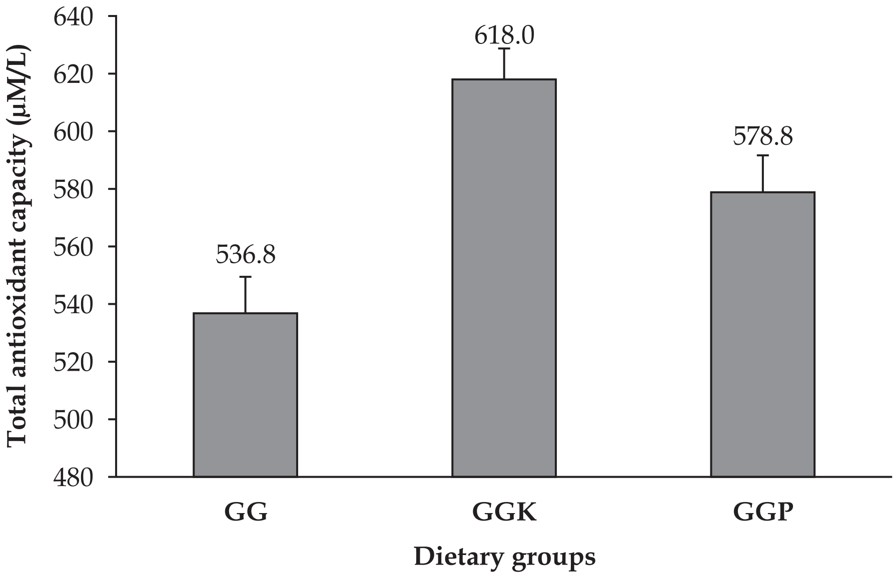


**Fig 2.** Total antioxidant capacity of milk serum of lactating camels in different groups
GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)
GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios
GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio.

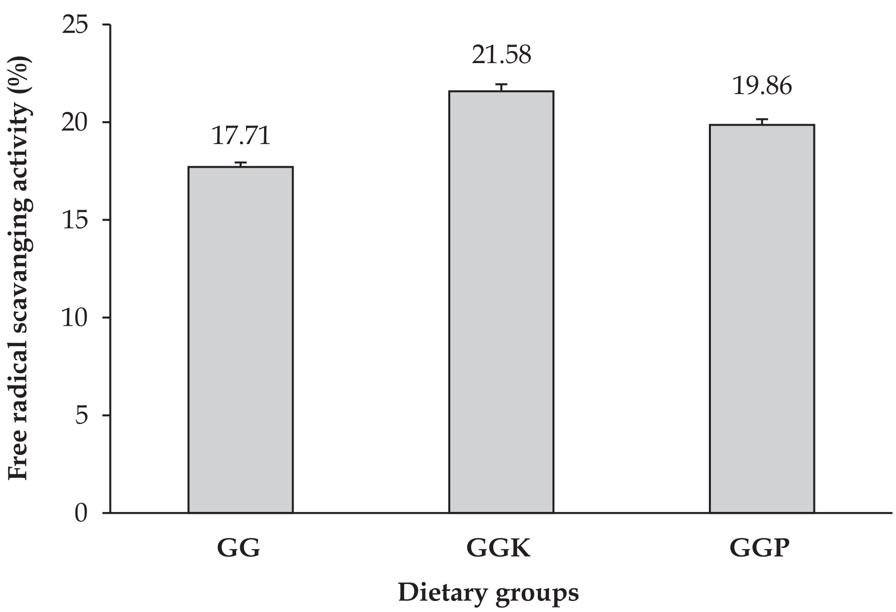


**Fig 3.** Free radical scavenging activity of milk serum of lactating camels in different groups.
GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)
GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios
GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio.

its role in host-microbe metabolism improve the efficiency of utilisation of dietary protein for milk protein synthesis (Jeronimo *et al,* 2016). The other nutritional constituents like total solids, fat, lactose, SNF and minerals were similar between the groups. Nagpal and Jabbar (2005) reported enhancement in total solids, protein and lactose content of milk when the nutritional profile of lactating camel was improved through complete feed block. But, a similar milk nutrient composition in all the three dietary groups would thus be indicative of adequate nutritional status of camels, while additional nutrient input supported increment in milk yield. Similar nutrient composition of milk in lactating camels has been reported earlier (NRCC, 2020).

Upon critical analysis, a decrease in fat: SNF ratio in tanniferous forage fed groups can be interpreted differently. This decline was due to non-significant decrease in fat content with a concomitant increase in SNF content of milk in response to phytochemical-rich diet in GGK and GGP. Milk fat, SNF:fat ratio and, to a lesser extent, total solids contents were strong individual predictors of cheese yield (Caro *et al,* 2011). It is suggested that standardisation of fat: SNF ratio is essential for production of uniform quality and economic aspects of manufacturing any dairy product (Chaudhary *et al,* 2015).

### *Antioxidant activity*

Regarding the effects of feeding regimen, the results revealed that the milk from camels fed on Khejri and Pala leaves exhibited significantly higher ($P < 0.05$) TAC and FRSA values than the milk from the control (Figs 2 and 3). It was obvious that the uptake of phytochemicals from these tree forages are metabolised by the camels that eventually secreted through the milk to exert higher antioxidant activities in these groups. De Feo *et al* (2006) reported positive relationships between forage intake and antioxidant compounds. Similarly, some other studies in small ruminants have reported similar findings with regards to milk quality (De Feo *et al*, 2006; Jordán *et al*, 2010). Between the two tree-forage fed groups, GGK exhibited higher FRSA activity than GGP and this difference was due to higher TP, TTP and CT content in Khejri leaves than that in the Pala leaves. An increase in milk protein content in tree-forage fed groups may be ascribed to a positive influence on availability of bioactive peptides to exert antioxidant properties to camel milk (Khan *et al*, 2021). Thus, our results showed that the phenolic constituents of tree forages can affect the antioxidant activities of milk through its secondary compounds or degraded metabolites that entered into milk and may provide additional value in terms of oxidative status and thus, incorporation of alternate tanniferous tree forage seems to be a promising strategy for improving milk quality.

Incorporation of tanniferous tree forages at 20% level by replacing the conventional crop residues in the basal diet of lactating camel could be a promising strategy to support or enhance milk yield besides improving the milk quality parameters and providing an impetus to milk processing for commercial cheese manufacturing. Additionally, an increase in antioxidant properties in the milk due to possible influence of polyphenolic metabolites may have a role in increasing the economic value of camel milk and its processed products. This will ultimately have far-reaching socio-economic impact in the life of camel rearers. The top-feed biomass of hot-arid deserts could be a cost-effective solution for nourish lactating camel during scarcity of crop fodder in extreme climatic conditions.


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