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Monthly variation in the nutritional and antinutritional composition of Morus alba leaves

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ABSTRACT

Marus alba is a multipurpose fodder tree recognized for its nutrient-rich foliage and its role in addressing green fodder deficits in livestock systems. The present study assessed the monthly variation in the nutritional, mineral, and anti-nutritional composition of M alba leaves sampled at monthly intervals from March to October 2016 under a mulberry-based agroforestry system in the mid-hill region of the North Western Himalayas. Leaf samples from fifteen randomly selected trees were analysed for proximate composition , fibre fractions (NDF, ADF, hemicellulose), minerals (Ca and P), and phenolic constituents (total phenols, condensed tannins, hydrolysable tannins) following standard analytical procedures. The results of the study reported that with advancing leaf maturity, crude protein (I 8.75-15.14%), nitrogen-free extract (50.45-43.09%), organic matter (87.75-85.34%), and phosphorus (0.35-0.21%) declined significantly ($p < 0.05$), whereas dry matter (25.54-33.50%), crude fibre (18.69- 25.81%), total ash (10.25-13.33%), calcium (1.18-2.74%), and condensed tannins (0.94- 1.98%) increased progressively. These findings indicate that early maturing M alba leaves harvested during March to April possess superior nutritive quality and lower anti-nutritional burden, and therefore represent the optimal harvest stage for maximizing forage value in livestockfeedingsystems in the North-Western Himalayan agroforestry landscapes.

INTRODUCTION

Livestock are vital to the global food system and rural livelihoods, supporting the livelihoods of about 1.3 billion people and contributing nearly 40% of global agricultural output (FAO, 2026). The global demand for livestock products has been steadily increasing due to rising incomes, population growth, and rapid urbanisation, and is projected to increase by nearly 20% by 2050 (FAO, 2023). Beyond the provision of milk, meat, and other animal products, livestock offer crucial economic, social, and agricultural benefits, including income generation, draught power, manure for soil fertility, and waste recycling. These functions make livestock an essential component of food security and poverty reduction strategies, particularly in developing countries.

In India, livestock farming forms an integral component of mixed farming systems and supports the livelihoods of rural households. Despite its importance, livestock productivity in many developing regions remains relatively low. One of the primary constraints affecting livestock production is the scarcity of quality feed and fodder resources. Increasing pressure on agricultural land, rapid urbanisation, and changing land use patterns have significantly reduced the availability of natural grazing lands and cultivated fodder resources (Alam *et al.* 2023).

To overcome the limitations associated with conventional fodder resources, the integration of fodder trees and shrubs into farming systems has gained considerable attention. Fodder trees are widely recognised as important components of sustainable livestock production systems due to their ability to provide nutrient-rich foliage during periods of feed scarcity. These multipurpose species are capable of producing substantial biomass and often possess high crude protein and mineral content, making them valuable supplements in livestock diets (Navale *et al.* 2022).

Among the various fodder tree species, *Marus alba* (mulberry) has received considerable attention because of its multipurpose nature and high nutritional value (Kandylis *et al.* 2009). Traditionally cultivated as the primary feed resource for sericulture, mulberry has increasingly been recognised for its potential as a valuable fodder resource for livestock. The leaves are highly palatable and possess a favourable nutritional profile characterised by relatively high crude protein content, moderate fibre levels, and significant concentrations of essential minerals. In addition, the species exhibits high adaptability to diverse agro climatic conditions (Rohela *et al.* 2020).

However, the nutritional quality of fodder leaves is not constant and varies with leaf maturity, season, and environmental conditions. Such variations influence the concentration of crude protein, fibre fractions, minerals, and secondary metabolites, including phenolic compounds and tannins, which ultimately affect feed quality and digestibility (Navale *et al.* 2022). Therefore, understanding the temporal changes in the nutritive and anti-nutritional composition of fodder leaves is essential for determining the optimal harvesting stage and improving their utilisation in livestock feeding systems. A comprehensive understanding of these variations can assist in improving fodder management practices and enhancing the efficient utilization of mulberry leaves in livestock feeding.

In this context, the present study was undertaken to evaluate the monthly variation in the nutritional, mineral, and anti-nutritional composition of *Marus alba* leaves under agroforestry conditions in the mid hill region of the north-western Himalayas

MATERIALS AND METHODS STUDY AREA

Study Area The study area located in the mid-hill zones of the North-Western Himalayas in India (30° 51' N, 76° 11' E, elevation 1250 m above mean sea level; Fig. I), having undulating and hilly terrain with elevation and depressions, gentle slopes, and south eastern aspect. The area comes under the subtropical belt, but slightly skewed towards the temperate climate, with temperatures ranging from 1°C in winter to 37°C during the summer, with a mean annual temperature of 19.8°C. The hottest months are May and June, while the coldest months are December and January. The area receives 1100-1150 mm of rain per year, with most of the rain falling during the monsoon season (July and August) and rarely experiencing snowfall.

Leaf sampling and pre-processing

To assess the monthly variation in the nutritive value, sampling of the leaves was done from March 2016 to October 2016 at a monthly interval. A total of 15 *Marus alba* trees were randomly selected from a Morus-based agroforestry system developed in

university premises based on phenology of the tree. Leaves were harvested from different canopy positions of each tree and pooled to form a composite sample per individual. Thus, 15 tree-wise composite samples were obtained for each sampling month. The representative samples were packed in the paper bag and each composite samples were washed, oven dried (60±5°C) in hot air oven until constant weight was obtained. The dried samples were ground using the Willey mill and sieved through a 40-mesh sieve in the laboratory for proximate and mineral content analysis. The pre-processed composite leaf samples were analysed to determine proximate components based on methods prescribed by AOAC (2000) *viz.*, dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), total ash (TA) and nitrogen-free extracts (NFE)

The crude protein (CP¾) content was determined by analysing the nitrogen content in the samples using the Micro Kjeldahl method as per AOAC (2000) methods and subsequently converting the nitrogen to CP (%) by multiplying it by a factor of 6.25. Soxhlet apparatus was used for extracting ether extract (EE%) from the samples using petroleum ether for 8 h. Ash content was determined by incinerating the samples at 560 °C for 8 h in a muffle furnace, and the resulting ash was analysed for calcium (Ca) using the flame photometer method (Jackson, 1967; Kalra , 1997) while phosphorus (P¾) was analysed using atomic absorption spectrophotometer method (Chapman and Pratt, 1962). The neutral detergent fibre (NDF¾) and acid detergent fibre (ADF %) were analysed by using Van Soest *et al.* (1994).

Total phenols (TP) were analysed using the Folin Ciocalteu method (Makkar, 2000). For condensed tannins (CT), the extract was treated with butanol-HCl in the presence of ferric ammonium sulphate, and the results were expressed as leucocyanidin equivalents (Porter *et al.* 1985). Non-tannin phenols (NTP) were determined after precipitating tannins with polyvinyl po lypyrro lidone. Total tannin phenols and hydrolysable tannins were calculated using the following formulas: Total tannin phenols (TTP) = Total phenols-Non tannin phenols; Hydrolysable tannins (HT) = Total tannins (TT) - Condensed tannin (CT).

Statistical analysis

The data for nutritive parameters were statistically analysed using analysis of variance (ANOVA) under a factorial randomized block design (RBD) (Gomez and Gomez, 1984) and means compared with Fisher's least significant difference (LSD) at the 5% level of significance through R software.

Statistical model for nutritional and antinutritional analysis

Yj , =μ+M,+Rj+ej , Where:

• Yi , =Observation for the i1h replication and j"

treatment

• μ=Overall mean

• M,=Effect of the ih' month

• = Random effect of the j1h replicat ion

• ej , =Experimental error

RESULT AND DISCUSSION

Monthly variation in the nutritional value of the

Morus alba leaves

The nutritive value of a feed is determined by its ability to provide the nutrients required by an animal for maintenance, growth and reproduction, and is a function of the feed intake and the efficiency of extraction of nutrients from the feed during digestion. The data pertaining to nutritional value is demonstrated in Table

I. The DM (%) exhibited an increasing trend with

advancing maturity of leaves, the mean dry matter percentage of October (33.50%) followed by September, August, July, June, May, April and March with 32.31, 30.66, 29.47, 27.79, 26.90, 26.07 and 25.54

(%), respectively. The monthly variation in dry matter content showed a progressive increase with advancing leaf age, reflecting the natural maturation process. This rise in dry matter is primarily associated with declined moisture content and greater lignification in mature leaves, particularly during the late months compared to earlier months (Ji *et al.*2021).

Contrastingly, CP content decreased from starting months to final months of the trial. The highest CP (%) was recorded in the month of March (18.75%) followed by April (18.33%) > May (17.36%) > June (16.82%) > July (16.30%) > August (16.09%) >

September(l5.53%) > October(l 5.14%). The decline in crude protein content with seasonal progression can be attributed to the retranslocation ofleaf nitrogen into branches prior to leaf fall, as well as a dilution effect resulting from leaf expansion and maturation (Singh and Todaria, 2012).

A similar trend has also been observed in the EE, CF, NDF, ADF and TA. The EE content increased steadily from 1.86 (%) in March to 2.63 (%) in October, with October showing significantly higher *(p* < 0.05) EE than all preceding months. Monthly CF, NDF and ADF values ranged from 18.69-25.81(%), 35.36-

43.23 (%) and 28.43-34.68(%), respectively, with overall means of 22.06(%), 39.50(%) and 31.82(%).

Hemicellulose content varied marginally (17.83-18.89%) acro ss months, averaging 18.29(%). TA also exhibited gradual increases, with overall means of 11.82 (%). The increase in crude fibre content with advancing leaf maturity is physiologically linked to the progressive development of lignocellulosic structures (Hashmi and Waqar, 2014). Additionally , elevated maturity of the leaves has been reported to reduce soluble carbohydrate levels in plants, thereby increasing fibre content and lowering digestibility (Amole *et al.* 2013). The increase in NDF andADF levels in leaves can be attribut e d to advancing maturity, which is primarily linked to enhanced lignification, driven by higher concentrations of enzymes involved in cell wall modification and structural reinforcement (Bakshi and Wadhwa, 2004). The rise in hemicellulose content in mulberry leaves with advancing maturity is attributed to a proportionally greater increase inADF than in NDF, reflecting enhanced formation of cellulose and lignin during leaf development (Navale *et al.* 2022). The decrease in organic matter content may be attributed to the increase in the ash content of the leaves. The Ash(%) was significantly higher (p<0.05) in the later months than the earlier months , The increase in ash content with advancing maturity indicates greater mineral accumulation in mature leaves (Kumar *et al.* 2017). For most parameters (DM, EE, CF, HC, NDF and TA) values recorded in March were statistically at par with those of April.

In contrast, NFE and OM showed a declining trend from March to October. The mean NFE content was 47.13 (%), decreasing progressively from 50.45 (%) in March to 43.09 (%) in October. Similarly, OM content declined with advancing leaf maturity, with an overall mean of86.64 (%). Overall, these results indicate that *M alba* leaves exhibit higher NFE and OM concentrations during early maturity stages, which gradually diminish as the leaves advance towards later growth stages. The results of the study reported that the NFE content decreased significantly from

March to October, corresponding with the progressive maturation of *M alba* leaves . The variation in nitrogen-free extract content attributed to corresponding fluctuations in other nutritional fractions, including CP, CF, ash, and EE.

Monthly variation in the minerals and anti-nutritional value *oftheMorus alba* leaves

The data related to minerals and anti-nutritional parameters demonstrated in Table 2. In the present study Calcium content increased from 1.18 (%) in March to 2.74 (%) in October, with an overall mean of 2.00 (%). The physiological basis for the rise in calcium concentration is because it is largely immobile within plant tissues and does not readily retranslocate from older to activ ely growing parts, it progressively accumulates in mature leaves as the plant advances **in** age (Singh and Todaria, 2012).

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While Phosphorus content decreased steadily, from 0.35 (%) in March to 0.21 (%) in October , resulting in a mean value of 0.27 (%). Since phosphorus is one of the most mobile minerals in plants, its retranslocation from older, senescing leaves to younger, actively growing leaves has been widely documented across numerous plant species (Marschner, 2012) .

The TP, NTP, and HT declined steadily from March to October, ranging from 7.57 (%) to 6.77 (%), 4.53 (%) to 3.46 (%), and 2.09 (%) to 1.33 (%), respectively. Their overall mean values were 7.31 (%), 4.09 (%), and 1.79 (%). The study reported there was a decline in the total phenols content in the leaves of *M alba,* the higher concentration of total phenolics in young leaves, compared with mature leaves, is likely due to the greater selective pressure experienced during early growth stages, which stimulates increased synthesis of defensive secondary metabolites such as phenolics (Naz *et al.* 2013). The reduction in hydrolysable tannins (HTs) may be attributed to their breakdown into dimers during biogenetic processes, followed by increased insolubilization of these dimers. As leaves mature, these dimers gradually diminish, leading to an overall decline in HT content. Consequentl y, HTs tend to accumulate predominantly in young leaves and decrease with advancing leaf maturity (Rana *et al.* 2006; Kumar *et al.* 2017). Conversely, TTP and CT increased with advancing leaf maturity, with overall mean values of3 .23 (%) and 1.43 (%), respectively. TTP increased from 3.03 (%)

in March to 3.31 (%) in September and remained stable in October, while CT progressively increased from 0.94 (%) in March to 1.98 (%) in October. The physiological basis for increase in the concentration of condensed tannins was due to drought and low temperature stress during summer. (Rana *et al.* 2006) reported that condensed tannins are synthesized mainly during winter. Together, these patterns indicate a shift in the phenolic profile of *M alba* leaves as they mature.

CONCLUSION

The present study demonstrates significant month wise variation in the nutritional and anti-nutritional composition of *Marus alba* leaves under mid-hill agroforestry conditions of the North-Western Himalayas. Progressive leaf maturation resulted in marked declines in crude protein, nitrogen free extract, organic matter, and phosphorus, with concurrent increases in fibre fractions (NDF, ADF), calcium and condensed tannins. A maturity-driven phenolic shift from hydrolysable toward condensed tannin dominance further elevated anti-nutritional burden in mature leaves. These findings collectively identify March-April as the optimal harvest time for maximising forage quality and sustaining livestock productivity in mid-hill Himalayan conditions.

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AUTHOR CONTRIBUTIONS

Conceptualization: R.B., I.Q. and C.L.T. Methodology: R.B. and C.L.T., Data Collection: I.Q., K.L.G. and R.B., Software: K.K., S.S. and S.K., writing and original draft preparation: I.Q., R.B., K.K., H.S., and S.S., Writing-review and editing, K.K., R.B., I.Q, K.L.G., H.S., and K.R. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY

No datasets were generated or analysed during the current study.

DECLARATIONS

Conflict of interest: The authors declare no competing interests.

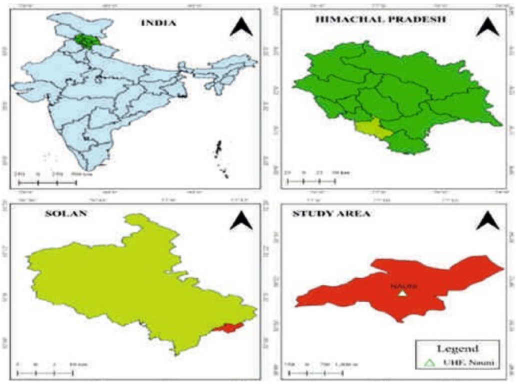

Figure 1. Fig.I: Map of the Study Site

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