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University of Eldoret, Kenya, Mount Kenya University, Kenya, Chukwuemeka Odumegwu Ojukwu University, Nigeria, Kyambogo University, Uganda and University of Makeni, Sierra Leone

Comparative Proximate Nutritional Quality of Selected Mulberry Varieties in Selected Agro-ecological Zones for Suitability in Silkworm (*Bombyx mori* L.) Rearing

*Joan Jeptum-Kiplagat[1], Pixley Kipsumbai[1], Kasino Muo[2] and Emily Jepyegon Chemoiwa[1]
[1]*Department of Biological Sciences, University of Eldoret, P.O. Box 1125-30100, Eldoret*
[2]*Kenya Agricultural and Livestock Research Organization (KALRO) crop protection, P.O. Box 14733-00800, Nairobi*

*Corresponding email – joankiplagat2013@gmail.com

Abstract

Mulberry tree (Morus spp.) leaf quality determines the growth performance and silk yield of Bombyx mori silkworm. The proximate composition of mulberry foliage varies across ecological zones. This study examined how difference in agro-ecological climatic factors influence mulberry leaf proximate nutrient composition across three contrasting environments in Kenya at lowland (Marigat); mid-altitude at University of Eldoret (UoE) and highland (Kaptagat). Two elite mulberry varieties, Ichinose and Kanva2, were established uniformly in all the sites. On maturity the leaves were harvested and leaf proximate composition quantified using standard analysis procedures. Rainfall amount, relative humidity and temperature were significantly different across the elevation (p < 0.001). Mulberry nutritional composition varied significantly by site and variety with Ichinose having significantly higher protein (8.69±0.07%) in UoE, but lowest in Marigat (4.98±0.72%), whereas fibre was higher in Kanva2 in Marigat (23.34±1.38%) and was significantly different across the sites. Crude Protein: Fibre ratio was highest in Kaptagat for both Ichinose (0.55±0.01) and Kanva2 (0.40±0.01). Pearson correlation coefficients showed crude protein correlated positively with humidity (0.63). Principal component analysis revealed higher temperature influenced crude fiber and lipids, whereas rainfall and humidity influenced crude protein and moisture content. Ichinose showed superiority in higher altitude producing higher quality leaves, suitable for promoting climate smart sericulture while Kanva2 showed suitability for low altitude regions.

Keywords: Mulberry, Variety, Proximate Composition, Agro-ecological zone, Sericulture

Introduction

Mulberry plant (*Morus* spp.) have been cultivated across the world for feeding silkworms (*Bombyx mori*), which is known for years to contribute significantly to rural economies, particularly in regions like South and East Asia which have favorable climates (Padhan et al., 2025; Altman & Farrell, 2022; Hasan et al., 2025). Through trade ties the production has spread to many regions of the world including Kenya. The silkworms depend mainly on mulberry plant leaves for feed. The mulberry leaf nutrient content is equally important because *B. mori* is strictly monophagous. The source for proteins needed for sericin and fibroin synthesis as well as carbohydrates to provide energy for growth and cocoon spinning, lipids and minerals for metabolic regulation is mulberry leaves. (Hăbeanu, 2024; Maenaka & Park, 2018). The main determinants of composition of mulberry leaf is cultivar, leaf maturity stage during harvesting and mulberry growing conditions like rainfall, humidity and temperature (Jayaramaiah et al., 2025). Leaves growing in drought stressed conditions develop higher lignin and structural fiber thus reducing digestibility and silk production efficiency (Saini et al., 2023; Sun et al., 2023; Chundang et al., 2020). Larval growth is therefore, strongly predicted by the protein to fiber ratio in the leaves which also determines silk output in sericulture (Liu, 2024; Bu et al., 2022; Thulasi & Sivaprasad, 2015). On the other hand, cooler and humid conditions increase crude protein and moisture content which in turn increase digestibility hence nutrient assimilation and cocoon yield (Sharma et al., 2023; Rahmathulla, 2012; Dahi et al., 2016).

There is use of improved mulberry varieties and silkworm strains adapted to local conditions to enhance leaf yield, stress tolerance, and cocoon output in sericulture practices in Africa (Terefe et al., 2018), although most of the evaluations remain at demonstration plot, with limited multi environment testing and comparisons across different ecological zones, and management strategies (Sujatha et al., 2024; Kumar et al., 2024; Tuigong et al., 2015). This has resulted in recommendations on rearing practices that are often generalized in all rearing regions even those where temperatures sometimes exceed 30 °C, relative humidity fluctuates and low mulberry protein to fiber ratios thus affecting the fifth instar larval physiology and general silk gland output (Mallikarjuna & Balasaraswathi, 2023; Bu et al., 2022). To optimize rearing practices for high cocoon yield and shell ratio, zone specific evidence comprising of silkworm strain and mulberry variety is therefore needed.

This study addresses this knowledge gap by evaluating the proximate composition of two widely cultivated mulberry varieties across three contrasting agro-ecological locations of Kenya. By linking environmental inclination to mulberry leaf biochemical components, the findings provide a basis for sericulture planning and optimization in potential areas under varied climatic conditions.

Materials and Methods

Study area

The study was carried out across three agro-ecological zones in Kenya's North Rift region which were selected to capture a clear gradient in temperature, humidity and elevation which are factors known to influence both mulberry nutrition for the performance of *Bombyx mori*. Site selection followed the agro-ecological zoning framework (Sombroek et al.,1982; Jaetzold et al., 2010) whose national classification system organizes landscapes according to humidity, elevation, and rainfall patterns (Figure 1). The first site located in Marigat, at the Kenya Agricultural and Livestock Research Organization (KALRO) station (0.47°N and 35.98°E on an elevation of about 1100 meters above sea level), falls within the semi-arid lowlands of Agro-ecological Zone V (Sombroek, 1982), experiences high temperatures during the day that frequently rise to between 29°C and 34°C during hotter months, low relative humidity typically 35 to 50 percent and low annual rainfall. The second site situated at about 0°34′45″N and 35°18′22″E, an elevation of about 2100 meters above sea level was situated at a farm near University of Eldoret. This location represented a sub humid mid-altitude environment classified as Agro-ecological Zone III. This is characterized by temperatures generally ranging from 21°C to 26°C, and relative humidity between 55 and 70 percent with well distributed seasonal rainfall. The third site was Kaptagat which lies at approximately 0°26′13″N and 35°27′21″E (0.43694°N, 35.45583°E), at an elevation of approximately 2400 meters above sea level, corresponding to Agro-ecological Zone II and characterized by cool temperatures ranging from 17°C to 22°C with consistently high relative humidity of 65 to 85 percent with rainfall that is evenly distributed throughout the year.

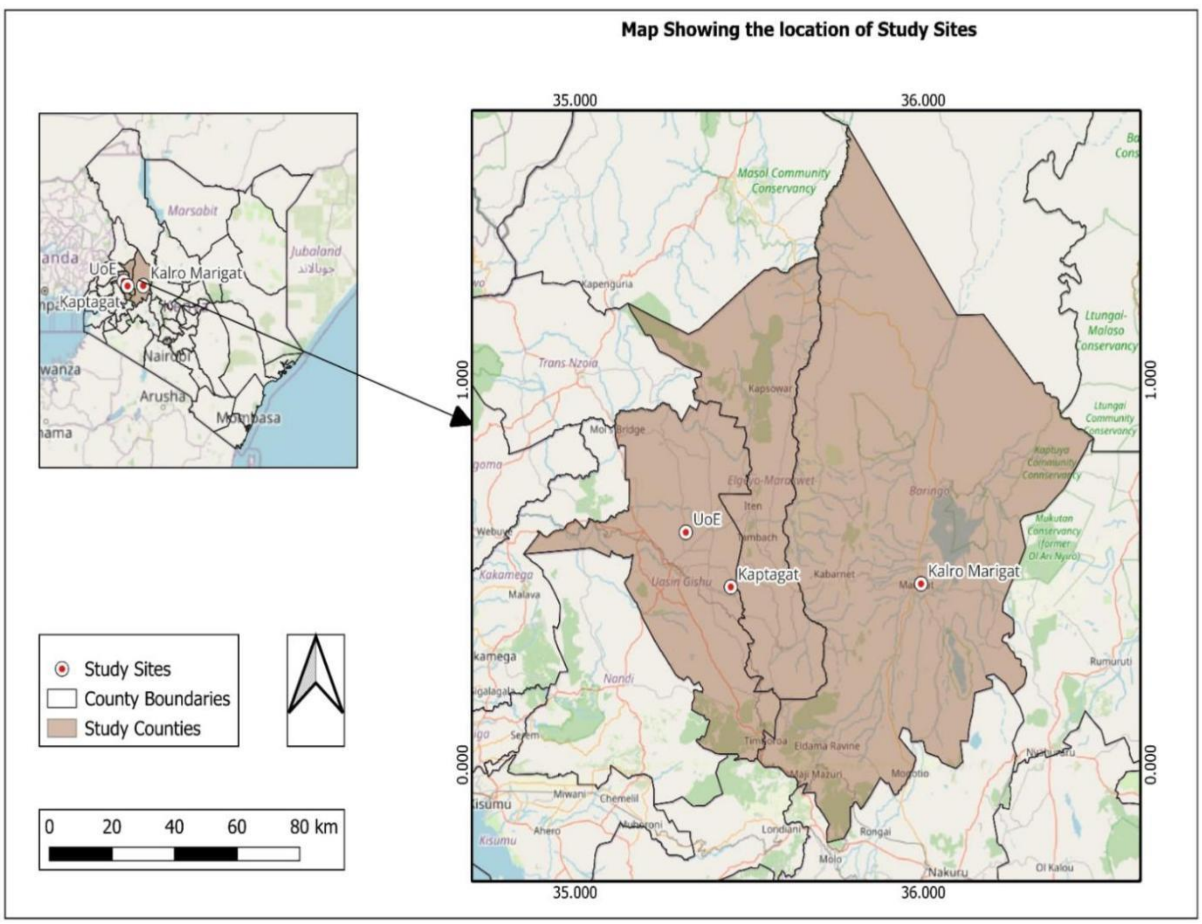

Figure 1. Geographical locations of the experimental sites

Mulberry establishment and leaf management

Two mulberry varieties, Ichinose and Kanva2, sourced from the Kenya Agricultural and Livestock Research Organization (KALRO), were selected because of their high leaf yield potential, and suitability for silkworm feeding. To minimize the effects arising from management differences across sites, both varieties were established from 10cm cuttings at a uniform spacing of 1.5 by 1.5 meters simultaneously at all three locations. The spacing adopted was as per Agronomic advice of KARLO and planting was done at the start of the rainy season.
Soil fertility management was standardized across the sites by applying well decomposed compost manure at the rate of four tonnes per acre and two spoonful of compound fertilizer nitrogen phosphorus potassium (N–P–K) fertilizer per hole. Average daily temperature, relative humidity and precipitation were recorded and their monthly means calculated for 8 months, to coincide with the maturity period of mulberry leaves to feed the silkworm (Rahmathulla, 2012). Recommended agronomic practices for the crop establishment and management were followed (Offor et al., 2014) from the end of eighth month though Marigat mulberry trees had more leaves compared to Kaptagat for results. Temperature and humidity were recorded using digital thermohygrometer smart Thermopro make TP359 and rainfall was measured using a rain gauge model CoCoRaHS.

Mulberry leaf sample collection

Mulberry leaf samples were collected by handpicking and put in a woven basket concurrently to assess nutritional composition and assess the environment influence. Leaves were harvested and processed following standardized protocols for determining moisture, crude protein, crude fiber, ash, and lipid content using the procedure described by Offor et al., (2014).

Mulberry leaf proximate nutritional composition analysis

The proximate composition analysis was conducted for Ichinose and Kanva2 leaves sampled from all study sites. Leaf sampling was done early in the morning between 8 a.m. to 9 a.m. to minimize diurnal moisture variation. Only fully grown, disease free 3^{rd},4^{th} and 5^{th} leaves from the trees growing in the mid-canopy were selected to ensure uniform physiological maturity. Approximately 500 g of fresh leaf material per replicate was collected and transported to the laboratory in aerated cool boxes to prevent dehydration and alteration of biochemical components.
In the laboratory, leaf samples were sorted, washed with distilled water, dried on the surface, and divided into 3 fresh sub-samples, put in a plastic tray and allowed to dry in cool conditions away from direct sunlight. After 7 days the dried samples were dried in an oven at 60°C to constant weight and ground to fine powder using a stainless steel Wiley mill (0.5 mm mesh). All analyses for proximate composition followed AOAC (2019) standard procedures. Apart from carbohydrate content which was calculated following procedure by Liang et al., 2012.

Data analysis

Data analysis was performed using R 4.5.1package vegan (R Core Team, 2025). Two-way ANOVA was used to test the effects of site and variety and their interaction on each nutrient parameter, with Tukey's HSD applied where assumptions of normality and homoscedasticity were met. Pearson correlation coefficients were used to assess relationship between environmental variables and nutrient composition. To examine multivariate structure and ecological patterns, Principal Component Analysis (PCA) was applied, and constrained ordination (RDA) was used to quantify the variance in nutrient composition as explained by environmental gradients (Legendre et al., 2012). Statistical significance was set at $p \leq 0.05$.

Results

Agro-ecological conditions of the sites
The study sites represented a clear elevation variation linked to environmental gradient. Marigat (~ 1100 m) was the hottest and driest site, University of Eldoret (UoE ~2100 m) showed moderate temperature and humidity, and Kaptagat (~2400 m) was the coolest and the most humid. The mean temperature was 29.81 ± 2.21°C in Marigat, UOE (22.79 ± 1.34°C) and Kaptagat (17.99 ± 2.37°C). Relative humidity and rainfall increased correspondingly with altitude (Table 1). This indicated significant site differences in temperature $F(2,69) = 257.37$, $p < 0.001$) and relative humidity $F(2,69) = 296.51$, $p < 0.001$). Rainfall also differed significantly among sites ($p < 0.001$). These showed a strong climate stratification driven by elevation across the study region indicating significant site × month interactions for temperature, humidity, and rainfall ($p < 0.001$).

Table 1. Spatial variation in agro-ecological conditions across the three experimental sites

Variable	Marigat (Mean ± SD)	UoE (Mean ± SD)	Kaptagat (Mean ± SD)	*p*-value
Temperature (°C)	29.81 ± 2.21[a]	22.79 ± 1.34[b]	17.99 ± 2.37[c]	< 0.001
Relative Humidity (%)	42.16 ± 3.01[c]	65.26 ± 5.09[b]	73.97 ± 7.21[a]	< 0.001
Rainfall (mm)	35.75 ± 17.51[c]	93.60±26.33[b]	159.39 ± 36.85[a]	< 0.001

Proximate nutritional composition of mulberry leaf varieties across sites

Mulberry leaf proximate composition varied significantly by both site and variety (Figure 2). Ichinose consistently showed higher crude protein and lower fiber, while Kanva-2 exhibited higher fiber and lipid content, particularly in the low altitude Marigat where temperatures were high. Crude protein was highest in UoE for Ichinose (8.69 ± 0.07%) and highest in Kaptagat for Kanva-2 (6.48 ± 0.16%). This showed significant effects of site to protein, moisture, lipid, carbohydrates, ash, and fiber ($p < 0.05$). Variety effects on proximate composition were prominent for fiber, lipid, and carbohydrates, while the site × variety interaction was significant for moisture, fiber, and carbohydrates, indicating that mulberry variety responded differently across environments. Environmental correlations supported these patterns with crude protein showing positive association with elevation, humidity, and rainfall, and negative association with temperature, whereas lipid and fiber showed the opposite trends.

Moisture content was highest in Kaptagat and lowest in Marigat, which reflects the effect of cooler and more humid conditions. Lipid content was greater in Kanva2 than in Ichinose, with the greatest differences observed in Marigat. Crude protein content showed a contrasting pattern, with Ichinose recording the highest protein levels in UoE, while Kanva2 had highest protein concentrations in Kaptagat. In contrast, crude fiber content was consistently higher in Kanva2 than in Ichinose across all sites, indicating that Kanva2 leaves were generally more lignified and tougher.

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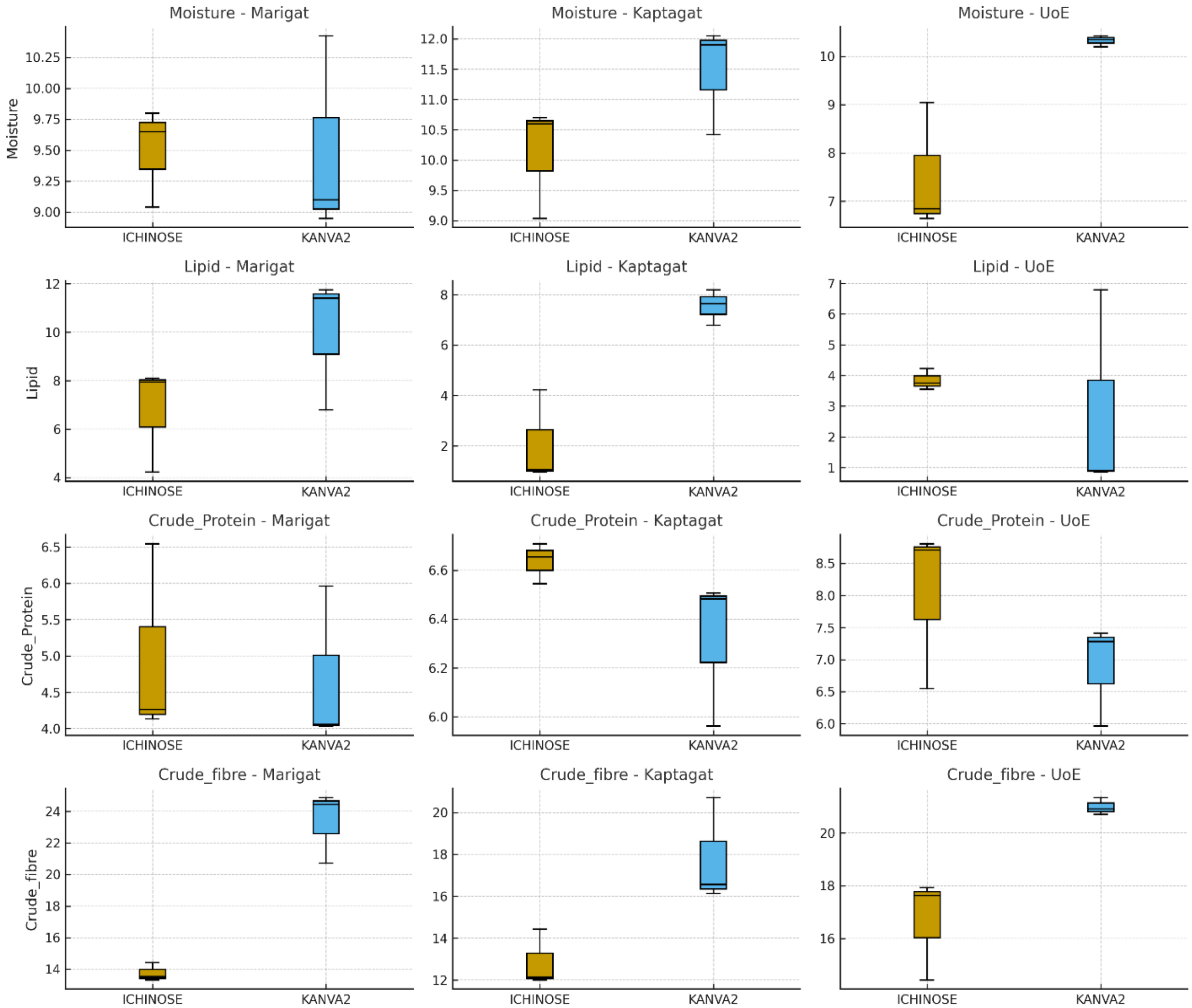

Figure 2. Box plots comparing moisture, lipid, crude protein, and crude fiber contents of mulberry leaves across agro-ecological sites and varieties.

Pearson correlation analysis revealed associations between mulberry leaf proximate composition and agro-ecological conditions across the three production sites (Figure 3). Protein content showed the strongest relationships with environmental conditions, with a significant positive correlation with elevation (r = 0.648, p = 0.0037), humidity (r = 0.627, p = 0.0054), and rainfall (r = 0.469, p = 0.049), but negatively correlated with temperature (r = –0.575, p = 0.0126).

Lipid concentration correlated positively with temperature (r = 0.502, p = 0.0337) and negatively with both elevation (r = –0.555, p = 0.0169) and humidity (r = –0.540, p = 0.0208). Fibre did not show statistically significant correlations (p > 0.05), but the coefficient signs indicated a positive association with temperature (r = 0.294, p = 0.237) and negative associations with rainfall (r = –0.338, p = 0.171) and humidity (r = –0.264, p = 0.289). Moisture content exhibited moderate positive correlations with rainfall (r = 0.395, p = 0.105) and humidity (r = 0.265, p = 0.288), although not statistically significant. Carbohydrates showed weak positive associations with humid and high elevation conditions, while ash exhibited minimal correlations with environmental conditions.

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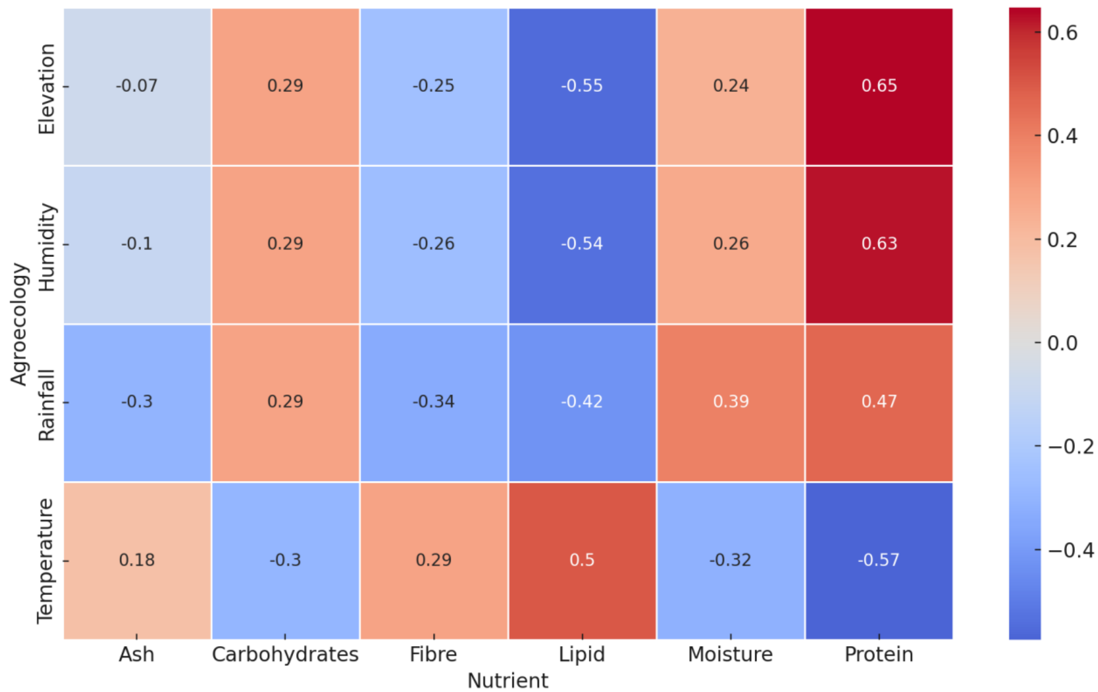

Figure 3: Correlation heat map showing Pearson correlation coefficients (r) between agro-ecological variables and mulberry leaf proximate nutrients.

The Principal Component Analysis (PCA) illustrating nutrient variation for Ichinose and Kanva-2 within Marigat, Kaptagat, and UoE showed patterns that indicate combined effects of site conditions and mulberry variety leaf nutritional quality for *Bombyx mori* rearing. The PCA (PC1 = 51.6%, PC2 = 24.6%) showed that the temperature was the main factors influencing lipid and fiber, whereas the humidity, elevation and rainfall highly influenced protein and carbohydrates, which segregated the hot and dry Marigat site from cool and more humid UoE and Kaptagat sites (Figure 4).

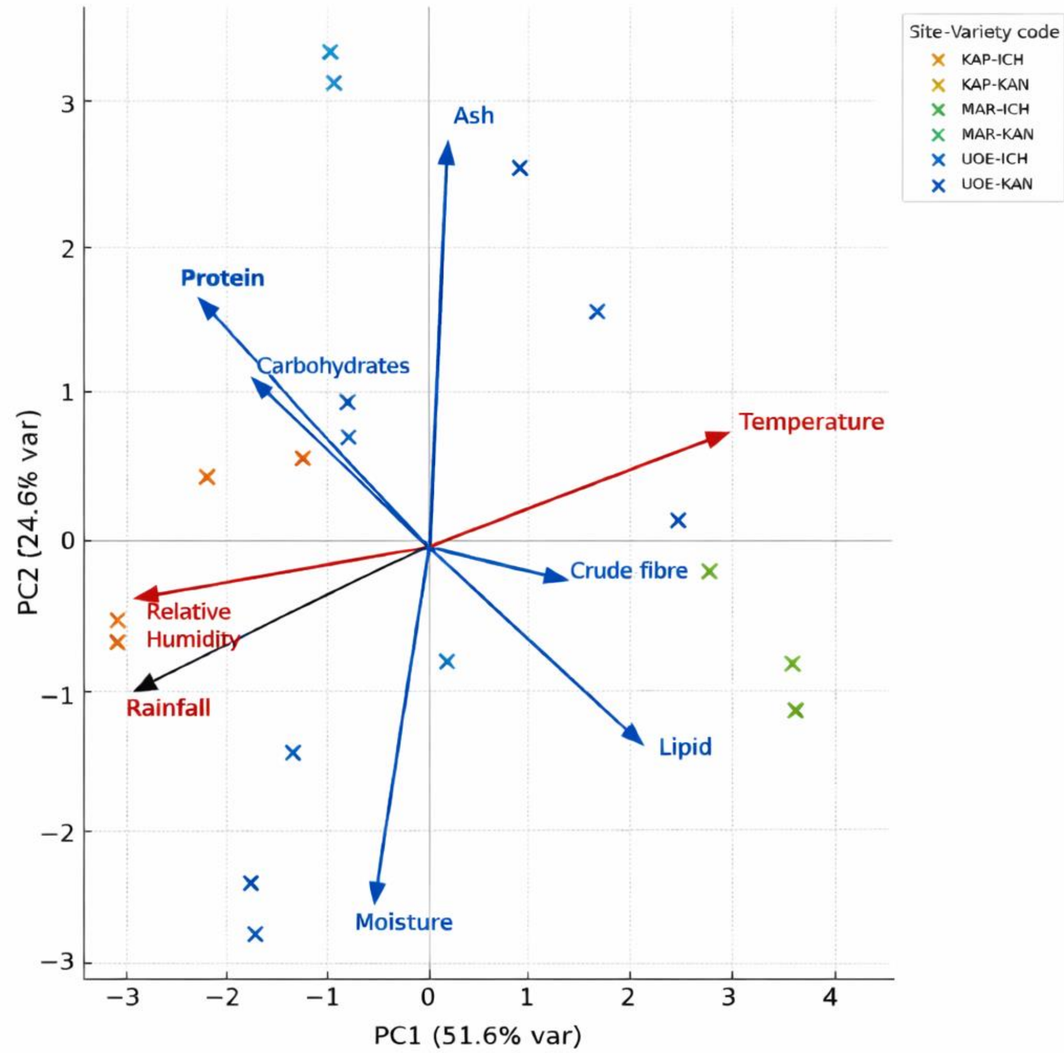

Figure 4. PCA biplot of mulberry leaf nutrients and agro-ecological variables. Arrows represent correlations of each variable with PC1 and PC2.

The Correlation-scaled Redundancy Analysis (RDA) biplot illustrating how mulberry leaf nutrients are constrained by agro-ecological variables showed that in Marigat the most prominent nutrients were the crude fibers, lipids and ash, on the other hand carbohydrates, protein and moisture were determinants in UoE and Kaptagat sites which were related to higher rainfall, humidity and higher (Figure 5) elevation.

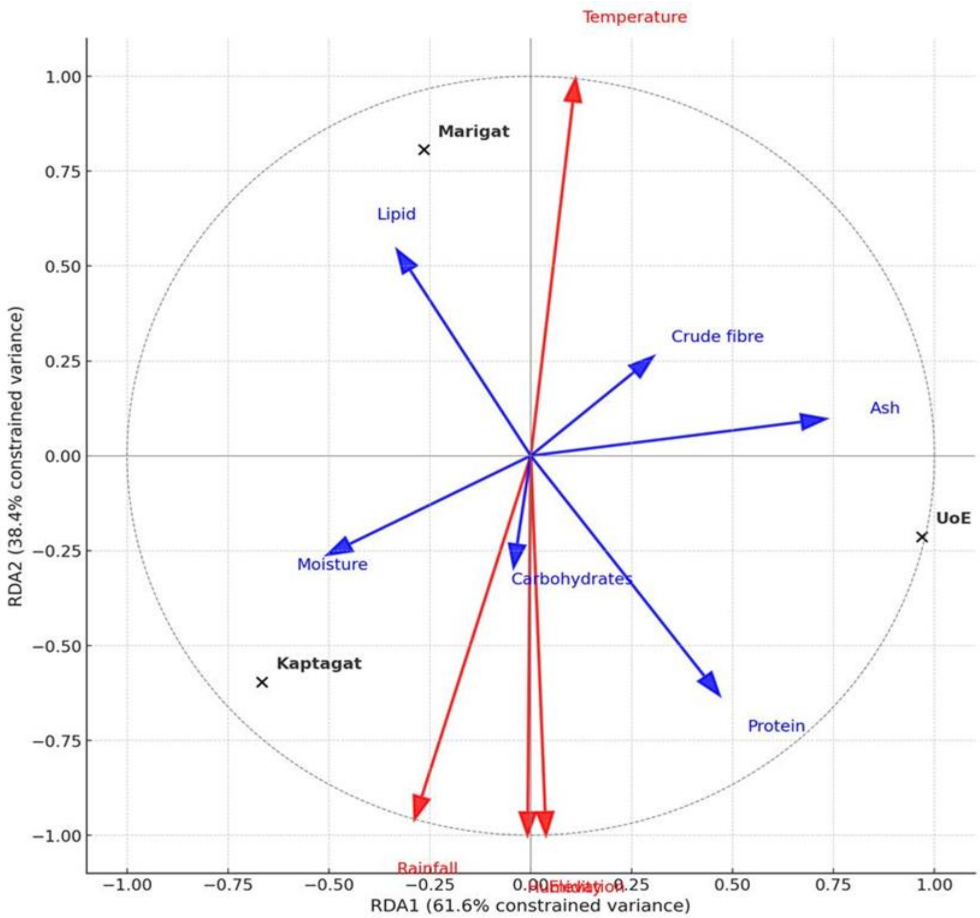

Figure 5. Constrained ordination of leaf nutrients on agro-ecological conditions (RDA1–RDA2)

Discussion

Variation of Agro-ecological conditions of the experimental sites

The agro-ecological differences observed across Marigat, UoE, and Kaptagat sites represent a gradient that is both ecologically consistent and physiologically significant. Marigat, l, consistently registered the highest mean temperatures and lowest relative humidity and rainfall values. UoE, at 2100 m, displayed intermediate environmental conditions, while Kaptagat exhibited the lowest temperatures, highest humidity and highest rainfall. The higher temperatures recorded in Marigat situated at approximately 1100 m above sea level, are consistent with the atmospheric lapse rate principle, which states that temperature decreases with increasing altitude due to reduced atmospheric pressure and density (Frierson, 2024). This temperature variation has significant physiological implications on mulberry growth. When there is increase in temperatures, the metabolic rate in plants accelerate, increasing respiratory demand thus diverting energy away from protein synthesis toward stress response pathways which in mulberry plants it manifests as reduced crude protein accumulation and increased fiber deposition. This lowers the nutritional quality of leaves available to silkworms (Liu, 2024; Bu et al., 2022; Hăbeanu et al., 2024). This has been shown by mulberry plants in lowland semi-arid zones producing leaves with reduced protein content compared to highland environments (Aleme, 2020; Sime & Siraj, 2020).

Kaptagat located 2400 m above sea level recorded high rainfall which is a characteristic of a highland environment. Rainfall is of ecological importance because it regulates soil moisture, nutrient mineralization, supports nitrogen assimilation and protein synthesis. Low rainfall in contrast induces drought stress, fiber and lipid accumulation (Sun et al., 2023; Guha et al., 2010). This agrees with the current report which established that rainy Kaptagat site produced leaves that were typically richer in protein. Studies by Terefe et al., (2018) and Tulu et al., (2022) similarly confirm that rainfall variability shapes

mulberry nutritional quality, with humid zones producing higher protein leaves and semi-arid zones producing fiber rich foliage.
The low relative humidity in Marigat accelerated leaf dehydration, increased fiber deposition, and resulted to leaves low in moisture and high in both fiber and lipids. Humidity is an important ecological factor because it directly influences leaf moisture content, regulation of stomata, and nutrient assimilation. In mulberry, high humidity supports efficient photosynthetic carbohydrate turnover and delays leaf senescence, thereby favoring protein retention and softer leaf texture that enhances digestibility for silkworm larvae. (Liu et al., 2024; Bu et al., 2022; Hăbeanu et al., 2024). However, the high humidity has been shown to predispose silkworms to microbial infections such as *Nosema bombycis* and Nuclear Polyhedrosis Virus, in Brazil, which therefore means that a need for ventilation management in humid highland rearing systems (Mandal., 2025; Chakrabarti & Manna, 2008; Tayal & Chauhan, 2017). These comparisons highlight that while high humidity generally enhances mulberry nutritional quality, it also introduces disease management challenges that must be addressed through proper husbandry practices

Proximate nutritional composition of mulberry leaf varieties across Agro-Ecological sites

The proximate nutritional composition of mulberry leaves across agro-ecological zones and between varieties revealed variation in moisture, lipid, crude protein, carbohydrates, ash, and fiber content which showed the combined influence of environmental differences and mulberry variety, with site effects generally stronger than differences in variety. These findings emphasize the importance of considering both climate and cultivar choice when evaluating mulberry quality for *Bombyx mori* rearing. Moisture content was consistently highest in the highland site of Kaptagat, intermediate in UoE, and lowest in Marigat. This pattern is consistent with the ecological principle that cooler, humid environments reduce evapotranspiration, allowing leaves to retain higher internal water content. High rainfall and relative humidity in Kaptagat sustain leaf hydration, delay cellular senescence, and support enzymatic activity necessary for nitrogen assimilation and protein synthesis. Hot and dry conditions of Marigat promote rapid evapotranspiration, leading to physiological drought stress even when soil moisture is present this causes mulberry plants to respond by thickening epidermal tissues and increasing lignification, which increases fiber and reduces leaf moisture and digestibility. González & Martín (2017) and Saini et al., (2023) reported that during rainy season leaves exhibit higher moisture and protein content compared to dry season foliage and highland mulberry leaves retained more moisture and supported superior silkworm growth.

The decline in protein content in Marigat can be explained by the sensitivity of protein metabolism to temperature. High temperatures accelerate protein catabolism, reducing accumulation in leaves (Peng et al., 2015, Li et al., 2024; Song et al., 2024). This shows that protein content is highest in humid sub-tropical zones and declines under arid climates (Saini et al., 2023; Li et al., 2024; Peng et al., 2015) which show that protein correlates positively with elevation and rainfall, and negatively with temperature (Tulu et al., 2022; Ebrahim et al., 2016; Feleke et al., 2025). This explains the high protein content in mulberry leaves grown in Kaptagat.

Variety differences were also clear, Ichinose consistent high protein to fiber ratio in all the three sites compared to Kanva2, reflects superior nutritional quality. In sericulture, this ratio is a stronger predictor of silk productivity than protein alone, as it integrates both digestible nutrient supply and indigestible structural fractions. However, even Ichinose experienced reduced ratios under Marigat conditions, indicating that environmental stress can override genetic advantages. Fiber content was highest in Marigat and lowest in Ichinose leaves across all sites. Kanva-2 consistently exhibited higher fiber levels than Ichinose, with the largest differences observed in Marigat. Fiber and lipid accumulation reflects adaptive thickening of cell walls under drought stress, increasing lignin deposition to strengthen leaf tissues because plants synthesize cell membrane structures to reduce cellular desiccation (Chandang et al., 2017; Song et al., 2024; Tuigong et al., 2015). The impact of this in silkworm is that, excessive fiber reduces digestibility and increases gut passage time, which lower feed conversion efficiency (Chaitanya et al., 2001; Ebrahim et al., 2016; Rhamathulla, 2012). While lipids are present in small amounts relative to protein and carbohydrates, they play important roles in hormone regulation and membrane synthesis. However, excessive lipid accumulation under stress conditions reduces leaf palatability and digestibility which could impact silkworms.

Multivariate associations between proximate nutritional composition of mulberry leaves and agro-ecological gradients

The clustering of Ichinose samples with high protein environments and Kanva2 samples with fiber rich profiles confirms genotype to environment interactions similar to Saini et al, (2023), Manjunatha et al., (2020) and Kumar et al., (2024) PCA studies that showed protein clustering with humid, high elevation conditions, while fiber aligned with semi-arid zones. Fiber deposition increases under drought, reducing feed conversion ratio, which collaborates with current observations (Sharma et al., 2023; Bista et al., 2008; Banjara, 2022). In the RDA biplot fiber further showed weaker associations but still clustered toward hotter, drier conditions indicating that mulberry nutritional quality depends on environment

Conclusion

Moisture, lipid, carbohydrate, and fiber showed significant contributions from both site and variety, while protein and ash content were driven by site. This shows that proximate nutritional composition of mulberry leaves is shaped by both environmental and variety differences, with site effects being stronger than varietal differences.

Acknowledgements

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