



## Research article

## Nutritional composition and cannabinoid concentrations of hemp leaf hay during 5 mth of storage

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### Abstract

**Importance of the work:** The leaves of hemp (*Cannabis sativa* L.) obtained as a by-product after fiber harvest represent a potential conserved-feed ingredient for ruminants; however, information remains limited on the changes in nutritive value and cannabinoid profiles during hay storage.

**Objectives:** To assess the nutritional value and cannabinoid content of hemp leaf hay during different storage durations compared to leucaena.

**Materials and Methods:** Hemp leaves and leucaena leaves were harvested, sun-dried and stored for 0, 1, 2, 3, 4 and 5 mth. Proximate composition and fiber fractions were analyzed using standard methods, while cannabinoids were quantified using high-performance liquid chromatography with ultraviolet detection.

**Results:** Initially, the hemp leaf hay contained 23.89% crude protein (CP), which declined significantly to 10.06% after 5 mth of storage. In contrast, the detergent fiber fractions and ash content remained statistically unchanged over storage (neutral detergent fiber, 42.97%; ash, 12.46%). Total cannabidiol (CBD) and total  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) did not change significantly across storage durations, although slight numerical increases were observed, whereas cannabinol (CBN) increased significantly (2.05–2.20 mg/g at month 3), consistent with oxidative conversion of THC to CBN under ambient storage. Compared with leucaena, the hemp leaves had lower CP and total fiber contents but higher levels of hemicellulose and ash. Correlation analysis showed that CBD was strongly correlated with CBN, while  $\Delta^9$ -THC was significantly correlated with CBN, suggesting possible co-variation among major cannabinoids during storage.

**Main finding:** The hemp leaf hay maintained statistically unchanged total CBD and total THC contents during 1–5 mth of storage, despite slight numerical increases, whereas CBN increased and CP declined markedly. Therefore, early utilization of the hay is recommended to preserve protein value. Hemp leaf hay may be used as a complementary feed ingredient for ruminants under farm-like storage conditions.

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## Introduction

The production of conserved forages, such as hay from agricultural residues, is a common practice in the livestock industry due to their extended shelf life and ease of handling, particularly during periods of fresh forage scarcity (Sandström et al., 2022). The leaves from hemp (*Cannabis sativa* L.), generated as a by-product after fiber harvest, represent a potentially valuable roughage resource for ruminant feeding systems. Often, the leafy fraction is retained during fiber-oriented production and processing and it may be utilized as hay owing to its relatively favorable nutritional characteristics (protein and structural carbohydrates) compared to the more lignified fractions. In addition, hemp leaves contain biologically active cannabinoids such as cannabidiol (CBD),  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), cannabigerol (CBG) and cannabinol (CBN), which necessitate compositional characterization to support safe and evidence-based use in ruminant diets (Kleinhenz et al., 2020; Vastolo et al., 2021).

Studies have reported that hemp leaves contained higher crude protein than the stems or other co-product fractions; furthermore, their fiber may be comparatively more digestible, supporting their consideration as an alternative protein- and fiber-containing ingredient for ruminants (Kleinhenz et al., 2020). When incorporated at appropriate levels, hemp leaves may contribute as a functional energy and protein source without apparent adverse effects (Della Rocca and Di Salvo, 2020). Furthermore, Vastolo et al. (2021) indicated that hemp leaves and related by-products provided substantial crude protein along with digestible fiber, with potential implications for rumen fermentation outcomes.

From a ruminant nutrition standpoint, interpretation of forage feeding value requires a clear understanding of plant cell wall composition, commonly quantified using the detergent fiber system (Van Soest et al., 1991). Neutral detergent fiber (NDF) broadly represents total cell wall constituents and is closely related to intake potential, whereas acid detergent fiber (ADF) and lignin are associated with reduced digestibility due to higher proportions of cellulose linked to lignification (Van Soest et al., 1991). In practical ration formulation, meeting fiber requirements relies not only on the concentration of NDF but also on its effectiveness in stimulating chewing activity and maintaining ruminal function (Mertens, 1997). Therefore, tracking NDF, ADF and lignin during storage is essential for interpreting changes in nutritive value and for predicting animal responses. Despite these advantages, information remains

scarce on the long-term stability of nutritional and cannabinoid profiles in hemp leaf hay. Prolonged storage may lead to the degradation of nutrients and phytochemicals through oxidation and storage-related heating effects, potentially decreasing crude protein and increasing apparent structural fiber fractions. Ribeiro et al. (2024) reported that extended storage of stargrass hay reduced ethanol-soluble carbohydrates while increasing the structural fiber components, including ADF and NDF. Similarly, Coblenz et al. (1996) observed that high-moisture alfalfa hay accumulated heat during storage, resulting in losses of water-soluble nutrients and elevated lignin levels. In addition, Feyissa et al. (2014) confirmed that extended storage of natural pasture hay increased fiber fractions (NDF, ADF, lignin) while decreasing protein content.

Regarding cannabinoid stability, cannabinoids can undergo time-dependent transformations during storage, particularly under conditions of heat, light and oxygen exposure. Grafström et al. (2019) reported that degradation of  $\Delta^9$ -THC was reduced under low-temperature and dark conditions, whereas exposure to heat and light accelerated conversion to CBN. Likewise, Zamengo et al. (2019) highlighted temperature, light and oxygen as critical factors influencing cannabinoid stability. However, studies remain limited specifically examining cannabinoid interrelationships in stored hemp leaf hay, even though such relationships can provide practical insight into degradation pathways and inform storage management for hemp-based feed materials.

Although hemp leaves obtained as a by-product after fiber harvest show promise for hay production, detailed information is still lacking on how storage duration influences nutritional composition—particularly plant cell wall fractions—and cannabinoid stability. Therefore, this study aimed to: 1) evaluate changes in nutritional composition, with emphasis on plant cell wall components (NDF, ADF, lignin), of hemp leaf hay across storage durations; 2) quantify major cannabinoids during storage; and 3) analyze the interrelationships among individual cannabinoids. The findings should support effective utilization and storage management of hemp leaf hay in ruminant feeding systems.

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## Materials and Methods

### *Hemp material acquisition and study site*

The research team coordinated with Thai Federal Hemp Corporation, a legally licensed hemp producer in Chiang Mai

Province, Thailand, to procure whole hemp plants for this study. The hemp cultivar used was accession 14-2, harvested at the flowering stage (approximately 120–130 d after sowing). All samples were obtained from a single production lot collected from one site in one growing season to minimize variability in plant material and to ensure consistency across storage treatments. The crop was cultivated by local farmers at the Doi Pui Research Station, Su Thep Subdistrict, Mueang Chiang Mai District, Chiang Mai Province, Thailand (18°48'40.4"N, 98°52'58.6"E; approximately 1,272 m above sea level). The soil at the site belongs to the Doi Pui series, characterized by deep, friable red loam with high fertility due to elevated organic matter content. The region receives an average annual rainfall of approximately 1,600 mm and has a mean annual temperature of approximately 16°C.

After harvest, representative whole-plant samples were collected randomly across the field within the same lot to form a composite sample representative of that production lot. The harvested material was transported to the Department of Agronomy Laboratory, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom Province, Thailand, for further processing and analysis.

#### *Plant sample preparation and experimental design*

The experiment was arranged in a completely randomized design (CRD) with three replications. Six storage durations of hemp leaf hay were evaluated after drying for 0 mth, 1 mth, 2 mth, 3 mth, 4 mth and 5 mth. Additionally, dried leaves of leucaena (*Leucaena leucocephala* (Lam.) de Wit), a common hay material produced by local farmers, were included as a comparative treatment. The hemp leaves used were derived from legally certified hemp fields in Chiang Mai Province, Thailand cultivated under official authorization using certified seeds.

After the composite hemp sample had been prepared, the dried material was subdivided into separate mesh-bag sample units assigned to each storage duration (0 mth, 1 mth, 2 mth, 3 mth, 4 mth, 5 mth). Therefore, each storage time point was analyzed from a separately stored sample unit rather than by repeated subsampling from a single bag over time.

For sample preparation, the hemp leaves were sun-dried for 3–4 d, with daily turning to ensure uniform drying. When the moisture content was below 15%, the samples were checked by hand-twisting, with leaves that showed no sign of residual moisture being considered sufficiently dry. Then, the dried leaves were packed in mesh bags and stored in

a well-ventilated shed for the designated storage periods (0–5 mth). Temperature, relative humidity and light exposure inside the storage shed were not continuously monitored during the storage period. Therefore, direct shed microclimate data were unavailable, which is acknowledged as a limitation of the study. The temperature and relative humidity ranges reported here represent general ambient environmental conditions during the storage period and are provided only as contextual background and do not represent direct measurements inside the storage shed. Ambient temperatures during storage were in the approximate range 26–30°C, with daily fluctuations in the range 23–36°C, while the relative humidity was in the range 66–80%.

At each storage time point (0 mth, 1 mth, 2 mth, 3 mth, 4 mth, 5 mth), the samples were oven-dried at 95°C for 72 hr using a hot air oven. This drying protocol was used to standardize sample preparation before chemical analyses; however, it may have affected heat-labile constituents and is therefore acknowledged as a methodological limitation of the study. In particular, prolonged heating at this temperature may have promoted thermal decarboxylation of acidic cannabinoids and may also have altered protein fractions through heat-related reactions. After drying, the samples were weighed to determine their dry matter concentration and moisture content. Then, the dried leaves were ground using a hammer mill fitted with a 1 mm sieve.

This standardized preparation process ensured uniformity of sample quality for evaluating the effects of storage duration on the nutritional composition of hemp leaf hay. The same procedure was applied to the leucaena hay samples used as the reference forage.

#### *Nutritional and chemical composition analysis*

The dried forage samples were collected randomly from each treatment and storage duration (0 mth, 1 mth, 2 mth, 3 mth, 4 mth, 5 mth) for the analysis of nutritional and chemical composition relevant to animal feed. The parameters analyzed were: crude protein (CP), ash content, neutral detergent fiber content (NDF), acid detergent fiber content (ADF) and acid detergent lignin content (ADL). From these, the cellulose, hemicellulose and lignin contents were calculated.

CP was determined using the Kjeldahl method based on analyzing the nitrogen content (%N), which was then multiplied by the standard conversion factor of 6.25. The ash content was determined following the method of Association of Official Analytical Chemists (1990) based on incinerating

samples at high temperature until all organic matter had been removed.

Fiber fractions (NDF, ADF and ADL) were determined using the sequential detergent analysis procedure with a model 200 Fiber Analyzer (ANKOM Technology Corp.; Fairport, NY, USA), following the Van Soest fiber fractionation system (Van Soest et al., 1991). Briefly, NDF was analyzed first to remove non-fiber components and to quantify total cell wall constituents (hemicellulose, cellulose and lignin), followed by ADF to quantify the acid detergent fraction (cellulose and lignin) and ADL to quantify the acid-insoluble lignin fraction. Accordingly, hemicellulose and cellulose were calculated as NDF – ADF and ADF – ADL, respectively. Acid detergent lignin (ADL) is an operationally defined fraction and does not represent total lignin; it refers to the acid-insoluble lignin fraction determined by the Van Soest method and, while potentially underestimating total lignin, remains a widely accepted indicator for forage evaluation (Van Soest et al., 1991; Jung et al., 1997, 1999).

These chemical analyses were conducted to evaluate changes in the nutritional quality and composition of the hemp leaf hay across the different storage durations and to compare the findings with those of the leucaena hay used as a reference forage.

### *Cannabinoid analysis*

Cannabinoid concentrations were determined in randomly selected hemp leaf hay samples after storage periods of 0 mth, 1 mth, 2 mth, 3 mth, 4 mth and 5 mth, with two replicates per storage period. The use of two replicates for cannabinoid analysis was due to analytical resource limitations, including instrument time and the cost associated with high-performance liquid chromatography with ultraviolet detection (HPLC-UV) determination. This lower replication level, relative to the three replicates used for nutritional analyses, is acknowledged as a limitation of the study. Whole hemp leaves, including the petioles, were ground into fine powder before analysis.

Sample preparation began by mixing 200 mg of ground hemp leaf powder with 25 mL of methanol, followed by ultrasonic extraction for 15 min. Then, the extract was centrifuged at 4,500 revolutions per minute for 2 min and subsequently passed through a 0.22  $\mu$ m nylon membrane filter. The resulting filtrate was transferred into 2 mL HPLC vials for analysis. Quantification was performed using HPLC-UV following the Association of Official Analytical Chemists Official Method 2018.10 using external standard calibration

(AOAC International, 2018). Based on method sensitivity and published validations under similar analytical conditions, the estimated limit of detection and the limit of quantification (LOQ) were approximately 0.001–0.01 mg/g dry matter and 0.003–0.03 mg/g dry matter, respectively. Compounds reported as not detected were below the LOQ.

In total, 11 cannabinoids were identified and quantified: cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidiol (CBD), tetrahydrocannabivarin (THCV), cannabinol (CBN),  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC),  $\Delta^8$ -tetrahydrocannabinol ( $\Delta^8$ -THC), cannabichromene (CBC) and tetrahydrocannabinolic acid (THCA). Certified cannabinoid standards were used to generate calibration curves and concentrations were calculated based on peak areas. The results were expressed as milligrams per gram of dry plant weight (mg/g DW) to ensure standardization and to allow comparisons across storage treatments and time points.

### *Statistical analysis*

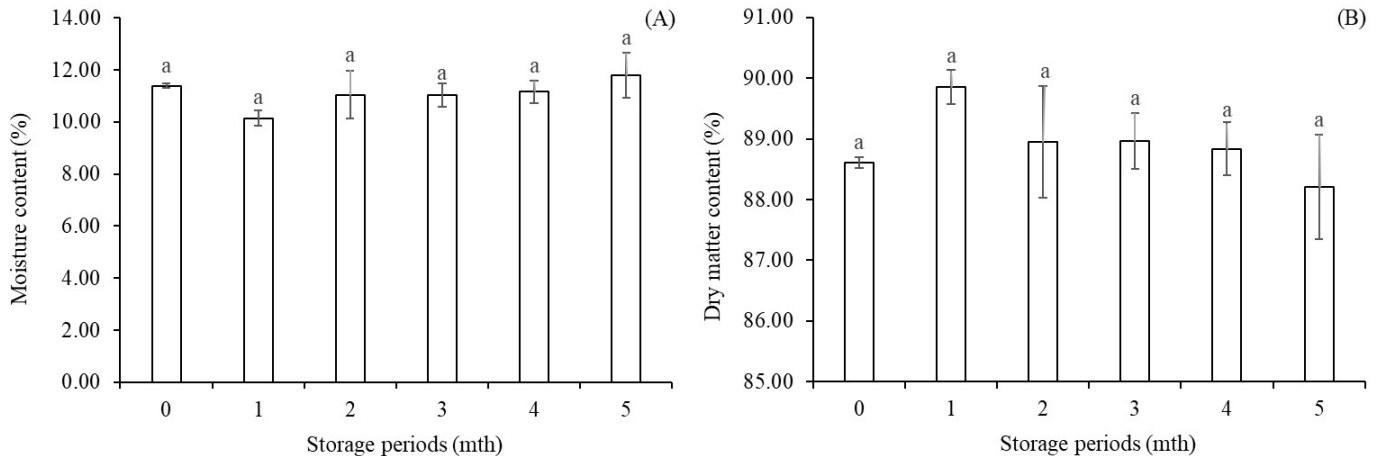
Experimental data were subjected to analysis of variance based on a CRD. Treatment means were compared using the least significant difference (LSD) test to evaluate differences among storage durations. A paired *t*-test was used to compare hemp leaf hay and leucaena hay parameters. Results were reported as mean  $\pm$  SD values. Additionally, Pearson's correlation coefficients were calculated to assess the relationships among individual cannabinoid concentrations in the hemp leaf hay. Because this analysis was based on only 10 observations (two replicates across five storage time points), the correlation analysis was considered exploratory and was interpreted with caution.

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## **Results**

### *Chemical composition and nutritional quality of hemp leaf hay*

The analysis of variance revealed no significant differences in the moisture content and dry matter across storage durations at the 95% confidence level (Figs. 1a and 1b). The approximate moisture content range was 10–12%, showing only minor fluctuations over time without statistical significance. The error bars ( $\pm$  SD) indicated similar variability throughout the storage period. Based on these results, the moisture content of hemp leaf hay remained stable during the studied storage period.



**Fig. 1** Hemp leaf hay across different storage periods: (A) moisture content; (B) dry matter content, where error bars represent mean  $\pm$  SD values ( $n = 3$ ), different lowercase letters above columns indicate significant differences among storage periods based on least significant difference test at  $p < 0.05$ , with columns sharing the same letter not significantly different

As shown in Table 1, the chemical composition and nutritional parameters of the hemp leaf hay were mostly unaffected by storage duration. There were no significant changes ( $p > 0.05$ ) in the NDF, ADF, hemicellulose, cellulose, lignin, or ash contents across the six storage time points. The average NDF content was 42.97%, ranging from 42.11% at month 0 to a peak of 43.41% at month 2, though this variation was not statistically significant. The ADF content averaged 27.99%, with only slight, not-significant fluctuations during storage.

The hemicellulose content averaged 14.98%, with a slight increase at month 2 (15.95%) and month 4 (15.90%). The cellulose content remained consistent, averaging 12.17%, with no significant differences across storage periods. The lignin and ash contents averaged 3.37% and 12.46%, respectively, with stable values throughout the study.

In contrast, the CP content was highly significantly ( $p < 0.01$ ) affected by storage duration. The highest CP value was recorded at month 0 (23.89%), followed by a sharp decline at month 1 (14.78%) and a continued decrease to the lowest value of 10.06% at month 5. These findings suggested that the major loss of CP may have occurred early, during drying and the initial storage phase, whereas the other chemical components, such as fiber fractions and minerals, remained relatively stable throughout storage.

These findings indicated that extended storage had a significant impact on the degradation of crude protein in hemp leaf hay, while other chemical components, such as fiber fractions and minerals, remained relatively stable. This suggested that although the structural and mineral composition of hemp leaf hay was resilient during storage, the protein content may decline substantially over time.

**Table 1** Chemical composition and nutritional value of hemp leaf hay under various storage periods

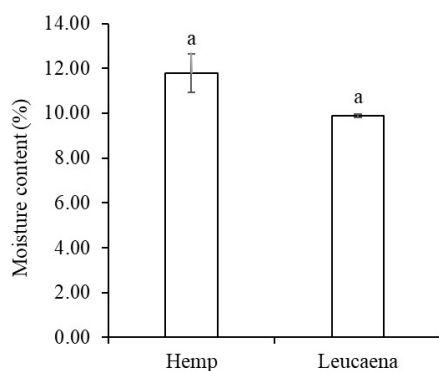
| Storage Period (mth)s | NDF (% DM)       | ADF (% DM)       | Hemicellulose (% DM) | Cellulose (% DM) | Lignin (% DM)   | Ash (% DM)       | CP (% DM)                      |
|-----------------------|------------------|------------------|----------------------|------------------|-----------------|------------------|--------------------------------|
| 0                     | 42.11 $\pm$ 0.53 | 28.77 $\pm$ 0.92 | 13.34 $\pm$ 1.39     | 13.02 $\pm$ 0.55 | 3.34 $\pm$ 0.44 | 12.41 $\pm$ 0.45 | 23.89 $\pm$ 0.92 <sup>a</sup>  |
| 1                     | 43.15 $\pm$ 0.33 | 28.95 $\pm$ 0.33 | 14.2 $\pm$ 0.36      | 12.90 $\pm$ 0.44 | 3.35 $\pm$ 0.13 | 12.70 $\pm$ 0.20 | 14.78 $\pm$ 0.74 <sup>b</sup>  |
| 2                     | 43.41 $\pm$ 0.52 | 27.46 $\pm$ 0.20 | 15.95 $\pm$ 0.39     | 11.36 $\pm$ 0.29 | 3.67 $\pm$ 0.43 | 12.43 $\pm$ 0.33 | 13.05 $\pm$ 1.15 <sup>c</sup>  |
| 3                     | 42.96 $\pm$ 1.69 | 27.47 $\pm$ 0.11 | 15.49 $\pm$ 1.67     | 11.70 $\pm$ 0.08 | 3.37 $\pm$ 0.07 | 12.41 $\pm$ 0.06 | 11.75 $\pm$ 0.77 <sup>cd</sup> |
| 4                     | 43.21 $\pm$ 0.31 | 27.31 $\pm$ 0.31 | 15.90 $\pm$ 0.59     | 11.85 $\pm$ 0.19 | 3.10 $\pm$ 0.19 | 12.36 $\pm$ 0.23 | 10.88 $\pm$ 0.17 <sup>de</sup> |
| 5                     | 42.55 $\pm$ 0.86 | 27.30 $\pm$ 1.38 | 15.24 $\pm$ 0.52     | 11.99 $\pm$ 1.49 | 3.03 $\pm$ 0.36 | 12.29 $\pm$ 0.15 | 10.06 $\pm$ 0.49 <sup>e</sup>  |
| <i>p</i> value        | 0.67             | 0.11             | 0.11                 | 0.16             | 0.40            | 0.75             | <0.001                         |
| Mean                  | 42.97            | 27.99            | 14.98                | 12.17            | 3.37            | 12.46            | 14.87                          |
| CV                    | 2.44             | 3.12             | 7.90                 | 6.96             | 11.32           | 2.65             | 6.71                           |

NDF = neutral detergent fiber content, ADF = acid detergent fiber content, CP = crude protein content, DM = dry matter content.

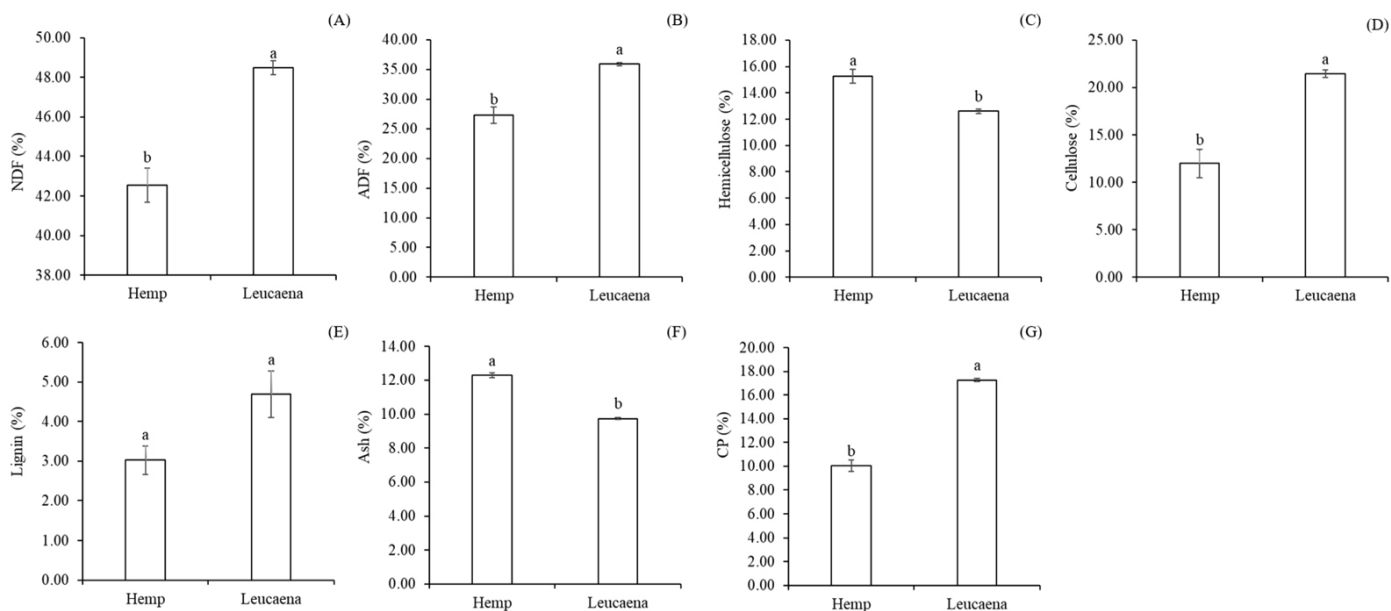
Mean  $\pm$  SD within the same column followed by different lowercase letters are significantly different based on least significant difference test ( $p < 0.05$ ).

### Comparative nutritional value of hemp leaf hay and leucaena leaf hay

The comparison of nutritional values between the hemp leaf hay and leucaena leaf hay using a paired *t*-test revealed significant differences in nearly all parameters, except for the moisture, dry matter and lignin contents, which were not significantly different. Fig. 2 presents the comparison of moisture after 5 mth of storage. The moisture content of the hemp leaf hay was approximately 12%, compared to 10% in the leucaena hay. The error bars ( $\pm$  SD) indicated slightly higher variability in the hemp than in the leucaena. Based on these results, the hay maintained comparable moisture levels after 5 mth of storage, with no significant differences.



**Fig. 2** Comparison of moisture content between hemp and leucaena leaf hay after 5 mth of storage, where error bars represent mean  $\pm$  SD ( $n = 3$ ). The same lowercase letters above columns indicate non-significant differences between hay types based on paired *t*-test ( $p > 0.05$ ).



**Fig. 3** Comparison between hemp and leucaena leaf hay after 5 mth of storage: (A) neutral detergent fiber (NDF); (B) acid detergent fiber (ADF); (C) hemicellulose; (D) cellulose; (E) lignin; (F) ash content; (G) CP content. Bars and error bars represent mean and  $\pm$  SD, respectively ( $n = 3$ ). Different lowercase letters above bars indicate significant differences between hay types based on paired *t*-test ( $p < 0.05$ ).

Fig. 3 compares the chemical composition of the hemp and leucaena hay after 5 mth of storage. The leucaena hay had significantly higher values for most parameters. The NDF and ADF contents (Figs. 3a and 3b, respectively) in the leucaena hay were approximately 48% and 35%, respectively, compared to 43% and 28%, respectively, in the hemp. The cellulose content (Fig. 3d) in the leucaena reached approximately 20%, which was significantly higher than the 12% found in the hemp. The lignin content (Fig. 3e) was slightly higher in the leucaena (about 5%) than in the hemp (about 3%), though the difference was not significant.

Notably, the hemp leaf hay had a higher hemicellulose content (Fig. 3c), averaging around 15%, compared to 13% in leucaena. In addition, the ash content (Fig. 3f) was greater in the hemp (12%) than in the leucaena (10%), while the CP content (Fig. 3g) was significantly higher in the leucaena hay (18%) than in the hemp (14%). The error bars ( $\pm$  SD) for all variables indicated low variability, with the leucaena samples having slightly more consistent values.

Overall, the leucaena leaf hay had higher nutritional quality than the hemp leaf hay after 5 mth of storage, particularly in the contents of the fiber fractions, cellulose and CP, indicating its superior potential as a high-quality ruminant feed.

### Cannabinoid content in hemp leaf hay

As presented in Table 2, various cannabinoids were detected in the hemp leaf hay stored for different durations. The main detected compounds were: cannabidiolic acid (CBDA), cannabidiol (CBD), cannabinol (CBN),  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), tetrahydrocannabinolic acid (THCA), cannabichromene (CBC) and the total concentrations of CBD and THC. However, certain cannabinoids—cannabidivarin (CBDV), cannabigerolic acid (CBGA), cannabigerol (CBG), tetrahydrocannabivarin (THCV) and  $\Delta^8$ -tetrahydrocannabinol ( $\Delta^8$ -THC)—were not detected throughout the storage period.

The CBDA content was in the range 1.25–1.37 mg/g, with no significant differences observed across storage times. Similarly, the CBD content remained statistically unchanged (4.02–4.61 mg/g). The CBN content showed a slight but statistically significant difference ( $p < 0.05$ ), peaking at 2.20 mg/g in month 3, compared to 2.05 mg/g in month 1. The  $\Delta^9$ -THC levels were in the range 3.43–3.67 mg/g, without any significant variation. The THCA and CBC concentrations were in the ranges 1.78–1.92 mg/g and 1.09–1.13 mg/g, respectively, with no significant changes over time. The average total CBD content was 5.49 mg/g DW (0.549% DW), while the total THC averaged 5.15 mg/g DW (0.515% DW), with no significant differences across the storage durations. These total cannabinoid values were calculated using the standard conversion formulas from their acidic precursors. The similar levels of total CBD and total THC observed in this study reflected the measured cannabinoid profile of the hemp leaf samples.

Based on these results, the total concentrations of CBD and THC in the hemp leaf hay remained statistically unchanged during the 1–5-mth storage period, although slight numerical increases were observed. In contrast, the CBN content increased significantly, suggesting that individual cannabinoids may still have transformed during storage, consistent with oxidative degradation pathways under the given storage conditions.

**Table 2** Cannabinoid contents of hemp leaf hay held for various storage periods.

| Storage period (mth) | CBDV (mg/g) | CBDA (mg/g) | CBGA (mg/g) | CBG (mg/g) | CBD (mg/g) | THCV (mg/g) | CBN (mg/g)               | $\Delta^9$ -THC (mg/g) | $\Delta^8$ -THC (mg/g) | THCA (mg/g) | CBC (mg/g) | Total CBD (mg/g) | Total THC (mg/g) |
|----------------------|-------------|-------------|-------------|------------|------------|-------------|--------------------------|------------------------|------------------------|-------------|------------|------------------|------------------|
| 1                    | ND          | 1.28±0.06   | ND          | ND         | 4.02±0.01  | ND          | 2.05±0.01 <sup>c</sup>   | 3.43±0.08              | ND                     | 1.80±0.05   | 1.11±0.01  | 5.14±0.05        | 5.00±0.12        |
| 2                    | ND          | 1.25±0.05   | ND          | ND         | 4.18±0.11  | ND          | 2.09±0.0 <sup>b</sup>    | 3.46±0.02              | ND                     | 1.92±0.17   | 1.09±0.00  | 5.28±0.15        | 5.14±0.12        |
| 3                    | ND          | 1.25±0.02   | ND          | ND         | 4.56±0.40  | ND          | 2.20±0.06 <sup>a</sup>   | 3.54±0.09              | ND                     | 1.84±0.06   | 1.10±0.03  | 5.65±0.41        | 5.15±0.13        |
| 4                    | ND          | 1.29±0.05   | ND          | ND         | 4.44±0.30  | ND          | 2.12±0.01 <sup>abc</sup> | 3.63±0.05              | ND                     | 1.78±0.02   | 1.11±0.00  | 5.58±0.26        | 5.19±0.07        |
| 5                    | ND          | 1.37±0.01   | ND          | ND         | 4.61±0.18  | ND          | 2.18±0.0 <sup>ab</sup>   | 3.67±0.00              | ND                     | 1.81±0.00   | 1.13±0.02  | 5.81±0.18        | 5.26±0.00        |
| <i>p</i> value       | NA          | 0.40        | NA          | NA         | 0.44       | NA          | 0.04                     | 0.10                   | NA                     | 0.79        | 0.48       | 0.39             | 0.55             |
| Mean                 | NA          | 1.29        | NA          | NA         | 4.36       | NA          | 2.13                     | 3.54                   | NA                     | 1.83        | 1.11       | 5.49             | 5.15             |
| CV                   | NA          | 4.67        | NA          | NA         | 7.82       | NA          | 1.81                     | 2.24                   | NA                     | 6.30        | 1.76       | 6.25             | 2.83             |

CBDV = cannabidivarin; CBDA = cannabidiolic acid; CBGA = cannabigerolic acid; CBG = cannabigerol; CBD = cannabidiol; THCV = tetrahydrocannabivarin; CBN = cannabinol; CBC = cannabichromene;  $\Delta^8$ -THC =  $\Delta^8$ -tetrahydrocannabinol;  $\Delta^9$ -THC =  $\Delta^9$ -tetrahydrocannabinol; THCA = tetrahydrocannabinolic acid; ND = not detected (below limit of quantification). Total CBD = CBD + (CBDA  $\times$  0.877); Total THC =  $\Delta^9$ -THC + (THCA  $\times$  0.877). All cannabinoid concentrations expressed as milligrams per gram of dry matter on a dry weight basis. Mean  $\pm$  SD within the same column followed by different lowercase letters are significantly different based on least significant difference test ( $p < 0.05$ ).

### Correlations among cannabinoid compounds in hemp leaf hay

As shown in Table 3, the Pearson's correlation coefficients ( $r$ ) among the cannabinoid concentrations in the hemp leaf hay across the different storage durations indicated several significant associations. CBD was strongly positively correlated with total CBD ( $r = 0.99, p < 0.01$ ) and also positively correlated with  $\Delta^9$ -THC ( $r = 0.76, p < 0.01$ ) and CBN ( $r = 0.80, p < 0.01$ ). CBDA showed no significant correlation with CBD ( $r = 0.21, p > 0.05$ ), but was moderately correlated with CBC ( $r = 0.63, p < 0.05$ ). CBN was significantly correlated with  $\Delta^9$ -THC ( $r = 0.67, p < 0.05$ ) and total CBD ( $r = 0.80, p < 0.01$ ), while  $\Delta^9$ -THC was positively correlated with total THC ( $r = 0.79, p < 0.01$ ) and total CBD ( $r = 0.81, p < 0.01$ ). In addition, total CBD and total THC were positively correlated ( $r = 0.83, p < 0.01$ ). Because these correlations were calculated from only 10 observations, the results should be regarded as preliminary and interpreted cautiously. Overall, based on the analysis of these data, there was possible co-variation among several cannabinoids during storage; however, these data should be treated as indicative and not subjected to deep interrogation.

Overall, based on these results, there was possible co-variation among several cannabinoids in the hemp leaf hay during storage, particularly CBD, CBN,  $\Delta^9$ -THC and the total cannabinoid groups (total CBD and total THC). However, these relationships should be interpreted with caution because the correlation analysis was based on a limited number of observations. Other cannabinoids showed weaker or not significant associations.

### Discussion

This study aimed to evaluate the nutritional composition and cannabinoid content of hemp leaf hay, assess the effects

of storage duration on these attributes and analyze correlations among individual cannabinoids. Hemp leaves (*Cannabis sativa* L.) have attracted increasing interest as a roughage-like co-product for ruminants because the leaf fraction can contribute protein and minerals while also containing bioactive compounds such as cannabinoids and terpenes (Andre et al., 2016; Della Rocca and Di Salvo, 2020). However, practical use requires careful consideration of both nutritive value and safety, particularly due to the presence of  $\Delta^9$ -THC and the potential for transfer into animal-derived products under certain feeding scenarios (European Food Safety Authority, 2011; European Food Safety Authority, 2015). A comparative analysis with leucaena leaf hay—commonly used as ruminant forage—provides a valuable context for evaluating the feeding potential of hemp leaves.

Compared with hemp leaf hay, leucaena leaf hay had a higher crude protein content and higher detergent fiber fractions (NDF and ADF), consistent with the general nutritional characteristics of leguminous forages and the relevance of cell wall fractions to intake and digestibility (Van Soest et al., 1991; Mertens, 1997). Because conventionally, the amount of cellulose is derived from detergent fiber values as ADF – ADL, accurate computation and reporting of these fractions are important for meaningful cross-study comparisons and interpretation of feeding value (Van Soest et al., 1991). The relatively higher hemicellulose and ash contents in the hemp leaf hay likely reflected the mineral-rich nature of leaf tissues and differences in structural carbohydrate composition relative to leucaena (Kleinhenz et al., 2020). From a practical feeding standpoint, the present findings suggested that leucaena may be better positioned as a primary protein forage, whereas hemp leaf hay may be considered as a complementary ingredient contributing minerals and a distinct fiber profile, provided cannabinoid-related considerations are appropriately managed.

**Table 3** Pearson's correlation coefficients among cannabinoid contents in hemp leaf hay for various storage periods ( $n = 10$ ).

|                 | CBD                | CBDA               | CBN                | $\Delta^9$ -THC    | THCA                | Total CBD          | Total THC          |
|-----------------|--------------------|--------------------|--------------------|--------------------|---------------------|--------------------|--------------------|
| CBC             | 0.38 <sup>ns</sup> | 0.63*              | 0.35 <sup>ns</sup> | 0.65*              | -0.03 <sup>ns</sup> | 0.46 <sup>ns</sup> | 0.53 <sup>ns</sup> |
| CBD             |                    | 0.21 <sup>ns</sup> | 0.80**             | 0.76**             | 0.21 <sup>ns</sup>  | 0.99**             | 0.77**             |
| CBDA            |                    |                    | 0.29 <sup>ns</sup> | 0.52 <sup>ns</sup> | 0.23 <sup>ns</sup>  | 0.35 <sup>ns</sup> | 0.58 <sup>ns</sup> |
| CBN             |                    |                    |                    | 0.67*              | 0.09 <sup>ns</sup>  | 0.80**             | 0.63*              |
| $\Delta^9$ -THC |                    |                    |                    |                    | -0.08 <sup>ns</sup> | 0.81**             | 0.79**             |
| THCA            |                    |                    |                    |                    |                     | 0.24 <sup>ns</sup> | 0.55 <sup>ns</sup> |
| Total CBD       |                    |                    |                    |                    |                     |                    | 0.83**             |

CBDA = cannabidiolic acid; CBD = cannabidiol; CBN = cannabinol; CBC = cannabichromene;  $\Delta^9$ -THC =  $\Delta^9$ -tetrahydrocannabinol; THCA = tetrahydrocannabinolic acid.

\*, \*\* = significant ( $p < 0.05$ ) and highly significant ( $p < 0.01$ ), respectively. <sup>ns</sup> = non-significant ( $p \geq 0.05$ ).

In the present study, compared with the hemp leaf hay, the leucaena leaf hay had a higher crude protein content, as well as higher NDF, ADF and cellulose contents. These differences may be explained by inherent variation in the botanical composition and structural carbohydrate characteristics between the two forage sources. Although leucaena is recognized widely as a high-quality leguminous forage, the proportions of leaf and stem tissues in the harvested material can still influence the measured fiber fractions. Therefore, the higher detergent fiber and cellulose values observed in leucaena in the present study most likely reflected the structural composition of the sampled material rather than analytical inconsistency.

A key finding of the present study was the marked decline in the CP content of the hemp leaf hay from 23.89% DM at 0 mth to 14.78% at 1 mth, followed by a continued decrease to 10.06% at 5 mth. The particularly sharp decline during 0–1 mth suggested that substantial protein-related losses may have occurred during sun-drying or the early stage of storage, or both, rather than only arising from gradual deterioration over the full storage period. In hay systems, such reductions may result from multiple interacting factors, including oxidative processes, microbial activity when drying is not completely uniform, localized heating or “hot spots,” and the physical loss of fine leaf particles that are relatively rich in nitrogenous compounds, which is consistent with reports on hay quality deterioration during storage (Coblentz et al., 1996; Feyissa et al., 2014). In addition, heat-related reactions may alter protein fractions and reduce nutritive value, even when Kjeldahl-derived CP still reflects total nitrogen (Coblentz et al., 1996). Therefore, the observed CP decline should not be attributed to a single mechanism but rather interpreted as the likely outcome of combined drying- and storage-related effects. Future studies should include additional indicators, such as neutral detergent-insoluble nitrogen, acid detergent-insoluble, or heating indices to clarify the relative contribution of these mechanisms.

Notably, the oven-drying step at 95°C for 72 hr should be considered when interpreting the present results. Although this standardized procedure allowed consistent sample preparation across storage durations, it may have influenced heat-labile constituents and therefore was a methodological limitation of the study. For protein, prolonged heating may alter protein fractions and reduce functional quality, even if the Kjeldahl-derived crude protein still reflects total nitrogen. For cannabinoids, acidic forms, such as CBDA and THCA, may undergo thermal decarboxylation during drying, potentially affecting the measured acid-to-neutral cannabinoid ratio. Because all samples were processed using the same protocol,

between-month comparisons remain informative; however, absolute values related to cannabinoid speciation and protein quality should be interpreted with caution. Future studies should consider milder drying conditions ( $\leq 40$ – $60^\circ\text{C}$  or freeze-drying) for cannabinoid-focused subsamples.

In contrast to CP, the fiber fractions (NDF, ADF, hemicellulose, lignin) remained statistically unchanged during storage. This pattern—substantial CP decline with stable detergent fiber fractions—is consistent with the relative chemical robustness of structural carbohydrates in well-dried hays, where microbial activity is limited once moisture falls below safe thresholds (Coblentz et al., 1996; Feyissa et al., 2014). In addition, it suggested that protein-related losses may have occurred without a measurable shift in bulk cell wall concentration, potentially because: 1) nitrogenous compounds were lost or transformed without substantially altering the total cell wall mass; or 2) any small increases in fiber concentration due to loss of soluble components were not large enough to be detected statistically; or 3) both of these. Nevertheless, the magnitude of the CP decline observed here was greater than would be expected for well-preserved hay, implying the contribution of handling and storage factors such as early drying losses, physical loss of fine, nitrogen-rich particles, or intermittent moisture/temperature excursions.

The hay was stored in a well-ventilated shed under ambient conditions. Temperature, relative humidity and light exposure inside the shed were not continuously monitored; therefore, direct microclimate data from the shed were unavailable, limiting mechanistic interpretation of storage effects. The meteorological information reported in the present study was provided only as contextual background to describe the general ambient environment during the storage period, not as a substitute for direct measurements inside the storage shed. These ambient conditions likely involved temperature fluctuations and some light exposure, which differed from the recommended low-temperature and dark conditions reported to better preserve cannabinoids, especially by slowing  $\Delta^9$ -THC degradation and related transformations (Grafström et al., 2019; Zamengo et al., 2019).

Regarding cannabinoids, “stability” in the present study should be interpreted specifically to mean that total CBD and total THC were statistically unchanged across 1–5 mth, even though individual cannabinoids may still have transformed during storage. In this dataset, total CBD and total THC were not significantly affected by storage time, whereas CBN increased significantly, consistent with the concept that oxidation of THC to CBN can proceed under less controlled storage conditions

(Grafström et al., 2019; Zamengo et al., 2019). Accordingly, the observed maintenance of total CBD and total THC supports practical feasibility under farm-like storage; however, it does not preclude concurrent decarboxylation of acidic cannabinoids to their neutral forms and oxidative conversion of THC to CBN.

Furthermore, the correlation analysis requires careful interpretation. Based on Table 3, CBDA was not strongly correlated with CBD ( $r = 0.21$ ,  $p > 0.05$ ), whereas CBD was very strongly correlated with CBN ( $r = 0.80$ ,  $p < 0.01$ ) and  $\Delta^9$ -THC was significantly correlated with CBN ( $r = 0.67$ ,  $p < 0.05$ ). These relationships indicated co-variation among the major cannabinoids during storage rather than a direct CBDA-CBD linkage in this dataset. The significant association between CBN and  $\Delta^9$ -THC was directionally consistent with THC oxidation to CBN, while the CBD-CBN relationship may have reflected shared co-variation patterns across samples and storage times, rather than a single-step conversion (ElSohly and Slade, 2005; Andre et al., 2016).

The correlation analysis should be interpreted with caution because it was based on only 10 observations derived from two replicates across five storage time points. Therefore, these relationships should be regarded as exploratory rather than definitive. In the present dataset, CBD was positively correlated with CBN, while  $\Delta^9$ -THC was significantly correlated with CBN, suggesting possible co-variation among the major cannabinoids during storage. However, these associations do not establish direct conversion pathways by themselves and larger datasets with greater replication will be required to confirm the robustness and biological meaning of these relationships.

Several practical implications can be drawn. First, if the objective is to retain higher protein value, the data indicated that hemp leaf hay should be utilized as early as feasible after drying, with the most substantial CP decline occurring by 1 mth. Second, if longer storage is unavoidable (up to 5 mth in the present study), rations may require protein supplementation or blending with higher-protein forages (such as leucaena) to maintain dietary CP targets, while fiber fractions appear relatively stable. Third, the stability of total CBD and total THC suggests that the functional phytochemical profile can be maintained over typical on-farm storage durations; however, use in ruminant diets should incorporate risk management, including monitoring THC-related exposure, considering regulatory guidance and avoiding feeding strategies that may increase transfer risk into milk or other animal products

(European Food Safety Authority, 2011; European Food Safety Authority, 2015). Finally, storage practice should prioritize rapid drying to a safe moisture content, minimizing temperature build-up and reducing light exposure where possible; where direct shed monitoring is not feasible, reporting nearby meteorological context can improve reproducibility and transparency.

In conclusion, the hemp leaf hay maintained relatively stable fiber fractions and mineral (ash) contents throughout the storage period, whereas the crude protein content declined sharply—particularly during 0–1 mth—and continued to decrease thereafter. Compared to the leucaena leaf hay, the hemp hay contained significantly lower contents of fiber and crude protein, but higher contents of hemicellulose and ash. Key cannabinoids showed different patterns during storage, with total cannabidiol (CBD) and total tetrahydrocannabinol (THC) remaining statistically unchanged during 1–5 mth, whereas cannabinol increased, consistent with likely oxidative pathways under ambient storage. Overall, the hemp leaf hay may be considered a complementary ruminant feed ingredient, with its overall contents of total CBD and total THC being maintained during storage. However, its practical adoption should account for: 1) protein losses during drying and early storage; 2) potential heat effects from sample drying protocols (as a limitation); and 3) cannabinoid-related safety and compliance considerations.

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### Conflict of Interest

The authors declare that there are no conflicts of interest.

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