Asian Journal of Plant Sciences

ISSN 1682-3974

OPEN ACCESS

Asian Journal of Plant Sciences

ISSN 1682-3974

DOI: 10.3923/ajps.2024.176.183

Research Article

Biomass Production, Nutrient and Prussic Acid Content of Sunn Hemp (*Crotalaria juncea* L.) at Different Cutting Time

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Abstract

Background and Objective: Sunn hemp (*Crotalaria juncea* L.) is a tropical and subtropical legume primarily used as a green manure, cover crop and forage legume due to its rapid growth and ability to increase soil fertility. The objective of this study was to determine biomass production, nutrient content and prussic acid content of leaves and stems in Sunn hemp as a forage for livestock at the different of cutting time. **Materials and Methods:** This study was conducted using completely randomized design consisted of three cutting time treatments (8, 10 and 12 weeks) with 3 replications. The seeds were planted in 2-3 cm depth of soil with 10×30 cm of spacing row. Plant in every plot were randomly selected and hand separated into leaves and stems. Each component was sampled approximately 1000 g. Data were analyzed using Analysis of Variance (ANOVA) and continued with Duncan's Multiple Range Test ($p<0.05$). The following parameters observed were biomass production, nutrient content and prussic acid content. **Results:** The results showed that cutting time had a significant effect ($p<0.05$) on biomass production, nutrient content and prussic acid content of Sunn hemp leaves and stems. The dry matter and crude fiber content were decreased significantly ($p<0.05$), while biomass production, ash, crude protein and ether extract were increased as the cutting time increased. Nonetheless, prussic acid content had inconsistent results. **Conclusion:** It is suggested that Sunn hemp leaf can maintain forage mass and greater quality than its stem to be an alternative to ruminant feed.

Key words: Biomass production, cutting management, forage legume, nutrient content, prussic acid, Sunn hemp

Citation: Dewanti, M.S., B. Suhartanto, N. Umami, A. Kurniawati and Y.S. Prasojo, 2024. Biomass production, nutrient and prussic acid content of Sunn hemp (*Crotalaria juncea* L.) at different cutting time. Asian J. Plant Sci., 23: 176-183.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Livestock production in Indonesia is constrained by the continuity of good-quality forages. Improving the quality and continuity of the forage is necessary to boost animal productivity and ensure the production of high-quality food. Therefore, the utilization of local plant sources as feed ingredients that are available throughout the year is important for livestock sustainability. One of them is by using the leguminous plants. Sunn hemp (*Crotalaria juncea* L.) is a tropical and subtropical legume that originated from India and spread widely throughout the world, including in Indonesia. It is used primarily as a green manure and cover crop, because of its rapid growth and ability to increase soil nitrogen (N) fertility through N fixation with the help of Rhizobium[1]. With an estimated N intake of 100 to 200 kg/ha N, Sunn hemp can produce between 18 to 27 ton/ha dry weight of biomass as a green manure[2,3]. Sunn hemp is adaptable in a wide range of soil types including sandy soils with low fertility, poor irrigation and drought resistance[4,5]. It has an optimal growth in neutral pH between 5.0 to 8.5 where phosphorus and calcium are available[6].

Nutrient content and biomass production are important attributes of quality forages. Sunn hemp has a high protein and other nutrient value that can be used as alternative feed for ruminants. Previous research reported that Sunn hemp has 23.16% dry matter, 19.52% crude protein, 2.87% crude fat and 43.29% neutral detergent fiber harvested at 50 days[7]. The use of Sunn hemp as animal feed has a limiting factor because of the presence of anti-nutritional compounds such as pyrrolizidine alkaloids[8], saponin, tannin and flavonoid[9]. However, the study about prussic acid content in Sunn hemp is unavailable. Prussic acid levels in feed ingredients at 500 ppm on a dry basis are toxic[10]. The nutrient and prussic acid content of the plant should depend on cutting time as a growth stage on legume plants[11]. Cutting plants at the early growth phase can reduce plant biomass production, but delaying it can cause high fiber accumulation and reduce forage quality[12,13]. However, study on soybean legume about cutting plants showed that legumes can be regrowth and provide optimum yield and nutrient for animal livestock under specific cutting level[14]. Regrowth ability provide by cutting system could be adding in intercropping with rhodes grass for improving dry matter yield of total harvest and increasing crude protein of mixed silage sorghum and legume with sowing and rowing method intercropping[15-17]. Thomas *et al.*[18] also reported that the amount of prussic acid in young plants was higher than in old plants.

The primary determinant of nutritional content and biomass production in forage crops is the proportionate contribution of plant components (leaf and stem) to total plant dry matter[19]. Information regarding the appropriate cutting time to optimize the nutritional value and quality of the plant component of Sunn hemp is very limited. Therefore, it is necessary to develop a comprehensive understanding of biomass production, nutrition and prussic acid content partitioning to Sunn hemp leaves and stem to adopt and use this legume as forage in livestock systems in Indonesia. The objective of this study was to determine the biomass production, nutrition and prussic acid content partitioning to leaves and stems of Sunn hemp at the different of cutting time for ruminant feed. The results will be useful as initial data for the use and preservation of Sunn hemp which are safe for consumption by ruminants.

MATERIALS AND METHODS

Study area: This research was conducted from February to July, 2023 at Gadjah Mada University, Karanggayam Village, Depok District, Sleman Regency, Special Region of Yogyakarta, Indonesia (7°46'09.8"S and 110°23'11.4"E), located at an altitude of 136 m above sea level. The research was conducted on regosol soil with a neutral pH of 7.3. Sunn hemp can grow well in the soil with 5-8.4 pH[20]. The C-organic soil is in the low category, while the availability of macronutrients such as N-total is low, P_2O_5 is medium and the availability of K is medium (Table 1). Those soil characteristics indicate that the soil conditions at the research site are categorized as less fertile soil.

Table 1: Soil characteristics and physical properties in the experimental site

Soil parameter	Value
pH (H_2O)	7.3
pH (KCl)	6.3
C-organic (%)	2.15
N-total (%)	0.17
C/N ratio	12.65
P_2O_5 (ppm)	15
K (cmol/kg)	0.48
Ca (cmol/kg)	13.07
Mg (cmol/kg)	1.82
Na (cmol/kg)	0.22
CEC (cmol/kg)	23.11
Texture	
Sand (%)	21
Dust (%)	35
Clay (%)	44

C/N: Carbon-nitrogen ratio, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium and CEC: Cation exchange capacity

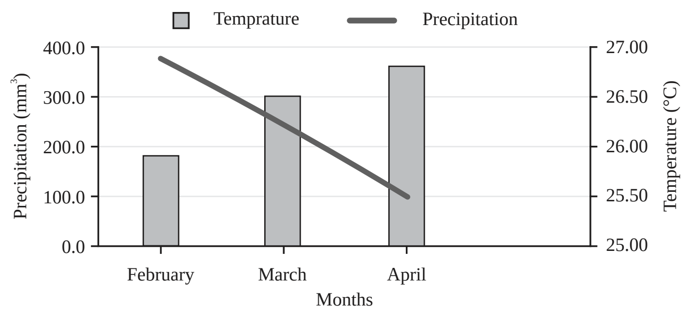

Fig. 1: Climate conditions in term of precipitation and temperature at the experimental site

The average precipitation in February, 2023 which is at the beginning of plantation and investigation was higher (375.1 mm) than in March and April, 2023 (243.7 and 100.9 mm, respectively) during the investigation (Fig. 1). The precipitation was ideal since Sunn hemp is a drought-tolerant that requires 100 to 250 mm of rainfall for its growth[21]. On the other hand, the temperature in February, 2023 was 25.9°C which was slightly lower than that in March and April, 2023 (26.5 and 26.8°C, respectively) (Fig. 1). The agroclimatic conditions during the plant cultivation were ideal for plant development. Sunn hemp grows well with the average temperature 18 to 27°C[22].

Cultivation and plant management: The land was processed using a cultivator and applied with organic fertilizer obtained by cattle, then divided into 18 plots. The size of each plot was 1×1.5 m with the distance between plots was 0.5 m. Sunn hemp seeds were planted on the land with a depth of 2-3 cm and spacing row of 10×30 cm[23]. Planting was done by making a hole on the soil manually, which consisted of 1 seed in every hole so that each plot contains 40 plants. Irrigation was done twice, in the morning and afternoon. According to da Silva *et al.*[24] Sunn hemp requires adequate moisture for germination and establishment. Soil samples were taken before planting for laboratory analysis. Weeding was done manually using hand every week until the plants reached 8 weeks old. This study was conducted using a Completely Randomized Design (CRD) which consisted of three cutting time treatments (8, 10 and 12 weeks). Every treatment was replicated 3 times.

Cutting management, sampling and yield measurement: Biomass samples for forage nutrient and prussic acid content determination were collected at 8, 10 and 12 weeks according to treatment. Sunn hemp in every plot were randomly selected and trimmed approximately 15 cm from the ground. In order to eliminate border effects, sampling within 0.5 m of the edges of each plot was avoided. Plants were hand separated into leaves and stems (hereafter referred to as "component") and weighed immediately to determine fresh weight production. Each component was sampled approximately 1000 g and put into a paper bag and then oven-dried at 55°C for 4 days and weighed back to obtain the dry weight. Dried samples were ground to pass a 1.0 mm screen using a Wiley mill and then stored individually inside the sealed plastic bag based on the treatment. Ground samples were used for further laboratory analysis. Total fresh weight is converted into tons/ha. To determine the total dry matter weight, total fresh weight was multiplied by dry matter weight.

Laboratory analysis: The chemical analysis of nutrient content included dry matter (DM), ash, crude protein (CP), crude fiber (CF) and ether extract (EE) was analyzed based on the AOAC method (2005). DM content was determined by oven-dried the sample at 105°C, where the moisture will fully evaporate so only the DM will remain. Ash content was determined using a furnace at a temperature of 600°C for 4 hrs. The CP content (N×6.25) was determined according to the Kjeldahl method including three steps (digestion, distillation and titration). The CF was determined using strong acid and alkaline solvents. The EE content was determined according to the Soxhlet extraction method using petroleum ether as the extract agent (60-80°C). The prussic acid content was analyzed using spectrophotometry method.

Statistical analysis: The data were analyzed statistically using Analysis of Variance (ANOVA). Significant differences among treatment means were compared by Duncan's Multiple Range Test (DMRT). Differences were considered significant when p-value was less than 0.05 ($p<0.05$). All statistical tests used SPSS 23.0 (SPSS Inc. USA).

RESULTS AND DISCUSSION

Biomass production: The biomass production of Sunn hemp can be affected by cutting the plant at different stages of its grows. The biomass production of Sunn hemp partitioning leaf and stem was shown in Table 2. The result showed that leaf and stem biomass in both fresh and DM forms were influenced ($p<0.05$) by the cutting time. The stem fresh yield was higher than the leaf with the highest fresh yield accumulation found in Sunn hemp harvested in 12 weeks (20.40 and 26.56 ton/ha, respectively). The stem dry matter content was higher than the leaf with the highest accumulation was found in Sunn hemp harvested over 12 weeks (20.91 and 29.65%, respectively). Cutting plants as it age can cause a rapid increase in cell wall structure, resulting in a higher dry matter in the plant[25]. The stem dry matter yield was higher than the leaf with the highest dry matter yield accumulation found in Sunn hemp harvested over 12 weeks (4.27 and 7.88 ton/ha, respectively). This result was higher than Lepcha and Naumann[20] report, that Sunn hemp leaf and stem dry matter mass accumulation in 8 weeks could reach 2.1 and 4.4 ton/ha, respectively. This could be because of the greater precipitation during the early stage of plant growth coupled with good soil permeability and drainage resulting in higher biomass production in this study. Sunn hemp in a whole plant can produce 43.24 ton/ha of dry matter yield and 10.78 ton/ha of fresh yield[21].

This study demonstrated that the cutting times at 12 weeks had a greater result compared with 8 and 10 weeks on the in both fresh and DM biomass of Sunn hemp. These results were in line with a previous study by Srisaikham and Lounglawan[7] that Sunn hemp harvested after 50 days is the best cutting age for harvest since it produces a greater dry matter and nutrient yield than after 30 and 40 days and, despite the low fiber, results in high protein. Biomass production in the plant is derived from the process of photosynthesis. During photosynthesis, plant captured light energy and used it to transform water and carbon dioxide into oxygen and organic compounds. The organic matter produced by the process of photosynthesis was the source of the plant's dry matter[26]. Therefore, longer cutting time, will give a plant more time to photosynthesize and produce photosynthate accumulate in plant tissues, leading to produced higher growth and yield of the plant. Plant dry weight is influenced by the growth rate and preparation time of the dry material itself[25].

The increased in the percentage of dry matter in Sunn hemp was also caused by a decrease in the percentage of water content in old plants. Young plants tend to have a higher water content, so that the percentage of dry matter is low, while old plant tend to have a lower water content that makes the proportion of cell content is lower than the cell walls. High cell wall content makes the plant dry matter content higher[25]. The increase of biomass production in Sunn hemp was related to its advantages as a green fertilizer to increase the accumulation of soil organic matter, which is directly related to biomass production per area[26]. Numerous factors, including planting periods that result in varying planting densities, soil type or fertility, Sunn hemp species and other environmental conditions, can also impact Sunn hemp biomass.

Overall, stem mass production increased at a greater rate than that on the leaf of the Sunn hemp. It was because as the plant biomass increases, the leaf-stem ratio will generally decrease[20]. During the early growth stages, the plant invests more energy into leaf production, which are the primary photosynthetic organ. As the plant matures, the plant begins to allocate more resources towards stem and reproductive growth (flowers and seeds). The stem, which supports the reproductive structures, continues to grow, while leaf production may slow down or stop altogether. Larger proportion of the forage mass was continuously partitioned to the stem as crop biomass increased and the leaf-stem ratio decreased. This in turn resulted in partitioning lower forage nutrient content to the stem than leaves[20]. Leaves of the forages can provide a high-quality feed that is easier for animals to digest, has more CP and contains less components of the cell wall than stems.

Stem mass dry matter content in Sunn hemp increased at a greater rate than that on the leaf. The dry matter content increases as the cutting age is delayed, which is caused by a decrease in air content and the lignification process which tends to occur in the stem cell walls[27]. Older plants will stop the vegetative phase and begin to enter the generative phase, where in this phase the lignification process occurs in the stem. The increase in structural components (cell walls) due to lignification causes a decrease in the leaf to stem ratio so that dry matter in the stem was higher than in the leaf. The leaf to stem biomass fraction ratio in tropical forage grasses is more important because it influences diet selection, forage quality and ruminant consumption.

Nutrient content: The nutrient content of Sunn hemp partitioning leaf and stem were shown in Table 3. The results showed that nutrient content was significantly influenced by the cutting times ($p<0.05$) except for the stem extract ether.

Table 2: Leaf and stem dry matter (%), fresh and dry matter yield (ton/ha) of Sunn hemp under different cutting time

Cutting time (weeks)	Fresh yield (ton/ha)		Dry matter (%)		Dry matter yield (ton/ha)	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
8	10.42±0.47[a]	12.68±0.73[a]	15.45±0.17[a]	15.15±0.83[a]	1.61±0.09[a]	1.93±0.17[a]
10	16.14±3.81[ab]	22.70±4.11[b]	19.38±0.63[b]	26.76±0.09[b]	3.07±0.70[ab]	5.90±1.08[b]
12	20.40±5.35[b]	26.56±2.67[b]	20.91±0.62[c]	29.65±0.21[c]	4.27±1.08[b]	7.88±0.82[c]
p-value	<0.041	<0.003	<0.001	<0.001	<0.014	<0.001
SE	1.78	2.20	0.82	2.21	0.44	0.90

Means in the same column with different superscripts in lowercase differ significantly (p<0.05)

Table 3: Leaf and stem nutrient content (%) of Sunn hemp leaf and stem under different cutting time

Cutting time (weeks)	Ash		CP		CF		EE	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
8	14.57±0.05	9.35±0.57	28.37±0.33[a]	7.66±0.03[b]	16.16±0.58[a]	52.82±0.71[a]	4.54±0.18[b]	1.63±0.05
10	14.09±0.02	5.94±0.15	24.56±0.45[b]	7.42±2.78[b]	18.08±0.26[b]	54.58±0.37[b]	4.47±0.12[b]	1.62±0.6
12	13.82±0.01	4.63±0.76	22.47±0.69[c]	3.76±0.57[a]	18.01±0.11[b]	58.37±0.51[c]	4.20±0.07[a]	1.57±0.05
p-value	<0.001	<0.001	<0.001	<0.047	<0.001	<0.001	<0.045	<0.455
SE	0.11	0.72	0.87	0.78	0.33	0.83	0.06	0.01

Means in the same column with different superscripts in lowercase differ significantly (p<0.05). CP: Crude protein, CF: Crude fiber and EE: Ether extract

Leaf and stem ash content decreased linearly with the increasing of cutting time until 12 weeks. Leaf ash content decreased at the rate of 14.09 and 13.82% in 10 and 12 weeks, respectively, while stem ash content decreased at the rate of 5.94 and 4.63% in 10 and 12 weeks, respectively. Halgerson *et al.*[28] reported that concentrations of most minerals were higher in leaves than in stems. The decrease in ash content as cutting age increases can be caused by several factors. One of them is the accumulation of more organic material and the decrease in mineral content as the plant grows. Apart from that, increasing the cutting age also increases the stem to leaf ratio which results in the plant becoming more fibrous and less nutritious. Therefore, the decrease in ash content can be considered as an indicator of the decrease in nutritional quality in plants as the cutting time increases.

Leaf EE content decreased linearly with the increasing of cutting time until 12 weeks, while stem EE content showed no difference. Leaf EE content decreased at the rate of 4.47 and 4.20% in 10 and 12 weeks, respectively. Leaves contain more protein and fat, so the leaf proportion value indirectly reflects the quality of forage. The leaf EE was higher than in the stem of Sunn hemp. It was because the older the plant, the more energy reserves in the form of crude fat are stored in the leaves.

Leaf and stem CP content decreased linearly with the increasing of cutting time until 12 weeks. Leaf CF content decreased at the rate of 24.56 and 22.47% in 10 and 12 weeks, respectively while stem CF content increased at the rate of 7.42 and 3.76 in 10 and 12 weeks, respectively (Table 3). The greatest leaf and stem CP content was observed at 8 weeks (28.37 and 9.35%, respectively) then decreased linearly as the cutting time increased (Table 3). It was due to a change in stem structure during development along with plant maturity which is associated with an increase in lignin content (during the lignification process) which caused a decreased in crude protein content. The longer the cutting time, the higher the fiber content, resulting in a decrease in the crude protein content.

The Sunn hemp leaf CP content was higher than that in the stem (Table 3). Stems of most plant species have a greater fiber content and less crude protein content than do leaf blades[29]. Leaf fractions usually contain more nitrogen than their stem and root fractions, which increases the amount of nitrogen in the entire plant and, consequently, the content of carbon at early maturity[30]. The result was similar to Lepcha and Naumann[20] report, where both leaf and stem CP contents decreased linearly with the increasing of cutting time. Legume feeds are used by ruminant producers because they need an easily digestible source of protein and energy for their diet and production. From the result, it was clear that Sunn hemp leaf can be used as a high nutrient feed. Based on the CP content, Sunn hemp leaf can be the alternative animal feed for various classes and sizes of ruminants.

Leaf and stem CF content increased linearly with the increasing of cutting time until 12 weeks. Leaf CF content increased at the rate of 18.08 and 18.01% in 10 and 12 weeks respectively, while stem CF content increased at the rate of 54.58 and 58.37 in 10 and 12 weeks, respectively (Table 3). The increased of CF content due to the cutting time was associated with the proportion of structural tissues in the stem and leaves. Structural tissue proportion occupies a larger

Table 4: Leaf and stem prussic acid content (ppm) of Sunn hemp leaf and stem under different cutting time

Cutting times (weeks)	Prussic acid	
	Leaf	Stem
8	119.33±4.21[a]	40.44±2.25
10	154.72±10.30[b]	40.85±7.40
12	147.73±7.56[b]	33.98±3.39
p-value	<0.003	<0.234
SE	5.85	1.79

Means in the same column with different superscripts in lowercase differ significantly (p<0.05)

portion on the stem than leaves, which is largely occupied by thin-walled mesophyll cells during the plant maturity[29]. High fiber content can lead to a decrease in degradability resulting less fermentable energy that is used by ruminants. Generally, leaf is more digestible, because it has fewer cell-wall constituents than stems so the increase in forage value depends on the proportion of plant parts.

The CF content in the stem was higher than in the leaf of Sunn hemp. It was due to the high lignification process during the plant development which mainly occurs in the stem resulting in higher CF in the stem of Sunn hemp. Lignin composition is very limited at the early stage of development and begin to mature as the plant ages. Lignin composition increased in the middle lamella's major wall area, which is the least digestible. The fraction of lignified tissue in legumes increases over time as the stem grows[29].

Prussic acid content: The prussic acid content of Sunn hemp partitioning leaf and stem are shown in Table 4. The results showed that leaf prussic acid content was significantly influenced by the cutting times (p<0.05), while stem prussic acid was not influenced (p>0.05). Nonetheless, the leaf prussic acid content showed an inconsistent effect in this study. Leaf prussic acid content at 10 weeks (154.72 ppm) showed an increase compared to 8 weeks (119.33 ppm), while at 12 weeks 147.73 showed a decrease compared to 10 weeks (Table 4). The variation of prussic acid content in Sunn hemp is strongly decided by genetic factors[31]. Prussic acid content is strongly influenced by environmental stress factors (pest and disease attacks, prolonged drought, low P and K content in the soil)[32].

Leaf prussic acid content in this study was higher than that in the stem of Sunn hemp. The results were in line with Handriati *et al.*[33] report, that prussic acid or cyanide is abundant in leaves compared to stems. The amount of leaf production followed the amount of prussic acid. The leaves of the plant contain more cyanogenic glycosides than the stems, resulting in higher prussic acid content. The highest prussic acid content was found in leaf with a cutting time of 10 weeks (154.72 ppm). This concentration is declared safe for consumption by livestock, because the concentration of forage that is safe for livestock is 0-500 ppm[10]. The HCN levels in feed ingredients at 220 ppm on a wet basis are dangerous and at 500 ppm on a dry basis they are toxic[10]. If the prussic acid content is more than 750 ppm, this is very dangerous and can cause death[34]. Based on the result, the Sunn hemp in both components are safe to be consumed by ruminant livestock.

Forage mass, nutrient and prussic acid value partitioning of Sunn hemp leaves and stems are influenced by the cutting time. Early harvest was recommended to maximize nutrition and the quality of Sunn hemp. In addition, Sunn hemp could be use as hay, similar as prior study on alfalfa for poultry feed has shown[35]. Based on the measure of plant components in this study, Sunn hemp leaves maintained significantly lower fiber contents with higher crude protein than stems which can be alternative feed sources to livestock production. Although biomass production was consistently higher in the stem, Sunn hemp demonstrated considerable potential for balanced forage. Even Sunn hemp contained prussic acid, the concertation's is still at the safe zone for livestock.

CONCLUSION

Twelve weeks of cutting time produced higher biomass production for both leaves and stems. The dry matter and crude fiber content were decrease as the cutting time increased. On the other hand, ash, crude protein and ether extract content were increased as the cutting time increased. Nonetheless, prussic acid content had inconsistent result. Both leaves and stems of Sunn hemp were safe and can be a good sources of feed forage. However, the leaves component provides high nutrient content than stems.

SIGNIFICANCE STATEMENT

This study discovered the importance of cutting time as a growth stage of the Sunn hemp and its effect to biomass and quality that can be beneficial for researchers and farmers to choose appropriate cutting time for Sunn hemp as a forage. Due to the toxicity problem of the prussic acid content in Sunn hemp, this study provides the information that Sunn hemp was safe and can be an alternative material for feed forage to ruminant.

ACKNOWLEDGMENT

The authors are very thankful for Universitas Gadjah Mada that already supports this research by providing funding through the Final Project Recognition Grant Universitas Gadjah Mada Number 5075/UN1.P.II/Dit-Lit/PT.01.01/2023.

REFERENCES

1. Stute, J.K. and D.E. Shekinah, 2019. Planting date and biculture affect sunn hemp productivity in the Midwest. Sustainable Agric. Res., 8: 26-35.
2. Wanapat, M., P. Totakul, B. Viennasay and M. Matra, 2021. Sunnhemp (*Crotalaria juncea* L.) silage can enrich rumen fermentation process, microbial protein synthesis, and nitrogen utilization efficiency in beef cattle crossbreds. Trop. Anim. Health Prod., Vol. 53. 10.1007/s11250-021-02628-z.
3. Schomberg, H.H., N.L. Martini, J.C. Diaz-Perez, S.C. Phatak, K.S. Balkcom and H.L. Bhardwaj, 2007. Potential for using sunn hemp as a source of biomass and nitrogen for the piedmont and coastal plain regions of the Southeastern USA. Agron. J., 99: 1448-1457.
4. Durairaj, E.S. and J.K. Stute, 2018. Sunn hemp: A legume cover crop with potential for the Midwest? Sustainable Agric. Res., 7: 63-69.
5. Eberle, C. and L. Shortnacy, 2021. Sunn hemp planting date effect on growth, biomass accumulation, and nutritive value in Southeastern Wyoming. Crop Sci., 61: 4447-4457.
6. Wang, C.L. and Y.L. Dai, 2018. First report of sunn hemp *Fusarium* wilt caused by *Fusarium udum* f. sp. *crotalariae* in Taiwan. Plant Dis., 102: 1031-1031.
7. Srisaikham, S. and P. Lounglawan, 2021. Nutrient yield of brown hemp and its utilization as protein source in concentrate on Brahman×Thai-native cattle performances. Chiang Mai Univ. J. Nat. Sci., Vol. 20. 10.12982/CMUJNS.2021.006.
8. Mosjidis, J.A., J.M. Burke and J.B. Hess, 2012. The facts about sunn hemp toxicity. Crop Sci., 52: 1469-1474.
9. Rawat, R. and C.S. Saini, 2022. Effect of soaking conditions in the reduction of antinutritional factors in sunnhemp (*Crotalaria juncea*) seeds. Food Chem. Adv., Vol. 1. 10.1016/j.focha.2022.100092.
10. Gensa, U., 2019. Review on cyanide poisoning in ruminants. J. Biol. Agric. Healthcare, 9: 1-12.
11. Prasojo, Y.S., G. Ishigaki, M. Hashiguchi and R. Akashi, 2021. Effect of different growth stages on biomass weight and forage quality of different growth type soybean (*Glycine max*). Asian J. Plant Sci., 20: 256-262.
12. Sathees, D. and S. Santhiralingam, 2022. Evaluation of growth and yield performances of Napier grass cultivar pakchong-1 under different spacial patterns in the Kilinochchi District, Sri Lanka. J. Agro-Technol. Rural Sci., 1: 1-5.
13. Onjai-Uea, N., S. Paengkoum, N. Taethaisong, S. Thongpea and B. Sinpru *et al*., 2023. Effect of cultivar, plant spacing and harvesting age on yield, characteristics, chemical composition, and anthocyanin composition of purple Napier grass. Animals, Vol. 13. 10.3390/ani13010010.
14. Prasojo, Y.S., G. Ishigaki, M. Hashiguchi, M. Muguerza and R. Akashi, 2021. Evaluation of regrowth ability of soybeans for forage utilization under two-cutting systems. Aust. J. Crop Sci., 15: 1452-1458.
15. Prasojo, Y.S., M. Niimi, M.M. Rahman, G. Ishigaki and R. Akashi, 2022. Effect of soybean (*Glycine max* L. Merr) intercropping into rhodesgrass (*Chloris gayana* Kunth.) on dry matter yield, crude protein, and silage characteristics grown in Southwest Japan. J. Anim. Plant Sci., 32: 460-465.
16. Prasojo, Y.S., M. Niimi, M. Hashiguchi, M. Muguerza and G. Ishigaki, 2023. The effects of the different sowing methods and planting density on the yield components of soybean (*Glycine max*) under intercropping condition with rhodes grass (*Chloris gayana* Kunth.). Aust. J. Crop Sci., 17: 770-775.
17. Prasojo, Y.S., G. Ishigaki, K. Fukuyama and R. Akashi, 2019. Development of soybean production technique by living mulch method with Rhodes grass in Southwestern Japan. IOP Conf. Ser.: Earth Environ. Sci., Vol. 387. 10.1088/1755-1315/387/1/012017.
18. Thomas, M.E., J.L. Foster, K.C. McCuistion, L.A. Redmon and R.W. Jessup, 2013. Nutritive value, fermentation characteristics, and *in situ* disappearance kinetics of sorghum silage treated with inoculants. J. Dairy Sci., 96: 7120-7131.
19. Hintz, R.W. and K.A. Albrecht, 1994. Dry matter partitioning and forage nutritive value of soybean plant components. Agron. J., 86: 59-62.
20. Lepcha, I. and H.D. Naumann, 2021. Partitioning of forage mass and nutritive value in sunn hemp leaf and stem components. Int. J. Agron., Vol. 2021. 10.1155/2021/5547120.
21. Dewanti, M.S., B. Suhartanto and Y.S. Prasojo, 2024. Morphology characteristic and biomass production of sunn hemp (*Crotalaria juncea* L.) at different cutting time. Asian J. Plant Sci., 23: 15-21.
22. Ahmed, A.T.M.F., M. Zahidul Islam, M.S. Mahmud, M.E. Sarker and M. Reajul Islam, 2022. Hemp as a potential raw material toward a sustainable world: A review. Heliyon, Vol. 8. 10.1016/j.heliyon.2022.e08753.
23. Tripathi, M.K., B. Chaudhary, S.K. Sarkar, S.R. Singh, H.R. Bhandari and B.S. Mahapatra, 2013. Performance of sunnhemp (*Crotalaria juncea* L.) as a summer season (pre-monsoon) crop for fibre. J. Agric. Sci., 5: 236-242.

24. da Silva, B.N.P., T.E. Masetto and L.C.F. de Souza, 2022. Changes in the physiological potential of sunn hemp seeds during storage. Pesqui. Agropecu. Trop., Vol. 52. 10.1590/1983-40632022v5272687.
25. Ernawati, A., L. Abdullah, I.G. Permana and P.D.M.H. Karti, 2023. Morphological responses, biomass production and nutrient of *Pennisetum purpureum* cv. Pakchong under different planting patterns and harvesting ages. Biodiversitas, 24: 3439-3447.
26. Zhang, H., Y. Wang, S. Yu, C. Zhou and F. Li *et al*., 2023. Plant photosynthesis and dry matter accumulation response of sweet pepper to water-nitrogen coupling in cold and arid environment. Water, Vol. 15. 10.3390/w15112134.
27. Balk, M., P. Sofia, A.T. Neffe and N. Tirelli, 2023. Lignin, the lignification process, and advanced, lignin-based materials. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms241411668.
28. Halgerson, J.L., C.C. Sheaffer, N.P. Martin, P.R. Peterson and S.J. Weston, 2004. Near-infrared reflectance spectroscopy prediction of leaf and mineral concentrations in Alfalfa. Agron. J., 96: 344-351.
29. Marković, J.P., R.T. Štrbanović, D.V. Terzić, D.J. Djokić, A.S. Simić, M.M. Vrvić and S.P. Živković, 2012. Changes in lignin structure with maturation of alfalfa leaf and stem in relation to ruminants nutrition. Afr. J. Agric. Res., 7: 257-264.
30. Müller, M.M., V. Sundman, O. Soininvaara and A. Meriläinen, 1988. Effect of chemical composition on the release of nitrogen from agricultural plant materials decomposing in soil under field conditions. Biol. Fertil. Soils, 6: 78-83.
31. Zahid, A., A. Khanum, M. Ansar and M.A. Malik, 2012. Effect of cutting and post-cutting intervals on hydrogen cyanide in sorghum forage grown under rain-fed conditions. Pak. J. Bot., 44: 955-960.
32. Ayanru, D.K.G. and V.C. Sharma, 1984. Changes in total cyanide content of tissues from cassava plants infested by mites (*Mononychellus tanajoa*) and mealybugs (*Phenacoccus manihoti*). Agric. Ecosyst. Environ., 12: 35-46.
33. Handriati, L.N., B. Suhartanto, S. Widodo, M.P. Dewi and N. Umami, 2019. Effect of sorghum varieties and molasses addition on prussic acid content and of silage quality. IOP Conf. Ser.: Earth Environ. Sci., Vol. 387. 10.1088/1755-1315/387/1/012062.
34. Asgar Ud Deen, V. Kumari, A.N. Sharma, G. Mondal and G.P. Singh, 2018. Understanding cyanogenic glycoside toxicity in livestock: A review. Int. J. Chem. Stud., 6: 1559-1561.
35. Suwignyo, B., A. Mustika, Kustantinah, L.M. Yusiati and B. Suhartanto, 2020. Effect of drying method on physical-chemical characteristics and amino acid content of tropical alfalfa (*Medicago sativa* L.) hay for poultry feed. Am. J. Anim. Vet. Sci., 15: 118-122.