JOURNAL OF AGROBIOTECHNOLOGY 2021, VOL 12(1S):157-165
e-ISSN: 2180-1983
http://dx.doi.org/10.37231/jab.2021.12.1S.281
https://journal.unisza.edu.my/agrobiotechnology/index.php/agrobiotechnology/index

Evaluating the Potential of *Tridax procumbens* and *Asystasia gangetica* as Ruminant Feed

Nurul Aini Kamaruddin[a, *], Najihah Ali[a], Nur Adilah Mohd Hanafee[a], Serene Liew[a], and Nur Yuhanis Yasin[a]

[a] School of Animal Science, Aquatic Science and Environment, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu Darul Iman, MALAYSIA.

***Corresponding author: nurulkamaruddin@unisza.edu.my**

ABSTRACT

Tridax procumbens and *Asystasia gangetica* are extensively prevalent and can be found from tropical Asia to Africa. Both plant samples were found to have a high nutritional value, especially in protein, and to be highly attractive to ruminants. Therefore, the objectives of the study were to measure and compare the nutrient composition of *Tridax procumbens* and *Asystasia gangetica* in terms of the nutritional value for ruminants. Both plants samples were collected at Besut campus, University of Sultan Zainal Abidin. The samples were then washed under tap water to remove foreign matter such as soil to prevent soil contamination in the analysis. Then, the samples were dried in a furnace below 60 - 70˚C and crushed prior to further analysis using proximate analysis. Seven parameters were measured using proximate analysis, which included dry matter (DM), moisture, ash, crude protein (CP), crude fiber (CF), ether extract (EE), and nitrogen-free extract (NFE). The findings in this study show that *A. gangetica* had significantly higher *($p < 0.05$)* in dry matter (DM) (18.84%), crude protein (CP) (22.27%), and nitrogen-free extract (NFE) (50.25%). However, *T. procumbens* showed the highest nutrient in moisture (88.70%), ash (12.15%), crude fibre (CF) (25.01%), and ether extract (EE) with 3.71%. Thus, this study revealed that *A. gangetica* to have a higher potential to be used as an animal feed than *T. procumbens.*

Keywords: *Asystasia gangetica, Tridax procumbens,* proximate analysis, ruminant feed, nutritive value

INTRODUCTION

In Malaysia, the ruminant sub-sector mostly operates in a small scale and is not well developed. Over the 1996-2002 period, the industry grew slowly while it grew rapidly in 2005-2012 because of the government's emphasis and initiatives. Even though the industry is growing, it cannot full fill the high demands and the self-sufficiency level still less than 30% (Mohamed et al., 2013). Apart from this, the low self-sufficiency of ruminant production is caused by a limited of grazing area. Malaysia has low productivity on natural grasslands due to competition from other agriculture and other industries in land use (Zahari and Wong, 2009). In addition, low self-sufficiency was also affected by higher feed costs. The higher cost of feed like pellets is usually due to raw materials such as corn, which we must import from other countries such as Argentina, Brazil, and Thailand (Fadhilah, 2015. Chisoro (2015) and Alagbe (2017) reported that there is evidence that some trees or wild plants possess nutritional properties that could be incorporated into

livestock feed. Some of these plants can also be a pellet substitute because they contain protein. *Tridax procumbens* and *Asystasia gangetica* are common tropical wild plants. This two plants species native to tropical Malaysia, India, and Africa, but has been introduced into tropical areas in North, Central and South America, Hawaii, West Indies, and Australia as an ornamental herb and eventually escapes into natural and disturbed areas. In addition, these two wild plants were widely used as a forage in Mexico and United Arab Emirates (UAE) (Shabana et al., 2020). The two plant species are highly protein rich. According to Kpodekon et al. (2015), the protein contents in the *T. procumbens* is an average 34.57% while in *A. gangetica,* the protein can reach 23.5% and the value can increase during the dry season (Adjorlolo et al., 2014 and Kumalasari et al., 2020).

A study of *T. procumbens* and *A. gangetica* is important for assessing the potential of wild plants in Malaysia for livestock feed. This study is important to help small farmers replace the higher cost of pellets with low-lying, nutrient-rich pastures. In addition, this study able to give an idea towards small farmers to cultivate *T. procumbens* and *A. gangetica* instead of common pasture grass like Napier grass in their place Furthermore, this study is being carried out because of the lack of research on the two plant samples in Malaysia compared to other countries. Furthermore, the different climate and weather also were influenced on result of this study. According to Dumont et al. (2014) the environmental factors such as climate and weather have significant affect to the nutritive value of forage. The findings of this study are important in introducing the ability of *T. procumbens* and *A. gangetica* into the diet of ruminants because of their nutritional characteristics. Therefore, the aims of this study were to measure the nutrient compositions of *T. procumbens* and *A. gangetica* in terms of nutritive value for ruminant feed and to compare the nutrient compositions between both samples

MATERIALS AND METHODS

Sample collection and sample preparation

Samples of *T. procumbens* and *A. gangetica* were collected from the Tembila area (5°75'29" N, 102°62'79" E), close to the Besut campus, University of Sultan Zainal Abidin (UniSZA). Both plant samples were placed in a plastic bag and transported to the UniSZA nutrition laboratory. Subsequently, the *T. procumbens* and *A. gangetica* were divided into several groups to perform proximate analysis. Next, the *T. procumbens* and *A. gangetica* plants were washed appropriately under tap water to make sure it cleans enough from any debris or soil before proceeding to the next process. The fresh weight of plant samples was approximately 200-300 g for full proximate analytical processes (Motsara and Roy 2008). After the samples were washed using tap water, the plant samples were dried in the oven around 24 - 48 hours at approximately 60 – 70˚C (Motsara and Roy, 2008). This step is to ensure that there is no water on the plants prior to the immediate analysis. After the drying process was completed, the plant samples were grounded with a 0.5mm stainless steel electric sieve. This process ensures that plant samples are powder-based. Next, the powdered plant samples were stored in tightly sealed plastic bags for further analysis

Nutritive Value Content Analysis

A proximate analysis was used to determine the qualitative and quantitative content of moisture (dry matter) and total solids, protein, ether extract, crude fibre, total ash, phosphorus and NFE. All samples were analyzed using the standard methods of Official Methods of Analysis, Association of Official Analytical Chemists, 18th edition (AOAC, 2005) in triplicate. Detailed procedures of each parameter are explained below:

Crude protein

The crude protein was analyzed using the Kjeldahl method which consisted of three processes: digestion, dilution, and filtration. In the digestion process, sulphuric acid digests proteins and other organic compounds in the presence of catalysts with organic nitrogen has been converted to ammonium sulphate. 1 g of sample was placed in a digestion tube, followed by the addition of the Kjeltabs Cu 3.5 catalyst. Subsequently, H_2SO_4 concentrate was added to the digestion tube and gently agitated to blend the sample with the acid. The rack loaded with the exhaust system into a digester block was then attached to the digester

tubes in the rack. Temperature was set to 420°C. Samples were digested for 60 - 90 minutes until it turned into clear with a green / blue solution.

In distillation process, the digested samples in the digestion tube were placed in the distillation unit. Before that, 25 ml of receiver solution consist of 25 ml of 2% boric acid with 10 drops of indicator solution has been filled into a conical flask hence been placed to the distillation unit. Then, 70 ml distilled water and 50 ml of 32% of NaOH was added into the digestion tube automatically. This process took around 4 minutes. The receiver solution in the distillate flask was changed into green color due to the presence of alkali (ammonia). In titration process, the distilled sample was titrated with standards hydrochloric acid 0.1 N. This process takes until it is switched to pink or red. The volume of HCI used was recorded. Calculation of % protein in sample using below equation (Eqn. 1):

$$\% \text{ N} = \frac{\text{A x (T} - \text{B) x 14.007 x 100}}{\text{Weight of sample (g) x 1000}} \qquad \textbf{Eqn. 1}$$

% Crude Protein = % nitrogen x *F*

Where: T= volume acid for sample
B= volume acid for blank
A= Normality of HCI
F= Protein factor, 6.25 / 5.7 / 6.38

Crude fiber

Crude fiber (CF) is measuring the indigestible parts in feed content. Crude fiber is commonly having such as lignin, chitin, pentosan and cellulose. Then, 1 g of sample was inserted in the fiber bag (W2) and weighed with an analytical balance. The empty fibre bag (W1) and crucible weighed as well (W6). Following that, glass spacer was inserted into the fiber bag and then inserted into the bag in the carousel. Next, the carousel was placed in the glass container which on the intended position of the plate before the machine will operate. Following the analysis, the fiber bags were removed from the carousel and placed in the crucible. The bags and crucible were dried for 4 hours at 105°C. Afterwards, it was cooled in a desiccant for 30 minutes. Then, the crucible and dried fiber bag was weighed using analytical weighing scale (W3). The crucible that contains with fiber bag placed in a furnace at temperature at 550°C and burn for 4 hours. Next, the crucible was removed and cool in desiccator again (W4). After crucible and ash of the empty fiber bag were cooled in a desiccator, it weighed to get the value of ash remained in the crucible (W7). Blank value (W5) could be got if the value of (W7) minus (W6).

The % of crude fiber was used using below equation (Eqn. 2):

$$\% \text{ Crude fibre} = \frac{[(\text{W3} - \text{W1}) - (\text{W4} - \text{W5})]}{\text{W2}} \text{ x } 100 \qquad \textbf{Eqn. 2}$$

Where,

W1 = Weight of fiber bag (g)
W2 = Weight of sample (g)
W3 = Weight of crucible and fiber bag after digestion (g)
W4 = Weight of crucible and ash (g)
W5 = Weight of blank value of empty fiber bag (g)
W6 = Weight of crucible (g)
W7 = Weight of crucible and ash of the empty fiber bag (g)

Moisture

Moisture refers to the amount of water in the feed, whereas dry matter refers to the remaining material after the water has been removed. Fresh samples were used in this study and the analysis was performed using

the oven drying method. According to this method, the dry crucible with cover was heated to 105ºC for 4 hours (W1). Next, 3 g of homogenized samples were weighed using an analytical scale and placed in the crucible (W2). After that, the sample in the crucible was heated at a temperature of 105°C for 6 hours. Then, it was cooled in a desiccator and weighed (W3).

Calculation of % moisture was calculated using below equation (Eqn. 3):

$$\% \text{ Moisture} = \frac{W2 - W3}{W2 - W1} \text{ X } 100 \qquad \textbf{Eqn. 3}$$

Where:

W1= Weight of crucible (g)
W2= Weight of crucible + weight of wet sample (g)
W3= Weight of crucible + weight of dried sample (g)

% Total solid = 100 - % Moisture

Ash

Ash is an inorganic residue remaining after water and organic matter has burnt away. Firstly, the crucibles were dried with the covers in an oven at 105ºC for four hours. The crucibles were cooled in a desiccator and weighed it after reach room temperature. The samples were weighed and placed into the crucible. The samples were dried in an oven for one day if samples were contained high moisture. The samples were placed in a muffle furnace, and the temperature was set to 550ºC overnight. The samples were removed and cooled in a desiccator, and then it was weighed after it reached room temperature. The percentage of ash was calculated by using a formula:

$$\text{Ash } \% = \frac{W3 - W1}{W2} \text{ X } 100 \qquad \textbf{Eqn. 4}$$

Where,

W1= Weight of crucible(g)
W2= Weight of sample (g)
W3= Weight of crucible + ash (g)

Ether extract

The extraction cups were dried in the oven at 105ºC for six hours and were cooled in desiccators on one-day prior experiment. The extraction cups were pre-dried, and the extraction cup holder were used to hold it to avoid error on the result and need to wear the gloves during this experiment. Three grams of the samples were weighed accurately, and the samples were wrapped with a piece of filter paper and were placed into the extraction thimble. The opening of the thimble was plugged loosely with cotton or the filter was folded and was plugged with cotton.

The petroleum ether volume was measured using the volumetric cylinder at 150 ml and was poured into the extraction cup. The extraction cups were attached to the Automated Soxhlet Fat Extractor. The desired program on the machine was selected and pressed the start button. The extraction cup containing petroleum ether was removed after the extraction complete. Then, the extraction cups were drawn into a desiccator to cool and were weighed. The percentage of fat was calculated by using the below formula:

$$\% \text{ Fat} = \frac{W3 - W2}{W1} \text{ X } 100$$ **Eqn. 5**

Where,

W1= Weight of sample (g)
W2= Weight of extraction cup (g)
W3= Weight of extraction cup + fat (g)

Nitrogen-free extract

Nitrogen free extract (NFE) is crucial as a source of energy for animals. Nitrogen-free extract (NFE) could be obtained after subtraction of the contents (%) including crude protein, ether extract, crude fiber, moisture and crude ash from the whole feed. NFE consist of soluble carbohydrates.
Calculation of nitrogen free extract by following a formula (Eqn. 6):

NFE % = 100 - (Ash content (%) + Crude Protein (%) Ether Extract (%) + Crude Fibre (%) **Eqn. 6**

Statistical analysis

Data were analysed using Independent T-Test and presented as mean ± SD and significance to determine the significant difference between nutritive values in both plant samples. The value $p<0.05$ was considered a significant difference. The Minitab version 17.0 software was used for the statistical analysis.

RESULTS AND DISCUSSION

Proximate analysis

The results of this study show that the percent proximate analysis between the two plant species which are *Tridax procumbens* and *Asystasia gangetica* shows a significant difference ($p < 0.05$). The proximate compositions of *T. procumbens* and *A. gangetica* were tabulated in the Table 1. The percentage of the crude protein (CP) and nitrogen-free extract (NFE) in *A. gangetica* was higher compared to *T. procumbens*. While the proximate analysis results in moisture, ash, crude fibre (CF), and ether extract (EE) of *T. procumbens* show a higher percentage compared to *A. gangetica.*

T. procumbens shows a higher percentage of moisture with 88.70% compared to *A. gangetica* which contains 81.16%. The moisture content of both species has significant differences ($p < 0.05$) in mean values. The highest percentage of ash was shown in *T. procumbens* (12.15%) compared to *A. gangetica* (9.67%). Both species show a significant difference ($p < 0.05$) for means value in ash. The crude protein (CP) in *A. gangetica* shown a higher percentage which is 22.27% compared to *T. procumbens* which content 16.77%. There are also significant differences ($p < 0.05$) for the mean value in crude protein content for both plant species. The percentage of crude fibre (CF) for *T. procumbens* shows a higher percentage than *A. gangetica* which are 25.01% and 15.36%, respectively. The means value for both plant species shows a significant difference ($p < 0.05$) in crude fibre content. The percentage of ether extract (EE) in *T. procumbens* shown a higher percentage compared to *A. gangetica* which are 3.71% and 2.44%, respectively. The means values for both plant species also showed a significant difference ($p < 0.05$) in ether extract content. Lastly, nitrogen-free extract (NFE) in *A. gangetica* has a higher percentage which 50.25% compared to *T. procumbens,* which contains 44.52% NFE and significantly difference ($p < 0.05$).

The dry matter (DM) content of *A. gangetica* has a higher percentage with 18.84% compared to *T. procumbens* with 11.30%. The result of DM content of *A. gangetica* in this study was comparable to the previous study by Khalil (2016) who has reported that dry matter content in *A. gangetica* was 14.6%. The dry matter content of *A gangetica* in this study is lower due to the fresh sample used. According to Khalil (2016) the fresh sample has lower nutrient content compared to a dry matter basis. Dry matter is the most important nutrient in a

feed that contains fiber, protein, carbohydrates, and minerals. The dry matter is one of the components that needed by livestock to maintain their health and production (Robert, 2013). The amount of dry matter required on a regular basis is determined by numerous factors such as weight, stage of development such as lactation, weaning, and finishing. For example, cows typically consume 1.8% of their body weight in dry matter, whereas lactating cows consume about 2% of their body weight (Robert, 2013). Therefore, it is important to determine the DM content in their feed.

Table 1. Proximate composition of *Tridax procumbens* and *Asystasia gangetica.*

Parameter (%)	**Plant samples** (mean ± SD)	
	A. gangetica	*T. procumbens*
Dry matter (FW)	$18.84^{a} \pm 0.29$	$11.30^{b} \pm 0.22$
Moisture	$81.16^{b} \pm 0.29$	$88.70^{a} \pm 0.22$
Ash	$9.67^{b} \pm 0.04$	$12.15^{a} \pm 0.02$
Crude Protein (CP)	$22.27^{a} \pm 0.29$	$16.77^{b} \pm 0.36$
Crude Fibre (CF)	$15.36^{b} \pm 0.69$	$25.01^{a} \pm 0.50$
Ether Extract (EE)	$2.44^{b} \pm 0.45$	$3.71^{a} \pm 0.15$
Nitrogen-Free Extract (NFE)	$50.25^{a} \pm 0.62$	$44.52^{b} \pm 1.06$

[ab] Means with common superscript are significantly different ($p < 0.05$)
SD: Standard Deviation
FW: Fresh Weight

A result of moisture content in *T. procumbens* shows a higher percentage (88.70%) than *A. gangetica* (81.16%) and it was comparable to a study by Jude et al., (2009) which has found 90.05% in their study. It is important to know the moisture content of the feed on account of its weight even though has no nutritional benefit to the animal (Cozzolino and Labendra, 2019). Next, *T. procumbens* has a higher percentage of ash (12.15%) compared to *A. gangetica* (9.67%). The percentage ash content of *T. procumbens* in this study was near to the previous findings by Akinmoladun et al., (2018) which has found 11.77% of ash content. Ash is the overall mineral content of a forage diet. It is important to determine the ash content because it is considered as minerals that contains of phosphorus, calcium, magnesium, and potassium which has a significant role in animal performance (Mike, 2018).

In addition, a crude protein (CP) of *A. gangetica* has a higher percentage (22.27%) than *T. procumbens*. (16.77%). The result of this study is comparable to the study by Sobayo et al., (2012) who has reported the value of crude protein in *A. gangetica* was 19.38 %. According to Hay (2021), certain animals are depending on the protein content in the forages for digestibility and feed intake. In addition, the protein is also crucial for the maintenance of the animal's body, reproduction, lactation, and growth (David, 2020). A study by David (2020) reported that the minimum of protein required by livestock for its maintenance is 7%. Therefore, nutrient requirements for proteins vary depending on the development and physiological phase and the level of production. For example, the protein needs of a goat is 11% (Luginbuhl, 2015). Thus, the protein content in A. *gangetica* is adequate to fulfil the protein requirement of livestock.

Next, *T. procumbens* has a higher percentage of crude fibre which contains 25.01% compared to *A. gangetica* (15.36%). In a previous study, Bamigboye and Oluwarinde (2017) reported that *T. procumbens* contain 18.5% of the crude fibre content. The finding of this study is comparable to the results of Bamigboye and Oluwarinde (2017). Crude fibres are important for livestock feed because they require a special amount of fibre in their diet to allow the rumen to function optimally. According to Moran (2014), the minimum percentage of crude fibre required by a cow's diet is 17%. Thus, the crude fibre content in *T. procumbens* and *A. gangetica* in this study shows an adequate percentage to fulfil the needed of the cow's diet. In terms of

ether extract (EE), *T. procumbens* has a higher percentage of ether extract (3.71%) compared to *A. gangetica* (2.44%). This result was comparable to the previous study by Akinmoladun et al., (2018), which reported *T. procumbens* contain 2.92 % of ether extract's content.
Fat is an important component that must be supplied in the correct amount in the diet to meet animal's need. It is a unique nutrient that provides benefits that are not achieved in other types of nutrients such as carbohydrates and fibre. The fat content of common dietary components will be around 2 to 3% (Eastridge, 2019). Linn et al., (2021) reported that total fat must not exceed 7% of the diet DM. Furthermore, overfed fat may cause diarrhea to occur in the animal (Marcondes et al., 2013). Lastly, nitrogen-free extract (NFE) in *A. gangetica* has a higher percentage (50.25%) compared to *T. procumbens* (44.52%). In a previous study, Sobayo et al., (2012) reported that NFE content in *A. gangetica* was a bit lower with 36.34% only. Hence, determine NFE is important because hemicellulose, lignin, starch, pectin, and other compounds can be found in the nitrogen-free extract (Maynard, 2020).

CONCLUSION

The study found that different pasture species have different nutrient contents. The means nutrient contents for the two pasture species which are *A. gangetica* and *T. procumbens* were significantly different. In addition, crude protein (CP), dry matter (DM), and nitrogen-free extract (NFE) in *A. gangetica* were higher compared to *T. procumbens*. This study suggested that *A. gangetica* has a higher potential to be as an animal feed than *T. procumbens*. As a recommendation, this study could be enhanced by performing mineral analysis for *A. gangetica* and *T. procumbens*. Thus, these two species can also be used in ruminants in the future to assess growth efficiency and can be compared to the present diet.

ACKNOWLEDGEMENTS

This study was funded by the Fundamental Research Grant Scheme by the Ministry of Higher Education, Malaysia (MOHE) FRGS/1/2019/WAB01/UNISZA/02//4.

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How to cite this paper:
Kamaruddin, N.A., Ali, N., Mohd Hanafee, N.A., Liew, S., Yasin., Y. (2021). Evaluating the Potential of *Tridax procumbens* and *Asystasia gangetica* for ruminant feed. *Journal of Agrobiotechnology, 12*(1S), 157-165