JOURNAL OF AGROBIOTECHNOLOGY 2021, VOL 12(1S):166-173
e-ISSN: 2180-1983
http://dx.doi.org/10.37231/jab.2021.12.1S.282
https://journal.unisza.edu.my/agrobiotechnology/index.php/agrobiotechnology/index

Nutritional Assessment of *Setaria sphacelata* and *Cleome gynandra* as Potential for Ruminant Feed

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ABSTRACT

Setaria sphacelata is a high and most prevalent tropical grass, while *Cleome gynandra is* a tropical annual herb which commonly used as vegetables in Africa and Asia. Both plant samples were found to be high in nutritive value especially in protein and very appetizing in ruminants. The main objectives of the study were to measure and compare the nutritional composition of *Setaria sphacelata* and *Cleome gynandra* as a function of their nutritional value for ruminants. The two plant samples were collected near the Tembila area, Besut Terengganu. The samples were dried in a furnace below 60 - 70 °C and crushed prior to further analysis using proximate analysis. Proximate analysis was used to measure values for moisture, dry matter (DM), ash, crude protein (CP), crude fibre (CF), ether extract (EF) and nitrogen-free extract (NFE). The result of this analysis shows that *Cleome gynandra* had much higher crude protein (CP) ($p < 0.05$) at 36.86% and ether extract (EF) at 5.50%. Crude protein (CP) is one of the most essential nutrients that ruminants need. Therefore, this study found that *Cleome gynandra* contains a higher nutritional value in terms of crude protein (CP) than *Setaria sphacelate*, which can be used as a feed for ruminants.
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Keywords: *Cleome gynandra, Setaria sphacelata,* proximate analysis, ruminant feed, nutritive

INTRODUCTION

Setaria sphacelata is a high grass and the most widespread in tropical and subtropical regions such as Africa, Australia, and Asia (primarily in Malaysia and Indonesia). *Setaria sphacelatea* is widely cultivated for grazing beef cattle. Instead, it is tolerant of cold temperatures and has the ability to survive in dry seasons and resist flooding and watering. *Setaria sphacelata* considerably as highly palatable grasses for all classes of livestock. It is mainly utilized as fodder. However, it also can be hay or silage. A cut-and-carry system is the types of grazing that a suitable use for this grass (Cook et. al, 2005). The *Setaria sphacelata* expect to have about 10 – 15 t/ha of dry matter per year and approximately 6 – 15% of crude protein. While *Cleome gynandra* is a tropical annual herb which commonly used as vegetables in Africa and Asia. *Cleome gynandra* also known as an African spider flower which can be found in a wide range of soils that mostly on sandy to clay loam. *Cleome gynandra* favors high organic matter and suitable mineral reserves. The nutritional values in a certain type of pastures were measured using the proximate analysis for measuring the properties of moisture or dry matter (DM), crude ash, carbohydrate, crude protein, crude fiber, and fats. The *Cleome gynandra* expect to have about 30 t/ha of dry matter per year (Tran et al., 2020).

In Malaysia, ruminant production still does not exceed self-sufficient demand. Currently, Malaysia's ruminant production is approximately 23% self-sufficient, while local production is 45,353 million tones This scenario, which accounts for about 90% of the ruminant industry, is still owned by smallholders (Mohamed et al., 2013). Most small farmers are unable to feed their animals with adequate amounts of nutrition. Animal feed is very important in the livestock industry, where critical nutrition plays an important role in livestock production. However, the cost of feedstuffs involved, such as commercialized pellets is extremely high that lead into inability towards smallholders to obtain it. Feed creates a big part of the production rate in the livestock sub sector. Consequently, the success of the ruminant industry is primarily dependent on locally available feed. Therefore, this study can help small farmers in Malaysia obtain high quality fodder at low cost for their animals just by using grass like *Setaria sphacelata* and *Cleome gynandra*.

The findings of this study are important in introducing the ability of *Setaria sphacelata* and *Cleome gynandra* into the diet of ruminants because of their nutritional characteristics. It can therefore solve the problem of feeding ruminants to small farmers who can replace the consumption of marketed pellets. In addition, this study able to give an idea towards small farmers to cultivate *Setaria sphacelata* and *Cleome gynandra* instead of common pasture grass like Napier grass in their place. Thus, the aims of this study were to measure the nutrient compositions of *Setaria sphacelata* and *Cleome gynandra* 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 *Setaria sphacelata* and *Cleome gynandra* were collected from the Tembila area, 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 *Setaria sphacelata* and *Cleome gynandra* were divided into several groups to perform proximate analysis. Next, the *Setaria sphacelata* and *Cleome gynandra* 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 105ºC 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:

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

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

Both *Setaria sphacelata* and *Cleome gynandra* showed a significant difference ($p < 0.05$) in the percent proximate composition result, as shown in Table 1. The percentage of moisture, crude protein (CP), ether extract (EE) and ash content were highest in the *Cleome gynandra* whereas, the dry matter (DM), crude fibre (CF) and nitrogen-free extract (NFE) were highest *Setaria sphacelate.*

Table 1 shows the highest moisture content in *Cleome gynandra* compared to *Setaria sphacelata* with 85.03% and 75.38%, respectively. In addition, the proportion of dry matter (DM) obtained in *Setaria sphacelata* is higher than *Cleome gynandra* at 24.62% and 14.97%, respectively. The percentage of crude protein (PC) displayed in *Cleome gynandra* was 36.86%, while *Setaria sphacelata* was 11.80%. For the percentage of ether extract (EE), *Cleome gynandra* obtained the highest value with 5.50% relative to *Setaria sphacelata* reported as 2.83%. However, the percentage of crude fibre (CF) in *Cleome gynandra* was lower than *in Setaria sphacelata* with 13.54% and 32.29%, respectively. The mean values for moisture content, DM, CP, EE and CF was a significant difference at $p < 0.05$.

The highest percentage of ash found in *Cleome gynandra* was 9.72%, compared with 7.44% for *Setaria sphacelata.* In addition, the highest proportion of nitrogen-free extract (NFE) in Setaria sphacelata was 45.63%, while Cleome gynandra was 35.38%. The mean values for ash and NFE showed that there was a significant difference at $p < 0.05$ for both samples, *Setaria sphacelata* and *Cleome gynandra.*

Table 1. Proximate composition of *Setaria sphacelata* and *Cleome gynandra.*

Parameters (%)	Samples Mean ± SD	
	Setaria sphacelata	*Cleome gynandra*
Dry Matter (FW)	$24.62^{a} \pm 0.42$	$14.97^{b} \pm 0.54$
Moisture	$75.38^{b} \pm 0.42$	$85.03^{a} \pm 0.54$
Ash	$7.44^{b} \pm 0.37$	$9.72^{a} \pm 0.20$
Crude Protein (CP)	$11.80^{b} \pm 0.36$	$36.86^{a} \pm 0.78$
Ether Extract (EE)	$2.83^{b} \pm 0.03$	$5.50^{a} \pm 0.19$
Crude Fibre (CF)	$32.29^{a} \pm 0.37$	$13.54^{b} \pm 0.05$
Nitrogen - Free Extract (NFE)	$45.63^{a} \pm 0.59$	$35.38^{b} \pm 0.68$

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

The proximate composition of *Setaria sphacelata* and *Cleome gynandra* on moisture, DM, CP, CF, EE, ash and NFE are presented in the Table 1. In terms of percentage of dry matter, *Setaria sphacelata* obtained the highest percentage (24.62%) compared to *Cleome gynandra* (14.97%) and significantly different at ($p < 0.05$). The result obtained almost similar with previous study from Thiollet et al., (2019) which reported that the average dry matter in *Setaria sphacelata* was 23.1%. The result obtained by *Cleome gynandra* on dry matter also almost similar with study from Agbo et al., (2014) which found to have 13.22% of DM. Both findings slight differences in results with previous study due to different seasonal between previous study areas (Africa) and Malaysia. In addition, the percentage of DM on both plants' samples seen to be low because high moisture content (fresh samples) was measured. Even though there was a low percentage of DM in *Cleome gynandra*, it still can be fed to animal in bulk to meet the requirement DM of ruminant animals. As general, dry matter determines the nutrient content such as protein, fat, carbohydrates, and fibre in animal feed after removal of moisture content (Samad, 2019).

Animals need to consume a certain amount of dry matter according to their needs to maintain production and health (Wilkin, 2000). Commonly, the amount of dry matter given to ruminant animals is between 1-3% of their body weight, but it depends upon on several other factors including stage of production (Samad, 2019). In addition, there was a significantly different at $p < 0.05$ on the percentage of ash, which *Cleome gynandra* obtained higher percentage than *Setaria sphacelata* with 9.72% and 7.44%, respectively. The result obtained by *Cleome gynandra* slightly different with study from Jinazali et al., (2017) which ash content found in *Cleome gynandra* was 6.1%. It may be due to some external factor such as environmental differences on the study site between previous study and the present study. According to Weiss (2019), ash content referred to the mineral compositions in plants such as potassium, calcium, magnesium and copper that were affected by the environmental conditions.

In terms of crude protein (CP), *Cleome gynandra* has highest percentage value with 36.86% compared to *Setaria sphacelata* with 11.80%. The result showed there was a significant difference at $p < 0.05$. In accordance with previous, the result obtained for *Cleome gynandra* similar with finding by Lebas et al., (2020) which *Cleome gynandra* found to have more than 24% of CP. In general, crude protein is one of the main essential elements on ruminant nutrition. According to Lardy (2018), crude protein includes with true protein and nonprotein compounds. It refers to the amount of nitrogen (N) that is needed to make acid amino blocks which are then utilised to makes the protein needed by ruminants' animals. Deficiency of CP in animal leads to improper function of vital organs and systems of animals. As general, Capelloza (2019) reported that more than 7% of CP needed by small ruminants while Ondarza (2004) mentioned that 16% of CP needed by large ruminants in purpose for maximal growth and activity of ruminal microorganisms. However, the requirements of CP varied with production stages (Capelloza, 2019). Thus, the result obtained from CP on both plants have ability to meet the requirements of crude protein needed by both small and large ruminants which it has great potential to be as animal feed.

Furthermore, there was a significant difference at $p < 0.05$ on CF results. *Setaria sphacelata* obtained the highest percentage (32.29%) compared to *Cleome gynandra* (13.54%). The result obtained nearly similar with study from Heuze et al., (2017) which found the percentage of crude fibre averagely 34.4%. The value of CF in animal feed is important, but it should be given to animal according to the specific requirements needed by the animals. CF help in maintaining hindgut health and microbial population in ruminants. Too much or too low on consumption of CF in the animal's diet may lead into several problematic health toward animals. According to Lardy (2018), CF refers to indigestible portions of plant material, but it can be partially digested by the microorganisms in the rumen of animals. It represents cellulose, hemicellulose, and lignin. For small ruminant, the minimum requirement of CF is 12% (Rashid, 2008). According to Moran (2005), reported that the minimum requirement of CF for large ruminant is 17%. However, *Setaria sphacelata* recommended to be given to ruminants in small portion to prevent too much excessive of CF among ruminants.

In terms of EE, *Cleome gynandra* has a higher percentage than *Setaria sphacelata* with 5.50% and 2.83% respectively. There was a significant difference ($p < 0.05$). The result obtained higher compared to previous study by Coulibaly et al., (2018) which reported that *Cleome gynandra* have 2.19% of EE. Ether extract is an organic compound that non-soluble in water, but soluble in organic solvents. It is also known as crude fat, which consist of triglycerides that commonly essential in animal nutrition. Too much excessive amount of ether extract in ruminant diets is detrimental which it causes unpalatable and cause a loss in rumen microbes

(Comeford, 2014). Minimum requirement of ether extract in ruminant reported by Esmail (2018) was between 2% – 3% of ether extract. This requirement varies with production stage. However, the amount of EE cannot over than 7% of diet dry matter if it is given to ruminants because its able cause negative side effects such as metabolic problems which can cause damage to the rumen health (Coulibaly et al., 2018).

In terms of Nitrogen – Free Extract (NFE) typically consists of readily digestible carbohydrates. The percentage of NFE was influenced by the values of CP, CF, total ash, and EE (Anita et al., 2016). Results of this study was showed there was a significant difference at $p < 0.05$ for the mean values of NFE component. Setaria *sphacelata* obtained higher percentage of NFE compared to *Cleome gynandra* with 45.63% and 35.38%, respectively. However, almost the whole proximate composition of the *Setaria sphacelate* was lower than that of *Cleome gynandra*. The result of this study was similar with the findings by Andree at al., (2009) found an average of 45% of NFE's. The NFE may be able to be as energy source for body process in ruminants.

CONCLUSION

This study showed the different pasture species have different nutrient contents. The means nutrient contents for the two pasture species which are *Cleome gynandra* and S*etaria sphacelate* were significantly different. In addition, crude protein (CP), ether extract (EE), moisture and ash in *Cleome gynandra* were higher compared to S*etaria sphacelate*. This study suggested that *Cleome gynandra* has a higher potential to be as an animal feed than S*etaria sphacelate*. Keeping view the above fact, further determination of mineral analysis must be taken out for *Cleome gynandra* and *Setaria sphacelata* for ensuring the accurate information regarding mineral composition that needed by the ruminants.

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//

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How to cite this paper:
Kamaruddin, N.A., Mohd Hanafee, N.A., Ali, N., Liew, S., Yasin, N.Y. (2021). Nutrition Assessment of *Setaria sphacelata* and *Cleome gynandra* as Potential for Ruminant Feed. *Journal of Agrobiotechnology, 12*(1S), 166-173