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Comparative Study on Nutritive Value of Different Legumes Species (*Leucaena leucocephala, Calopogononium muconoides and Stylosanthes guianensis*)

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ABSTRACT

Legumes are the plant that contains high protein content that has been used as supplementary for animal feed and has a great potential to increase the productivity of livestock. However, no much study has been measured on the nutritive value of legume species. Thus, the aims of this study were to measure the nutritive content in the leaves of three different legume species (*Leucaena leucocephala*, *Calopogonium mucunoides* and *Stylosanthes guianensis*) and to compare the species that contain high of nutritive value. The samples were collected in Tembila area, Besut, Terengganu, then were brought to the Plant Physiology laboratory at UniSZA Kampus Besut for sample preparation. The samples were washed under tap water for 30 seconds to prevent nutrient leaching, then were dried and ground before analyzed by proximate analysis and mineral analysis using Induces Coupled Plasma Optical Emission Spectrometry (ICP-OES) machine. The parameters that were measured by using the proximate analysis consist of dry matter (DM), crude protein (CP), crude fibre (CF), ash, ether extract (EE) and Nitrogen Free Extract (NFE). For mineral analysis, the samples were analyzed for Calcium (Ca), Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn). The results of this study showed that *Leucaena leucocephala* had significantly higher ($p < 0.05$) of CP and EE. While, *Stylosanthes guianensis* had significantly higher ($p < 0.05$) of the micro-elements which are Zn, Fe and Mn. Therefore, this study revealed that *L. leucocephala* contained the highest nutritive value of CP, EE and Calcium.

Keywords: *Leucaena leucocephala*, *Stylosanthes guianensis*, *Calopogonium mucunoides*, mineral analysis, proximate analysis

INTRODUCTION

Legumes are the most important plant species used for forage. The forage plants contain different quantities of fibre, lignin, minerals and protein, and vary in the proportion of their tissue that can be digested by ruminants which the herbaceous legumes contained the highest of protein (Lee, 2017). Legumes are not only useful for

human, but it can be as an animal feed and improving soil component of agriculture (Misra, 2016). Humphreys (1991) reported that legumes have an important role in farming system of the tropical area through their contributions to enhance the nutritive value of animal diet, biological nitrogen (N) fixation and landscape stability. Legumes have great potential to increase the productivity of livestock due to its high nutrient content (Warly et al., 2004b). Legumes have several advantages in the context of animal nutrition which are widely recognized that contain higher protein content compare to grasses and cereals (Mello, 1992).

In Malaysia, the current livestock producer face problems in providing high quality and sufficient feed throughout the year. The basal feeds for ruminant production in Malaysia are the native grasses and shrubs. These tropical forages, which are also the sole feed in most ruminant production systems. By the years, the local grazing grass become fibrous and have lower digestibility values as compared with their temperate counterpart. The nitrogen content of these grasses is just marginally above 1% and their potential for animal production is low (Zahari & Wong, 2009). The net result is a poor-quality feed and in some situations such as during dry seasons and insufficient supply as well for the animals. The shortage of green feed is significant during the flooding season. The farmer face problem to feed livestock low-cost, readily available and high-quality feedstuffs in a suitable manner without affecting the productivity. The availability of feed that containing imbalanced chemical composition and metabolize energy is a major problem in ruminant production the world over. Most of the available feed contains high fibre content with low protein content. This situation makes the farmers need to depend on the pallets as the supplement to increase the nutrient value in the feeds which is much costing.

Thus, the legumes have been introduced in Malaysia as supplementary feed for ruminant due to feed shortage during dry and flooding season and low quality of other forages (Zahari & Wong, 2009). Local legumes are shown to be of better quality because it contains high protein content and low fibre (Warly et al., 2004a). The average of nitrogen and energy contents of these native forages is reported to be comparable to those recorded for the introduced tropical grasses and legumes. It showed that legumes contain more nutrient (Amiri & Shariff, 2012). Through some research, the legumes are more suitable as a supplement rather than as a sole feed for ruminants. The ruminant must consume less than 50% of legumes because of some effects, but it depends on the species of the legumes (Dewhurst, 2013). The species of legumes that were used in this study are *Leucaena leucocephala, Calopogonium mucunoides* and *Stylosanthes guianensis*. The aims of this study were to measure the nutritive value of different species of legumes (*Leucaena leucocephala, Calopogonium mucunoides* and *Stylosanthes guianensis*) and to compare the nutritive value between these three legume species.

MATERIALS AND METHODS

Sample collection and sample preparation

Leaves from three legume species, *L. leucocephala*, *C. mucunoides* and *S. guianensis* were collected in Tembila, Besut area (5°45'00"N, 102°37'59"E). Then, the plant samples were placed into plastic bags and transferred to the Plant Physiology Laboratory of University Sultan Zainal Abidin (UniSZA), Besut Campus for providing materials for this study. The samples were washed under the tap water for 30 seconds to avoid the nutrient leaching. The samples of fresh leaves *Leucaena leucocephala*, *Calopogonium muconoides* and *Stylosanthes guianensis* were dried in the incubator at 65 °C for 48 hours (Portela et al., 2007).

Nutritive value content analysis

Proximate analysis was used to determine qualitative and quantitative measurement content of moisture (dry matter) and total solids, protein, ether extract, crude fibre, total ash, phosphorus and NFE. All the samples were analyzed according to the standard methods of Official Methods of Analysis (AOAC, 2005) in the triplicate. Details procedures of each parameter were explained below.

Dry matter

The fresh samples were used in this analysis. The crucibles were dried with cover for four hours in an oven at 105°C. The crucibles were cooled until it was reached the room temperature. Five grams of the fresh samples were weighed and, then it was placed into the crucibles. The samples were placed uncovered in oven at 105°C for six hours. The samples were removed and were cooled in desiccator. The crucibles were weighed after it reaches room temperature. Below the formulation for dry matter:

$$\%\, Moisture = \frac{W_2}{W_2} - \frac{W_3}{W_3} \times 100 \qquad \textbf{Eqn. 1}$$

Where,

W_1 = Weight of crucible (g)
W_2 = Weight of crucible + weight of wet sample (g)
W_3 = Weight of crucible + weight of dry sample (g)

% Dry matter = 100 - % Moisture

Ash analysis

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 105°C 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 550°C for overnight. The samples were removed and cooled in a desiccator, then it was weighed after it reached room temperature. The percentage of ash was calculated by using a formula:

$$\%\, Ash = \frac{(W_3 - W_1)}{W_2} \times 100 \qquad \textbf{Eqn. 2}$$

Where,

W_1 = Weight of crucible (g)
W_2 = Weight of the sample (g)
W_3 = Weight of crucible + ash (g)

Crude protein

All protein contains about the same amount of nitrogen (16%). There are three steps which were digestion, dilution and filtration process. According to Kjeldahl method, Nitrogen x 6.25 = crude protein. It is because of 16% of N in protein. It is called crude protein because not all N comes from the amino acid in a protein (Ahmed & Hasan, 2014). The first process is the digestion operation by using the Kjeldahl method, in Gerhardt system. The samples were prepared and weighed one gram into a digestion tube. Then 2 tablets of a catalyst Kjeltabs Cu 3.5 were added into a digestion tube. 12 ml of concentrated sulphuric acid (H_2SO_4) were added carefully and shook gently to wet the sample with the acid. The rack was loaded with exhaust system into a digestion tube in the rack. The tap water was turned on and switched on the scrubber unit. The control was switched on and set the temperature at 400°C. The samples were digested until all samples were clear with a green or blue solution. This normally be over 60 to 90 minutes, depending on the sample. The rack of tubes was removed by the exhaust system still in place and were cooled for 10 to 20 minutes.

For distillation process, the power system of the distillation unit was switched on. 25 ml of boric acid was filled with five drops of indicator solution into a conical flask. The conical flasks were placed into distillation unit and

platform was closed so that the distillate outlet is submerged in the receiver solution. The digestion tubes were placed in the distillation unit and closed the safety door. The desired program was pressed and 70 ml distilled water were dispensed into the tube automatically and followed by 50 ml of 32% sodium hydroxide (NaOH). The distillation process was taking approximately four minutes. The receiver solution in the distillate flask turned to green indicating the presence of alkali (ammonia). The last operation is titration. The distillates were titrated with standardized hydrochloric acid (HCl) 0.1 N until the color of mixture turn to pink or red. The volume of hydrochloric acid was recorded that used for sample and blank. Below the equation for protein content.

$$\%\, Nitrogen = \frac{A \times (T-B)X\ 14.007 \times 100}{Weight\ of\ sample\ (g) \times 100} \qquad \textbf{Eqn. 3}$$

Percentage of crude protein = Percentage of nitrogen x F

Where,

T = Volume acid for sample
B = Volume acid for blank
A = Normality of HCL
F = Protein factor, 6.25

Ether extract

The extraction cups were dried in the oven at 105°C 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 were 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 removed into a desiccator to cool and were weighed. The percentage of fat was calculated by using the below formula:

$$\%\, Fat = \frac{(w_3 - w_2)}{w_1} \times 100 \qquad \textbf{Eqn. 4}$$

Where,

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

Crude fibre

From this method, the amount fat – free organic substances which are insoluble in acid and alkaline media was determined. At the first step, the fibre bags were weighed. The samples were weighed for one gram into the fibre bags. The glass spacers were put into the fibre bags and insert the bags in a carousel. If the fat content more than 10%, defatting was done by immersing the carousel three times into 100 ml 40/60 (boiling range) petroleum ether. By turning it as well as moving up and down the samples was defatted. The fibre bags were dried up for approximately two minutes. The carousel was placed into the axis carousel before placing into the glass container. The glass container was placed on the previewed position of the hotplate. The container was pushed all way back to the catch at the rear end. The program method was started. Then the fibre bags were removed from the carousel and were put it into the crucible. The fibre bags were dried up for four hours or

overnight at 105°C. Then it was cooled in the desiccator for 30 minutes. The crucibles and fibre bags were dried after digestion. It then was placed in the furnace at temperature 550°C and ignited for four hours. The crucibles containing ash were removed and cooled in a desiccator. It then was weighed right after reach room temperature. For the blank value, the empty crucible was weighed. Then the crucible and ash of the empty fibre bag was weighed. The percentage of crude fibre was calculated using below equation:

$$\% \, Crude \, Fiber = \frac{[(w_2 - w_1) - (w_4 - w_5)]}{w_2} \times 100 \qquad \textbf{Eqn. 5}$$

Blank value (W_5) = $W_7 - W_6$

Where,

W_1= Weight of fibre bag (g)
W_2=Weight of sample (g)
W_3= Weight of crucible (g) + fibre bag after digestion (g)
W_4= Weight of crucible and ash (g)
W_5= Weight of blank value empty fibre bag (g)
W_6= Weight of crucible (g)
W_7= Weight of crucible + ash of empty fibre bag (g)

Nitrogen-free extract (NFE)

NFE supposedly represent soluble carbohydrate of feed such as starch and sugar. This fraction may also contain solubilized hemicellulose and lignin. Calculation of NFE was determined by using the formula:

$$Percentage \, of \, NFE = 100 - (\% \, EE \, + \% \, CP \, + \% \, ash \, + \% \, CF) \qquad \textbf{Eqn. 6}$$

Statistical analysis

Data were analyzed using a single factor one-way ANOVA and presented as mean ± SD along with the level of significance to determine the significant difference between nutritive values in different species of legumes. The value $p < 0.05$ was considered as significant differences. The SPSS 2.0 statistical software was used for the statistical analysis.

RESULTS AND DISCUSSION

Proximate analysis

The different species of legumes have a significant difference ($p < 0.05$) on the percentage of the result of the proximate analysis. The percentage of the crude protein (CP) and ether extract (EE) were the highest in *L. leucocephala*, which are 27.49% and 7.70%, respectively. The percentages of ash and crude fibre (CF) were the highest in *S. guianenis,* which are 10.14% and 18.50%, respectively. The *Calopogonium muconoides* had the highest percentage in the analysis of dry matter (DM) and nitrogen-free extract (NFE) which are 34.02% and 56.26%, respectively. The highest percentage of dry matter (DM) showed in *C. muconoides,* which is 34.02% where in *S. guianenis* and *L. leucocephala* were 27.83% and 28.38%, respectively. There is a significant difference ($p < 0.05$) for mean values of DM for the three species of legumes. The percentage of ash in *S. guianenis* was 10.14% higher than *L. leucocephala* (5.54 %) and *C. muconoides* (5.52 %). *Leucaena leucocephala* showed the highest percentage of CP, which is 27.49% meanwhile *S. guianensis* and *C. muconoides* were 21.71% and 17.86% respectively. The mean value for CP showed that with a significant difference at $p < 0.05$. Other than that, the mean value of CF also showed that there is the significant difference at $p < 0.05$ for *S. guianensis* (18.50%), *C. muconoides* (14.90%) and

L. leucocephala (7.36%). For a percentage of EE, *L. leucocephala* showed the highest result, which is 7.70%, followed by *C. muconoides* (5.45%) and *S. guianenis* (3.27%). *Calopogonium muconoides* showed the highest result for a percentage of NFE, with 56.26%, where 51.82% and 46.39% for *L. leucocephala* and *S. guianensis*. The mean value for NFE showed that there is a significance difference at $p < 0.05$.

Table 1. Proximate composition of different legume species.

Parameters (%)	Samples		
	L. leucocephala	*C. muconoides*	*S. guianenis*
Dry matter	$28.38^{b} \pm 0.10$	$34.02^{a} \pm 0.17$	$27.83^{c} \pm 0.15$
Ash	$5.54^{b} \pm 2.97$	$5.62^{b} \pm 0.06$	$10.14^{a} \pm 0.07$
Crude Protein	$27.49^{a} \pm 0.00$	$17.86^{c} \pm 0.18$	$21.71^{b} \pm 0.35$
Crude Fibre	$7.36^{c} \pm 0.56$	$14.90^{b} \pm 0.29$	$18.50^{a} \pm 0.41$
Ether Extract	$7.70^{a} \pm 0.10$	$5.45^{b} \pm 0.03$	$3.27^{c} \pm 0.12$
NFE	$51.82^{b} \pm 0.63$	$56.26^{a} \pm 0.20$	$46.39^{c} \pm 0.23$

^{abc} Means in the same row with different superscripts are significantly different ($p<0.05$).

The proximate compositions of *Leucaena leucocephala, Calopogonium mucunoides* and *Stylosanthes guianensis* are presented in the Table 1 above. *Calopogonium mucunoides* showed the highest percentage of dry matter (34.02%), where the result showed a significant difference ($p < 0.05$). This is because it contains low moisture and hairy leaves. The lower of DM also make the lower nutrient density in the fresh feed. Also, high moisture may decrease the keeping quality of a feed (Duponte, 1998). *Calopogonium mucunoides* showed that there is no significant difference with *L. leucocephala* in term of ash. But *S. guianensis* showed that there is a significant difference ($p < 0.05$) with the highest percentage (10.14%). This is different from the study by Geleti et al. (2013), where the ash of *S. guianensis* is 5.3%. Ash is a measure of the total mineral content of the feed, but it does not give the specifics of each mineral is present (FAO, 2011). The high ash content of feeds may dilute the amount of nutrients available to the animal. It needs by ruminant where it is essential for their health (Duponte, 1998).

In term of CP, *L. leucocephala* showed the highest one which is 27.49%. Based on the previous report by Muamba et al. (2014), *L. leucocephala* is one of the legumes that contain the highest protein value. The CP includes both true protein and non-protein nitrogen which can be used most effectively by ruminant animals. Among the non-structural carbohydrates, starch, fructose and sugars are important components for the ruminant (Duponte, 1998). Leaves of *Leucaena sp.* had the highest nutrient content, whereas others part of this plant has low nutrient content (Lie et al., 2015). Warly et al. (2004b) reported that the CP content of *L. leucocephala* is 29.1%, higher than other types of legumes. The higher percentage of crude fibre (CF) was showed by *S. guianensis* and the lowest percentage showed by *L. leucocephala* which are 18.50% and 14.90%, respectively. This because CF mainly consists of cellulose, a linear polymer of glucose and further of lignin, a polymer of phenolic acids. The hemicellulose and cellulose are partly digestible by the ruminants but almost not digestible for monogastric animals where their digestibility mainly depends on the lignification degree (Duponte, 1998).

For a percentage of EE, *L. leucocephala* also show the highest percentage which is 7.7%. Ether extract mainly contains fatty acids and sometimes pigments and waxes that the former provide energy, but also building blocks for the formation of animal fat and are a source of vitamins (Barbosa et al., 2017). If a feed is high in fat, it may be susceptible to rancidity, which causes off-flavours, low palatability, and potential toxic effects. Fat is also helpful in lubricating and maintaining feed mixing equipment (Duponte, 1998). Muamba et al. (2014) reported that *L. leucocephala* forage presented higher CP and ether extract levels. The Nitrogen-free extract (NFE) in *C.*

muconoides was the highest among the three species. The NFE is a calculated value when the original sample weight minus the sum of weights of water, ether extract, crude protein, crude fibre, and ash (FAO, 2011). NFE is made up primarily of readily available carbohydrates, such as sugars and starches; this fraction may also contain solubilised hemicellulose, and lignin exclude the protein content (Rodrigues et al., 2014).

Mineral analysis

The mineral composition of the *Leucaena leucocephala, Calopogonium mucunoides* and *Stylosanthes guianensis* showed that there were differences between these species. Table 2 shows the mean values of mineral composition of *Leucaena leucocephala, Calopogonium mucunoides* and *Stylosanthes guianensis*.

Table 2. Mineral composition of different legume species

Parameters (mg/kg)	Samples		
	L. leucocephala	*C. muconoides*	*S. guianenis*
Calcium	$143.15^{a} \pm 1{,}521.62$	$105.10^{a} \pm 1{,}487.90$	$168.03^{a} \pm 237.62$
Zinc	$12.78^{c} \pm 0.39$	$19.38^{b} \pm 1.38$	$39.08^{a} \pm 1.87$
Iron	$109.33^{b} \pm 2.72$	$246.08^{ab} \pm 39.70$	$285.23^{a} \pm 49.18$
Manganese	$12.03^{c} \pm 0.25$	$125.85^{b} \pm 7.35$	$191.78^{a} \pm 9.58$
Copper	$43.15^{a} \pm 8.28$	$33.83^{a} \pm 2.65$	$55.45^{a} \pm 17.11$

^{abc} Means in the same row with different superscripts are significantly different ($p<0.05$).

The result showed that there is no significant difference ($p > 0.05$) of Ca content among these three species. Calcium content in *L. leucocephala, C. mucunoides* and *S. guianensis* were showed by 143.15 mg/kg, 105.10 mg/kg and 168.03 mg/kg respectively. According to Warly et al. (2010), the Ca content in legumes are mostly highest and not have different concentration of macronutrient.

The results showed that there is a significant difference ($p < 0.05$) of zinc (Zn) content among these three species of legumes. The result showed that the Zn content was highest in *S. guianensis* 39.08 mg/kg, where *C. mucunoides* contain 19.38 mg/kg, and the lowest result was *L. leucocephala* contain 12.78 mg/kg. Zinc is necessary to the plant where it is essential for the transformation of carbohydrate, regulates the consumption of sugar and also regulate the plant growth. Zinc concentration in all species in this study was within the recommended range of 12 to 20 mg/kg, which is sufficient for growing ruminants (Gill et al., 2004).

Manganese (Mn) content in *S. guianensis* (191.78 mg/kg) was higher than *C. mucunoides* (125.85 mg/kg) and *L. leucocephala* (12.03 mg/kg). The result showed that there is a significant difference ($p < 0.05$) of Mn content among the three species. Manganese is necessary for normal fertility in ruminants. If feeding with low-manganese ration can cause depresses conception rates.

Stylosanthes guianensis has the result of iron (Fe) which is 285.23 mg/kg higher than *C. mucunoides* (246.08 mg/kg) and *L. leucocephala*. (109.33 mg/kg). There is a significant difference ($p < 0.05$) between *S. guianensis* and the other two species. Iron is another important mineral composition that needs by the plant and also ruminant. It is important for ruminant reproduction. Low availability in certain instances could affect highly ruminant reproduction. Therefore, it is necessary to select the forage that rich in iron. In this study, Fe content was above the level of 50 mg/kg in the three species which was proposed as adequate for grazing animals (Gill et al., 2004; Khan et al., 2005).

The result showed that there is a significant difference ($p < 0.05$) of copper (Cu) content among the three species. *Stylosanthes guianensis* contains the highest concentration, with 55.45 mg/kg. C*alopogonium*. *muconoides* show the lowest percentage of the Cu with 33.83 mg/kg where *L. leucocephala* contain 43.15 mg/kg. According to Ward (2005), if the plant contains the high Ca, Cu availability will be reduced. It is commonly suggested that the dietary requirements of ruminants for Cu range from 8 to 14 mg/kg (Khan et al., 2006). High content of cell wall constituent (crude fibre) has been associated by attachment more minerals into the cell wall. However, most of the mineral elements were found in the cell contents and should be available to the ruminants (Warly et al., 2010).

CONCLUSION

This study has shown that different species of legumes have different on the nutritive value. The nutritive value content in the *Leucaena leucocephala, Calopogonium mucunoides* and *Stylosanthes guianensis* were measured by proximate analysis and also mineral analysis by using the ICP-OES. The result showed that there was a significant difference ($p < 0.05$) in the mean of the nutritive value among the three species of legumes which are *Leucaena leucocephala, Calopogonium mucunoides* and *Stylosanthes guianensis*. The species of legume that contain the highest of nutritive value were determined after comparing the nutrient content between the three different species. The highest nutritive value is *L. leucocephala* compared to *C. muconoides* and *S. guianensis*. This study revealed that the *L. leucocephala* contained highest nutritive value of CP, EE and calcium. It has the potential to be used as feedstuffs for livestock. In future recommendation, these three species can be applied to the ruminant to know the effectiveness of the growth and can be compared with available feed.

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