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Evaluation of the nutrient composition of *Azolla microphylla* and *Azolla pinnata*

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Azolla is an aquatic free-floating fern known to have a high nutritional value and very productive plant, which is potential for feedstuff. However, the nutritional composition of the Azolla species varies. This study aims to determine the nutritional compositions in two different species of Azolla: *Azolla pinnata* and *Azolla microphylla.* This study was conducted at the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus. The samples of both Azolla species were collected in Bachok, Kelantan and transferred to the campus for culturing. Both species were cultured in separate tanks in triplicate and harvested in the following weeks. The fresh samples of both species were dried and ground for proximate analysis and mineral composition using dry ashing methods and Induced Couple Plasma Optical Emission Spectrometry (ICP-OES). The results show that A. *microphylla* contains the highest value for both analyses, proximate and mineral composition, and significant difference ($p<0.05$). The A. *microphylla* contains 95% of moisture, 39% of nitrogen-free extract (NFE), 30% protein, 13% ash, 11% fibre and 5% ether extract (EE). The mineral concentration of iron (Fe), Manganese (Mn), and Zinc (Zn) were highest in the *A. microphylla,* which are 21%, 15% and 8%, respectively. This study revealed that *A. microphylla* is more suitable for animal feed. It contains high nutritional value compared to *A. pinnata,* especially for crude protein and NFE essential for ruminant diet.

Keywords: Aquatic plants, proximate analysis, mineral composition, ruminant diet, *Azolla sp.*

INTRODUCTION

The deficit and fluctuating quality and quantity of feedstock are major constraints to livestock production in developing countries (Sihag et al. 2018). It is a significant challenge to sustain the excellent quality and quantity of feedstock. To solve this problem, the farmers substituted the roughages with concentrated pellets or conventional feed ingredients, which are costly and not always available at affordable prices, such as protein supplements. Since the cost of feeding is a crucial factor in determining the economic viability of the livestock sector (Senthil et al. 2020), the use of them must be minimised by implementing a new ration formulation. Thus, there is a significant requirement to use affordable alternative feedstuffs to make livestock production profitable.

There are varieties of affordable and highly nutritious feedstuffs for livestock. In Asia, the farmers have cultivated native aquatic plants for various uses, including animal feed, human food, and green manure (Chatterjee et al. 2013). One of the aquatic plants is Azolla, a free-floating aquatic fern in shallow water and the only genus belonging to the Azollaceae family.

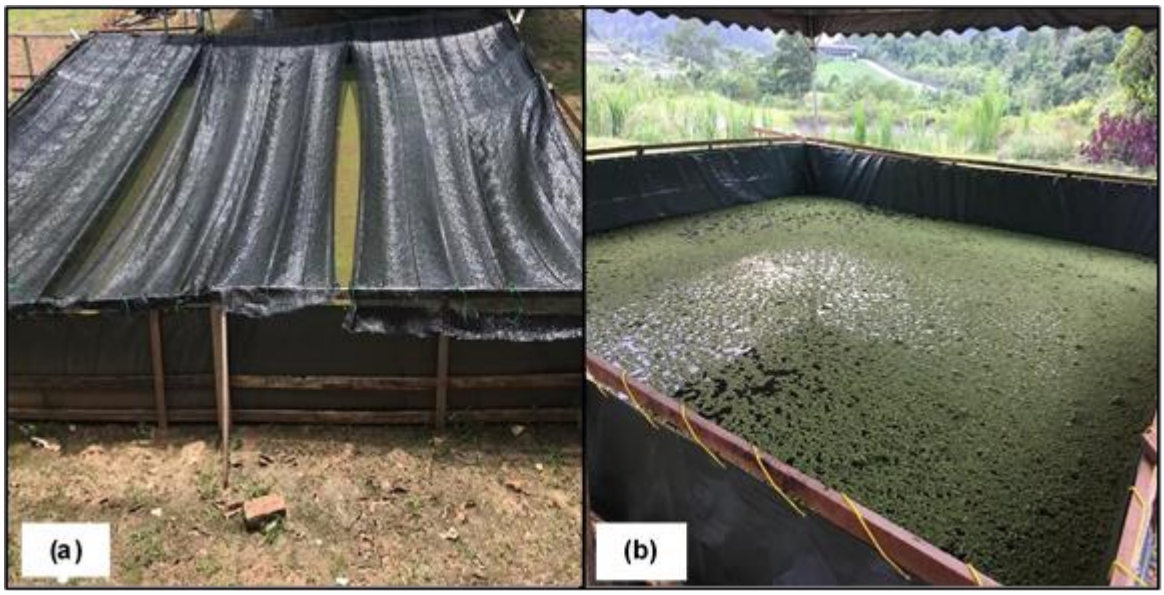

Figure 1: Acclimatisation and cultivation of Azolla species (a) *Azolla pinnata (b) Azolla microphylla*

It is commonly known as mosquito fern, duckweed fern, fairy mass and water fern and widely distributed throughout the tropical, sub-tropical and temperate freshwater systems (Ara et al. 2015). Wagner (1997) stated that it is also called Green Gold Mine and Super Plant as it contains a high nutritional value and fast growth. There are seven species of Azolla, and the most cultivated locally is *A. pinnata* and *A. microphylla*. According to Van Hove and Lejeune (2002), *A. pinata* commonly found in India, *while A. microphylla* in Tropical and Subtropical America. They also stated that these two species could be green manure, food, water purifier and some more (Chatterjee et al. 2013).

Anitha et al. (2016) reported that Azolla has a great source of protein and contains nearly all amino acid and micronutrients required for animal nutrition. These facts suggest that Azolla can be used as a non-conventional protein supplement for livestock, including ruminants, poultry, pigs, and fish (Hossiny et al. 2008). Due to the ease of cultivation, high productivity, and better nutritive value, it has been used as a beneficial feed supplement (Prabina and Kumar, 2010).

However, the reported nutrient composition of Azolla species varied depending on the environmental conditions, including temperature, light intensity, and soil nutrients (Chatterjee et al. 2013). These factors would therefore have an impact on growth morphology and its nutrient composition. Given these facts, this present study

is to determine the nutritional composition between two species of Azolla, which are *A. pinnata* and *A. microphylla*.

MATERIALS AND METHODS

Study site

This study has been carried out in Biochemistry Laboratory, Faculty of Bioresources and Food Industry in Universiti Sultan Zainal Abidin and Pasir Akar Farm in Besut.

Acclimatisation and cultivation of Azolla species

Three tanks for each species with even bottom and 1.5 ft width × 3 ft length × 2 ft height capacity were selected for this study. These tanks were filled with water and maintained the level of water at 15 cm from the bottom. Approximately 100-200 g of compost was dissolved in the water. Two-hundred-gram of *Azolla sp*. were put into each tank. The compost was added weekly to avoid nutrient deficiency.

Collection and preparation of Azolla species

A week after an acclimatisation and cultivation, Azolla sp. covered the surface of the tanks entirely. Azolla sp. were harvested and washed thoroughly under tap water and oven-dried at

80°C overnight or until constant weight (AOAC, 2005). The samples were ground using a glass laboratory blender till powdery. The samples were stored in zipper bags for further analysis.

Proximate composition 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 analysed according to the standard methods of Official Methods of Analysis, Association of Official Analytical Chemists 18th edition, (AOAC, 2005) in the triplicate. Detail 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 ºC. The crucibles were cooled until it was reached room temperature. Five grams of the fresh samples were weighed and, then it was placed into the crucibles. The samples were placed uncovered in the oven at 105 ºC for six hours. The samples were removed and were cooled in a 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 ºC 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 ºC 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:

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

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. 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 two 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 ºC. The samples were digested until all samples were clear with a green or blue solution. This normally is 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 the 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 a distillation unit. Then, the platform was closed so that the distillate outlet is submerged in the receiver solution. The digestion tubes were placed in the distillation unit and shut the safety door. The desired program was pressed, and 70 ml distilled water was automatically dispensed into the tube 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 standardised 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.

$$\% Nitrogen = \frac{A \times (T-B)X\ 14.007 \times 100}{Weight\ of\ sample\ (g) \times 100}$$ **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 ºC 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:

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

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

Crude fibre

The amount fat – free organic substances that are insoluble in acid and alkaline media was determined from this method. 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 were defatted. The fibre bags were dried up for approximately two minutes. The carousel was placed into the axis carousel before putting it 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 into the crucible. The fibre bags were dried up for four hours or overnight at 105 ºC. 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 ºC 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 were weighed. The percentage of crude fibre was calculated using the below equation:

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

Blank value (W_5) =$W_7 - W_6$

Where,

W_1= Weight of fibre bag (g)
W_2=Weight of the 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 solubilised hemicellulose and lignin. Calculation of NFE was determined by using the formula:

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

Mineral analysis

The mineral composition of Ca, Zn, Fe, Cu and Mn were determined using the dry ashing method according to AOAC (2005) procedures and were analysed using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

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 different species of Azolla. The value *p<0.05* was considered a significant difference. The SPSS 2.0 statistical software was used for the statistical analysis.

RESULTS AND DISCUSSION

Proximate analysis

The results of the proximate composition analysis between two *Azolla sp.* are presented in the Table 1. It showed that there is a significant difference (*p<0.05*) in proximate composition between *A. pinnata* and *A. microphylla*. *A. pinnata* shows lower percentages of crude protein, ether extract, moisture, and total ash compared to *A. microphylla*. In terms of crude protein content, *A. pinnata* showed lower percentages with only 19.16% than *A. microphylla* with 30.50%.

The crude protein content in *A. pinnata* is almost similar to the results obtained by Kavya et al. (2014), which is 21%. Higher crude protein values ranged from 22.06% to 28.24% reported by Indira and Ravi (2014), Kumar et al. (2015) Anitha et al. (2016), Ara et al. (2015) and Gupta et al. (2018). Our finding on the crude protein of *A. microphylla* aligns with the previous study reported by Prawitasari et al. (2012) with 31.25%. Others have shown that the percentage of crude protein of *A. microphylla* was below 30%. From the results, it is clear that *A. microphylla* has higher CP due to the nitrogen-fixing bacteria, Anabaena-azollae that lives in Azolla symbiotically. The higher crude protein content has potential as food sources, especially for livestock, as it is an essential component of optimum health and well-being.

For crude fibre, 15.05% and 11.77% were recorded in *A. pinnata* and *A. microphylla,* respectively. The percentage of crude fibre was significantly 4% lower in *A. microphylla* in comparison to *A. pinnata*. The crude fibre content of *A. pinnata* was close with Kavya, 2014 (15.5%), and Ara et al. 2015 (14.3%). Mohamed et al. (2018) reported lower crude fiber content ranging from 11-13%, while Shukla et al. (2018) reported higher crude fibre content with 17.29%. The crude fibre content of *A. microphylla* varies from 11-15% (Chatterjee et al. 2013; Prawitasari et al. 2012; Datta., 2011), and the present study was the lowest. Chatterjee et al. (2013) stated that feeding livestock with high fibre content feed would reduce palatability, hence, reduced body weight.

Proximate analysis indicated that ether extract in *A. pinata* is 4.59% and in *A. microphylla* is 5.51%. According to Mohamed et al. (2018), ether extract values varied between 6-5%. The ether extract content in *A. pinnata* was in close agreement with the value reported by Anitha et al. (2016) with 4.50%. On the contrary, Ghodake et al. (2011) and Gupta (2017) reported less value of ether extract. Other results were broadly higher published by Mandal et al. (2012) and Cherryl et al. (2014). A slight variation was observed of ether extract content in *A. microphylla*. The results from Prawitasari et al. (2012) and Fiogbé et al. (2014) were highest among the others, which above 7%. The results from Lukiwati et al. (2008) agreed with findings (2.9-3.3%) from other studies, Sujatha et al. (2013) and Chatterjee et al. (2013). Ether extract represents the amount of fat. Fat consumption in ruminants' diet is essential, especially when in need of high energy. Ruminants fed with high-fat content diets could improve fertility by the increase of ovulation rate, reduce heat stress and less affected by adverse effects (Çetingül & Yardımcı, 2008).

Table 1: Mean ± standard error of the mean of proximate composition of two Azolla species

Elements (%)	*Azolla sp.*	
	A. microphylla	***A. pinnata***
Moisture	94.86 ± 0.21	92.30 ± 0.33
Nitrogen Free Extract	39.01± 0.20	50.87± 0.53
Crude Protein	30.50 ± 0.06	19.15 ± 0.03
Total Ash	13.20 ± 0.02	10.33 ± 0.03
Crude Fiber	11.78 ± 0.24	15.05 ± 0.53
Ether Extract	5.52 ± 0.03	4.60 ± 0.04

This study revealed that total ash in A. pinnata is 10.33% and nearly 3% higher than *A. microphylla* which is 13.20%. Compared to the previous study, the percentage of total ash was lower than Alalade and Iyayi, 2016 (16.2%) and the highest total ash value was recorded by Bhattacharyya et al. (2016), which is 32.25%. The percentage of total ash of *A. microphylla* in this study was the lowest compared to the earlier reports. The closest value was reported by Datta, 2011 (16.30%), while the highest value was recorded by Chatterjee et al. (2013) and Sankar et al. (2020), which is 19.47% and 24.35%, respectively. The wide variability in total ash values in Azolla could be due to mineral inputs in the ingredients used for cultivation (Anitha et al. 2016).

There is slightly difference of moisture percentage in *A. pinnata* and *A. microphylla*. The *A. pinnata* recorded only 92.30% of moisture while *A. microphylla* was marginally higher with 94.86%. Gupta, 2018 (90%) and Bhatt et al. 2020 (90.05%) recorded a nearly similar value. The highest moisture percentage among the previous study was indicated by Anitha et al. (2016) which is 95.30%. The moisture content of *A. microphylla* is still less recorded. Bhaskaran and Kannapan (2015) have recently reported 92.25% of moisture in *A. microphylla*. The slight variation in moisture content may be due to the environment and soil condition in which Azolla has been cultivated (Sanginga and VanHove, 1989; Sankar et al. 2020).

NFE typically consists of readily digestible carbohydrates. The percentage of NFE was influenced by the values of CP, CF, total ash, and EE. Nearly all the proximate composition of *A. pinnata* were lower than *A. microphylla*. However, the NFE values were higher in *A. pinnata than A. microphylla*. According to the previous study by Anitha et al. (2016), Parashuramulu et al. (2013), Kumar et al. (2015), Lukiwati et al. (2018) and Ara et al. (2015), they were also stated that NFE percentage in *A. pinnata* was higher than in *A. microphylla.*

Minerals composition

Minerals are inorganic nutrients, typically required in small quantities from less than 0.001 to 2.5 g/kg/day (Soetan et al. 2010). The health and growth of livestock are driven by both macronutrients and micronutrients (Sordilo, 2016). However, the requirements for minerals differ by animal species. The average mineral compositions in *A. pinnata* and *A. microphylla* are presented in Table 2. Most of the mineral contents in the *A. microphylla* was higher compared to the *A. pinnata*.

Overall, K value was the highest, followed by P, CA, Mg, Mn, Fe, Zn and Cu.

The results demonstrated by Anitha et al. (2018) agreed with the current results where K was the highest among the minerals. The average value of K in *A. microphylla* was 1.22 g/kg, while *A. pinnata* has only 0.18g/kg. The present study showed that *A. microphylla* was approximately 1 g/kg higher than *A. pinnata* in terms of K. Bhatt et al. (2020) reported a much higher K in Azolla compared to the current study which is 24.1 g/kg. However, Chatterjee et al. (2013) obtained the highest K value of almost 50 g/kg. K is unique amongst the most essential minerals required by animals as dietary deficiencies of these elements are very unrecognised. All animals are likely never to be deficient in K (Habib et al. 2013).

The *phosphorus (P)* value in *A. microphylla* has significantly differed to *phosphorus* value in *A. pinnata*. 0.62 g/kg of phosphorus was found in *A. microphylla* while *A. pinnata* with only 0.07 g/kg. The obtained results in this study showed

considerably different compared to previous findings, where 3.4 g/kg and 12.9 g/kg by Anitha et al. (2016) and Alalade et al. (2006), respectively. Soetan et al. 2010 stated that P is an essential mineral in the plant involved in transferring energy, while in animals, it involves bones, teeth, and numerous metabolic reactions.

Table 2: Mean ± standard error of the mean of mineral composition of two Azolla species.

Minerals (g/kg)	***Azolla sp***	
	A.microphylla	*A.pinnata*
Potassium (K)	1.22 ± 0.62	0.18 ± 0.45
Phosphorus (P)	0.62 ± 0.03	0.07± 0.02
Calcium (Ca)	0.03 ± 0.01	0.06± 0.14
Magnesium (Mg)	0.02 ± 0.02	0.03± 0.17
Minerals (mg/kg)	*A.microphylla*	*A.pinnata*
Iron (Fe)	21.00 ± 0.01	1.10 ±0.00
Manganese (Mn)	15.90 ± 0.01	2.5 ± 0.00
Zinc (Zn	8.50 ± 0.00	0.70 ±0.00
Copper (Cu)	2.40 ± 0.00	0.30 ±0.00

This is supported by Karn (2001), plus, P is also vital for a vast array of enzyme reactions, in particular energy metabolism and genetic information transfer.

A. pinnata showed 0.06 g/kg of C content which is higher and doubled the amount screened in *A. microphylla*. This result was contradictory with the finding of Cherryl et al. (2014), which found 25.8 g/kg of C content in *A. pinnata.* Cherryl et al. (2014) and Srinivas et al. (2012) also found a higher content of C in *A. pinnata* than P content which dissimilar from the results of this study. It is necessary to feed a sufficient amount of C since C is essential for animal bodies. Other than that, C also important information and stability of cell walls for plants system. Meanwhile, it presents in bones, teeth and acts as a regulator for functioning nerve and muscle in the animal body (Soetan et al. 2010).

Mg content in *A. microphylla* was slightly higher than *A. pinnata,* which is 0.02 g/kg and 0.03 g/kg, respectively. Chatterjee et al. (2013) reported that Mg content in *Azolla sp.* was 1.79 g/kg, which dissimilar to the results of this study. Katole et al. (2017) obtained 2.5 g/kg of Mg, 2% higher than the present study. According to Soetan et al. (2010), Mg is an essential element in the chlorophyll molecule that makes the plant yellowing when it is insufficient of Mg and can be found in animal body skeleton and acts as a co-factor for functioning enzyme reaction.

Iron (Fe) is essential minerals in the formation of chlorophyll and the photosynthesis process. Fe as haemoglobin is essential for oxygen transportation in red blood cells in animal bodies (Soetan et al. 2010). The Fe content was highest in *A. microphylla* and followed by *A. pinnata* which recorded 21 mg/kg and 1.1 mg/kg. Chatterjee et al. (2013) recorded a different Fe content which is 2.5 g/kg. This study found the content of Fe were lower than Mn. That is different from Kumar et al. (2018) and Parashumulu et al. (2013) reported as the value of Fe higher than Mn, Zn and Ca.

Zinc (Zn) is found widely in plant and animal. It is essential for regulating sugar and involved in many enzyme reactions for plant growth and necessary in the animal hair growth and wound healing process (Fisher, 2008; Soetan et al. 2010). The values of Zn were higher in *A. microphylla* (8.5 mg/kg) followed by *A. pinnata* (0.70 mg/kg). Chatterjee et al. (2013) recorded that Zn content in *Azolla sp.* of 0.718 g/kg, which higher than the Zn content recorded in the present study.

Manganese (Mn) is an essential trace mineral required by plant and animal in small quantities. The deficiency can cause stunted growth, acute newborn ataxia, and reproductive failure in livestock (Fisher, 2008). It is involved in plant and animal life as an enzyme activator (Kumar et al. 2011). *A. microphylla* showed a higher value of Mn than *A. pinnata* which recorded at 15.9 mg/kg and 2.5 mg/kg, respectively. This is dissimilar compared to a previous study by Chatterjee et al. (2013) that reported the highest mean concentration in *Azolla sp.* is 2.7 g/kg.

Tan et al. (2006) reported that Copper (Cu) is a vital micro-mineral necessary for the hematologic, neurologic systems, growth, and bone formation in animal bodies. This study revealed that Cu content was 2.4mg/kg in *A. microphylla* and 0.3 mg/kg in *A. pinnata*. Chatterjee et al. (2013) got higher Cu content of 17.6 mg/kg than the current study. Among the mineral, Cu showed the lowest concentration compared to other minerals. Chatterjee et al. (2013) supported this finding, which stated that *Azolla sp.* has the lowest concentration of Cu than other minerals.

CONCLUSION

These studies have shown that different types of aquatic plants have a significant difference in the chemical composition for both *Azolla* (*A. microphylla* and *A. pinnata*). Based on the proximate analysis and mineral composition, there were statistically significant difference ($p < 0.05$) in the mean of both *A. microphylla* and *A. pinnata*. By these results, both *A. microphylla* and *A. pinnata* chemical composition were compared. This study revealed that *A. microphylla* are more suitable to be used as a source of fodder mixture to the ruminant because it has more nutritional value in terms of crude protein and ether extract that are essential for ruminant diet compared to *Azolla pinnata*. To encourage more growth and proliferation of *A. microphylla*, a small amount of fertiliser may be applied to boost rapid growth.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NAK devised the project, the main conceptual ideas, manuscript preparation and proof outline. NZAR and NA were involved in the field sampling and lab analysis.

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