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Nutritional Evaluation of *Azolla pinnata* and *Azolla microphylla* as Feed Supplements for Dairy Ruminants

Ting Jia Yee, Nurul Aini Kamaruddin* and Siti Syahirah Safiah Mohamad

School of Animal Science, Aquatic Science and Environment, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia

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

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ABSTRACT

Azolla is an aquatic plant that has the potential to be used as animal feed due to its high nutritive value and very productive plant. Thus, the objectives of this study are to determine and compare the nutrient and mineral composition of *Azolla pinnata* and *Azolla microphylla*. *A. pinnata* and *A. microphylla* are aquatic plants that have a high potential to be introduced as a new alternative feed supplement to ruminants. Samples were collected at Ladang Lembah Kaprima Hulu Seladang, Tenox Agribusiness (M) Sdn Bhd, Kampung Seladang, Setiu, Terengganu. The collected samples were dried and ground before the samples were analysed by using proximate analysis and microwave digestion for Induced Coupled Plasma Optical Emission Spectrometer (ICP-OES). Six parameters were measured for the proximate analyses which were dry matter, ash, crude protein, ether extract, crude fibre and nitrogen-free extract. The mineral compositions were analyzed for the contents of copper, zinc, iron, calcium and manganese using the ICP-OES. The result shows that *A. microphylla* contains the highest protein and ether extract content compared to *A. pinnata*. As in mineral composition, *A. pinnata* and *A. microphylla* showed that there were significant differences ($p < 0.05$) between the two plants. This study revealed that *A. microphylla* is more suitable to be used as a supplement for dairy ruminants as it contains a more nutritive value in terms of higher crude protein and ether extract that are essential for the ruminant diet.

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

INTRODUCTION

Azolla pinnata is locally distributed in its native range of Africa and Madagascar, India, Southeast Asia, China, Japan, Malaysia, Philippines, New Guinea mainland and Australia. *A. pinnata* is found on the surface of small, still ponds or backwaters without wave action, at low to middle latitudes. It becomes abundant in water with high nutrient levels, such as ponds in cattle paddocks and farm ponds, where it can completely cover the water surface. It has the ability to survive on moist soil in and around rivers, ditches and ponds. *Azolla microphylla* is common in the Indian subcontinent. It grows naturally in stagnant water in drains, canals, ponds, rivers and water bodies including marshy lands.

The deficit and fluctuating quality and quantity of feedstock are major constraints on livestock production in developing countries (Sihag et al., 2018). Floating freshwater ferns of the genus *Azolla* are being considered as an alternative crop to produce protein feed. *Azolla* is of interest as a protein feed due to the high protein content of its biomass. In recent years, *Azolla* has attracted the attention of scientists as a feed resource for livestock and is even called a green gold mine or super plant due to its high nutritive value and high biomass yield. The commercial feeds are not economical for dairy milk production thus *Azolla* as a low-cost green fodder feed ingredient is explored (Kumar et al., 2020).

Azolla thrives without the addition of nitrogen fertiliser to sustain its growth because it habors symbiotic nitrogen-fixing cyanobacteria (Brouwer et al., 2014). This makes the *Azolla* tend to contain relatively high levels of nitrogen and be an attractive protein source for animal feed. Cultivating *Azolla* can function very well as it can be grown at minimum labour costs, using minimum land and producing nutrients of high quality all through the year. The use of nitrogen fertiliser pollutes the environment but cultivating *Azolla* does not need the use of nitrogen fertiliser. 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 aims to determine and compare the nutrient and mineral composition of *A. pinnata* and *A. microphylla.*

MATERIALS AND METHODS

Study site

This study has been carried out in Tenox Agribusiness (M) Sdn Bhd, Ladang Lembah Kaprima Hulu Seladang, Kampung Seladang, Setiu, Terengganu.

Acclimatisation and cultivation of *Azolla* species

Three canvases for each species with an even bottom and 12 ft width × 7 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. Both *Azolla* sp. were cultivated using AB fertiliser. Two hundred grams of *Azolla* sp. were put into each tank. Two weeks after acclimatisation and cultivation, *Azolla* sp. covered the surface of the canvas entirely.

Sample preparation and chemical analysis

Azolla sp. were harvested and washed thoroughly under tap water and oven-dried at 105°C for 4 hours (Azhar et al., 2018). The plant samples were ground by using Waring Blender to have a fine size to obtain a homogenous powder for analysis. Samples were then kept in a small clear plastic bag and labelled. The powdery samples were analyzed for the proximate and minerals composition at the Nutrition Laboratory, Universiti Sultan Zainal Abidin, Besut Campus using AOAC (2005) method. The proximate compositions were analyzed for moisture, ash, crude protein, crude fiber and fat while the mineral compositions were determined as calcium, iron, copper, manganese and zinc.

Statistical analysis

The data of nutrient composition and mineral composition from both *A. pinnata* and *A. microphylla* were analysed by using Independent T-Test Analysis via Statistical Package for the Social Science (SPSS) version 27.0 statistics software to determine the significant difference ($p < 0.05$).

RESULTS AND DISCUSSION

Proximate analysis

Nutritional analysis of *A. pinnata* and *A. microphylla* were analysed for the moisture, ash, crude protein, ether extract, crude fibre and nitrogen-free extract. *A. microphylla* showed a higher percentage of crude protein, ether extract, crude fibre and nitrogen-free extract whereas *A. pinnata* showed a higher percentage in terms of moisture and ash. The results of the proximate composition analysis between two *Azolla* species are presented in Table 1.

There was a slight difference in moisture percentage in *A. pinnata* and *A. microphylla*. *A. microphylla* recorded only 92.58% of moisture while *A. microphylla* was 94.62%. Based on previous studies by Azhar et al. (2018) have reported 93.8% moisture in *A. microphylla* while Kumar et al. (2019) reported 94.6% of moisture in *A. pinnata*. The slight variation in moisture content may be due to the environment and soil condition in which *Azolla* has been cultivated (Sankar et al., 2020). There is a significant difference in mean values in the moisture content of both species ($p < 0.05$).

Ash is defined as the total inorganic and mineral content in a material. Determination of ash content is crucial as it presents the mineral content in materials for nutrition labelling, quality assessment, microbiology stability and food processing (Kamaruddin et al., 2019). This study revealed that total ash in *A. pinnata* is 16.51% and nearly 3% higher than *A. microphylla* which is 13.35%. There are significant differences in mean values in the ash content of both species. Compared to the previous study by Roy et al. (2016), the percentage of ash is 15.9% in *A. pinnata* and according to Kamaruddin et al. (2019), the percentage of ash is 13.20% in *A. microphylla*. 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).

The percentage of crude protein of *A. microphylla* was 25.32% which was higher compared to *A. pinnata* which was 24.14%. However, there is no significant difference in the mean value in the crude protein content of both species ($p > 0.05$). From the results, it is clear that *A. microphylla* has higher crude protein due to the nitrogen-fixing bacteria, *Anabaena azollae* that lives in *Azolla* symbiotically (Kamaruddin et al., 2021). The possible reason for this crude protein variation may be due to temperature, the nutritional content the of soil, pH, pest growth and the mineral mixture obtained from an external source that may have affected its growth and composition (Bhatt et al., 2020). The higher crude protein content has potential as a food source especially for livestock, as it is an essential component of optimum health and well-being and increase milk production in dairy ruminant.

Proximate analysis indicated that ether extract in *A. pinnata* is 3.34% and in *A. microphylla* is 2.49%. There are significant differences in the mean value in ether extract of both species ($p<0.05$). The results of ether extract content obtained in *A. pinnata* and *A. microphylla* were in close agreement with the value reported by Kumar et al. (2018) with 2.45% and Chatterjee et al. (2013) with 3.27%. 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, reducing heat stress and less affected by adverse effects (Çetingül & Yardımcı, 2008).

For crude fibre, 12.24% and 13.29% were recorded in *A. pinnata* and *A. microphylla*, respectively. There are significant differences in the mean value in crude fibre of both species ($p<0.05$). The results of crude fibre content obtained in *A. pinnata* and *A. microphylla* were in close agreement with the value reported by Kumar et al. (2018) with 11.19%% and Chatterjee et al. (2013) with 13.44%. The slight difference between the result obtained and previous studies in the crude fibre values may be due to a change in dry matter content and maturity level of the *Azolla* that was collected for estimation at different intervals (Bhatt et al., 2020).

Nitrogen Free-Extract (NFE) typically consists of readily digestible carbohydrates. The percentage of NFE was influenced by the values of crude protein, crude fibre, total ash, and ether extract. The percentage of NFE for

A. pinnata is 44.61% while of *A. microphylla* is 44.70%. These results are not significantly different from the mean value in the NFE content of both species ($p>0.05$).

Table 1. Proximate composition of *Azolla pinnata* and *Azolla microphylla*

Parameters (%)	Samples Mean ± SD	
	A. pinnata	*A. microphylla*
Moisture	94.62 ± 0.95[b]	92.58 ± 0.64[a]
Ash	16.51 ± 0.44[b]	13.35 ± 0.82[a]
Crude Protein	24.14 ± 0.14[a]	25.32 ± 1.72[a]
Ether Extract	2.49 ±0.06[a]	3.34 ±0.15[b]
Crude fibre	12.24 ±0.30[a]	13.29 ±0.30[b]
NFE	44.61±0.60[a]	44.70±1.47[a]

Note: Means within the same column followed by the different letters differ significantly according to Tukey at $p < 0.05$.

Mineral analysis

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 (Sordillo, 2016). The mineral composition of both *A. pinnata* and *A. microphylla* were shown in Table 4.2. Based on mineral composition in terms of calcium, *A. pinnata* showed higher content of calcium with 60.02mg/kg compared to *A. microphylla* with only 30.05mg/kg. These results are significant differences in the mean value of calcium of both species ($p<0.05$). Kamaruddin et al. (2021) have reported that *A. microphylla* contains 60.02mg/kg calcium while *A. pinnata* contains 30.00mg/kg of calcium. The major function of calcium in reproduction is assisting in parturition, muscle contractibility, maintaining the muscle tone of the uterus and uterine involution. Low blood calcium levels will lead to delay in involution, increase the chances of retained placenta, dystocia and higher incidence of uterine prolapse (Ahuja & Pamar, 2017).

In terms of iron, *A. microphylla* has higher iron content with 1327.36 mg/kg compared to *A. pinnata* with only 1100.31 mg/kg. There are significant differences in the mean value in the iron of both species ($p<0.05$). Compared to the previous study by Kumar et al. (2020), the iron content of *A. microphylla* is 1327.6 mg/kg and according to Bhatt et al. (2020), iron content is 1100 mg/kg in *A. pinnata*. Iron is essential for the synthesis of haemoglobin and myoglobin and various other enzymes that help in the formation of ATP through the electron transport chain. It helps in the transport of oxygen to tissues, and the maintenance of various oxidative enzyme systems (Khillare, 2007).

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. When the level of Cu is below physiological needs problems like early embryonic deaths, fetal resorption, necrosis and increased chances of retention of the placenta develop. In dairy cows, they may show delayed or suppressed estrous, impaired ovarian function and infertility (Ahuja & Pamar., 2017). In copper composition between both plant samples, *A. microphylla* shows a high composition of copper with 47.95 mg/kg compared to *A. pinnata* with only 18.61 mg/kg. There are significant differences in the mean value of copper of both species ($p<0.05$).

Manganese (Mn) is an essential trace mineral required by plants and animals in small quantities. The deficiency can cause stunted growth, acute newborn ataxia, and reproductive failure in livestock (Fisher, 2008). *A. microphylla* has higher manganese content with 313.19 mg/kg compared to *A. pinnata* with only 205.23 mg/kg.

There are significant differences in the mean value in the manganese of both species (*$p<0.05$*). These manganese results were almost to Kumar et al., (2019) with 313.23 mg/kg in *A. microphylla* and 205.31 mg/kg in *A. pinnata*.

Zinc act as a cofactor and coenzyme of many enzymes and various reproductive hormones. Zinc plays an essential role in the maintenance and repair of the uterine lining after calving and helps in early involution. Abnormal levels of zinc are associated with decreased conception rate, abnormal estrous and abortion (Ahuja & Pamar, 2017). *A. pinnata* has higher zinc content of 230.04 mg/kg while *A. microphylla* with only 54.71 mg/kg. These zinc results were almost to Kumar et al. (2019) with 230 mg/kg in *A. pinnata* and 54.71 mg/kg in *A. microphylla*. There are significant differences in the mean value in zinc of both species (*$p<0.05$*).

Table 2. Mineral composition of *A. pinnata* and *A. microphylla*

Parameters (mg/kg)	**Samples Mean ± SD**	
	A. pinnata	*A. microphylla*
Calcium	60.02 ±0.03[b]	30.05±0.08[a]
Iron	1100.31±5.85[a]	1327.36±1.59[b]
Copper	18.61 ±0.10[a]	47.95 ±0.23[b]
Manganese	205.23 ±0.19[a]	313.19±0.17[b]
Zinc	230.04 ±0.06[b]	54.71±0.04[a]

Note: Means within the same column followed by the different letters differ significantly according to Tukey at $p < 0.05$.

CONCLUSION

There are statistical differences in the proximate analysis results of both *A. pinnata* and *A. microphylla*, whereas, in mineral composition, there were statistical differences (*$p<0.05$*) between *A. pinnata* and *A. microphylla.* This study revealed that *A. microphylla* is more suitable to be used as feed supplements for dairy ruminants because it has more nutritive value in terms of crude protein, ether extract, crude fibre, and nitrogen-free extract, which are essential for the ruminant diet compared to *A. pinnata*.

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