THE EFFECT OF DIFFERENT HARVESTING DAYS ON YIELD AND NUTRITIVE VALUE OF AZOLLA (*AZOLLA MICROPHYLLA*)

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ABSTRACT. *Azolla microphylla*, often reffered to as mosquito fern or water fern, is a small free-floating aquatic fern species native to Asia, Africa, and America that belongs to the Azollaceae family. This unique unconventional super-fast growth plant can be easily grown in stagnant water such as ponds, rivers, drains, canals, and available water bodies at 15-35 ºC temperature range. This study aims to find the quality production of *Azolla microphylla* and its nutritional benefits for animal feeding by conducting experiments with different period of harvesting. The result from this study showed that *Azolla microphylla* can be used as fresh basis or dry basis for daily animal feed due to its high nutritious value, fast growing with low production cost and easily managed plant species. The present study found that a significant improvement of total dry matter yield and higher digestible nutrients were obtained through harvesting *Azolla microphylla* at day 14. Although at day 14 the protein content is lower compared with day 7 harvest, it still fulfills the required livestock feeding nutrient. Thus, the uniqueness of this easily grown and nutritious plant can significantly benefit animal feeding usage by incorporating it in the daily mixed ration.

Keywords: water fern, matter yield, nutritive values, protein content, green manure

INTRODUCTION

Azolla sp. is a free-floating water fern that quickly fills space on the water's surface. It floats on the water's surface thanks to a slew of small, closely overlapping scale-like leaves with roots that dangle in the water (Meena *et al.,* 2017). This plant belongs to the *Azollaceae* family which is indigenous to Asia, America, and Africa region. To date, there are at least eight species of *Azolla sp.* identified including *Azolla circinata, Azolla caroliniana*, *Azolla mexicana*, *Azolla japonica, Azolla microphylla, Azolla pinnata, Azolla nilotica,* and *Azolla rubra* (Bhutia *et al.,* 2020). This plant species can grow naturally in static water bodies such as ponds, canals, drains, rivers, and swampy lands under the temperature of 15-35 ºC which makes it an uncustomary rapid growing with high protein source for animal feed (Chatterjee *et al*., 2013; Meena *et al*., 2017). Symbiotic relationship is formed with *Anabaena Azollae* or blue green algae, which leads to fix atmospheric nitrogen that provides the plant access to the essential nutrient. Due to Azolla's association with blue green algae *Anabaena Azollae*, this can be considered as easy to grow plant with high productivity, good nutritive value which is able to be a highly potential source of nutrients and also has a great deal of high feeding value (Hossiny *et al*., 2008; Prabina & Kumar, 2010). In Asia, *Azolla sp.* has been utilized for a long time as green manure fertilizer for crop production, rice fields, and supplements in livestock diet, mainly for poultry and pigs (Hasan & Chakrabarti 2009; Meena *et al.,* 2017). According to Meena *et al.* (2017), under optimum conditions it grows

rapidly, doubling its biomass in every three days. *Azolla sp.* can produce more than 4 to 5 times of outstanding and excellent quality compared to hybrid napier and lucern. According to Ghodake *et al.* (2012), up to 1-3 kg of nitrogen/ha/day can be fixed by *Azolla sp.* which produce around 730 tonnes/ha annual fresh yield that can be used as animal feed. The aquatic rooting system can absorb surrounding nutrients either directly from the water or in the soil for shallow water environment. Basically, depending on best conditions, *Azolla sp.* can double its biomass within 3-10 days. Previous studies have shown that this plant can reach 8-10 tonnes/ha fresh weigh as standing crop in rice fields in Asia. Meanwhile, in India, production up to 37.8 tonnes/ha fresh weight (2.78 tonnes/ha dry weight) was reported specifically for *A. pinnata* (Pullin & Almazan, 1983; Hasan & Chakrabarti., 2009). According to Ferentinos *et al.* (2002), Azolla produces dry matter in the range of 39-390 tonnes/ha, in crop cycles of 40-365 days.

Azolla sp. is rich in important vitamins (vitamin A, vitamin B12, Beta Carotene), amino acids, growth promoter intermediaries, and minerals such as phosphorus, calcium, ferrous, potassium, magnesium, and, copper. Other than that, *Azolla* sp. has been identified as one of the most efficient and cost-effective feed alternatives for cattle based on its low lignin content and high protein, which eases the digestion. It is noted that protein content of *Azolla* sp. can be commensurate to or higher than other known aquatic macrophytes including water hyacinth and seaweed (Vinu, 2017). Generally, during optimal growing conditions, the crude protein level is typically about 19-30 % dry matter basis and frequently near to 20-22 % under natural conditions (Hasan & Chakrabati, 2009). Furthermore, present situation stated by Nazli *et al.* (2018) shows a rapid growth of animal feed cost worldwide especially in Malaysia. This study helps to understand the importance of *Azolla sp.* that can be considered as an alternative for animal feed. Based on the above discussions, the study was undertaken to evaluate the yield and nutritive value of *Azolla microphylla* at different harvesting age as a potentially valuable animal feed and good alternative protein source mainly for ruminant industry, taking into some consideration of current issues in animal feeding such as animal food scarcity and expensive animal feed. Based on farmer's experience through random surveys, 7 and 14 days are selected as the best period of harvesting to easily differentiate their outcomes.

MATERIALS AND METHODS

The study was conducted for six (6) months from April to August 2019 at Veterinary Institute Malaysia, Kluang, Johor (2 ° 01› North latitude and 103 ° 19› East longitude). The experimental plot is in a tropical humid zone with the average minimum and maximum temperature of 24.0 °C and 32.3 °C, respectively, with a mean 27.1 °C and the average relative humidity of 80.6 % with an average rainfall of 1930 mm per annum. Fresh Azolla (*Azolla microphylla*) was used and cultivated in poly tanks under natural light. The 6 poly tanks used are 120 x 50 cm (diameter x depth) each. Three poly tanks for each interval different treatment were used. Cow dung was equally deposited at a depth of 1 to 2 inches at the bottom of all poly tanks, followed by a second coating of fine topsoil at the same depth of 1 to 2 inches. The poly tanks were filled with three quarters (3/4) of chlorine free water. To provide the optimal conditions for Azolla growth, the pH level of the water employed in this study was kept between 5.8 and 6.0. Once the growing media was ready, each poly tank was inoculated

Figure 1. *Azolla microphylla.*

Figure 2. *Azolla microphylla* in poly tank.

with 200 g/m^2 fresh Azolla culture and allowed to rest for seven days before harvesting, with subsequent harvests taking place on the seventh and fourteenth days. Every two weeks, 3 kg addition of cow dung per tank was required to ensure an active multiplication phase during the growing period. The harvests were carried out at 7th and 14th day intervals, and the process was repeated up to four times.

Dry Matter Yield

Fresh harvests were done at 7th and 14th day intervals regularly for four times. Azolla was harvested on the entire top surface of the poly tank for each treatment and sieved for half an hour to obtain fresh Azolla yield. Fresh weight was determined using an electronic balance. All samples collected were sent to the Feed Laboratory, Veterinary Institute Malaysia for dry matter and chemical composition test. Samples received were pre-dried using a forced-air drying oven at 60 ˚C overnight. Once dried, samples were then grounded to pass a 1-mm sieves before going through a forced-air drying oven at 103 ± 2 ˚C over 4 hours (AOAC, 2000) to be used in the computation of dry matter composition as well as overall dry matter per hectare.

Chemical Composition

The ground samples were also used to determine the chemicals composition of the Azolla samples. The crude protein content (*N x 6.25*) was determined after digestion in sulphuric acid and distillation by the Kjeldahl method using *Kjeltec*™ methods (FOSS). The crude fiber was measured after treated with boiling dilute sulphuric acid and with boiling sodium hydroxide solution using *Fibertec*™ methods (FOSS). Finally, the Total Digestible Nutrient (TDN) was calculated using the formula according to Devendra (1979), while the metabolized energy (ME) for ruminant was calculated using the Close and Menke equation (1986).

Statistical Analysis

All data were subjected to an independent sample T-test using IBM SPSS statistical program (Version 20).

RESULTS AND DISCUSSION

The dry matter (DM) and dry matter yield (DMY) production of Azolla are presented in Table 1. The DM percentage of different Azolla species

Table 1. Dry matter and dry matter yield for *A. microphylla* at the different harvesting age.

Parameter	Harvesting age (days)	
	7	14
Dry Matter (DM), %	4.28 ± 0.10**	7.67 ± 0.38**
Dry matter yield, tonnes/ha/ harvest	0.49 ± 0.55**	1.42 ± 0.53**

Note: **Indicates significant differences at probability level (P ≤ 0.01)

Table 2. Nutrient composition of *A. microphylla* at different harvesting intervals. (mean ± SE).

Parameter	Harvesting age (days)	
	7	14
Crude Protein (CP), %	36.28 ± 0.88**	30.19 ± 0.57**
Crude Fiber (CF), %	14.08 ± 0.39**	16.61 ± 0.39**
Total Digestible Nutrient (TDN), %	35.23 ± 0.83**	48.39 ± 1.70**
Metabolized Energy (ME), MJ/kg	6.93 ± 0.15	7.51 ± 0.24

Note: **Indicates significant differences at probability level (P ≤ 0.01)

varies greatly, and there is no consistency among published data; however, the numbers range from 5 % to 7 %, which are reasonable estimations (Hasan & Chakrabarti, 2009). The DM of Azolla obtained from this study on the 7th day was 4.28 %, which agrees with the finding of Anitha *et al.* (2016) with 4.70 % DM content. The present study also reveals that the DM content is significantly higher at 14th day harvesting age by 7.67 %. This result is in agreement with the finding of Giridhar *et al.* (2012), Parashuramulu *et al.* (2013), and Mohamed *et al.* (2018) with almost double DM content compared to the 7th day harvesting age.

Dry matter yield of harvested Azolla also increased significantly aligned with the increase of additional harvesting age as shown in Table 1. Hasan and Chakrabarti (2009), also mentioned the ability of Azolla to double its biomass in 3 to 10 days, depending on conditions. The favourable conditions for optimum growth of Azolla species are a strip of water that is not more than a few centimetres deep because it provides good mineral nutrition, with the root not too far from the soil as it also reduces the wind effects (Hasan & Chakrabarti, 2009). Strong winds can cause the Azolla to accumulate to one side of the poly tank and create overcrowded conditions thus slowing the growth. Another important element for optimum growth of Azolla is the pH level of the water used, in which previous studies suggested for it to range from 3.5 to 10, as the optimum growth was reported to occur at pH 4.5 to 7.0. This study has maintained the pH around 5.8-6.0 to ensure the best condition for Azolla growth.

According to Anitha *et al.* (2016), even though Azolla has a lower DM concentration, it can still be utilised as a supplement to meet DM requirements in animal feeds. Meanwhile, Giridhar *et al.* (2012) also stated that it is difficult to rely solely on Azolla as the livestock feed resource because the DM content is only about 7 %. However, it is suitable to be used as

a nutritive feed supplement because its feeding can improve the milk production by 15 % to 20 % (Gauri *et al.*,2012; Chatterjee *et al.,* 2013; Mathur *et al.,* 2013; Khare *et al.,* 2014.; Rawat *et al.,* 2015; Meena *et al*., 2017; Kumar *et al.,* 2020). Several studies also reported that integrating Azolla into ruminant feed improves digestibility, daily gain, and feed efficiency (Samanta & Tamang 1995; Indira *et al.,* 2009; Ghodake *et al.,* 2012; Ahmed *et al.,* 2016; Sireesha *et al.,* 2017; Sihag *et al.,* 2018).

The crude protein (CP) content of *Azolla* in the present study as presented in Table 2 is 6 % significantly lower at the 14th day harvesting age compared to the 7th day harvesting age. However, the CP value at the 7th day and 14th day is still higher than the other studies ranging between 21 % to 24 % (Giridhar *et al.,* 2012; Ghodake *et al.,* 2012; Chatterjee *et al.,* 2013; Cherryl, 2014; Anitha *et al.,* 2016). While, according to Hasan and Chakrabarti (2009), generally the CP content of Azolla is about 19 % to 30 % during the optimum growth conditions. The amount of CP required for livestock varies according on the stage of production, the animal's size, and the desired performance. To sustain muscle growth and milk production, young and growing cattle, notably dairy livestock require relatively high levels of CP in their diets. For rations of dairy cows on early lactation, a CP content of 17 % to 19 % (Nutrient Requirements of Dairy Cows, National Research Council, 2001) is recommended while according to Nutrient Requirements of Small Ruminants, National Research Council (2007), for lactating goat the recommended CP content should be 17%. Azolla has been used successfully as a protein supplement to dairy animals. It significantly increases monthly milk production and improves animal health condition (Ambade *et al.,* 2010; Chatterjee *et al.,* 2013; Giridhar *et al*., 2014; Menna *et al.,* 2017; Senthil *et al.,* 2020; Upendra *et al.,* 2020). Azolla meal is also proven to be suitable to be utilized as a replacement for the concentrate mixture in the diet of goat male kids (Sharma *et al.,* 2021) and heifer (Roy *et al.,* 2016) that require a diet containing high protein content. These studies suggested that Azolla could be used as a natural protein source in cattle feed. In the present study, it is found that the CF content obtained is 14.08 % at the 7th day harvesting age and 16.61 % at the 14th day harvesting age. Crude fibre content at the 14th day harvesting age is 2.53 % significantly higher compared to CF content at the 7th day harvesting age. According to Chatterjee *et al.* (2013), in general, CF content of Azolla is between 9.07 % to 22.25 %. Crude fibre content obtained on the 7th day is found to be in accord with the values obtained by Samantha and Tamang (1995), Cherryl (2014) and Anitha *et al.* (2016), higher than values obtained by Giridhar *et al.* (2012), and lower compared to the value reported by Bolka (2011). While CF content at the 14th day harvesting age obtained from this study is higher than CF content obtained by Parashuramulu *et al.* (2013) and Kumar *et al.* (2018). Ruminants need a certain amount of fibre in their diet to ensure that the rumen functions properly, the minimum amount of CF that cows need in their diet is 17 %. As for goats, the CF content must maintain at least 12 % in the diet as stipulated by National Research Council (2007). Thus, the low CF content in Azolla sp. in comparison with other legumes forages shows that *Azolla* sp. is a good source of protein base additive for animal feed with low fibre content.

The TDN at the 14th day harvesting age is significantly higher than the 7th day harvesting age, while there is no significant difference in the ME contents between the 7th and 14th day harvesting age. The study by Samantha and Tamang (1995) showed a higher TDN value

on the 7th day harvesting age with a value of 51.8 %. While Parashuramulu *et al.* (2013)'s study obtained 7.36 MJ/kg ME at the 14th day harvesting age which is close to the ME value from the present study. Actual requirements of TDN and ME for livestock will vary depending on breed, productivity, and environment. The energy requirements of most goats stay the same, except for dairy kids, who require 21 % more energy than the average (Rashid, 2008). It is also crucial to feed high-energy rations during breeding, late pregnancy, and lactation. Lactation periods have the largest energy consumption, which is between 53 and 66 percent TDN, according to the NRC (2007). Furthermore, TDN requirement for lactating dairy cows from the early to late lactation period is between 66 % to 74 % (NRC, 2001).

CONCLUSION

According to the current study, Azolla can be utilized as an alternative protein supplement or as supplementary protein supplement to ruminants with 30.19 % crude protein, a moderate supply of energy (7.51 MJ/kg), and a low CF (16.61 %). Thus, this study gives some impact towards improvement of animal feed quality in Malaysia and will help to increase the quality and quantity of our livestock productions in line with our National Beef Industry Development Strategic Plan BIF PLAN) 2021-2025. Despite the reduced CP content, the present study shows that harvesting *Azolla microphylla* at 14-day intervals results in a considerable increase in total DM output and better digestible nutrients. Despite the decreased CP concentration on 14th day, it still meets the nutrient criteria for livestock feeding. Incorporating *Azolla microphylla* in daily animal feed ration shows high potential as this crop is easily grown and most importantly this unique plant accommodates high nutrient value. In addition, some recommendations can be taken into considerations for future improvements, as for planting material which can be prepared with various parameters such type of fertilizers, type of soils, and types of planting seed. Despite that, providing good abiotic factors can also help to improve the quality and yield productions of plants especially *Azolla microphylla* which can help in feeding of our livestock.

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