COMPREHENSIVE GROWTH AND NUTRITIONAL STUDY OF *Azolla filiculoides* Lam. AS SOURCE OF FIBER AND PROTEIN FOR RUMINANT'S DIET

By

MOHAMMAD FITRI RIMI BIN HAMIDAN

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fullfilment of the Requirement for the Master of Science

January 2023

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree Master of Science

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January 2023

Chair : Mohd Noor Hisham Mohd Nadzir, PhD

Faculty : Science

Constraints in Napier grass production such as limited land use, labor shortages and long cutting intervals had become the factors that cause the underdeveloped situation on the ruminant industry. As an alternative, the use of water fern species known as mosquito fern (*Azolla filiculoides* Lam.) as a source of fiber and protein in livestock diets is seen as suitable due to its adequate composition of crude protein and crude fiber that required by ruminants which are in the range of 19-25% DM^{-1} and 12-18% DM^{-1} respectively. Furthermore, this species is very practical and economical to be self-produced by the farmers in their farms. This study was carried out to determine the potential of *A. filiculoides* as an alternative feedstuff in ruminant diet. An objective of Study 1 was to evaluate the potential of different fertilizer (NPK 15:15:15, broiler manure, sheep manure and cow manure) with increasing concentrations of 0.25 g/L (C1), 0.50 g/L (C2), 0.75 g/L (C3), 1.00 g/L (C4) and 1.25 g/L (C5) on growth and nutritive value of *Azolla filiculoides*. Objective of study 2 was to determine the practical procedures between dried (T1), burned (T2) and freshly used (unprocessed) (T3) in preparing a livestock manure according to an optimum concentration for *A.filiculoides* cultivation based from the finding of the first study. Study 3 was conducted to analyze the effectiveness of Azolla meal as an inclusion of 0% (control), 6% (TMR 1), 10% (TMR 2), 17% (TMR 3) and 23% (TMR 4) inclusion in the Napier silage TMR based on digestibility and VFA profile. All experiment was designed according to Complete Randomized Design (CRD) with four replication each. The analysis was carried out according to the method that had been developed in AOAC and the data was analyzed using two or one-way ANOVA according to the factors tested and significant differences were determined through Duncan's Multiple Range Test (DMRT). As a result, sheep manure was able to produce a higher fresh weight (136 g m^{-2}). In fact, the application of C4 (1.00 g/L) sheep manure in the *Azolla filiculoides* cultivation was able to produce dry

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matter: 7.3% kg DM^{-1}, ash: 19.6% kg DM^{-1}, crude protein: 21.2% kg DM^{-1}, ether extract: 4.3% kg DM^{-1} and crude fiber: 14.4% kg DM^{-1}. Subsequently, the application of T3 procedure for *A.filiculoides* cultivation was able to produce higher yields ($p<0.05$) with lower fiber components (NDF: 33.02% kg DM-1, ADF: % kg DM-1 and ADL: 8.13% kg DM- 1) compared to the application of T2 procedure for manure preparation. The value of IVDMD and IVOMD as 82.9% and 43.7% proved that digestibility increased by using Azolla meals in the ruminant dietary feed. Although the IVDMD and IVOMD were decrease with increment of the inclusion, Azolla meal could supply 14.1 MJ/kg ME at 6% inclusion in the ruminant diet. To conclude, *A. filiculoides* at 6% inclusion has show a potential as a practical and economic source of fiber and protein by direct cultivation in the farm employing sheep manure (T3) at 1.00 g/L concentration (C4).

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Sebagai memenuhi keperluan untuk ijazah Master Sains

KAJIAN KOMPREHENSIF TERHADAP PERTUMBUHAN DAN NUTRISI *Azolla filiculoides* Lam. SEBAGAI SUMBER PROTIN DAN SERAT UNTUK RUMINAN

Oleh

MOHAMMAD FITRI RIMI BIN HAMIDAN

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Kekangan-kekangan di dalam pengeluaran rumput Napier seperti penggunaan tanah yang terhad, kekurangan tenaga kerja dan pusingan pemotongan yang panjang telah menjadi faktor kepada kemunduran di dalam industri ruminan. Sebagai alternatif, penggunaan spesies pakis air yang dikenali sebagai mosquito fern (*Azolla filiculoides* Lam.) sebagai sumber serat dan protein dalam diet ternakan dilihat sesuai disebabkan komposisi protein kasar dan serat kasar yang mencukupi yang diperlukan oleh ruminan yang masing-masingnya berada di dalam julat 19-25% DM^{-1} dan 12-18% DM^{-1}. Tambahan pula, spesies ini sangat praktikal dan menjimatkan untuk dihasilkan sendiri oleh penternak ladang. Kajian ini dijalankan untuk mengenalpasti potensi *Azolla filiculoides* sebagai bahan alternatif di dalam diet ruminan. Objektif kajian 1 adalah untuk menilai potensi penggunaan baja berbeza (NPK 15:15:15, tinja ayam, tinja bebiri dan tinja lembu) dengan peningkatan konsentrasi 0.25 g/L (C1), 0.50 g/L (C2), 0.75 g/L (C3), 1.00 g/L (C4) dan 1.25 g/L (C5) terhadap pertumbuhan dan nilai nutrisi A. *filiculoides.* Objektif kajian 2 adalah untuk menentukan prosedur proses yang paling praktikal di antara pengeringan (T1), pembakaran (T2) dan penggunaan terus (tanpa proses) (T3) di dalam penyediaan tinja haiwan untuk mengikut konsentrasi untuk pengeluaran *A.filiculoides* berdasarkan penemuan daripada kajian 1. Akhir sekali, kajian 3 adalah bagi menganalisa kesesuaian penggunaan mil Azolla sebagai inklusi di dalam TMR silaj Napier pada kadar 0% (control), 6% (TMR 1), 10% (TMR 2), 17% (TMR 3) dan 23% (TMR 4) terhadap kebolehcernaan dan pemprofilan VFA. Semua eksperimen telah direka bentuk mengikut Complete Randomized Design (CRD) dengan empat replikasi setiap kajian. Analisis dijalankan mengikut kaedah yang telah dibangunkan di dalam AOAC dan data telah dianalisis menggunakan ANOVA dua atau satu hala mengikut faktor yang diuji dan perbezaan signifikan ditentukan melalui Duncan's Multiple Range Test (DMRT). Hasilnya, penggunaan baja daripada sisa tinja ternakan bebiri (F3)

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dapat menghasilkan berat basah yang lebih tinggi (136 g/m^2). Di samping itu, konsentrasi C4 (1.00 g/L) tinja bebiri dalam penanaman *A.filiculoides* mampu menghasilkan DM: 7.3% kg DM^{-1}, abu: 19.6% kg DM^{-1}, CP: 21.2% kg DM^{-1}, EE: 4.3% kg DM^{-1} dan CF: 14.4% kg DM^{-1}. Seterusnya, penggunaan prosedur T3 untuk penanaman *A.filiculoides* mampu menghasilkan hasil yang lebih tinggi ($p<0.05$) dengan komponen fiber yang lebih rendah (NDF: 33.02% kg DM^{-1}, ADF: % kg DM^{-1} dan ADL: 8.13% kg DM^{-1}) berbanding penanaman yang menggunakan baja yang diproses dengan prosedur T2. Nilai IVDMD dan IVOMD masing-masing sebanyak 82.9% dan 43.7%. telah membuktikan terhadap peningkatan tahap kebolehcernaan melalui penggunaan mil Azolla di dalam diet ternakan ruminan. Walaupun peningkatan peratus inklusi Azolla mil ke dalam campuran TMR telah menyebabkan penurunan nilai IVDMD dan IVOMD yang signifikan, namun dengan hanya 6% inklusi mil Azolla ke dalam campuran TMR telah cukup untuk menghasilkan sebanyak 14.1 MJ/kg ME. Kesimpulannya, mil Azolla berpotensi digunakan pada tahap inklusi 6% sebagai sumber serat dan protin yang ekonomi dan praktikal. Ini kerana tumbuhan ini boleh dihasilkan sendiri di ladang melalui penggunaan terus (T3) tinja ternakan bebiri (F4) pada tahap konsentrasi 1.00 g/L (C4).

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LIST OF ABBREVIATIONS

DM	Dry matter
CP	Crude Protein
CF	Crude Fiber
EE	Ether Extract
NDF	Neutral Detergent Fiber
ADF	Acid Detergent Fiber
ADL	Acid Detergent Lignin
IVDMD	*In vitro* Dry Matter Digestibility
IVOMD	*In vitro* Organic Matter Digestibility
ME	Metabolizable Energy
VFA	Volatile Fatty Acid
KH_2HPO_4	Monopotassium phosphate
Na_2HPO_4	Disodium phosphate
$MgSO_4$	Magnesium Sulphate
$MnCl_2$	Manganese Cloride
$CaCl_2$	Calcium cloride
$FeCl_3$	Ferum cloride
$NaHCO_3$	Sodium bicarbonate
$NH4HCO_3$	Ammonium bikarbonate
Na_2SO_4	Sodium bicarbonate
NaOH	Sodium hyroxide
FFAP	Free Fatty Acid Phase
FID	Flame Ionzation Detector
CRD	Complete Randomized Design
A.O.A.C	Association of Official Agriculture Chemist
N.P.K	Nitrogen Phosphorous Kalium compound
S.E.M	Standard Error of Means
µl	Microlitre
°C	Degree Celsius
g	Gram
kg	Kilogram
ml	Millilitre
m	Meter
MARDI	Malaysia Agriculture Research and Development Institute
%	Percent
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CHAPTER 1

INTRODUCTION

1.1 Research Background

In Malaysia, agriculture had become one of the important industries since its independence from Colonization Era. Contribution of this industry had become a profitable employment opportunity to supply a domestic need such as food and other requirement goods for the population. An inception of this industry had secured local's food production and ensures dependency on imported food source could be reduced. Despite of agriculture, livestock industry had also been emphasised by the government in line to access this mission. Hence, agriculture in Malaysia had been reported to impart approximately 7.1% from total GDP in 2013 and the livestock's sector had contributed 16.2% (Mustafa et al., 2021). As expected by Malaysian National Agro-food Policy 2011-2020, demand, and production of meat in Malaysia had been emphasized to be increase. An increment into 1.8 million MT with growth 2.4% per annum and 2.1 million MT with 2.7% per annum had been forecast for 10 years periods from 2010 on local's demand and production for meat respectively (Sheng et al., 2010; Zainalabidin et al, 2019). Besides, demand of other livestock's products such as milk and eggs had also expected to rise consequent from the increased of an economic level and affordability of the community whereby had bring to diet alteration. Unfortunately, until now the sector is still raised in a small scale (Wong et al., 2020). Even though effort and positive progress had been observed to establish local livestock industry, our domestic demand and production are still imbalanced (Adnan et al., 2018). Therefore, imported goods from India, Australia and New Zealand such as frozen meats and dairy products were allowed to penetrate into local market as a solution for the shortage (Buda et al., 2021). Currently in 2018, our local's self-sufficient level (SSL) was still low on beef, mutton and milk (Abdullah et al., 2018). The underdeveloped situation of the local ruminant's sector is normally associated with several factors such as the lack of land resources, high feed price, cheaper import substitutes, poor private sector involvement, disease prevention with control and lack of quality breeds, expertise, and workforce (Dardak, 2019). The insufficient local protein sources for domestic market and high dependency on imported meats are hereby are associated feeds production systems for Malaysian ruminant industry. Eventually, research and development should be conducted on the unusable local available sources such as plants or by product to be utilized as an alternative feedstuff for the ruminants and to sustain this industry in Malaysia.

Fiber and protein had become the important components for ruminant diet. For centuries, our farmers had used local forage such as Napier and Guinea grass to feed their goat, sheep, and cattle (Aswanimiyuni et al., 2018). However, fodder sources had become another limitation to our farmers to raise their livestock towards a sustainable production. Most of our land usages have been

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transformed into commodity plantations this has reduced available pastureland to support our ruminant's fodder needs (Banu & Fazal, 2016). As a result, farmers could not produce their own forage and tend to a use commercial animal feed such as pelleted feed, which can lead to high cost of meat and milk production (Tamizhkumaran & Rao, 2016). Alternatively, certain farmers used the local agricultural by-product such as oil palm frond (OPF), corn stalk and bagasse (Rusli et al., 2021; Agus & Widi, 2018). In fact, palm kernel cake (PKC) or palm oil sludge (POS) were also used as an alternative source of protein and energy for animal (Arief & Satria, 2019; Saminathan et al., 2020). Even though most of our farmers had managed to transform their activities towards semi-intensive and fully intensive systems, by-product from plantations and food industries such as PKC and soybean meal have become more expensive and high-demand. Furthermore, the availability of these products was limited to supply and could not be sustained due to higher price was offered by exporter to support a huge and is seasoned to other more lucrative industries such as construction, papers and cosmetics (Kumar et al. 2020; Sahota. 2014; Moghadase & Hussein. 2012).

Napier grass had become one of the renewable fibers sources, but it requires areas and much manpower to ensure an adequate amount of quality fodder is produced. Shortages of labor and unable to manage the cutting interval at 6 to 8 weeks had decreased the fodder nutritional quality (Zailan et al. 2016b). Thus, alternative fodder source with adequate fiber and protein content is needed to partially replace Napier grass and soybean meal in ruminant diet. Therefore, efforts are taken to discover alternative ruminant feed source rich in fiber and protein that is practical and affordable to farmers.

Meanwhile, Malaysia is well endowed with varieties of aquatic ecosystems including rivers, lakes, reservoirs, swamps, mangroves, estuaries, lagoons and the seas. Other local aquatic plants such as *Eichhornia crassipes, Monochoria vaginali*s (pickerel weed), *Pistia stratiotes* (water lettuce), *Salvinia molesta, Lemna minor* (common duckweed) and *Azolla spp* had been lushly occupy most of these areas. The forested wetland in Malaysia is estimated to cover about 4.39 million ha and 2.5% of the area which is around 1.1 million ha are belong to freshwater swamp (Ministry of Natural Resources and Environment, 2014). However, the uncontrolled population of the floating aquatic plants has been reported to cause a negative impact on aquatic ecology. Therefore, previous researchers have realized the potential of these plants as a source of additional fiber and protein for livestock. Hence, studies related to utilization of an aquatic plant as an animal feed was conducted many years prior. However, *A. filiculoides* species was found to be more suitable than other aquatic plant species due to its growth potential and nutrient content (Kamarudin et al. 2019). This species prevailed in irrigated areas and able to propagate at 2.9 g/m^2 per day due to a shorter doubling time. In fact, this species able to produce 22.48% kg/dm crude protein (CP), 14.70% kg/dm crude fiber (CF), 37.6% kg/dm neutral detergent fiber (NDF) and 8.03% kg/dm lignin (ADL) in the wild environment (Kollah et al., 2016). An ability to absorbed 2.6 t N/ha and 0.43 t P/ha was made this species a good bio-phytoremediation agent as reported by Costa et al. (1999). This allows farmers to fully utilize the available

resources on the farm to reduce the cost of their livestock. Even though, there is a perception that labelled this species as an invasive species or in some area they were recognized as one of the noxious weeds (Barreto et al., 2000). This species has a great potential to produce as a high-quality fodder for livestock (Nasir et al., 2022). *Azolla filiculoides* is a species of fern, it is an aquatic plant that is grow in slow-moving water bodies such as pond. Thus, this will help farmer that raising the animal farming in urbanization area to produce fodder. Next, the fronds of this plant have angular overlapping shape and usually each frond is 1 or 2 millimeters long. Moreover, the leaves contain cyanobacteria that known as *Anabaena azollae*. Symbiotic relationship between *Azolla filiculoides* and *Anabaena azolla* is one of the factors that related with phosphate autogenesis ability from existent phosphorus in the enviroment. Hence, the growth rate of *Azolla filiculoides* was good in the area with lack phosphorus element. In addition, presence of *Anabaena azolla* helps to fixes nitrogen from the atmosphere to the form that can be utilized by the *Azolla filiculoides* (Bhuvaneshwari & Singh, 2015).

The nitrogen element that contains in *Azolla filiculoides* can be transferred to the animal as they eat the fodder which consists of Azolla. Whereby, the nitrogen is very crucial for the building blocks of protein, namely amino acids. Eventually, a comprehensive study is required to discover the potential of this species from the production stages to the nutritional aspect including their fiber component and finally their digestibility in ruminants when used in the diets.

1.2 Problem Statement

Morphologically, *Azolla filiculoides* may seem like just ordinary floating plant on water, but it has huge potential to be use as fodder. The *Azolla filiculoides* has been recommended for feeding the livestock as it has potential feeding ingredient (Lumpkin, 1984). This because the *Azolla filiculoides* have high nutritive value and may be able to produce four to five times amount of protein compared to other Napier grass. *Pennisetum purpureum* is an example of Napier grass that is only able to produce about 14.62% of crude protein (Haryani et al., 2018) while *Azolla filiculoides* have 21.4% of crude protein (Alalade and Iyayi, 2006). Other than that, *Azolla filiculoides* is also excellent fodder to feed chicken, duct and Japanese quails as 20% of commercial feed can be replace by inclusion of fresh *Azolla filiculoides* in their diet (Subudhi & Singh, 1978). Unfortunately, studies on the growth performance of *Azolla filiculoides* by using different type of fertilizers and their optimum concentration to culture the Azolla are very limited.

People in Asian countries such as China and Vietnam extensively use *Azolla filiculoides* in rice paddy cultivation and as high-protein sources of food for their livestock (Pereira, 2017; Bhujel & Rizal, 2022). In addition, Malaysian was also use *Azolla filiculoides* as a source of protein to feed their livestock. However, this species was still not widely used in Malaysia due to our farmers unawareness about Azolla supplementation to improve herd quality. Other

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than that, farmers also do not aware that feeding their livestock with Azolla can lower the feeding cost as Azolla are highly propagated in short period of time. However, there are limited information regarding literature on the propagation of Azolla in short period of time by using different type of fertilizer and concentration. The null hypothesis of this study is *Azolla filiculoides* was not practical and economical to be produced by using livestock manure and unable to be utilized as source of fiber and protein for ruminant. Oppositely, as an alternative hypothesis this species is practical to be cultivated with minimum cost by using fertilizer from manure and suitable to be used in ruminant's diet.

1.3 Significance of study

The sustainable availability of the fodder for livestock is one of the major concerns among the farmer and agencies such as Malaysia Agricultural Research and Development Institute (MARDI). This study will not only give significantly benefit to Malaysia but also for other countries that native range of distribution of *Azolla filiculoides* such as India, China, Japan, Africa and Australia in cultivating practice of *Azolla filiculoides*

In Malaysia, this study also give benefit to the agencies that involve in the animal husbandry and rearing such as Department of Veterinar, Malaysia Agricultural Research and Development Institute (MARDI), Farmers' Organization Authority (FOA), Department of Veterinary Services (DVS), Department of Agriculture (DOA The researcher from those agencies can have more understanding about the nutritional composition of Azolla. Agencies can have more understanding about the nutritional composition of Azolla. Thus, can advise farmers to practice cultivation of Azolla and substitute into their livestock diet.

Hence, the result of this study can help farmers to cultivate and process the *Azolla filiculoides* to produce an alternative fodder that is high in protein and fiber for livestock. In addition, *Azolla filiculoides* is easy to cultivate, low cost and readily available which will ease the farmer in *Azolla filiculoides* cultivation. Moreover, the cultivation of *Azolla filiculoides* in one hectare can produce about 820-1220 g of fresh Azolla per day (Aziz, 2012). Therefore, probably *Azolla filiculoides* one of the more efficient feed substitutions for livestock.

1.4 Objective of study

This research was conducted to provide optimal requirement and information through the cultivation and nutrition study of cultivated *Azolla filiculoides*. Thus, the objectives of this study were:

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i. To evaluate the growth performance, fresh weight, nutrient composition, and fiber component of cultivated *Azolla filiculoides* from different fertilizers with increasing concentrations.
ii. To compare different procedures on sheep manure preparation as a fertilizer for *Azolla filiculoides* cultivation on fresh weight, growth performance, nutrient composition, fiber component and digestibility.
iii. To identify the optimum inclusion level of *Azolla filiculoides* meal in the Napier silage total mixed ration (TMR) on the digestibility and volatile fatty acid profile for ruminant's diet.

It is hoped that this study will provide information on the potential of aquatic macrophyte *Azolla filiculoides* as a alternative source for fiber and protein for ruminant's diet, which will improve our local ruminants industry.

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