การเปรียบเทียบองค์ประกอบและคุณค่าทางโภชนาการของแหนแดง (*Azolla pinnata*) ในช่วงอายุการเก็บเกี่ยวต่างกัน เพื่อประเมินศักยภาพในการใช้เป็นอาหารสัตว์ต้นทุนต่ำ

Comparative Nutritional Composition of *Azolla pinnata* at Different Harvest Stages for Low-Cost Animal Feed Development

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลผลิตและคุณค่าทางโภชนาการของแหนแดง (*Azolla pinnata*) ที่เก็บเกี่ยวในช่วงอายุต่างกัน (7, 14 และ 21 วัน) เพื่อหาช่วงอายุที่เหมาะสมสำหรับใช้เป็นอาหารสัตว์ต้นทุนต่ำ การทดลองใช้การออกแบบแบบสุ่มสมบูรณ์ (Completely Randomized Design: CRD) ประกอบด้วย 3 ทรีทเมนต์ ทรีทเมนต์ละ 3 ซ้ำ โดยเก็บข้อมูลผลผลิต ได้แก่ น้ำหนักสด น้ำหนักแห้ง และวัตถุแห้ง พร้อมวิเคราะห์องค์ประกอบทางเคมี ได้แก่ โปรตีนหยาบ ไขมัน (Ether extract) เถ้า วัตถุอินทรีย์ เยื่อใย NDF และ ADF ตามวิธี AOAC International (2016); Van Soest et al. (1991) ผลการศึกษาพบว่า ปริมาณผลผลิตสดของแหนแดงที่เก็บเกี่ยวในอายุต่างกันไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$) แต่คุณค่าทางอาหารแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) โดยปริมาณโปรตีนรวมลดลงเมื่ออายุเก็บเกี่ยวเพิ่มขึ้น (จาก 19.78 % ที่ 7 วัน เหลือ 14.90 % ที่ 21 วัน) ขณะที่ค่า ADF เพิ่มขึ้นจาก 10.93 % เป็น 54.85 % ปริมาณเถ้าลดลง ส่วนวัตถุอินทรีย์เพิ่มขึ้นเมื่ออายุมากขึ้น และพบปริมาณไขมันสูงสุดที่อายุ 14 วัน (1.66 %) สรุปได้ว่า แหนแดงที่เก็บเกี่ยวในช่วงอายุ 7 – 14 วัน มีคุณค่าทางโภชนาการสูงกว่า เนื่องจากมีโปรตีนมากและเยื่อใยน้อยกว่าการเก็บเกี่ยวที่อายุ 21 วัน โดยไม่กระทบต่อผลผลิตสด ดังนั้น ช่วงอายุ 7 – 14 วันจึงเป็นช่วงที่เหมาะสมที่สุดในการเก็บเกี่ยวแหนแดง เพื่อใช้เป็นแหล่งอาหารโปรตีนราคาถูกสำหรับปศุสัตว์ โดยเฉพาะในระบบการผลิตแบบเกษตรกรรายย่อยที่มุ่งสู่ความยั่งยืน

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คำสำคัญ: *Azolla pinnata* อายุเก็บเกี่ยว องค์ประกอบทางโภชนาการ โปรตีนหยาบ อาหารสัตว์ยั่งยืน

Abstracts

This study aimed to evaluate the yield and nutritional composition of *Azolla pinnata* harvested at different growth stages (7, 14, and 21 days) to determine the optimal harvest age for use as a cost-effective livestock feed. A completely randomized design (CRD) with three treatments and three replications was applied. Fresh weight, dry weight, and dry matter content were measured, and chemical composition—including crude protein, ether extract, ash, organic matter, neutral detergent fiber (NDF), and acid detergent fiber (ADF)—was analyzed using standard AOAC International (2016); Van Soest et al. (1991) methods. Results showed no significant differences in yield parametersacross treatments ($p > 0.05$); however, significant changes in nutritional quality were observed ($p < 0.05$). Crude protein decreased with increasing harvest age (19.78% at 7 days to 14.90% at 21 days), while ADF increased significantly (10.93 % to 54.85 %). Ash content declined, whereas organic matter increased over time. The highest lipid content was recorded at 14 days (1.66 %). This study confirms that *Azolla pinnata* harvested at 7–14 days provides a superior nutritional profile with higher protein and lower fiber content compared to 21-day-old plants, without compromising biomass yield. This suggests a more precise harvest window for optimizing both feed quality and production efficiency. These findings support the use of early-harvested azolla as a viable alternative protein-rich feed resource for livestock, particularly in smallholder farming systems seeking low-cost, sustainable solutions.

Keywords: *Azolla pinnata*, harvest age, nutritional composition, crude protein, sustainable livestock feed

Introduction

Azolla pinnata, commonly known as mosquito fern, is a fast-growing aquatic plant found in tropical and subtropical regions. Due to its symbiotic relationship with the nitrogen-fixing cyanobacterium anabaena azollae, azolla has the ability to accumulate significant amounts of protein and other nutrients, making it a promising resource for sustainable agriculture and livestock feeding. Studies have shown that azolla contains 20–33 % crude protein on a dry matter basis, along with essential amino acids, minerals, β-carotene, and vitamins such as A and B12 (Tadavi et al., 2023) Rising feed costs, especially for protein-rich ingredients like soybean meal and fishmeal, have driven the search for alternative, cost-effective, and locally available feed resources. Azolla has been widely studied for its potential as an alternative feed supplement in livestock diets due to its high productivity, environmental adaptability, and low input requirements. In Pakistan, Khan et al. (2023) reported that partial replacement of dietary protein with *Azolla pinnata* (containing 32.8% CP and 13.6% CF) in Sahiwal calves improved feed efficiency and growth performance. Similarly, in Egypt, Fayed et al. (2023) found that replacing up to 25 % of sunflower meal protein with sun-dried azolla in the diets of Zaraibi goats did not compromise milk yield, nutrient digestibility, or animal health. In Thailand, Sirilak and Prapai (2014) evaluated the nutrient composition of *Azolla microphylla* and reported that the optimal protein and mineral content occurred when harvested at 7–14 days of age. Another study by Tadavi et al. (2023) found that inclusion of 9 % azolla meal in layer diets resulted in performance comparable to conventional protein sources, demonstrating the economic potential of azolla-based feed formulations for poultry.

Despite growing interest, one key limitation in azolla utilization is the variation in its nutritional composition across different growth stages. Younger azolla may have higher crude protein and lower fiber content, while more mature plants tend to develop fibrous structures that reduce digestibility and palatability. However, there remains limited information comparing the nutrient composition of azolla harvested at different growth stages under Thai cultivation conditions, where climate and water quality may influence its growth and chemical composition. In this context, the present study was designed to evaluate the yield and nutritional composition of *Azolla pinnata* harvested at three growth stages at 7, 14, and 21 days representing early, mid, and mature phases of development. These harvest ages were selected based on prior studies indicating that protein concentration declines and fiber content increases as azolla matures beyond two weeks of growth. Understanding the influence of harvest age on azolla's nutritional value is essential for optimizing its inclusion in livestock diets and reducing overall feed costs. Therefore, this study aims to evaluate the nutritional composition of *Azolla pinnata* harvested at different growth stages and assess its potential as a cost-effective feed ingredient. The results will provide practical recommendations for smallholder farmers and contribute to the development of sustainable livestock feeding systems using locally available aquatic resources.

Objective

To evaluate the differences in nutritional values of azolla harvested at varying ages

Materials and Methods

Experimental Design

The experiment was arranged in a completely randomized design (CRD) comprising three treatments, each with three replications. The treatments were based on the harvest age of *Azolla pinnata*, as follows; T1: azolla harvested at 7 days of growth, T2: azolla harvested at 14 days of growth and T3: azolla harvested at 21 days of growth. All experimental units were cultivated under uniform environmental conditions, including light intensity, water depth, and nutrient supplementation, to minimize environmental variation.

Cultivation and Harvesting

Azolla was sourced from a local aquatic plant nursery and propagated in plastic trays [specify dimensions 14.5″ x 12″] filled with nutrient-enriched water. The trays were maintained under natural sunlight, with regular monitoring of water temperature and pH to ensure consistent growing conditions.

At the designated harvest intervals (7, 14, and 21 days), azolla was harvested from each replication. Fresh weight (FW) was measured immediately after harvesting using a digital balance. The samples were then sun-dried to a constant weight to obtain dry weight (DW). The yields were expressed in grams per square meter (g/m^2), and the dry matter content (DM%) was calculated using the following formula:

$$DM\ (\%) = (\text{Dry weight}/\text{Fresh weight}) \times 100$$

Chemical Analysis

The dried azolla samples were finely ground using a 1-mm sieve and analyzed for proximate composition. The analyses included crude protein (CP), crude fiber (CF), ether extract (EE), and ash, following the standard procedures of the Association of Official Analytical Chemists (AOAC International, 2016). Nitrogen-free extract (NFE) was calculated by difference. For fiber fractionation, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the method of Van Soest et al. (1991).

Statistical Analysis

All collected data were subjected to one-way analysis of variance (ANOVA) to evaluate the effect of harvest age on yield parameters and nutritional composition. When significant differences ($p < 0.05$) were observed, treatment means were compared using Duncan's Multiple Range Test (DMRT). Statistical analyses were performed using SAS package (SAS Institute Inc, 1996) software version.

Results

The yield performance of *Azolla pinnata* at different harvest ages is presented in Table 1. Although there were observable numerical differences among treatments, statistical analysis revealed no significant differences ($p > 0.05$) in any of the measured parameters. Specifically, the total fresh weight tended to decrease with increasing harvest age, with values of 751.9 ± 126.3, 714.1 ± 98.1, and 644.4 ± 137.5 g/m^2 at 7, 14, and 21 days, respectively. Similarly, the net fresh weight gain followed a decreasing trend from 474.1 ± 126.3 g/m^2 at 7 days to 368.6 ± 137.5 g/m^2 at 21 days.

Table 1 Yield performance of *Azolla pinnata* at different harvest ages

Parameter	Harvest age (days)			P-value
	7	14	21	
Total fresh weight (g/m^2)	751.9 ± 126.3	714.4 ± 98.1	644.4 ± 137.5	0.57
Net fresh weight gain (g/m^2)	474.1 ± 126.3	436.8 ± 98.1	368.6 ± 137.5	0.57
Dry weight (g/m^2)	52.9 ± 17.8	40.8 ± 15.0	25.0 ± 9.40	0.13
Dry matter content (%)	5.64 ± 0.12	5.26 ± 0.24	5.26 ± 0.60	0.41

1 kg/rai = 0.625 g/m^2; mean ± SD (g/m^2)

The dry weight yield also decreased with longer growth duration, from 52.9 ± 17.8 g/m^2 at 7 days to 25.0 ± 9.40 g/m^2 at 21 days; however, the difference was not statistically significant ($p = 0.13$). For dry matter content, the values remained relatively constant across all harvest ages, ranging from 5.26% to 5.64 %, with no significant difference observed ($p = 0.41$). These results suggest that while earlier harvesting may numerically provide higher biomass yield, the differences in yield parameters were not statistically significant under the conditions of this study.

The chemical composition

The chemical composition of azolla harvested at 7, 14, and 21 days is presented in Table 2. The results showed that harvest age had a statistically significant effect on most chemical parameters ($p < 0.05$), except for neutral detergent fiber (NDF). The organic matter (OM) content increased significantly with plant age, ranging from 84.89 % at 7 days to 92.90 % at 21 days ($p = 0.004$). This trend is inversely related to ash content, which significantly decreased with age from 13.48 % at 7 days to 7.21 % at 21 days ($p = 0.003$). This indicates that the proportion of mineral matter declines as the plant matures, likely due to dilution effects or changes in tissue composition. The crude protein (CP) content showed a clear and statistically significant decrease with increasing harvest age ($p = 0.001$). The highest CP level was recorded at 7 days (19.78 %), followed by 18.61 % at 14 days, and 14.90 % at 21 days. This decline reflects the reduction in metabolic protein content and increased in structural biomass as the plant ages. For ether extract (EE), the values at 7 and 14 days were not significantly different (1.36 % and 1.66 %, respectively), but both were significantly higher than at 21 days (0.63 %) ($p = 0.010$). This suggests that lipid content is maximized in the early stages of growth and declines as the plant matures.

Table 2 Chemical composition of *Azolla pinnata* harvested at different growth stages

Parameter	Harvest age (days)			P-value
	7 days	14 days	21 days	
Organic matter	$84.89^{b} \pm 2.16$	$88.20^{b} \pm 0.73$	$92.90^{a} \pm 0.90$	0.004
Crude protein	$19.78^{a} \pm 0.14$	$18.61^{b} \pm 0.14$	$14.90^{c} \pm 0.08$	0.001
Ether extract	$1.36^{a} \pm 0.12$	$1.66^{a} \pm 0.15$	$0.63^{b} \pm 0.50$	0.010
Ash	$13.48^{a} \pm 2.11$	$10.86^{a} \pm 0.64$	$7.21^{b} \pm 0.82$	0.003
NDF	34.65 ± 24.03	43.07 ± 3.79	58.21 ± 21.85	0.364
ADF	$10.93^{b} \pm 1.96$	$26.43^{b} \pm 5.96$	$54.85^{a} \pm 19.02$	0.009

Different superscript letters $^{a, b, c}$ within the same row indicated statistically significant differences ($p < 0.05$)

Although NDF values increased from 34.65 % at 7 days to 58.21 % at 21 days, the difference was not statistically significant ($p = 0.364$), possibly due to high variation among replications. In contrast, acid detergent fiber (ADF) showed a significant increase with age from 10.93 % at 7 days to 54.85 % at 21 days ($p = 0.009$), indicating a higher accumulation of cellulose and lignin in older plants. Early-harvested azolla (7–14 days) had significantly higher protein and lipid content and lower fiber and lignin levels. Late-harvested azolla (21 days) had higher organic matter and ADF, but lower nutritional value for livestock feed. These results suggest that harvesting between 7 – 14 days provides the most favorable balance of nutrient quality for feed formulation.

Discussion

Yield Performance

Although the yield parameters of *Azolla pinnata* harvested at 7, 14, and 21 days showed numerical differences, statistical analysis indicated no significant differences ($p > 0.05$) in total fresh weight, net fresh weight gain, dry weight, or dry matter content. This suggests that azolla can maintain relatively stable productivity over the 7–21-day harvest period under consistent growing conditions. The highest total fresh weight was recorded at 7 days (751.9 ± 126.3 g/m^2), followed by a gradual decline to 644.4 ± 137.5 g/m^2 at 21 days. A similar trend was observed in net fresh weight gain and dry weight, with the latter decreasing from 52.9 ± 17.8 g/m^2 at 7 days to 25.0 ± 9.4 g/m^2 at 21 days. Although these changes were not statistically significant, they may reflect physiological shifts in biomass composition during maturation, such as increased fiber content and reduced water accumulation. Dry matter content remained relatively constant across all treatments, ranging from 5.26 % to 5.64 %, indicating that the proportion of structural biomass did not vary significantly with age. This observation aligns with findings from Haryani et al. (2022) who reported similar dry matter stability in *Azolla microphylla* grown under tropical conditions.

The lack of statistical significance in yield metrics contrasts with some previous studies. For example, Basak et al. (2022) reported that biomass yield peaked at 10–12 days and declined thereafter, particularly under suboptimal nutrient conditions. However, the present study's uniform water and nutrient management may have minimized such variability. Although the numerical yield at 7 days was the highest, from a practical perspective, harvesting between 7 – 14 days may offer an optimal balance between biomass productivity and nutritional quality, as later harvests tend to result in increased lignification and decreased crude protein, as evidenced in Table 2. Moreover, early harvesting allows for faster production cycles and higher cumulative yields over time.

Nutritional values

The results of this study revealed that the nutritional composition of azolla was significantly influenced by its harvest age. As the plant matured from 7 to 21 days, a consistent decline in CP content was observed, while organic matter and fiber content increased. These findings align with those reported by Basak et al. (2022) who noted that younger azolla contains higher levels of protein and lipids, whereas older azolla tends to accumulate structural carbohydrates and lignified tissues that reduce digestibility. The highest CP content in this study was observed at 7 days of growth (19.78 %), which is comparable to the values reported by Anitha et al. (2016); Alalade and Iyayi (2006) who found protein levels ranging from 21 % to 25 % in young azolla. As the plant aged, protein content decreased to 14.90 % at 21 days, likely due to dilution effects and an increase in cell wall constituents. Mechanistically, the reduction in CP and EE content with increasing age may result from the biosynthetic shift toward complex carbohydrates and fibrous compounds in the cell wall during structural development.

A similar trend was found in ash content, which declined significantly with age, from 13.48 % at 7 days to 7.21 % at 21 days. This observation is consistent with findings from Sirilak and Prapai (2014) who reported that essential mineral concentrations, such as calcium and potassium, were highest in azolla harvested between 7 – 14 days. Ether extract content peaked at 14 days (1.66 %) and was significantly lower at 21 days (0.63 %). This is in line with studies conducted in India, which demonstrated that lipid content in azolla tends to be higher during early to mid-growth stages (Basak et al., 2022) The sharp increase in acid detergent fiber (ADF) with increasing harvest age from 10.93% at 7 days to 54.85 % at 21 days further indicates the accumulation of indigestible fiber in mature plants. These results are supported by Fiogbé et al. (2004), who cautioned against using mature azolla in high proportions due to reduced nutrient availability, especially in non-ruminants.

Overall, the findings suggest that harvesting azolla at 7–14 days provides optimal nutritional quality, particularly in terms of CP, EE, and ash. In contrast, harvesting at 21 days may yield lower-quality feed with higher fiber content, making it more suitable for compost or soil amendment rather than inclusion in high-performance livestock diets. Practically, this implies that azolla harvested within two weeks could serve as a partial substitute for conventional protein sources such as soybean meal or fishmeal in ruminant and aquaculture diets. Therefore, for use in ruminant or poultry feed, azolla should be harvested before significant lignification occurs preferably within 14 days of growth to maximize its nutritional benefits and minimize anti-nutritional factors.

Conclusion

This study evaluated the yield and nutritional composition of azolla harvested at three different growth stages at 7, 14, and 21 days to determine the optimal harvesting time for use as a low-cost and sustainable livestock feed. The results showed that while harvest age had no significant effect on total fresh yield, net biomass gain, or dry matter content, it had a pronounced impact on nutrient composition. CP content significantly declined as the harvest age increased, while ADF increased substantially, indicating reduced feed quality in mature azolla. The highest nutritional value characterized by high protein, low fiber, and balanced mineral content was observed in azolla harvested between 7 and 14 days. OM content increased with age, while ash and ether extract contents decreased, further reinforcing the nutritional superiority of early-harvested azolla.

These findings suggest that harvesting azolla at 7 – 14 days offers the best balance between nutritional quality and biomass productivity. Early harvest not only ensures higher feed value but also supports frequent cropping cycles and efficient land use, making it suitable for smallholder and sustainable livestock production systems. Azolla holds strong potential as a climate-smart, low-input, and nutritionally valuable feed resource. Its adoption in smallholder systems can contribute significantly to reducing feed costs, improving animal productivity, and enhancing the sustainability of livestock farming in tropical regions.

Suggestions

To fully realize the benefits of azolla as a practical feed solution, the following recommendations are proposed; (1) Farmers should adopt a 7 – 14 day harvest cycle to obtain high-protein, digestible biomass; (2) Further in vivo feeding trials are necessary to assess animal performance and feed efficiency; (3) Research on preservation methods such as drying and silage is needed to extend shelf life and availability; (4) Economic feasibility studies should be conducted to support broader adoption among small-scale producers; and (5) azolla cultivation should be integrated into circular farming systems to promote nutrient recycling and ecological resilience.

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