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INTEGRATED EFFECTS OF AZOLLA INCORPORATION AND INTERCROPPING ON RICE GROWTH, AND BIOMASS NUTRITIONAL VALUE

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Abstract
The excessive reliance on chemical fertilizers in rice cultivation has raised concerns regarding environmental sustainability and nutrient inefficiency, highlighting the need for alternative biofertilizer strategies. Although Azolla has been widely studied, most studies have focused primarily on soil properties without integrating crop performance and biomass utilization. This study aimed to evaluate the integrated effects of Azolla incorporation and intercropping on rice growth, nitrogen uptake, and the nutritional value of Azolla biomass. A greenhouse experiment was conducted using a completely randomized design with eight treatments and three replications, including control (no Azolla), Azolla incorporation at 240, 280, and 320 g pot^{-1} with and without intercropping, and chemical fertilizer treatment. The results showed that Azolla application significantly improved rice growth parameters, with plant height, leaf number, and tiller number increasing by up to 15–30% compared to the control. Higher Azolla doses (320 g pot^{-1}) consistently produced the best performance. In addition, Azolla biomass exhibited high nutritional value, with crude protein content exceeding 20%, suggesting its potential as a supplementary livestock feed. These findings demonstrate that Azolla can function as a multifunctional bioresource, enhancing crop performance while supporting biomass valorization within integrated agricultural systems.

Keywords: *Azolla, biomass utilization, nitrogen uptake, rice growth, sustainable agriculture*

Abstrak
Pemanfaatan pupuk kimia secara berlebihan dalam budidaya padi menimbulkan permasalahan keberlanjutan lingkungan dan efisiensi penggunaan hara, sehingga diperlukan alternatif pupuk hayati. Meskipun Azolla telah banyak diteliti, sebagian besar penelitian masih berfokus pada sifat tanah tanpa mengintegrasikan respons tanaman dan pemanfaatan biomassa. Penelitian ini bertujuan untuk mengevaluasi pengaruh terintegrasi pemendaman dan tumpang sari Azolla terhadap pertumbuhan tanaman padi, serapan nitrogen, serta nilai nutrisi biomassa Azolla. Penelitian dilakukan di rumah kaca menggunakan rancangan acak lengkap dengan delapan perlakuan dan tiga ulangan, meliputi kontrol (tanpa Azolla), pemendaman Azolla sebesar 240, 280, dan 320 g pot^{-1} dengan dan tanpa tumpang sari, serta perlakuan pupuk kimia. Hasil penelitian menunjukkan bahwa aplikasi Azolla secara signifikan meningkatkan pertumbuhan tanaman padi, dimana tinggi tanaman, jumlah daun, dan jumlah anakan meningkat hingga 15–30% dibandingkan kontrol. Dosis Azolla tertinggi (320 g pot^{-1}) memberikan hasil terbaik. Selain itu, biomassa Azolla memiliki nilai nutrisi tinggi dengan kandungan protein kasar lebih dari 20%, sehingga berpotensi sebagai pakan tambahan ternak. Hasil ini menunjukkan bahwa Azolla berperan sebagai sumber daya hayati multifungsi yang mampu meningkatkan kinerja tanaman sekaligus mendukung pemanfaatan biomassa dalam sistem pertanian terintegrasi.

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Kata kunci: Azolla, biomassa, pertanian berkelanjutan, pertumbuhan padi, serapan nitrogen

1. INTRODUCTION

Sustainable agricultural development requires integrated nutrient management strategies that enhance crop productivity while maintaining environmental quality. In rice-based agroecosystems, nutrient availability and organic matter inputs play a critical role in determining plant growth and long-term system sustainability. Organic amendments, including compost and biofertilizers, have been widely reported to improve nutrient cycling, enhance soil fertility, and support plant growth through increased availability of essential nutrients (Agegnehu et al., 2017; Ravindran et al., 2018).

The utilization of agricultural and livestock wastes as organic inputs has gained increasing attention as a sustainable approach to improve soil quality while reducing environmental pollution. Compost derived from plant residues and animal manure has been shown to significantly improve organic carbon, total nitrogen, and C/N ratio, indicating better decomposition and nutrient availability for plant uptake (Bernal et al., 2009; Awasthi et al., 2014; Di Lonardo et al., 2017). In addition, locally available organic materials have been proven to produce compost with improved stability and nutrient quality, supporting environmentally friendly agricultural practices (Bimantara et al., 2025).

Nutrient dynamics, particularly carbon (C) and nitrogen (N), are strongly influenced by agricultural management practices and land-use changes. Previous studies have demonstrated that changes in land use and vegetation can significantly alter C and N stocks, as well as their cycling processes, which ultimately affect plant productivity and ecosystem sustainability (Wang et al., 2016; Yang et al., 2023). For example, long-term land-use conversion has been shown to influence soil organic carbon and total nitrogen dynamics, including their distribution and stability in different soil layers (Kusumawardani et al., 2022; Hosogoe et al., 2024).

In addition, organic-based management practices can enhance nutrient availability and improve plant growth through biological processes. The incorporation of biofertilizers such as Azolla has been reported to increase soil organic carbon and total nitrogen while improving nutrient retention and cycling (Adnyana et al., 2025). Such improvements contribute to better plant growth performance and reduced dependence on synthetic fertilizers.

Despite these advances, there is still limited quantitative evidence linking organic-based treatments directly with rice growth performance under controlled experimental conditions. Many studies have focused primarily on soil properties, while fewer have explicitly evaluated plant growth indicators such as plant height, number of tillers, and nitrogen uptake in response to different organic treatments. Furthermore, studies integrating both nutrient dynamics and plant growth responses remain limited.

Therefore, this study aims to evaluate the effects of different organic-based treatments on rice growth performance and selected agronomic parameters. The study specifically quantifies growth responses, including plant height, number of tillers, and nitrogen-related indicators under defined

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treatment conditions. The results are expected to provide scientific evidence for optimizing sustainable nutrient management strategies in rice cultivation systems.

2. MATERIALS AND METHODS

2.1 Materials

The experiment was conducted using paddy soil, rice (Oryza sativa L.) seedlings, Azolla biomass, and chemical fertilizers. Soil samples were collected from a paddy field located in Pemecutan Klod, Denpasar, Bali, Indonesia, representing typical lowland rice soil conditions.

The soil was air-dried, homogenized, and sieved through a 5 mm mesh prior to use. A total of 28 kg of soil was prepared, and 7 kg of soil (equivalent to 5 kg dry weight) was placed in each pot. Rice seeds were sown in nursery trays (three seeds per hole), and seedlings were transplanted into pots at five weeks after sowing.

Fresh Azolla biomass was obtained from local cultivation and applied according to treatment levels. Chemical fertilizers used in this study included urea, SP-36, and KCl, applied based on recommended fertilization rates and converted into pot-scale units.

Water was maintained at approximately 5 cm above the soil surface throughout the experimental period to simulate flooded paddy conditions.

2.2 Methods

The experiment was conducted under greenhouse conditions at the experimental field of the Faculty of Agriculture, Udayana University, Denpasar, Bali, Indonesia, from February to July 2024. The study employed a Completely Randomized Design (CRD) consisting of eight treatments with three replications, resulting in a total of 24 experimental units.

2.2.1. Experimental Design and Treatments

The treatments applied in this study are presented in **Table 1**.

Table 1. Experimental treatments applied in the study

Code	Chemical fertilizer	Azolla incorporation (g pot^{-1})	Intercropping
K0	None	0	No
K1A	SP-36 (0.35 g), KCl (0.35 g)	240	Yes
K2A	SP-36 (0.35 g), KCl (0.35 g)	280	Yes
K3A	SP-36 (0.35 g), KCl (0.35 g)	320	Yes
K1AT	SP-36 (0.35 g), KCl (0.35 g)	240	No
K2AT	SP-36 (0.35 g), KCl (0.35 g)	280	No
K3AT	SP-36 (0.35 g), KCl (0.35 g)	320	No
KA	Urea (0.52 g), SP-36 (0.35 g), KCl (0.35 g)	0	No

Notes: Fertilizer application rates were based on recommendations from the Department of Agriculture of Buleleng Regency (2020) and converted into pot-scale units.

2.2.2 Crop Management and Treatment Application

Azolla was applied either through incorporation into the soil prior to transplanting or maintained as a surface cover in intercropping treatments. In intercropping treatments, Azolla was introduced after transplanting and allowed to grow alongside rice plants.

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Chemical fertilizers were applied according to the treatment design. All treatments were maintained under flooded conditions with a water depth of approximately 5 cm. Standard crop management practices were applied uniformly across all experimental units.

2.2.3 Observation Time and Parameters

Observations were conducted periodically at 7, 13, 27, 41, 55, 69, 83, and 97 days after transplanting (DAT), corresponding to approximately two-week intervals throughout the growing period.

The observed plant growth parameters included plant height (cm), number of tillers, number of leaves, and chlorophyll content measured using a SPAD meter. These parameters were used to evaluate the vegetative growth response of rice plants under different treatments. Yield parameters were measured at harvest (97 DAT), including number of panicles, grain weight, total grain number, 1000-grain weight, and plant dry weight, to assess the effect of treatments on crop productivity.

2.2.4 Nutritional Analysis of Azolla

Azolla biomass collected from the experimental treatments was analyzed for its nutritional composition at the Laboratory of Animal Nutrition and Feed, Faculty of Animal Husbandry, Udayana University.

The analyzed parameters included dry matter, organic matter, crude protein, crude fiber, crude fat, total digestible nutrients (TDN), nitrogen-free extract (NFE), ash content, and gross energy.

2.2.5 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) at a 5% significance level. When significant differences among treatments were detected, mean comparisons were performed using Duncan's Multiple Range Test (DMRT). In addition, Relative Agronomic Efficiency (RAE) was calculated based on grain dry weight to evaluate the effectiveness of Azolla treatments compared to the conventional fertilizer treatment, using the following equation:

$$RAE\ (\%) = (Yt - Yc) / (Yr - Yc) \times 100$$

Where Yt is the grain dry weight of the treatment (g pot^{-1}), Yc is the grain dry weight of the control (K0), and Yr is the grain dry weight of the reference treatment (KA). Grain dry weight per pot was used as a proxy for yield.

3. RESULT AND DISCUSSION

3.1 Rice Growth Response under Azolla Treatments

Rice growth responded significantly to Azolla-based treatments as presented in **Figure 1**. Plant height, number of tillers, number of leaves, and chlorophyll content increased progressively throughout the observation period, with more pronounced differences appearing after 27 days after transplanting (DAT). Treatments with higher Azolla doses, particularly K3A and K3AT, consistently exhibited superior growth compared to the control (K0) and conventional fertilization (KA).

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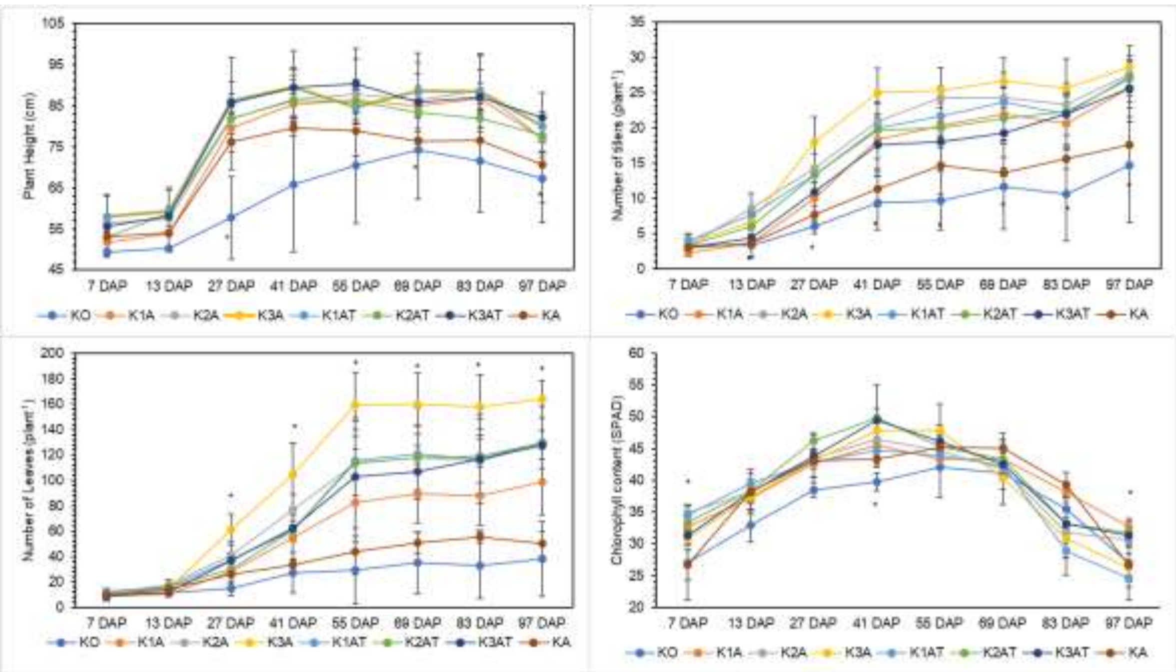

Figure 1. *Temporal dynamics of rice growth under different Azolla-based and fertilizer treatments measured at 7–97 days after transplanting (DAT): (a) plant height, (b) number of tillers, (c) number of leaves, and (d) chlorophyll content (SPAD). Error bars indicate standard deviation. Asterisks (*) denote significant differences among treatments at P < 0.05*

This improvement in vegetative growth indicates that Azolla provides a sustained supply of nutrients, especially nitrogen, through biomass decomposition and biological nitrogen fixation. Azolla forms a symbiotic relationship with Anabaena azollae, enabling the conversion of atmospheric nitrogen into plant-available forms (Adnyana et al., 2025). In addition, Azolla incorporation has been shown to increase soil organic carbon and cation exchange capacity (CEC), thereby enhancing nutrient retention and availability.

These findings are consistent with previous studies demonstrating that increased organic matter inputs improve soil microbial activity and nutrient cycling, ultimately enhancing plant growth performance (Hosogoe et al., 2024; Kusumawardani et al., 2022).

3.2. Yield Components and Productivity

Yield components were significantly influenced by Azolla treatments, as shown in **Table 2**. The highest values for number of panicles, grain number, grain dry weight, and plant dry weight were consistently observed under K3AT treatment, indicating that higher Azolla application combined with appropriate management resulted in optimal productivity.

The increase in yield components can be attributed to improved vegetative growth, which enhances photosynthetic capacity and assimilate production. The higher number of tillers led to increased panicle formation, ultimately contributing to greater grain production.

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Table 2. Effects of different Azolla-based and fertilizer treatments on yield components of rice

Treatments	Number of panicles (panicles pot^{-1})	Plant dry weight (g)	Grain dry weight (g)	Number of grains (grains pot^{-1})	1000-grain weight (g)
KO	11.33 ± 1.15 c	10.10 ± 1.00 c	0.32 ± 0.01 e	391.00 ± 406.20 d	16.73 ± 5.65 b
K1A	50.00 ± 17.78 a	136.60 ± 1.00 a	14.01 ± 0.58 b	1392.00 ± 687.00 bcd	27.74 ± 3.24 a
K2A	61.67 ± 3.51 a	100.15 ± 1.00 b	14.58 ± 1.00 ab	1667.33 ± 213.16 abc	26.62 ± 8.08 a
K3A	60.33 ± 9.02 a	133.49 ± 1.00 a	15.23 ± 1.00 a	2664.00 ± 1372.75 a	29.58 ± 4.53 a
K1AT	60.33 ± 14.01 a	104.66 ± 1.00 b	8.95 ± 0.10 c	1579.33 ± 220.84 abcd	29.87 ± 2.39 a
K2AT	63.67 ± 10.12 a	119.40 ± 1.00 ab	11.77 ± 1.00 bc	1566.00 ± 432.57 abcd	29.10 ± 5.69 a
K3AT	65.33 ± 6.81 a	142.13 ± 48.87 a	17.27 ± 1.00 a	1901.33 ± 627.93 ab	29.63 ± 3.98 a
KA	29.33 ± 10.21 b	16.50 ± 1.00 c	1.31 ± 0.10 d	625.67 ± 301.14 cd	23.64 ± 9.86 ab

Values are presented as mean ± standard deviation (n = 3). Different lowercase letters within the same column indicate significant differences among treatments according to Duncan's Multiple Range Test (DMRT) at $P < 0.05$

Relative Agronomic Efficiency (RAE), illustrated in **Figure 2**, further confirms the superior performance of Azolla-based treatments compared to conventional fertilization. The highest RAE value observed in K3AT indicates a substantial improvement in nutrient use efficiency.

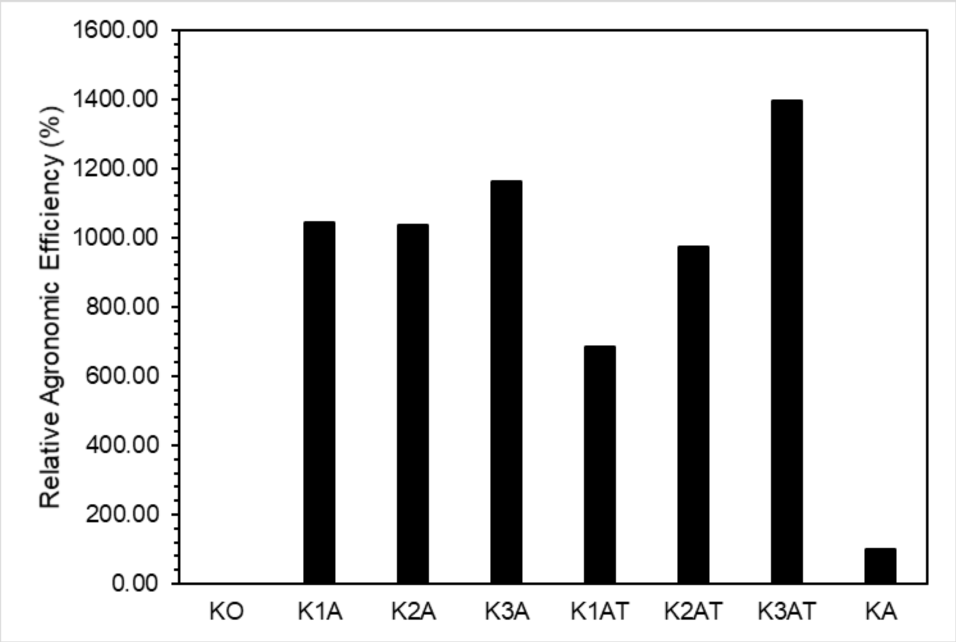

Figure 2. *Effects of different Azolla application methods and fertilizer treatments on Relative Agronomic Efficiency (RAE, %) based on grain dry weight*

These results are in agreement with previous studies reporting that Azolla can contribute approximately 40–60 kg N ha^{-1} per growing season and significantly enhance rice yield through improved nitrogen availability and efficiency (Akhtar et al., 2021; Seleiman et al., 2022). Furthermore, the use of organic amendments has been shown to improve agronomic efficiency by enhancing soil fertility and nutrient dynamics (Bimantara et al., 2025).

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3.3 Relationship between Soil Properties and Plant Performance

The correlation matrix presented in **Table 3**. reveals strong positive relationships between soil properties and plant performance. Soil organic carbon and cation exchange capacity (CEC) showed very strong correlations with grain yield, plant dry weight, and number of panicles.

Table 3. Correlation matrix showing relationships between soil properties, plant nitrogen uptake, and rice yield components

Parameter	pH H_2O	EC	Soil Organic Carbon	Total-N	Total-P	Total-K	CEC	BS	Plant N Absorption	Number of Panicles	Dry Weight of Rice Plant	Dry Grain Weight	Number of Grains	Weight of 1000 Grains
pH H_2O	1.00													
EC	-0.01	1.00												
Soil Organic Carbon	0.27	0.27	1.00											
Total-N	0.44	0.38	0.48	1.00										
Total-P	0.05	-0.26	0.13	0.05	1.00									
Total-K	-0.06	0.06	-0.03	0.10	-0.44	1.00								
CEC	0.27	0.30	0.82	0.63	0.09	0.11	1.00							
BS	-0.31	-0.46	-0.04	-0.07	0.10	-0.14	0.04	1.00						
Plant N Absorption	0.43	0.20	0.76	0.63	0.20	0.05	0.86	0.10	1.00					
Number of Panicles	0.23	0.10	0.82	0.47	0.07	0.13	0.81	0.25	0.73	1.00				
Dry Weight of Rice Plant	0.17	0.30	0.83	0.55	0.08	0.05	0.94	0.12	0.88	0.79	1.00			
Dry Grain Weight	0.27	0.32	0.84	0.76	0.09	0.10	0.93	0.08	0.88	0.82	0.92	1.00		
Number of Grains	0.34	0.08	0.63	0.44	0.01	0.06	0.71	0.27	0.76	0.81	0.65	0.71	1.00	
Weight of 1000 Grains	0.10	0.22	0.55	0.32	-0.13	0.48	0.58	-0.19	0.35	0.54	0.53	0.53	0.33	1.00

Soil variables (pH, EC, soil organic carbon, total N, total P, total K, cation exchange capacity (CEC), and base saturation (BS)) were obtained from Adnyana et al. (2025), while plant and yield parameters were measured in the present study. Color gradients indicate correlation strength ranging from very low to very strong. Red color represents very low – low; yellow color represents medium; green color represents strong – very strong.

This indicates that improvements in soil fertility directly contribute to enhanced crop productivity. Soil organic carbon plays a critical role in nutrient cycling, microbial activity, and soil structure, all of which are essential for plant growth. Previous studies have emphasized that higher SOC and total nitrogen levels are closely associated with improved soil fertility and crop productivity (Hosogoe et al., 2024).

In addition, increased CEC enhances the soil's ability to retain essential nutrients such as ammonium (NH_4^+) and potassium (K^+), reducing nutrient losses and improving nutrient use efficiency. Similar findings have been reported in long-term land-use studies, where stable or increased organic matter inputs maintained soil fertility and supported plant productivity (Kusumawardani et al., 2022).

3.4 Nutritional Quality of Azolla Biomass

The nutritional composition of Azolla biomass used in this study is presented in **Table 4**. The results indicate that Azolla biomass contains high organic matter (83.50%) and crude protein (36.78%), highlighting its strong potential not only as a biofertilizer but also as a high-quality supplementary feed for livestock. In addition, the biomass exhibited moderate crude fiber (13.33%), low crude fat (1.79%), and relatively high gross energy (4098.99 kcal kg^{-1}), indicating its suitability as an energy and protein source in animal feed formulations.

The high crude protein content is primarily attributed to biological nitrogen fixation through the symbiotic association between Azolla and Anabaena azollae, which continuously enriches the

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biomass with nitrogen (Adnyana et al., 2025). This characteristic makes Azolla a multifunctional resource, capable of enhancing soil fertility while simultaneously contributing to livestock nutrition.

Compared to previous studies, the nutritional quality observed in this study is relatively higher. For instance, Kumar et al. (2018) reported lower values of crude protein (22.25%), organic matter (74.50%), and dry matter (91.78%), indicating that the Azolla biomass used in this study has superior nutritional characteristics. These differences may be influenced by environmental conditions, cultivation methods, and biomass maturity at harvest.

Table 4. Nutritional composition of Azolla biomass

No	Parameter	Value
1	Dry matter (%)	95.32 ± 0.58
2	Moisture content (%)	4.68 ± 0.58
3	Ash content (%)	16.50 ± 0.10
4	Organic matter (%)	83.50 ± 0.10
5	Crude protein (%)	36.78 ± 0.72
6	Crude fiber (%)	13.33 ± 0.13
7	Crude fat (%)	1.79 ± 0.05
8	Total Digestible Nutrients (TDN, %)	49.59 ± 0.27
9	Nitrogen-Free Extract (NFE, %)	26.81 ± 0.12
10	Gross energy (kcal kg^{-1})	4098.99 ± 69.06

Values are presented as mean ± standard deviation. Parameters include dry matter, moisture content, ash, organic matter, crude protein, crude fiber, crude fat, total digestible nutrients (TDN), nitrogen-free extract (NFE), and gross energy.

The relatively balanced composition of organic matter and nitrogen in Azolla biomass suggests a favorable C/N ratio, which facilitates rapid decomposition and nutrient release when applied to soil. Organic materials with balanced C/N ratios are known to decompose more efficiently and enhance nutrient availability in agricultural systems (Kartini et al., 2026). This explains the dual role of Azolla as both a fast-releasing organic amendment and a nutrient-rich biomass resource.

From a livestock perspective, the high protein content of Azolla (36.78%) contributes significantly to meeting protein requirements in animal diets. However, the relatively high crude fiber content (13.33%) may act as a limiting factor, particularly for monogastric animals. To overcome this limitation, processing methods such as fermentation can be applied to improve digestibility and nutritional quality. Fermentation facilitates the breakdown of complex organic compounds into simpler forms through microbial activity, thereby enhancing feed efficiency.

Previous studies have demonstrated that fermented Azolla can be safely incorporated into livestock feed without negative effects on performance. For example, the inclusion of fermented Azolla up to 15% in poultry diets did not adversely affect growth performance or carcass production (Daud et al., 2022). Similarly, supplementation of Azolla at 1.5 kg day^{-1} per head in buffalo diets increased milk production by 16.25% (Meena et al., 2017).

Overall, the findings of this study confirm that Azolla biomass possesses high nutritional value and multifunctional potential, supporting its use in integrated farming systems. The ability of Azolla to serve as both a biofertilizer and a livestock feed resource strengthens its role in promoting sustainable and circular agricultural practices.

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3.5 Implications for Sustainable Agriculture

The integration of Azolla into rice cultivation systems provides multiple agronomic and environmental benefits. In addition to improving plant growth and yield, Azolla reduces dependence on synthetic fertilizers, which are associated with soil degradation and environmental pollution.

The results of this study support the concept of integrated nutrient management, where organic inputs are used to enhance soil fertility and crop productivity. Azolla contributes to improved nitrogen cycling, increased soil organic matter, and enhanced nutrient use efficiency.

Furthermore, the dual function of Azolla as both a biofertilizer and biomass resource aligns with the principles of circular agriculture, where agricultural inputs and outputs are efficiently recycled within the system.

Overall, Azolla represents a promising strategy for developing sustainable rice production systems, particularly in tropical regions such as Bali.

4. CONCLUSION

The application of Azolla-based treatments significantly improved rice growth and yield components compared to the control and conventional treatments. Enhanced vegetative growth, reflected in increased plant height, tiller number, and leaf development, was followed by substantial improvements in yield, particularly in grain number and grain dry weight. Among the treatments, K3AT consistently showed the best performance, indicating that the combination of Azolla application and treatment intensity plays a critical role in maximizing productivity. Relative Agronomic Efficiency (RAE) values further demonstrated that Azolla-based treatments were more effective than conventional fertilization, highlighting their potential as a sustainable alternative input in rice production systems. The strong correlations between soil organic carbon, cation exchange capacity, and yield components confirm that improved soil fertility plays a key role in enhancing rice productivity. Overall, Azolla biomass, characterized by high organic matter and crude protein content, has strong potential as a sustainable nutrient source. The findings suggest that integrating Azolla into rice farming systems can improve productivity while reducing dependence on synthetic fertilizers, thereby supporting more sustainable agricultural practices.

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