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Quail (*Coturnix coturnix japonica*) Fermented Manure as a Fertilizer to Support *Azolla microphylla* Growth Rate

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Abstract

Azolla (*Azolla microphylla*) is a potential livestock feed due to its fast-growing and relevant nutrient content. Quail farming generates manure as a by-product that can be used as fertilizer to support plant growth. This study aims to determine the dose effect of fermented quail manure on the growth rate of Azolla, as well as the nutritional content of the resulting Azolla biomass. The data were obtained through observation in 20 Azolla culture ponds according to the design method throughout the research for the Azolla growth rate parameters and laboratory tests to analyze manure and Azolla proximate content at the end of the study. The results showed that treatment of 50, 100 and 150 g m^{-2} of quail manure gave similarly good effects on the Azolla growth parameters, indicating that the application of quail manure can significantly increase the production of Azolla biomass in culture ponds. The resulting Azolla biomass from all treatments gave a value of 93% to 95% moisture content, 18% to 19% ash content, 36% to 42% protein, 20% to 37% fiber, 2.8% to 4% fat and 5% to 6% carbohydrates. Looking at the big picture, therefore, 100 g m^{-2} manure treatment is considered the most optimal among the rest. Based on the result obtained, this study can provide an overview of the dose of quail manure that can be used to support the growth of Azolla.

Keywords: *Azolla microphylla*; fertilizer; growth speed; quail manure

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INTRODUCTION

As an agricultural country, Indonesia relies heavily on the agricultural sector to maintain its resilience through plant and animal cultivation. The two are inseparable, mainly due to livestock's needs for nutritious feed from feed plants. Quality feed is the most significant expenditure component in the livestock industry, reaching 60% to 70% of the total cost (Mallick et al., 2020). Various plants can be used as alternative feed and feed additives for livestock, one of which is Azolla (*Azolla microphylla*) (Bhaskaran and Kannapan, 2015). Based on Utama et al. (2015), Azolla associates with the blue-green algae *Anabaena azollae*, which allows it to fix nitrogen from the atmosphere. This symbiosis is responsible for the relatively high protein content of Azolla biomass, 13% to 30% of its dry weight (Indarmawan et al., 2012; Sudadi and Suryono, 2016). In addition, the use of Azolla as a feed additive is supported by the fact that the growth of Azolla tends to be fast and is easy to maintain compared to other plant-based feed alternatives. As an invasive aquatic weed, Azolla has prolific growth with an exceptionally high reproduction rate to produce bioenergy (Kaur et al., 2018).

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It only requires around 10 to 15 days to mature, with a doubling time of merely 4 to 5 days, depending on the availability of nutrients and environmental conditions during cultivation (Azhar et al., 2018; Adzman et al., 2022). According to an earlier study (Nuraini et al., 2022), feed added with Azolla increased feed consumption, body weight gain, and feed conversion of broiler chickens. Azolla is also functional as a substitute for urea in fish and livestock feed (Yuniati, 2013), green manure in paddy fields to reduce the use of chemical fertilizers (Brouwer et al., 2018; Bharali et al., 2021; Ssenku et al., 2022) and phytoremediator of heavy metals in water bodies (Sundararaman et al., 2021). As stated by Kollah et al. (2016), the use of Azolla may also help regulate N_2O emission and NH_3 volatilization on agroecosystems also creates a new source of income for farmers. Nasir et al. (2022) addressed that Azolla impacts several environmental causes, including lower greenhouse gas emissions, carbon footprint and land requirements; additionally, it produces feedstuffs enriched in amino acid. In addition to providing the various benefits above, cultivating Azolla can also help overcome the problem of environmental pollution due to animal waste, which can be used as manure to aid its growth (Kusuma, 2012).

On the other hand, animal waste products are potential fertilizers for plant cultivation. In Indonesia, quail is a widely cultivated livestock with a growing population yearly (Nugraha et al., 2017). Apart from producing eggs and meat, quail farms produce by-products, like manure in abundance, which may pollute the environment if not handled or allocated correctly (Sumendap et al., 2019). Quail manure contains high slow-releasing nutrients, is easy to decompose, and is easily absorbed by plants to meet their needs; therefore, it has the potential to be used as organic fertilizer to improve crop productivity in the long run (Agustin et al., 2017). Although contained in sufficient quantities, the nutrients in manure are usually not immediately available for plants to be used directly. One way to ensure its availability to plants is through the fermentation process, in which the organic matter undergoes decomposition or overhaul by microorganisms into a simpler and readily available form for the plants to absorb. Fermentation of quail manure can be done using EM4 probiotic, which contains Lactobacillus to decompose carbohydrates in organic matter into simple fatty acids (Cahyono et al., 2015). In addition to *Lactobacillus casei*, EM4 probiotic also includes the *Saccharomyces cerevisiae* bacterium, which plays a role in fermenting organic matter such as quail droppings (Cahyono et al., 2015). Using quail manure as organic fertilizer can prevent land degradation, improve the fertility of the growing media and avoid pollution due to synthetic fertilizers (Haryadi et al., 2015). Recent studies by Bibiano et al. (2019) also concluded that fertilization with poultry manure, such as quail, has been proven to increase plant growth, dry matter and even yields in higher essential oil content of medicinal plants compared to cattle manure. Similar results were obtained by the application of quail manure as ameliorants to aid phosphorus availability in alfisols thus increasing soybean production in Indonesia (Triatmoko et al., 2020).

The availability of macro and micronutrients in growing media greatly influences a plant's growth and development (Mashud et al., 2018). While aided by the fact that it can fixate nitrogen from the atmosphere, Azolla is very dependent on phosphate available in the growing media to be later transformed into pyrophosphate as the primary energy source for the plant (Utama et al., 2015). Fortunately, quail manure contains an adequate amount of phosphate based on an experiment by Agustin et al. (2017), which is around 0.209% to 1.37%. This supports the use of quail manure in increasing Azolla productivity. Surdina et al. (2016) stated that the productivity of Azolla can then be measured using final weight, weight gain, relative growth rate, individual density and doubling time. Based on the multifunctional nature of Azolla and an abundant source of nutrients for it in the form of quail manure, there is a need for an integrated cultivation system between the two which can be very helpful in supporting sustainability in the production system of feed crops and livestock. This can also minimize external input from outside the system while simultaneously overcoming the problem of livestock waste. Based on these facts, authors hypothesize that the application of quail manure can accelerate Azolla's growth when compared to production relying on natural standing water. For this, the objectives were to determine the dose effect of fermented quail manure on the growth rate of Azolla, as well as the nutritional content of the resulting Azolla biomass.

MATERIALS AND METHOD

Research location and time

The research was conducted from August 2020 to March 2021 in Ujungberung, Arcamanik Sub-district, Bandung City, Indonesia, with the coordinate points of 06°54.510' S, 107°41.272' E located 688 m above sea level. The minimum air temperature is 24.33 °C and the maximum is 32.82 °C, accompanied by general sunny and cloudy weather conditions. Environmental conditions are essential for successful Azolla cultivation (Anggraini et al., 2017). The research area's environmental state follows the optimal requirements for Azolla growth, namely at a temperature of 20 to 35 °C (Sadeghi et al., 2013; Surdina et al., 2016).

Research design

The study used a completely randomized design with five treatment doses of quail manure in Azolla culture ponds, which will be described further with each treatment replicated four times. Observations were made on the entire colony of Azolla in each study pond separately based on the specified period. Azolla biomass harvest was carried out 21 days after application. Based on Raifannur et al. (2017), maximum Azolla biomass growth can be achieved in the span of 14 to 28 days after inoculation.

Experimental setup

The unit is a black UV plastic pond measuring 0.5 x 2 x 0.3 m. As many as 20 ponds were placed close together in the location. Each treatment added mixtures of soil and quail manure to the ponds, then filled with water as high as 10 cm. A roof made out of transparent UV plastic was used to protect the ponds from direct rainfall. The mixture was then left for three days with daily stirring to ensure that the water, fertilizer and soil were homogeneously mixed, and ready to be used as Azolla growing media. Surdina et al. (2016) stated that the manure needed for continuous Azolla production is 200 to 400 g m^{-2}. About 20 g m^{-2} of Azolla culture, purchased from a local Azolla farm, was placed on the water surface of each pond in the morning. The composition of quail manure and soil mixture applied in this study were as follows, with four replicates for each combination: Treatment A (Control) 0 g m^{-2} quail manure; Treatment B 50 g m^{-2} quail manure; Treatment C 100 g m^{-2} quail manure; Treatment D 150 g m^{-2} quail manure and Treatment E 200 g m^{-2} quail manure. The mixture was applied once prior to Azolla planting, and the water level was continuously maintained to encourage optimum growth.

Azolla growth observation

A digital scale was used to measure the fresh weight of Azolla biomass. A ruler was used to measure the size of the growth area while the transect method was applied to the estimated total number of individuals. These observations were all carried out once every seven days. The dry weight of Azolla was measured at harvest, 21 days after inoculation, by drying in an oven (temperature 105 °C for 24 hours) and weighing on a scale.

Data analysis

Observation variables in this study include initial, final and fresh weight gain (Equation 1), relative growth rate (Equation 2), growth rate (Equation 3), doubling time (Equation 4), as well as individual density (Equation 5). Dry weight data were used to describe the total dry weight of Azolla.

$$\text{Fresh weight gain} = \text{Final fresh weight} - \text{Initial fresh weight} \quad (1)$$

$$\text{Relative growth rate (RGR)} = \frac{\ln(\text{Fresh weight t2}) - \ln(\text{Fresh weight t1})}{\text{t2} - \text{t1}} \quad (2)$$

$$\text{Growth rate (Gri)} = \frac{\ln(\text{cover area on ti}) - \ln(\text{cover area on to})}{\text{ti} - \text{to}} \quad (3)$$

$$\text{Doubling time (Ti)} = \frac{\ln 2}{\text{Gri}} \quad (4)$$

$$\text{Individual density (D)} = \frac{\text{Individual number}}{\text{Area of sampling (m}^2\text{)}} \quad (5)$$

Where: t1 = former time, t2 = later time, to = initial time, ti = final time.

The results of the processed data were statistically tested using IBM SPSS Statistics 25 32bit software. The ANOVA test with the Shapiro-Wilk Normality test and Homogeneity of Variance test requirements. If the results show significance, further tests were then carried out in the least significant difference test (LSD) and Duncan multiple range test (DMRT).

Table 1. Minimum technical requirements for pure granule/pellet solid organic fertilizer

Parameter	Unit	Pure granule/pellet quality standard
C-organic	%	> 15
C/N ratio		15-25
Water Content	%	8-20
pH		4-9
Macronutrients (N + P_2O_5 + K_2O)	%	> 4
Micronutrients:		
Total Fe	ppm	max 9,000
Mn	ppm	max 5,000
Zn	ppm	max 5,000

Source: Ministry of Agriculture Regulation Number 70/Permentan/SR.140/10/2011

Analysis of quail manure nutrient and Azolla proximate content

Nutritional content analysis of the used quail manure and the resulting Azolla proximate were carried out at the Integrated Testing Laboratory of the Vegetable Crops Research Institute, Lembang, following the methods described by AOAC (2007). After that, the nutrient content that had been analyzed was compared with the minimum technical requirements for pure granule/pellet solid organic fertilizer from the Regulation of the Ministry of Agriculture Number 70/Permentan/SR.140/10/ 2011 concerning organic fertilizers, biological fertilizers and soil improvements as listed in Table 1.

RESULTS AND DISCUSSION

Quail manure nutrient content analysis

Assessing quail manure nutrient content and availability to plants is crucial to understanding its potential use as a fertilizer, allowing more efficient application recommendations. The nutrient contents of the quail manure used in this study are available in Table 2.

Nitrogen is not often a limiting factor in the growth of Azolla due to its capacity to fix it from the atmosphere through symbiosis with blue-green algae, even though nitrogen is used to produce energy and photosynthate. However, the lack of nitrogen in growing media will produce heterosis cells to fix N from the atmosphere (Handajani, 2011). This process uses energy, which causes inhibition of the development of other organs due to energy limitation. Phosphorus availability, especially phosphate, can increase the productivity of Azolla and the nitrogen-fixing activity carried out by *A. azollae* (Sadeghi et al., 2013; Utama et al., 2015).

Table 2. Quail manure content analysis

Parameter	Value	Unit
pH H_2O	8.3	-
pH KCl	7.9	-
Water content	13.30	%
C-organic	25.33	% fresh weight
Total-N	2.98	% fresh weight
C/N	9	-
P_2O_5	4.84	% fresh weight
K_2O	2.32	% fresh weight
SiO_2	2.39	%
Ash content	43.03	%
$N\text{-}NH_4$	0.37	% fresh weight
$N\text{-}NO_3$	0.77	% fresh weight
CaO	41.39	% fresh weight
MgO	1.32	% fresh weight
S	1.17	% fresh weight
Na	0.25	% fresh weight
Cl	0.46	% fresh weight
Fe	3,041	ppm
Mn	325	ppm
Cu	40	ppm
Zn	461	ppm
B	22	ppm
Al	926	ppm

Based on the comparison with minimum technical requirements for pure granule/ pellet solid organic fertilizer according to Ministry of Agriculture Regulation Number 70/Permentan/SR.140/10/2011, quail manure meets the minimum technical requirements for pure granule/pellet solid organic fertilizer through C-organic parameters, water content, pH, macronutrients (N, P, K) and micronutrients (Fe, Mn, Zn). Meanwhile, the C/N ratio parameter of 9 is outside the range of technical requirements, namely 15 to 25. The higher value indicates that the organic matter decomposition was not completed. In the meantime, the lower C/N ratio of quail manure indicates that the fertilizer is more mature or the decomposition has been completed (Ismayana et al., 2012). In addition, other macronutrients such as K, Ca and Mg encourage the growth of Azolla. The presence of micronutrients, even though they are only needed in small amounts, dramatically affects the productivity of Azolla. The analysis results of quail manure nutrient content indicate that the fertilizer can be used to substitute synthetic fertilizers for Azolla production. The macronutrient contents in quail manure can replace the usage quantity of 1 g of urea N fertilizer in 13.5 g, 1 g of P SP-36 fertilizer 36% P_2O_5 in 6 g, and 1 g of K fertilizer KCl Mahkota 65% K_2O in 24 g (Anas et al., 2012).

Final weight

In this study, from four ponds of Group A, only two ponds produced comparable numbers of Azolla. The average weight of the Azolla on the pond inundated with quail manure was significantly bigger than the control pond, where no manure was added (P = 0.036). The total weight of Azolla harvested was positively correlated to the amount of manure added to the cultivation pond (Treatment E produced the highest average total harvested weight of 1401.81±367.51 g). However, the differences were insignificant among all treatment groups (Figure 1a). On the other hand, the dry weight of harvested biomass of the control group was also significantly lower than those of the treatment groups (P = 0.1), which were insignificant (P = 0.10) (Figure 1b).

The total dry weight of Azolla ranged from 4.964% to 6.213% of total biomass. Similar results were obtained by Anitha et al. (2016), where the dry weight of Azolla is known to be 4.7%. The dry weight of the Azolla produced in this study was higher than that of Azolla cultivation in earthen ponds reported by Utomo et al. (2021). Based on this comparison, it can be hypothesized that a water-resistant pond is better applied to produce Azolla as it prevents the infiltration and percolation process, which may remove nutrients like phosphorus from the water body (Bali and Gueddari, 2019).

Relative growth rate

Observation of the change in Azolla weight showed that the Azolla population was still in the growing stage (Figure 2a). However, the relative growth rate pattern showed the population's maturity, indicating the necessity to harvest the product (Figure 2b). This study disclosed that the final relative growth rate of the control group (Group A) was significantly lower than those of the treatment groups (ANOVA, P = 0.021). The relative growth rate among treatment groups was similar, with Group E showing a slightly higher relative growth rate than the others (Figure 2c).

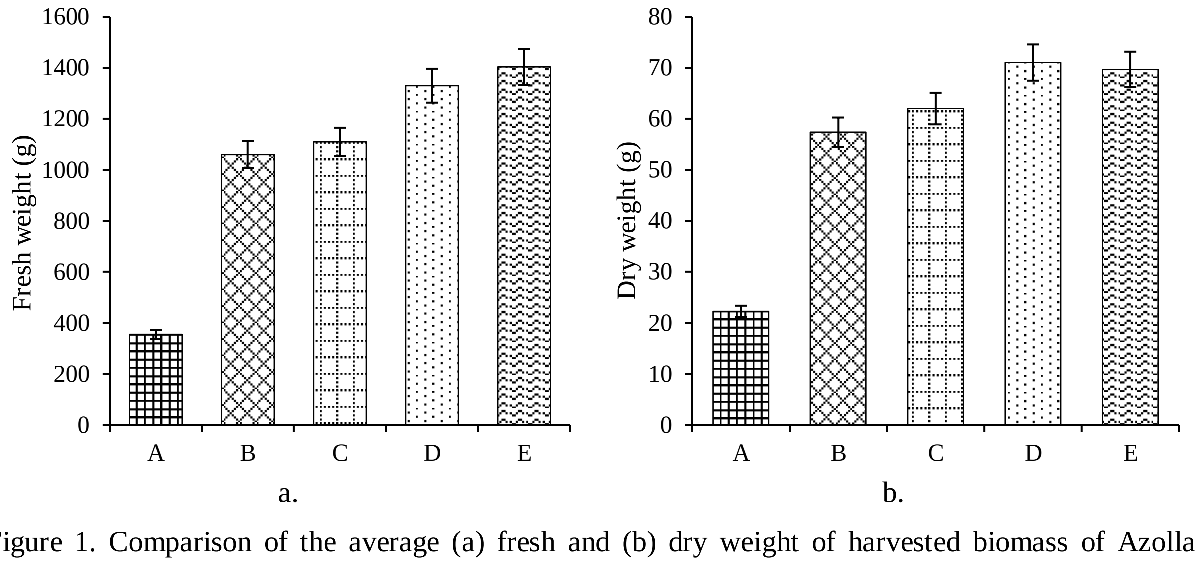

Figure 1. Comparison of the average (a) fresh and (b) dry weight of harvested biomass of Azolla in control dan treatment groups. Significant at P < 0.05

Note: Treatment quail manure: A = 0 g m^{-2} (Control); B = 50 g m^{-2}; C = 100 g m^{-2}; D = 150 g m^{-2}; E = 200 g m^{-2}

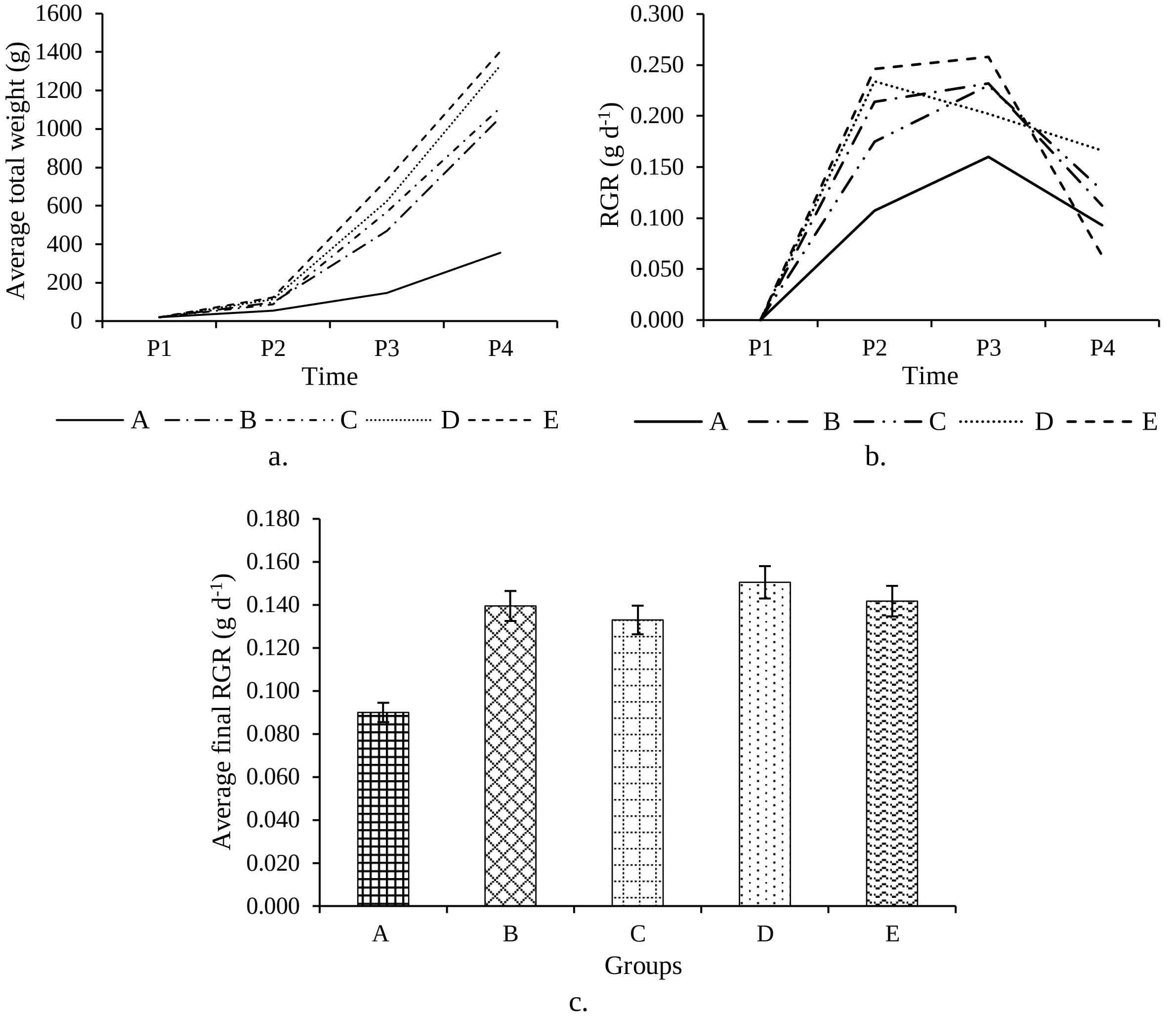

Figure 2. Comparison of the (a) growth pattern of Azolla, (b) change in relative growth rate, and (c) final relative growth rate in control (Group A), and treatment groups (Groups B-E). Significant at $P < 0.05$

Note: Treatment quail manure: A = 0 g m^{-2} (Control); B = 50 g m^{-2}; C = 100 g m^{-2}; D = 150 g m^{-2}; E = 200 g m^{-2}; P1 = Period 1 (day 0); P2 = Period 2 (day 7); P3 = Period 3 (day 14); P4 = Period 4 (day 21)

The relative growth rate of Azolla observed in this study forms a sigmoid curve that describes the three main phases of plant growth: the logarithmic or exponential phase, the linear phase, and the aging phase (Nafi'ah and Karuniawan, 2016). The aging phase in Azolla cultivation started at P3 (14 days from planting) due to competition for available nutrients to support Azolla growth and reproduction. The growth rate of Azolla in this study was slightly lower than the result of Hossain et al. (2021) and da Silva et al. (2022), who applied synthetic fertilizer in their research. However, it was higher than the results of research by Nordiah et al. (2012), by growing Azolla only on the water without fertilizer, and those of Kösesakal and Yildiz (2019), with the application of Hoagland solution as fertilizer. This study further confirms that the requirement of various nutrition for Azolla production and it's availability in quail manure seem to match in order to grow Azolla under suitable environmental conditions.

Doubling time

The fastest doubling time was recorded in Treatment E. In contrast, the longest doubling time was in Group A (Figure 3). High variation of doubling time for Group A caused insignificant differences among groups (P = 0.52). Loss of a significant amount of Azolla of Group A caused by pest disturbance in the form of snails, frogs and toads, as well as lack of nutrients in the water medium, significantly increases the doubling time. In general, the application of quail manure produced the doubling time of Azolla recorded between 3.46 and 4.07 days (Figure 3).

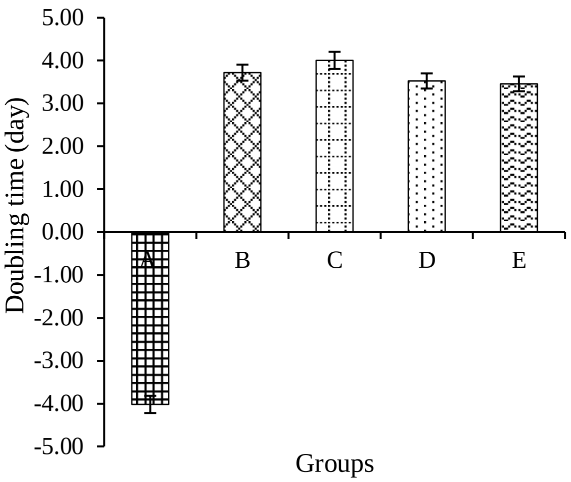

Figure 3. Comparison of the doubling time in control (Group A) and treatment groups (Groups B-E)

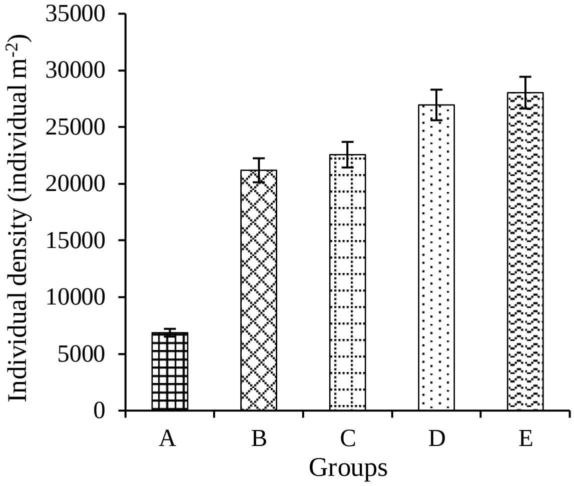

Figure 4. Comparison of the individual density in control (Group A) and treatment groups (Groups B-E)

Note: Treatment quail manure: A = 0 g m^{-2} (Control); B = 50 g m^{-2}; C = 100 g m^{-2}; D = 150 g m^{-2}; E = 200 g m^{-2}

Following Raifannur et al. (2017), the result reported that Azolla's doubling time ranges from 3 to 10 days. Interestingly, the doubling time recorded in this study from most treatment groups was shorter than that of Hossain et al. (2021), who applied synthetic phosphorus to Azolla's outdoor cultivation. The variation of the multiplication time may be influenced by the environmental conditions in which it is cultivated. Environmental conditions that increasingly support the optimal growth of Azolla can result in shorter doubling times.

Individual density

The highest final density was in Treatment E of 28,705 individuals m^{-2} and the lowest was in Treatment A of 7,108 individuals m^{-2}. The individual density of the control group (Group A) was significantly lower than the treatment groups (P = 0.03), while it was insignificant among treatment groups (Figure 4).

Raifannur et al. (2017) stated that individual density could be influenced by the availability of different nutrients between the treatments that were given fertilizer and those that were not given fertilizer. One of the crucial nutrients in stimulating the growth of Azolla tillers is phosphorus, which can be more available when fertilizing Azolla growth media (Raifannur et al., 2017). The density of Azolla recorded in this study was significantly higher than that of Surdina et al. (2016) on the effect of manure, including poultry, on Azolla productivity. It seems the differences in the feed provided to poultry were the possible explanation for them. In this study, quail were fed on commercial feed, which mainly consisted of phosphorus, and most of the phosphorus were released into manure (Li et al., 2016; Lokapirnasari, 2017), while Surdina's study applied chicken manure originated from chicken that fed on non-commercial feed.

Proximate composition of Azolla

The results of the proximate analysis of Azolla, namely the water content, ash content, protein, fiber, fat and carbohydrates, were obtained as shown in Table 3. Azolla has been touted as a possible low-cost feed for quail (Lakshmi et al., 2019; Putri et al., 2019; Varadharajan et al., 2019; Nasir et al., 2022). As an example, based on the comparison between the Azolla sample and the SNI 01-3907-2006 (Indonesian National Standard, 2006) on feed requirements of laying quail poultry (quail layer), Azolla produced through quail manure fertilizing fulfills the protein and fat requirements for the quail layer. Some of the parameters discussed as the nutritional needs of laying quails include maximum water content of 14% dry weight, maximum ash of 14% dry weight, minimum protein 20% to 22%, maximum fiber 7%, maximum fat 7% and minimum metabolic energy 2,800 kcal kg^{-1}.

Table 3. Proximate analysis results of Azolla due to variations in quail manure application amount

Treatment	Water content	Ash content	Protein	Fiber	Fat	Carbohydrate
(g m^{-2})	% Fresh weight		% Dry weight			
A (0)	95.80	19.23	39.52	20.24	2.86	6.67
B (50)	93.68	18.12	40.71	31.60	3.10	6.19
C (100)	93.02	19.61	39.52	37.14	4.29	5.24
D (150)	93.96	19.42	36.43	36.43	3.81	6.19
E (200)	93.70	18.06	42.86	34.76	4.29	5.24

Higher crude fiber content may reduce the digestibility of Azolla due to the absence of enzymes, in poultry, to break down cellulose and hemicellulose. Thus, further processing is needed to break down the fiber components into digestible carbohydrates, for example, by applying enzymes that hydrolyze cellulose (Nuraini et al., 2022). Other processes are also required to reduce its biomass's water content to a suitable level as feed for the quail layer.

CONCLUSIONS

Quail manure can be applied as a substitute to replace synthetic fertilizer for Azolla production, with 50, 100 and 150 g m^{-2} application of quail manure giving similarly good results on the Azolla growth parameters. This shows that the application of quail manure can significantly increase the production of Azolla biomass in culture ponds. The resulting Azolla biomass from all treatments gave a value of 93% to 95% moisture content, 18% to 19% ash content, 36% to 42% protein, 20% to 37% fiber, 2.8% to 4% fat, and 5% to 6% carbohydrates. Looking at the big picture, therefore, 100 g m^{-2} manure treatment is considered the most optimal among the rest. Furtherly, the resulting biomass is suitable to be applied as part of quail feeding material even though some additional processes are required regarding the level of crude fiber. The results of this study may be used as a baseline for sustainable agriculture through the integration of quail manure management and Azolla cultivation while providing an appropriate solution for possible environmental problems due to quail manure accumulation.

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