JWPR
Journal of World's Poultry Research

2026, Scienceline Publication
J. World Poult. Res. 16(3): 295-304, 2026
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2026.26
PII: S2322455X2600026-16

ISSN: 2322-455X

Effects of Fungal Bioconversion of Azolla-Enriched Diets on Nutritional Quality and Economic Efficiency in ZamPen Native Chicken

Gether Pacatang Enario

College of Agriculture, Mindanao State University – Buug Campus, Zamboanga Sibugay, Philippines

*Corresponding author's email: enario2017@gmail.com

Received: June 09, 2026, Revised: July 12, 2026, Accepted: August 06, 2026, Published: September 25, 2026

ABSTRACT

Rising feed prices have driven interest in local feed alternatives, even though these typically offer lower nutritional quality than standard commercial feeds. The present study aimed to evaluate the effects of an Azolla-enriched diet fermented with and without *Pleurotus (P.) ostreatus* on nutrient composition, growth performance, and economic efficiency in ZamPen native chickens. A 4 × 2 factorial experiment under a completely randomized design with 8 treatments and three replications was conducted using 168 two-month-old straight-run ZamPen chickens with an average weight of 665 g. Treatments consisted of 8 diets containing 0%, 5%, 10%, and 15% Azolla, with and without *P. ostreatus* fermentation. Results of nutrient analysis indicated that the fermented Azolla-enriched diet influenced crude protein, ash, and metabolizable energy compared to the non-fermented Azolla-enriched diet. Increasing Azolla levels increased crude protein content, moisture, fats, and metabolizable energy at 15%, 10%, and 5%, respectively, compared to 0% Azolla. Growth performance, including final body weight, weight gain, and feed conversion efficiency, was not significantly affected by the Azolla-enriched diet. However, feed consumption decreased significantly with increasing Azolla levels (10% and 15%), with animals consuming less feed than those fed 0% Azolla. Fermentation affected weight gain and feed conversion ratio, with non-fermented diets resulting in greater weight gain and better feed conversion than fermented diets. Significant interactions between Azolla and fermentation were observed for final body weight, feed conversion ratio, and feed conversion efficiency. Economic analysis revealed significant reductions in feed cost and total production cost at 10% and 15% Azolla, while gross sales, net income, and return on investment were not significantly affected compared to 0% Azolla. Fermentation with or without *P. ostreatus* did not affect economic performance, and no interaction of Azolla and fermentation was observed. These results suggested that Azolla can be incorporated up to 15% in native chicken diets as a cost-effective alternative feed ingredient without compromising performance. In addition, fermentation with *P. ostreatus* increased the moisture and fat contents of the diets and improved final body weight, weight gain, and feed conversion ratio, but reduced crude protein and ash contents.

Keywords: Alternative feed, Azolla meal, Feed conversion ratio, Fermentation, *Pleurotus ostreatus*

INTRODUCTION

The poultry industry in the Philippines is an important part of the economy because it supports people's livelihoods and helps ensure food supply (Ortega et al., 2021). It is also one of the fastest-growing areas in agriculture, and includes broiler, layer, and native chickens. In 2023, the total chicken inventory reached 202.82 million, with layer chickens accounting for 22.3%, broilers for 34.7%, and native chickens for 43% (PSA, 2023).

However, the industry faces challenges due to high feed ingredient prices (Shirisha and Mohanty, 2017; Miraflor, 2021; Tan, 2022), with the Philippines still heavily dependent on imports of feed raw materials (Tan, 2021). In 2018, the Philippines imported approximately 4 million metric tons of animal feed ingredients, including corn, soybean meal, and fishmeal (Cruz et al., 2015; OEC, 2022; Statista Research Department, 2024; Tan, 2024). Rising feed prices have put pressure on poultry farmers and consumers, leading to higher prices of livestock and poultry products (Miraflor, 2021; Reeve, 2021; Eardly, 2022).

The current crisis has encouraged farmers to use alternative feed ingredients for poultry to help lower feed costs (Ito, 2024; Nadgauda, 2024). Feed accounts for about 70% of the total production cost (Oladokun and Johnson, 2012; Mallick et al., 2020), so using alternative feeds can help reduce expenses and improve farmers' income. Locally available feed sources such as corn, azolla, moringa, and copra meal can be used as substitutes by being included in formulated rations. However, several factors must be considered in feed formulation, including nutrient availability of the feed ingredients (PCAARRD-DOST, 2000). One challenge in using alternative feed is that it often contains lower nutrient levels than conventional feed ingredients (BCRC, 2024).

Fungal fermentation is a biological process that uses fungi to break down and transform feed ingredients, improving their nutritional quality and digestibility (Imelda et al., 2008; Sun et al., 2021). During this process, fungi produce enzymes that degrade complex carbohydrates and other organic compounds that are otherwise difficult for poultry to digest (Sun et al., 2023). These complex carbohydrates include non-starch polysaccharides (NSP), such as cellulose, pectins, gums, glucans, mucilages, inulin, and chitin, as well as lignin, which make up a large portion of the dietary fiber component in plant-based feed ingredients (Choct, 2015; Eunjoo et al., 2022; Nguyen et al., 2022). These dietary fibers are the least digestible constituents of poultry feed, as birds lack endogenous NSPs and the enzymes required to degrade lignocellulose (such as cellulases, xylanases, glucanases, amylase, and protease) (Devi and Diarra, 2019; Eunjoo et al., 2022). Non-starch polysaccharides and lignin can affect gut physiology and function by altering the secretion of water, lipids, electrolytes, and proteins within the digestive tract. These compounds are considered anti-nutritional factors because they can reduce nutrient digestion and absorption in poultry (Nguyen et al., 2021). Through fermentation, however, fungi produce metabolites and enzymes that help break down these complex compounds, thereby improving nutrient availability and utilization in the feed (Sun et al., 2023). As a result, fermentation enhances the nutritional value of the feed and can improve the growth and production performance of poultry birds (Chu et al., 2016; Chuang et al., 2020; Ahmad et al., 2023; Ibrahim et al., 2023).

Pleurotus ostreatus (*P. ostreatus*), known as the oyster mushroom, is a fungal species widely used in the feed industry as a white-rot fungus for fermentation and for degrading lignin, cellulose, and hemicellulose (Bánfi et al., 2015; Heidari et al., 2022). A study by Nuraini et al. (2022) showed that fermentation using *P. ostreatus* improved the nutrient quality of Azolla. Combining fungal fermentation with the mechanical treatment of canola meal is a suitable strategy to improve its nutritional quality and significantly reduce its antinutrient content (Heidari et al., 2022). Another study showed that *P. ostreatus* improved the nutritive value of corn stover by increasing its energetic values, crude protein, microbial mass, and total volatile fatty acid concentrations (Olagunju et al., 2023).

However, fermentation using *P. ostreatus* in locally available feed ingredients for native chicken diets is scarce. Most previous studies have focused on common commercial animals or controlled laboratory experiments, leaving a gap in studies on its effects on the growth performance of native chickens. Thus, the present study aimed to use *P. ostreatus* to ferment Azolla-incorporated diets and evaluated the nutritive value, economic efficiency, and production performance in ZamPen native chickens.

MATERIALS AND METHODS

Ethical approval

The current study followed the ethical standards outlined in the Philippines' Animal Welfare Act (RA 8485, as amended by RA 10631). Proper care, handling, and housing were ensured to minimize animal stress and discomfort in the chickens.

Study area and experimental design

The study was conducted in Ozamiz City, Misamis Occidental, Philippines, using a 4 × 2 factorial experiment arranged in a Completely Randomized Design (CRD) with three replications. The experimental factors consisted of fermentation and an Azolla-enriched diet. The fermentation factor included diets prepared either with or without *P. ostreatus* fermentation, while the Azolla factor consisted of four dietary levels, including 0% Azolla (A1), 5% Azolla (A2), 10% Azolla (A3), and 15% Azolla (A4). A total of 168 two-month-old straight-run ZamPen native chickens with an average initial body weight of 665 g per chicken were used in the study. Seven chickens were randomly assigned to each replication, resulting in 21 chickens per treatment combination. The chickens were randomly allocated to the eight treatment combinations and reared for a 30-day experimental period.

Feed formulation

The feed formulation process followed a trial-and-error method (PCAARRD-DOST, 2000). Nutrient composition values for the feed ingredients were obtained from published feed composition tables and relevant literature (PCAARRD-DOST, 2000; Seid, 2023). The proportions of yellow corn, Azolla meal, soybean meal (SBM), rice bran, limestone, dicalcium phosphate (Dicaphos), salt, molasses, coconut oil, and vitamin-mineral premix were adjusted iteratively until the calculated nutrient levels met the nutrient requirements of growing native chickens. The diets were balanced for metabolizable energy (ME), crude protein (CP), crude fiber (CF), calcium (Ca), and phosphorus (P), while maintaining the desired Azolla inclusion levels (0%, 5%, 10%, and 15%). Microsoft Excel (version 2019) was used to calculate the nutrient contribution of each ingredient and the total nutrient composition of the formulated diets (Table 1).

Table 1. Feed Ingredients and diet chemical composition of ZamPen native chickens for a 30-day experimental period

Feed ingredients	Treatments							
	Fermented with *P. ostreatus*				Fermented without *P. ostreatus*			
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Corn	46.3	47.75	51.2	54.95	46.3	47.75	51.2	54.95
Azolla meal	0	5	10	15	0	5	10	15
Soybean meal	22.1	20	18	16.5	22.1	20	18	16.5
Rice bran D1	21.85	18	11.8	4.8	21.85	18	11.8	4.8
Calcium carbonate	2	1.5	1.5	1.5	2	1.5	1.5	1.5
Dicalcium phosphate	1	1	0.75	0.5	1	1	0.75	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Molasses	3	3	3	3	3	3	3	3
Coconut oil	3	3	3	3	3	3	3	3
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Chemical analysis								
Moisture (%)	9.59	9.04	9.91	9.96	10.56	9.26	13.47	10.79
Fat (%)	2.78	5.87	2.00	2.12	3.19	5.11	2.48	4.04
Crude Protein (%)	17.42	18.18	17.91	18.07	17.05	17.45	17.03	18.30
Ash (%)	9.20	9.48	9.69	10.00	9.36	9.73	8.95	8.68
ME (kcal)	3386	3552	3316	3308	3363	3496	3227	3423

Diet formulation, nutrient composition, and native chicken nutrient recommendations were based on PCAARRD-DOST (2000), while Azolla nutrient composition was obtained from Seid (2023). T_1-T_8: Different treatments with and without *Pleurotus ostreatus* fermentation. Vitamin-mineral premix composition: Vitamin A, D, E, potassium iodide, zinc, copper, manganese, iron, cobalt, sodium chloride, and magnesium oxide according to the recommendation of PCAARRD-DOST (2000).

Fermentation of the formulated azolla-enriched diets

Feed fermentation was carried out using *P. ostreatus* and compared with non-fermented azolla-enriched diets. Prior to fermentation, the formulated feeds were sterilized for 6 hours and allowed to cool to room temperature. The sterilized feeds were then inoculated with *P. ostreatus* tissue at 3% (w/w) and subjected to solid-state fermentation for 15 days at 60% moisture content. Upon completion of the fermentation period, the fermented feeds were heated at 80°C for 2 hours to inactivate the fungal culture and then dried at 60°C for 8 hours. The non-fermented feeds underwent the same processing conditions, except for inoculation with *P. ostreatus* (Nuraini et al., 2022).

Nutrient analysis of formulated and fermented feeds

The nutrient analysis of the formulated and fermented feeds, with and without *P. ostreatus*, was conducted at the Department of Science and Technology–Regional Standards and Testing Laboratory (DOST-RSTL), Region 10, Philippines. Moisture content was determined using the AOAC Official Method 930.15 (Oven Drying Method), ash content was analyzed using AOAC Official Method 942.05 (Gravimetric Furnace Method), crude protein was determined through AOAC Official Method 991.20 using the Automated Kjeldahl Method, and total fat was measured following AOAC Official Method 932.06 through acid hydrolysis and solvent extraction. All analyses were performed in accordance with the Official Methods of Analysis (OMA) of the Association of Official

Analytical Chemists (AOAC), 21st Edition (AOAC, 2023).

Growth parameters

The growth performance was measured using several parameters, including initial weight (IW), final weight (FW), and weight gain (WG). The initial body weight (g) was measured by individually weighing the chickens at the start of the experiment to establish their baseline weight. Final body weight (g) was measured at the end of the study to assess overall growth. Weight gain (g) was subsequently determined by subtracting the initial body weight from the final body weight, as shown in Formula 1. Feed consumption (FC) was measured as the total amount of feed consumed per chicken throughout the study period. FCR and FCE were computed using Formulas 2 and 3 (Shamsudin et al., 2022).

Weight gain (g) = Final Weight (g) – Initial Weight (g) (Formula 1)

Feed conversion ratio = Feed Consumption (g) / Weight Gain (g) (Formula 2)

Feed conversion efficiency = Weight Gain (g) / Feed Consumption (g) (Formula 3)

Economic analysis

The cost-and-return analysis was conducted to evaluate the cost-effectiveness and profitability of feeding strategies for ZamPen chickens. The parameters include calculations of feed cost (FC), total production cost (TPC), gross sales, net income, and return on investment (ROI). Feed cost was determined by summing all feed-related expenses incurred throughout the experiment. Total production cost was calculated by summing all production-related costs, including housing, feed, chicks, healthcare, labor, and other operational needs. Gross sales referred to the total income generated from selling the chickens, calculated at a standard market price of ₱350 (Philippine peso) per kilogram based on carcass weight. Net income was calculated by subtracting total production costs from gross sales, as shown in Formula 4. Return on investment was used to measure profitability by comparing net income to total production cost, indicating the efficiency of the production system, as shown in Formula 5 (PSA, 2022).

Net Income = Gross Sales (₱) – Total Production Cost (₱) (Formula 4)

Return On Investment = Net Income (₱) / Total Production Cost (₱) x 100% (Formula 5)

Statistical analysis

A two-way ANOVA under a factorial CRD was computed using the IBM SPSS Software version 26 to evaluate the significant effects of varying Azolla levels and fermentation treatments (with and without *P. ostreatus*) on the measured parameters. Where significant differences were observed, Tukey's test was used for multiple comparisons to identify specific differences among treatment means. Statistical significance was declared at the 5% probability level ($p < 0.05$).

RESULTS AND DISCUSSION

Nutrient analysis

The nutrient analysis of Azolla-enriched diets with and without *P. ostreatus* fermentation is presented in Table 2. Moisture content differed significantly among the Azolla-enriched diets, with the highest observed in the 10% Azolla diet (11.69%, $p < 0.05$). The variation in moisture content may be associated with Azolla's inherent water-retention capacity (Shamsudin et al., 2022) and its fibrous structure, which promotes moisture retention (Singh and Kim, 2021). Fermentation also significantly affected moisture content, with higher values observed in the treatment without *P. ostreatus* ($p < 0.05$). The moisture result may reflect differences in water utilization during fungal growth, as optimal *P. ostreatus* fermentation requires controlled moisture conditions (Wiesnerova et al., 2023). The interaction effect of azolla and fermentation suggests relatively stable moisture at lower Azolla inclusion levels but variation at higher levels (10%), with non-fermented azolla-enriched diets exhibiting greater moisture retention. The observed decrease in moisture content of the inoculated diets at 10% Azolla inclusion may indicate a partial reduction in fiber-associated water-holding capacity following fungal degradation (Yasar et al., 2020; Zhang et al., 2024). Fat content was significantly influenced by Azolla inclusion, with the highest at 5% Azolla (5.49%), while 10% and 15% Azolla showed reduced fat content ($p < 0.05$). The decrease in fat at higher Azolla levels (10% and 15%) may be due to a dilution effect from Azolla's low lipid content. Fermentation resulted in lower fat content, particularly in *P. ostreatus*-treated feeds, likely due to microbial lipolysis and lipid utilization during fermentation (Boukid et al., 2022; Heidari et al., 2024). The significant interaction between Azolla and fermentation indicates that lipid reduction was more pronounced at higher Azolla levels, suggesting substrate-dependent effects.

Table 2. Nutrient analysis of azolla-enriched diets fermented with or without *P. ostreatus* in ZamPen native chickens for a 30-day experimental period

Treatments	Moisture	Fat	Crude protein	Ash	Metabolizable energy
Azolla-enriched diet					
A_1	10.07^{b}	2.99^{b}	17.24^{a}	9.28	3375.25^{b}
A_2	9.15^{a}	5.49^{c}	17.81^{ab}	9.60	3524.22^{c}
A_3	11.69^{c}	2.24^{a}	17.47^{a}	9.32	3271.88^{a}
A_4	10.38^{b}	3.08^{b}	18.18^{b}	9.34	3365.32^{b}
P-value	$.001^{**}$	$.001^{**}$	$.005^{**}$	$.389^{ns}$	$.001^{**}$
Fermentation					
B_1	9.63	3.19	17.89	9.59	3390.94
B_2	11.02	3.71	17.46	9.18	3377.39
P-vale	$.001^{**}$	$.001^{**}$	$.018^{*}$	$.011^{*}$	$.127^{ns}$
A x B	$.001^{**}$	$.001^{**}$	$.125^{ns}$	$.003^{**}$	$.001^{**}$
CV	13.2	39.7	3.3	5.4	3.0

[a,b,c,d] Different superscript letters within a column showed a significant difference ($p < 0.05$). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: Not significant, A_1: 0% Azolla, A_2: 5% Azolla, A_3: 10% Azolla, A_4: 15% Azolla, B_1: with *Pleurotus ostreatus*, B_2: without *Pleurotus ostreatus*. A × B: Interaction of Azolla-enriched diet and fermentation, CV: Coefficient of variation.

Similar reductions in ether extract following fungal fermentation have been reported in different feed substrates, although responses vary with feed composition (Yalchil and Hajieghrari, 2011). Crude protein content was significantly affected by Azolla inclusion and fermentation ($p < 0.05$). Crude protein increased with higher Azolla inclusion levels, reaching 18.18% at 15% inclusion, consistent with Azolla's relatively high protein content (Swain et al., 2022). Fermentation with *P. ostreatus* resulted in a slight increase in CP, which may be attributed to microbial degradation of fiber and the resulting concentration effects following substrate breakdown (Wang et al., 2021; Heidari et al., 2022). However, the observed increase in crude protein was marginal. Ash content was not significantly affected by Azolla inclusion, indicating stable mineral contributions across diets ($p > 0.05$). In contrast, fermentation significantly increased ash content ($p < 0.05$). The increase is possibly due to the release of bound minerals during microbial degradation of complex feed matrices (Yasar and Tosun, 2020). The interaction between Azolla inclusion and fermentation significantly affected ash content ($p < 0.05$). In the absence of *P. ostreatus* fermentation, ash content decreased with increasing Azolla inclusion, suggesting alterations in the mineral composition of the diets. Fermentation may have moderated this response through fungal degradation of organic substrates and subsequent changes in nutrient distribution within the feed matrix (Heidari et al., 2022). Metabolizable energy was significantly affected by Azolla inclusion level ($p < 0.05$). The diet containing 5% Azolla exhibited the highest ME value (3524.22 kcal/kg), which was significantly higher than those of the 0% (3375.25 kcal/kg), 10% (3271.88 kcal/kg), and 15% Azolla diets (3365.32 kcal/kg, $p < 0.05$). The reduction in metabolizable energy at higher Azolla inclusion levels may be attributed to increased fiber content, which can limit nutrient digestibility and reduce the availability of dietary energy (Mtei et al., 2019; Jha and Mishra, 2021). Fermentation did not significantly influence metabolizable energy, although diets fermented with *P. ostreatus* (3390.94 kcal/kg) showed a numerically higher metabolizable energy than non-fermented diets (3377.39 kcal/kg). Azolla inclusion level and fermentation interacted significantly to influence metabolizable energy ($p < 0.05$). This interaction indicates that different Azolla inclusion levels modified the effect of fermentation on dietary energy utilization. The chemical composition and fiber content of the substrate likely influenced the nutritional effects of fermentation by affecting nutrient availability and energy release during digestion (Olukomaiya et al., 2019).

Growth performance

Growth performance data are presented in Table 3. Results on the initial weight showed no significant differences among treatments, confirming uniformity at the start of the experiment ($p > 0.05$). The lack of significant difference in initial weight supports the validity of subsequent comparisons, as initial weight is a key determinant of growth response in chicken (Khulel and Sabri, 2020). Final weight ranged from 1125.09 g to 1230.64 g and was not significantly affected by Azolla levels, fermentation, or their interaction ($p < 0.05$). The non-significant results for final weight indicated that Azolla can be incorporated at levels up to 15% without negatively affecting growth performance, consistent with the findings of Subramanian (2021). The lack of a fermentation effect indicates limited improvement under the present conditions, in contrast to reports of enhanced growth following fungal fermentation (Ahmad et al., 2023). Weight gain was not significantly affected by Azolla inclusion levels or by the Azolla-fermentation interaction ($p > 0.05$). Although numerical reductions in weight gain were observed at higher Azolla levels, this trend may be attributed to Azolla's increased dietary fiber content, which can reduce nutrient digestibility and energy utilization (Jha and Mishra, 2021). In contrast, fermentation significantly affected weight gain, indicating that the fermentation treatment influenced growth performance ($p < 0.05$). Feed consumption was significantly affected by Azolla inclusion, with reduced consumption at higher inclusion levels ($p < 0.05$). Fermentation and interaction effects were not significant ($p > 0.05$). The reduction in feed consumption is likely associated with Azolla's high fiber content, which may reduce palatability and voluntary feed intake (Singh and Kim, 2021; Swain et al., 2022). Feed conversion ratio ranged from 5.09 to 5.89 and showed no significant main effects of Azolla or fermentation ($p > 0.05$). However, a significant interaction effect indicates that fermentation influenced feed efficiency depending on Azolla level ($p < 0.05$). The improved feed conversion ratio observed at moderate Azolla inclusion levels (5-10%) suggests more efficient nutrient utilization under these conditions. However, this advantage was not maintained at higher inclusion levels (15%), likely due to Azolla's increased dietary fiber content. While moderate amounts of fiber may support normal digestive function, excessive fiber can reduce nutrient digestibility, increase digesta passage time, and limit the availability of energy and other nutrients for growth. Consequently, chickens fed higher levels of Azolla may have utilized feed less efficiently than those receiving moderate inclusion levels, resulting in diminished improvements in feed conversion ratio (Kumar, 2018; Katu et al., 2025). Feed conversion efficiency was not significantly affected by any factor ($p > 0.05$). The decrease observed at higher Azolla inclusion levels supports the notion that excessive dietary fiber can impair nutrient utilization efficiency (Joysowal et al., 2018; Kheravii et al., 2018). The limited effects of fermentation on performance parameters suggest that any improvements in digestibility were insufficient to produce substantial changes in overall performance (Zentek and Boroojeni, 2020).

Table 3. Growth performance of ZamPen native chicken fed fermented diets with or without *P. ostreatus* for a 30-day experimental period

Treatments	IW (g)	FW (g)	WG (g)	FC	FCR	FCE
Azolla-enriched diet						
A_1	662.93	1230.64	567.712	2826.95^{b}	5.09	19.98
A_2	679.13	1218.23	539.10	2749.74^{ab}	5.16	19.52
A_3	650.93	1136.01	485.08	2625.79^{a}	5.45	18.44
A_4	670.96	1125.09	454.1233	2601.04^{a}	5.89	17.32
P-value	.434^{ns}	.137^{ns}	.088^{ns}	.036*	.057^{ns}	.088^{ns}
Fermentation						
B_1	664.29	1172.04	507.75	2706.88	5.50	18.58
B_2	667.68	1182.94	515.26	2694.87	5.29	19.04
P-value	.001**	.001**	.018*	.833^{ns}	.011*	.127ns
A x B	.001**	.001**	.125ns	.079^{ns}	.003**	.001**
CV	13.2	39.7	3.3	6.3	5.4	3.0

^{a,b,c,d} Different superscript letters within a column showed a significant difference ($p < 0.05$). *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, NS: Not significant, A_1: 0% Azolla, A_2: 5% Azolla, A_3: 10% Azolla, A_4: 15% Azolla, B_1: with *Pleurotus ostreatus*, B_2: without *Pleurotus ostreatus*, IW: Initial weight, FW: Final weight, WG: Weight gain, FC: Feed consumption, FCR: Feed conversion ratio, FCE: Feed conversion efficiency, A × B: Interaction of Azolla-enriched diet and fermentation, CV: Coefficient of variation.

Table 4. Economic efficiency of ZamPen native chickens fed fermented diets, with or without *P. ostreatus* for a 30-day experimental period

Treatments	FC	PC	GS	NI	ROI
Azolla-enriched diet					
A_1	590.09[b]	2245.18[b]	2804.43	559.25	24.81
A_2	548.87[b]	2203.95[b]	2775.03	571.09	25.82
A_3	494.80[a]	2149.89[a]	2588.83	438.95	20.37
A_4	458.64[a]	2113.73[a]	2563.11	449.38	21.18
P-value	.001[**]	.001[**]	.137[ns]	.479[ns]	.591[ns]
Fermentation					
B_1	524.94	2188.36	2670.70	482.35	21.83
B_2	521.26	2168.01	2695.00	526.99	24.25
P-vale	.745[ns]	.085[ns]	.780[ns]	.562[ns]	.474[ns]
A x B	.115[ns]	.115[ns]	.138[ns]	.150[ns]	.133[ns]
CV	11.2	2.7	8.9	39.6	36.9

[a,b,c,d] Different superscript letters within a column showed a significant difference ($p < 0.05$). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: Not significant, A_1: 0% Azolla, A_2: 5% Azolla, A_3: 10% Azolla, A_4: 15% Azolla, B_1: w/ *Pleurotus ostreatus*, B_2: w/out *Pleurotus ostreatus*, FC: Feed cost, PC: Production cost, GS: Gross sales, NI: Net income, ROI: Return on investment, A × B: Interaction of Azolla-enriched diet and fermentation, CV: Coefficient of variation.

Economic performance

Table 4 presents the economic efficiency of ZamPen native chickens fed Azolla-enriched diets with or without *P. ostreatus* fermentation. Increasing Azolla inclusion significantly reduced feed cost, with the lowest cost observed at 15% inclusion, followed by the 10%, 5%, and 0% Azolla diets ($p < 0.05$). The reduction result confirmed the cost-reducing potential of Azolla as a locally available feed ingredient (Kumar et al., 2018; Swain et al., 2022). The reduction in feed cost also significantly reduced production cost, with the 15% Azolla diet recording the lowest production cost, followed by the 10%, 5%, and 0% diets ($p < 0.05$). This finding agrees with Sun et al. (2023), who identified feed as the major component of poultry production costs. *P. ostreatus* fermentation did not significantly influence feed or production costs, suggesting that fermentation neither increased nor reduced overall production expenses. Dai et al. (2020) similarly reported that fermentation treatments did not substantially alter the economic cost of feed production. Likewise, Azolla inclusion, fermentation, and their interaction did not significantly affect gross sales, net income, and return on investment ($p > 0.05$). Although the differences were not significant, the 5% Azolla diet numerically recorded the highest net income and return on investment among the Azolla treatments. Similarly, diets fermented with *P. ostreatus* numerically produced higher net income and return on investment than non-fermented diets. These trends suggest that moderate Azolla inclusion and fermentation may offer economic advantages; however, further studies are needed to confirm these effects.

CONCLUSION

The present study demonstrated that the fungal bioconversion of Azolla-enriched diets using *P. ostreatus* influenced the nutritional quality and affected selected growth performance and economic efficiency of ZamPen native chickens. Azolla inclusion increased crude protein content and metabolizable energy while reducing feed consumption, feed cost, and production cost. Fermentation with *P. ostreatus* altered moisture, fat, crude protein, and ash contents and affected final weight, weight gain, and feed conversion ratio. Furthermore, the interaction between Azolla inclusion and fermentation significantly influenced several nutritional and performance traits, indicating that the response to fermentation depended on the level of Azolla in the diet. However, the combined use of Azolla and *P. ostreatus* fermentation did not significantly affect economic returns. Thus, Azolla can be incorporated into diets for ZamPen native chickens at up to 15%, while a 5% inclusion level provided the most favorable balance among nutritional quality, growth performance, and economic return under the conditions of this study. Future studies should investigate higher Azolla inclusion levels, improved fermentation strategies, and longer feeding periods in native chickens.

DECLARATIONS

Acknowledgments

The author gratefully acknowledges the professors of the University of Science and Technology of Southern

Philippines, Claveria for their guidance and support throughout this study.

Authors' contributions

Gether Pacatang Enario designed the study; conducted the experiment; performed data collection, statistical analysis, and interpretation; and prepared the original draft. The author also reviewed, edited, and approved the final manuscript.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the author upon reasonable written request.

Competing interests

The author declares no competing interests.

Ethical considerations

The author assures that this manuscript complies with ethical standards and that no issues related to plagiarism, consent to publish, research misconduct, data fabrication and/or falsification, double publication and/or submission, or redundancy are present. Grammarly was used solely for English language editing and proofreading. The author reviewed and approved all revisions and accepts full responsibility for the content of the manuscript before submission and befor publication.

Funding

This study received financial support from the Commission on Higher Education (CHED) under the Scholarship for Instructors' Knowledge Advancement Program (SIKAP).

REFERENCES

Ahmad F, Rafi U, and Imran A (2023). Effects of fungal fermented feeds on broiler chicken growth performance, gut morphology, and gastrointestinal tract microecology: A review. BioScientific Review, 5(2): 91-102. DOI: https://www.doi.org/10.32350/bsr.52.09

Association of official analysis chemists (AOAC) (2023). Official methods of analysis of the association of official analytical chemists: Official methods of analysis of AOAC international, 21st Edition, AOAC, Washington DC. Available at: https://www.aoac.org/official-methods-of-analysis/

Bánfi R, Pohner Z, Kovács J, Luzics S, Nagy A, Dudás M, Tanos P, Márialigeti K, and Vajna B (2015). Characterization of the large-scale production process of oyster mushroom (*Pleurotus ostreatus*) with the analysis of succession and spatial heterogeneity of lignocellulolytic enzyme activities. Fungal Biology, 119(12): 1354-1363. DOI: https://www.doi.org/10.1016/j.funbio.2015.10.003

Beef cattle research council (BCRC) (2024). Alternative feeds. Available at: https://www.beefresearch.ca/topics/alternative-feeds/

Boukid F, Pera J, Javier P, and Castellari M (2022). Fungal bioconversion of brewery by- products: assessment of fatty acids and sterols profiles. Quality Assurance and Safety of Crops and Foods, 14(4): 202-211. DOI: https://www.doi.org/10.15586/qas.v14i4.1198

Choct M (2015). Fibre-chemistry and functions in poultry nutrition. LII Simposio Científico de Avicultura, Málaga, 28: 113-119. Available at: https://www.scirp.org/reference/referencespapers?referenceid=3315850

Chu YT, Lo CT, Chang SC, and Lee TT (2016). Effects of *Trichoderma* fermented wheat bran on growth performance, intestinal morphology and histological findings in broiler chickens. Italian Journal of Animal Science, 16(1): 82-92. DOI: https://www.doi.org/10.1080/1828051X.2016.1241133

Chuang WY, Hsieh YC, and Lee TT (2020). The effects of fungal feed additives in animals: A review. Animals, 10(5): 805. DOI: https://www.doi.org/10.3390/ani10050805

Cruz WM, Villanueva J, and Janeo EG (2015). Status development and use of alternative dietary ingredients in aquaculture feed formulation in the Philippines. In: M. R. Catacutan, R. M. Caloso, and B. O. Acosta (Editors), Development and use of alternative dietary ingredients or fish meal substitutes in aquaculture feed formulation. Proceedings of the ASEAN Regional Technical Consultation, pp. 23-29. Available at: http://hdl.handle.net/10862/2987

Dai Z, Lu C, Ju L, Wang B, Guo L, Wu Z, Zhu W, and Wu G (2020). Fermentation techniques in feed production. Animal agriculture, Chapter 24, pp. 407-427. DOI: https://www.doi.org/10.1016/B978-0-12-817052-6.00024-0

Devi AS and Diarra SS (2019). Maximum utilization of available resources for efficient poultry feeding in the South Pacific: Major issues and prospects. World's Poultry Science Journal, 75(2): 219-234. DOI: https://www.doi.org/10.1017/S0043933919000217

Eardley F (2022). Rising cost of agricultural fertilizer and feed: Causes, impacts and government policy. House of Lords Library. Available at: https://lordslibrary.parliament.uk/rising-cost-of-agricultural-fertiliser-and-feed-causes-impacts-and-government-policy/

Eunjoo K, Morgan NK, Moss AF, Li L, Ader P, and Choct M (2022). The flow of non-starch polysaccharides along the gastrointestinal tract of broiler chickens fed either a wheat- or maize-based diet. Animal Nutrition, 9: 138-142. DOI: https://www.doi.org/10.1016/j.aninu.2021.11.004

Heidari F, Øverland M, Hansen JØ, Mydland LT, Urriola PE, Chen C, Shurson GC, and Hu B (2022). Solid-state fermentation of *Pleurotus ostreatus* to improve the nutritional profile of mechanically-fractionated canola meal. Biochemical Engineering Journal, 187: 108591. DOI: https://www.doi.org/10.1016/j.bej.2022.108591

Heidari F, Øverland M, Hansen JØ, Mydland LT, Urriola PE, Chen C, Shurson GC, and Hu B (2024). Enhancing the nutritional value of canola meal through solid culture with *Pleurotus ostreatus*. Animal Feed Science and Technology, 309: 115893. DOI: https://www.doi.org/10.1016/j.anifeedsci.2024.115893

Ibrahim D, El-sayed H, Mahmoud E, El-Rahman G, Bazeed S, Abdelwarith A, Elgamal A, Khalil S, Younis E, Taha A et al. (2023). Impacts of solid-state fermented barley with fibrolytic exogenous enzymes on feed utilization, and antioxidant status of broiler chickens. Veterinary Sciences, 10(10): 594. DOI: https://www.doi.org/10.3390/vetsci10100594

Imelda J, Paulraj R, and Bhatnagar D (2008). Effect of solid-state fermentation on nutrient composition of selected feed ingredients. Indian Journal of Fisheries, 55(4): 327-332. Available at: https://epubs.icar.org.in/index.php/IJF/article/view/7018

Ito D (2022). Feed prices on the rise, can alternative feed materials be used for chicken diets?. Hendrix Genetics. Available at: https://layinghens.hendrix-genetics.com/en/articles/feed-prices-rise-can-alternative-feed-materials-be-used-chicken-diets/

Jha R and Mishra P (2021). Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: A Review. Journal of Animal Science and Biotechnology, 12(51): 1-16. DOI: https://www.doi.org/10.1186/s40104-021-00576-0

Joysowal M, Aziz AH, Mondal AM, Singh SM, Siddhnath SB, Chirwatkar B, and Chhaba B (2018). Effect of Azolla (*Azolla pinnata*) feed on the growth of broiler chicken. Journal of Entomology and Zoology Studies, 6(3): 391-393. Available at: https://www.entomoljournal.com/archives/2018/vol6issue3/PartF/6-2-252-137.pdf

Katu JK, Tóth T, and Varga L (2025). Enhancing the nutritional quality of low-grade poultry feed ingredients through fermentation: A review. Agriculture, 15(5): 476. DOI: https://www.doi.org/10.3390/agriculture15050476

Kheravii SK, Morgan NK, Swick RA, Choct M, and Wu SB (2018). Roles of dietary fibre and ingredient particle size in broiler nutrition. World's Poultry Science Journal, 74(2): 301-316. DOI: https://www.doi.org/10.1017/S0043933918000259

Khulel RMT and Sabri MA (2020). Effect of initial weight of chicks on performance of Iraqi local chickens. Plant Archives, 20(2): 5686-5690. DOI: http://www.doi.org/10.13140/RG.2.2.19298.96967

Kumar M (2018). Effect of inclusion of sun dried Azolla (*Azolla pinnata*) at different levels on the growth and performance of broiler chicks. Journal of Animal Research, 8(4): 629-632. DOI: http://www.doi.org/10.30954/2277-940X.08.2018.13

Mallick P, Muduli K, Biswal JN, and Pumwa J (2020). Broiler poultry feed cost optimization using linear programming technique. Journal of Operations and Strategic Planning, 3(1): 31-57. DOI: https://www.doi.org/10.1177/2516600X19896910

Miraflor MB (2021). Rising prices of animal feed ingredients to push meat prices higher. Manila Bulletin. Manila, Philippines. Available at: https://mb.com.ph/2021/2/22/rising-prices-of-animal-feed-ingredients-to-push-meat-prices-higher

Mtei AW, Abdollahi MR, Schreurs N, Girish CK, and Ravindran V (2019). Dietary inclusion of fibrous ingredients and bird type influence apparent ileal digestibility of nutrient and energy utilization. Poultry Science, 98(12): 6702-6712. DOI: https://www.doi.org/10.3382/ps/pez383

Nadgauda SS (2022). Rising poultry feed costs challenges and possible solutions. The magazine of the feed industry. Available at: https://benisonmedia.com/rising-poultry-feed-costs-challenges-and-possible-solutions/

Nguyen HT, Bedford MR, and Morgan NK (2021). Importance of considering non-starch polysaccharide content of poultry diets. World's Poultry Science Journal, 77(3): 619-637. DOI: https://www.doi.org/10.1080/00439339.2021.1921669

Nguyen HT, Bedford MR, Wu SB, and Morgan NK (2022). Dietary soluble non-starch polysaccharide level influences performance, nutrient utilisation and disappearance of non-starch polysaccharides in broiler chickens. Animals, 12(5): 547. DOI: https://www.doi.org/10.3390/ani12050547

Nuraini N, Mirzah M, Nur YS, and Harnentis H (2022). Improving Azolla microphylla through fermentation with lignocellulolytic fungi and its application in broiler feed. Advances in Animal and Veterinary Sciences, 10(5): 1090-1100. DOI: http://www.doi.org/10.17582/journal.aavs/2022/10.5.1090.1100

Observatory economic complexity (OEC) (2022). Soybean meal in the Philippines. Available at: https://oec.world/en/profile/bilateral-product/soybean-meal/reporter/phl

Oladokun V and Johnson A (2012). Feed formulation problem in Nigerian poultry farms: A mathematical programming approach. American Journal of Scientific and Industrial Research, 3: 14-20. DOI: https://www.doi.org/10.5251/ajsir.2012.3.1.14.20

Olagunju LK, Isikhuemhen OS, Dele PA, Anike FN, Ike KA, Shaw Y, Brice RM, Orimaye OE, Wuaku M, Essick BG et al. (2023). Effects of the incubation period of *Pleurotus ostreatus* on the chemical composition and nutrient availability of solid-state-fermented corn stover. Animals, 13(16): 2587. DOI: https://www.doi.org/10.3390/ani13162587

Olukomaiya O, Fernando C, Mereddy R, Li X, and Sultanbawa Y (2019). Solid-state fermented plant protein sources in the diets of broiler chickens: A review. Animal Nutrition, 5(4): 319-330. DOI: https://www.doi.org/10.1016/j.aninu.2019.05.005

Ortega AD, Mujitaba MA, Xayalath S, Gutierrez WM, Soriano AC, and Szabo C (2021). Perspectives of the livestock sector in the Philippines: A review. Acta Agraria Debreceniensis, 1: 175-188. DOI: http://www.doi.org/10.34101/actaagrar/1/9101

Philippine council for agriculture aquatic and natural resources research and development – Department of Science and Technology (PCAARRD-DOST) (2000). The Philippines recommends for livestock feed formulation. Los Baños, Laguna. Philippines Recommends Series No. 64-A. Available at: https://elibrary.pcaarrd.dost.gov.ph/full-description/PCRD-H000360

Philippine statistics authority (PSA) (2023). Chicken situation report. Available at: https://psa.gov.ph/content/chicken-situation-report-july-september-2023

Philippine statistics authority (PSA) (2022). Production costs and returns of selected agricultural commodities. Available at: https://psa.gov.ph/content/production-costs-and-returns-selected-agricultural-commodities

Reeve C (2021). How the current high feed prices are impacting on livestock sectors. Agriculture and Horticulture Development Board. Available at: https://ahdb.org.uk/news/how-the-current-high-feed-prices-are-impacting-on-livestock-sectors

Seid A (2023). Effects of inclusion of graded levels of Azolla (*Azolla pinnnata*) meal in layer's diet on productive performance and egg quality parameters. Livestock Research for Rural Development, 35(3): 24. Available at: https://www.lrrd.org/lrrd35/3/3524said.html

Shamsudin R, Azhari NAA, Kasim S, and Rahmat MAS (2022). Effects of formulation of soilless growth media from Azolla pinnata sp on water absorption, water loss, and water retention. Advances in Agricultural and Food Research Journal, 3(1): 1-11. DOI: https://www.doi.org/10.36877/aafrj.a0000196.

Shirisha R, Mohanty PP, and Umesh BU (2017). Concepts in various poultry feeding procedures and methods for optimal production: A review. International Journal of Science, Environment, 6(4): 2674-2682. Available at: https://www.ijset.net/journal/1876.pdf

Singh AK and Kim WK (2021). Effects of dietary fiber on nutrients utilization and gut health of poultry: A review of challenges and opportunities. Animals, 11(1): 181. DOI: https://www.doi.org/10.3390/ani11010181

Statista Research Department (2024). Import volume of animal feed ingredients Philippines 2009-2018. Available at: https://www.statista.com/statistics/1170358/philippines-import-volume-animal- feed-ingredients/

Subramanian V (2021). Effect of Azolla feeding on the growth performance of improved native chicken breed (Giri Raja) under intensive system of rearing. The Pharma Innovation Journal, 10(8S): 161-163. Available at: https://www.thepharmajournal.com/special-issue?year=2021&vol=10&issue=8S&ArticleId=7201

Sun X, Devi NB, Urriola PE, Tiffany DG, Jang JC, Shurson GG, and Hu B (2021). Feeding value improvement of corn-ethanol co-product and soybean hull by fungal fermentation: Fiber degradation and digestibility improvement. Food and Bioproducts Processing, 130: 143-153. DOI: https://www.doi.org/10.1016/j.fbp.2021.09.013

Sun H, Kang X, Tan H, Cai H, and Chen D (2023). Progress in fermented unconventional feed application in monogastric animal production in China. Fermentation, 9(11): 947. DOI: https://www.doi.org/10.3390/fermentation9110947

Swain BK, Naik PK, and Beura CK (2022). Nutritive value of Azolla as poultry feed – A review. Indian Journal of Animal Nutrition, 39(1): 1-11. DOI: https://www.doi.org/10.5958/2231-6744.2022.00001.9

Tan IQ (2021). Philippine feedmillers continue their reliance on imports. Asian Agribusiness Media. Available at: https://www.asian-agribiz.com/2021/11/05/philippine-feedmillers-continue-their-reliance-on-imports/

Tan IQ (2022). Higher feed costs hike meat prices in the Philippines. Asian agribusiness media. Available at: https://www.asian-agribiz.com/2022/12/01/higher-feed-costs-hike-meat-prices-in-the-philippines/

Tan IQ (2024). The Philippines uses more corn in feeds with favorable pricing. Asian agribusiness media. Available at: https://www.asian-agribiz.com/2024/04/02/philippines-uses-more-corn-in-feeds-withfavorablepricing/

Wang Y, Lou Y, Lou L, Zhang H, Liao Y, and Gou C (2021). Enhance of the nutritional value of fermented corn stover as ruminant feed using the fungi *Pleurotus* spp. Scientific Reports, 11: 11961. DOI: https://www.doi.org/10.1038/s41598-021-90236-0

Wiesnerova L, Hrebeckova T, and Jablonsky MK (2023). Effect of different water contents in the subsrate on cultivation of *Pleurotus ostreatus* jack. P. Kumm. Folia Horticulturae, 35(1): 25-31. DOI: https://www.doi.org/10.2478/fhort-2023-0002

Yalchi T and Hajieghrari B (2011). Effect of fungal treatment on chemical composition and in vitro ruminal digestibility of some agricultural residues. African Journal of Biotechnology, 10(85): 19707-19713. DOI: https://www.doi.org/10.5897/AJB11.2855

Yasar S and Tosun R (2020). Improving nutritional qualities of tomato pomace by *Pleurotus ostreatus* and Phanerochaete chrysosporium fermentation. Kahramanmaraş Sütçü İmam Üniversitesi Tarımve Doğa Dergisi, 23(2): 527-535. DOI: https://www.doi.org/10.18016/ksutarimdoga.vi.629347

Yasar S, Tosun R, and Sonmez Z (2020). Fungal fermentation inducing improved nutritional qualities associated with altered secondary protein structure of soybean meal determined by FTIR spectroscopy. Measurement, 161: 107895. DOI: https://www.doi.org/10.1016/j.measurement.2020.107895

Zentek J and Boroojeni FG (2020). (Bio)Technological processing of poultry and pig feed: Impact on the composition, digestibility, anti-nutritional factors and hygiene. Animal Feed Science and Technology, 268: 114576. DOI: https://www.doi.org/10.1016/j.anifeedsci.2020.114576

Zhang Y, Liu M, Wu F, Li J, Azi F, Yu K, He Z, Wang P, Wu C, Lu B et al. (2024). Fungal fermentation improves the nutritional quality, flavor, characteristics and physicochemical property of highland barley bran. Journal of Cereal Science, 120(4): 104055. DOI: https://www.doi.org/10.1016/j.jcs.2024.104055