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REGULAR ARTICLE

Comparative Nutritional Profiles and Fermentation Characteristics of Selected Tropical Legumes Subjected to Intensive Coppicing

ARTICLE HISTORY

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Abstract: This study evaluates the nutritional profiles and fermentation characteristics of three selected tropical legumes, *Flemingia macrophylla*, *Indigofera zollingeriana*, and *Gliricidia sepium*, from fresh states through post-ensiled fermentation under a repeated coppicing regime. A Randomized Complete Block Design was employed across four three-month coppicing periods. Samples were evaluated for DM, CP, EE, ash, NDF, ADF, GE, and silage pH and temperature. Forages were ensiled for 21 days with a 5% molasses additive. The analysis was done through ANOVA and Linear Mixed Model using R software. Post hoc Tukey's HSD for significant differences ($\alpha = 0.05$). Repeated coppicing reduced pre-ensiled DM across all species ($p<0.0001$), reflecting progressive succulence in late-period regrowths. Increasing defoliation increased CP content in *I. zollingeriana* ($p=0.0121$) and *G. sepium* ($p<0.0001$) through maximization of leaf-to-stem ratios, while *F. macrophylla* had low initial CP levels and accumulated NDF ($p=0.0035$). In the post-ensiling period, *G. sepium* maintained a very stable pH level of 4.30 in its silage in the third phase ($p<0.0001$), which indicates almost perfect homofermentation. *Indigofera zollingeriana* had moderate silage acidification (pH 5.56-6.64) and underwent considerable proteolysis (CP loss: 10.50%), although acid hydrolysis of NDF brought down NDF content to 28.65%. The pH value in *F. macrophylla* did not show any steep drop (4.75-6.11) because of high buffering capacity. However, it was completely stabilized in terms of temperature to avoid aerobic spoilage. *Gliricidia sepium* produced the most chemically stable and easily digestible silage, thereby making it an ideal forage for tropical ruminants. A coppicing period of 90 days, before ensiling with wilting at a dry matter level of 30%, and addition of 5% molasses are suggested.

Keywords: coppicing; fermentation; forage legumes; *Flemingia macrophylla; Indigofera zollingeriana*; *Gliricidia sepium*

1. Introduction

Feeding strategies for ruminants in year-round tropical production have inherent limitations owing to the seasonality of changes in forage quality. During long dry periods, there is a quick maturation of native grasses, which leads to increased lignification and decreased CP levels, ultimately resulting in reduced voluntary intake and reduced digestibility of nutrients. The use of tropical legumes in basal diets offers the advantage of drought resistance, high biomass yield, and provision of amino acids due to their ability to fix nitrogen symbiotically. Some good options for such species in this regard are *F. macrophylla*, *I. zollingeriana*, and *G. sepium*. *Flemingia macrophylla* grows well on acid aluminum-toxic soil conditions and gives a high yield of fresh biomass per hectare per annum. It does have relatively high levels of condensed tannins (6–11%) and saponins, which might result in an increase in structural fibers, but the positive aspect of using this plant is its ability to reduce enteric methane production. *I. zollingeriana* has an excellent leaf-to-stem ratio, fast re-sprouting after defoliation, and high CP levels, giving high dry matter per hectare per annum. *Gliricidia sepium* is a multipurpose leguminous fodder tree renowned for its adaptability across wide tropical geographic ranges, excellent symbiotic nitrogen-fixing capacity, and high leaf crude protein content (20–30% under optimized management), though its utilization involves consideration of anti-nutritional factors like coumarins (Silva et al., 2024; Tahuk et al., 2022).

Although possessing many agronomical advantages, the continuous conservation of these protein-rich legumes via anaerobic ensiling poses major biochemical obstacles. For effective fermentation in silage-making, epiphytic lactic acid bacteria must quickly metabolize water-soluble carbohydrates into lactic acid to reduce the silo pH to less than 4.5 to arrest the cellular respiration process and block the activity of putrefying bacteria like Clostridia and Enterobacteria (McDonald et al., 1991). But legumes create a double whammy for the silo fermentation process: the presence of very little WSC and extremely high natural buffering ability due to the abundance of organic acids, mineral cations, and ammonia formation.

Thus, the biomass of legumes exhibits a strong resistance to sudden reductions in pH, which typically result in large amounts of nitrogen gas loss, extreme reduction in the dry mass of the crop, and the creation of an undesirable and poisonous silage high in butyric acid (Figueiredo et al., 2025). Repeated coppicing increases tissue succulence (lowering dry matter), reduces water-soluble carbohydrates, and alters the inherent buffering capacity through increased protein concentration, directly influencing subsequent fermentation outcomes. This issue is overcome through the use of additional WSC sources, such as molasses, that would activate the homofermentation pathways of lactic acid bacteria (LAB) to surpass the buffer threshold of the legume (Zi et al., 2022). Moreover, the agronomic practices involving the timing and frequency of coppicing greatly impact the physio-morphological state of the plant before ensiling.

Therefore, the primary objectives of this study are: (1) to assess the impact of successive coppicing periods on the pre-ensiling nutritional composition of the selected legumes; (2) to assess the impact of successive coppicing periods on post-ensiling fermentation quality; and (3) to identify which of the selected legumes will be most suitable for long-term forage conservation under tropical conditions.

2. Materials and Methods

2.1 Planting and Establishment

The research location was situated in Baybay, Leyte, Philippines, on campus at Visayas State University (10.74° N, 124.79° E). Baybay has a type IV climate, receiving an average of 2600 mm of rain per year, with an average temperature of 27.5°C. Soil in this area is classified as clay loam. Baseline soil analysis revealed a slightly acidic pH ranging from 6.05 to 6.14, total organic matter of 1.92–2.30%, total nitrogen of 0.17–0.21%, available phosphorus of 26.20–49.20 mg/kg, and exchangeable potassium of 74.66–120.66 mg/kg. The forages *F. macrophylla* and *I. zollingeriana* were planted using seeds, while the

G. sepium plant was planted using stem cuttings. The planting materials were cultivated in polythene bags containing fertile soil for three months in a nursery, and were watered every day before transplanting. Preparation of the land was done in order to enhance soil conditions, and the plant materials were transplanted three months after seed planting. At the age of one year, all forages were harvested for the first time through coppicing. Four other coppices were done every three months. These harvests occurred from March 2024 to February 2025, capturing one dry season (Period 1: 228.5 mm rainfall, 74% humidity) and three wet seasons (Periods 2–4: 592.9–1,029.6 mm rainfall, 80–86% humidity).

2.2 Ensiling Procedure

All forages were wilted and air-dried before ensiling to achieve a dry matter content of 30%, thereby preventing excessive anaerobiosis associated with too much moisture content, promoting the growth of clostridia rather than lactic acid bacteria (FAO, 2020). The forage was then chopped into pieces of 2 to 4 cm in length to aid in compaction and promote ruminal peristalsis. An exogenous water-soluble carbohydrate, molasses, was added at 5% of the fresh weight to stimulate the growth of homofermentative LAB due to the high buffering power of legume forages (Zi et al., 2022). This 5% molasses addition was based on the weight of the wilted and air-dried biomass, and no water was added.

2.3 Chemical Analysis

The fresh and ensiled samples were analyzed in the Nutrition Laboratory of the Department of Animal Science, Visayas State University, using the AOAC methods for proximate and fiber analyses on a dry matter basis. Leaf-to-stem ratios were calculated using dry matter weights, and all reported post-ensiling DM values refer strictly to oven-dried silage DM, not as-fed DM. Dried samples were milled using a 1-mm mesh sieve. Dry Matter was estimated according to AOAC method 930.15; Crude Protein by AOAC method 990.03; Ether Extract by the procedure of Thiex et al. (2003); Total Ash by AOAC method 942.05; and Gross Energy by adiabatic bomb calorimetry. The contents of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were estimated by AOAC method based on the Sequential Detergent Procedure of Van Soest et al. (1991). Silage temperature was recorded immediately upon silo opening at day 21, measured at the Nutrition Laboratory of the Department of Animal Science, VSU.

2.4 Experimental Design and Statistical Analysis

The experimental design followed a Repeated Measures Randomized Complete Block Design without sub-sampling. The field layout evaluated three legume species subjected to four coppicing period treatments, replicated across four spatial blocks oriented horizontally along a field moisture gradient from a wetter to a drier area. The three-month intervals of coppice harvests made up the repeated-measures factor. The analysis was done through ANOVA and Linear Mixed Model to address the violation of the assumption of sphericity revealed by Mauchly's test using R software. Post hoc Tukey's HSD for significant differences ($\alpha = 0.05$).

3. Results and Discussion

3.1 Chemical Profile of F. macrophylla Pre- and Post- Ensiling

The nutritional analysis of fresh forage for *F. macrophylla* showed that its chemical composition was impacted by the repeated coppicing on multiple fronts (see Table 1). The content of DM fell from 38.10% in Period 1 to 27.50% in Period 4 ($p<0.0001$), indicating how the mobilization of non-structural carbohydrates stored in the roots occurred to maintain vigorous regenerative growth after each new defoliation event (Figueiredo et al., 2025; Nadir et al., 2024). Since the juvenile plants are active and consist of primary, thin-walled cells with plenty of water inside them, their DM content is bound to be lower at each defoliation cycle.

Crude protein remained unaffected by coppicing periods (p = 0.5817), remaining stable at 6.70-9.61%, indicating the biological hardiness of *F. macrophylla* symbiotic nitrogen-fixing root nodules, with continuous nitrogen fixation efficiency regardless of any defoliation severity (Mashudi et al., 2023). The relatively higher CP levels recorded in Periods 2 and 4 are indicative of the greater leaf-to-stem proportion of new regrowth biomass because of the natural tendency of young leaves to have more photosynthetic enzymes like Rubisco (Azzahra et al., 2025). However, *F. macrophylla* remains to have a lower protein content compared to other similar tropical leguminous plants (Asare, 1985).

Ether extract increased from 2.00% in Period 1 to 8.51% in Period 3, sustained throughout Period 4 (p<0.0001). Such an EE increase is attributed to the increasing dominance of leaves in harvested biomass, following the impact of intense coppicing on the formation of secondary woody stems. Epicuticular waxes and galactolipids that form part of chloroplast membranes are synthesized exclusively by leaves (Nadir et al., 2024). The ash content underwent a steep peak in Period 2 up to 7.05%, followed by steady levels of approximately 4.20-4.55%, which can be linked to the activation of mineral absorption after canopy damage (Ernakovich et al., 2014). NDF increased steadily from 56.31% to 73.70% (p=0.0035), associated with rapid synthesis of hemicellulose in newly formed cell walls (Meel et al., 2025). ADF exhibited a brief peak at 65.74% during Period 2, subsequently decreasing to 45.85% by Period 4 (p=0.0012), thus indicating the occurrence of a stress-induced temporal lignification process which later resulted in perpetual juvenility of regrowth tissues with lower lignification. On the other hand, GE values stayed constant at 4,121-4,164 cal/g (p=0.489), thus confirming that the caloric content of *F. macrophylla* is a remarkably stable property determined by the basic bonds of its carbon structure (Viennasay & Wanapat,2020). The substantially higher gross energy of *F. macrophylla* in relation to its low protein is explained by its high ether extract. Since Ether extract possesses approximately 2.25 times the caloric density of carbohydrates, this EE (%) accumulation accounts for the elevated total energy profile compared to the other legumes.

Table 1. Chemical profile of *F. macrophylla* pre-ensiling (fresh) across four coppicing periods

Parameters	Coppicing Period				SEM	p-value
	1	2	3	4		
DM, %	38.10a	30.80b	30.00b	27.50b	1.13	<0.0001
CP, %	6.70	9.61	8.06	9.54	0.17	0.5817
EE, %	2.00b	6.81a	8.51a	7.45a	0.71	<0.0001
Ash, %	1.24c	7.05a	4.55b	4.20b	0.30	<0.0001
ADF, %	53.07b	65.74a	46.37b	45.85b	3.21	0.0012
NDF, %	56.31b	72.02ab	73.70a	72.45a	4.05	0.0035
GE (cal/g)	4125	4121	4129	4164	24.4	0.489

Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)

After ensiling, DM was affected by coppicing period (p=0.0048), with Period 2 recording the highest value (80.83%). This reflects both the pre-ensiling wilting protocol and Period 2's morphological transitional state, where plants began depositing structural carbohydrates, while the lower DM in Periods 3 and 4 (67.78% and 70.04%) reflects the plant's increasing reliance on root reserves to produce succulent vegetative regrowths (Alam et al., 2026). CP was not affected by coppicing period post-ensiling (p=0.1619), though values declined markedly relative to fresh forage (4.60–11.90%). This reduction is attributable to plant-mediated protease activity and clostridial deamination: plant enzymes cleave proteins into soluble non-protein nitrogen (NPN) before anaerobic acidification is achieved, and if pH remains elevated, putrefactive clostridia further deaminate free amino acids into volatile ammonia (NH_3-N), which volatilizes during oven-drying (Wulandari et al., 2023).

Silage pH was not affected by coppicing period (p=0.1199), ranging from 4.75 to 6.11. A critical finding was that *F. macrophylla* consistently failed to achieve the standard preservation threshold of pH 4.5 even with 5% molasses supplementation. This is because of the high organic acid concentration and mineral cation base of the crop, which act as effective buffers to the buildup of hydrogen ions (Viennasay & Wanapat, 2020). The temperature during the ensiling process was significantly influenced by the coppicing period (p<0.0001). This is because Period 1 had higher DM content (38.10%), making it physically tough forage that could not compact easily due to the presence of air, thereby causing prolonged heat production through aerobic respiration (Borreani et al., 2018; Kung et al., 2018). On the other hand, the succulent regrowth of Periods 2-4 compacted easily due to the absence of air and consequently created anaerobic conditions.

Table 2. Chemical Profile of *F. macrophylla* Post-ensiling (Silage) Across Four Coppicing Periods

Parameters	Coppicing Period				SEM	p-value
	1	2	3	4		
DM, %	70.42ab	80.83a	67.78b	70.04b	4.90	0.0048
CP, %	4.60	11.90	5.70	8.58	1.80	0.1619
EE, %	1.07c	5.28ab	3.50b	7.39a	0.16	<0.0001
Ash, %	3.72c	7.33a	8.02ab	7.09b	0.50	0.0006
ADF, %	51.62b	57.12ab	60.06a	51.93b	2.10	0.005
NDF, %	57.31	55.60	59.03	61.73	1.87	0.1385
GE (cal/g)	4140	4139	4114	4132	34.00	0.3499
pH	5.23	6.11	4.75	5.20	0.34	0.1199
Temp.	30.61a	30.27b	30.23b	30.36b	0.04	<0.0001

Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)

3.2 Chemical Profile of I. zollingeriana Pre- and Post- Ensiling

Fresh DM percentage in *I. zollingeriana* reduced significantly from 40.50% in Period 1 to 19.20% in Period 4 (p<0.0001), highlighting morphological plasticity in response to leaf removal pressure in this plant (Table 3). Due to repeated cutting, this plant is forced to grow succulent, water-containing vegetation again, and the time gap of three months ensures that no further woody growth occurs in between (Figueiredo et al., 2025; Antari et al., 2023). This very low percentage of fresh DM poses serious problems for ensiling, as stringent wilting is needed to avoid putrefaction.

Unlike *F. macrophylla*, the CP of *I. zollingeriana* showed a directional increase with successive coppicing periods, varying from 9.45% in Period 1 to 20.54% in Period 4 (p=0.0121). This shows that *I. zollingeriana* possesses the greatest CP responsiveness among the three species studied. Continuous defoliation inhibited the growth of nutrient-deficient woody stems, hence achieving an optimal leaf-to-stem ratio, as photosynthetic protein production, such as Rubisco, occurred only in the harvested part (Azzahra et al., 2025; Nadir et al., 2024). The observed figures were comparable with those recorded by Antari et al. (2023), where CP was greater than 20% in repeatedly clipped *I. zollingeriana*, making it the forage plant with the highest amount of protein content among those tested. The ash content increased significantly from 1.65% in Period 1 to more than 9% in other periods (p<0.0001), indicating that the activation of mineral transport in the post-canopy defoliation phase is highly active (Ernakovich et al., 2014). GE exhibited a decline after Period 1 (3,888 cal/g) to a new lower plateau level (p=0.0008).

Table 3. Chemical profile of *I. zollingeriana* pre-ensiling (fresh) across four coppicing periods.

Parameters	Coppicing Period				SEM	p-value
	1	2	3	4		
DM, %	40.50^{a}	25.80^{b}	20.80^{bc}	19.20^{c}	0.06	<0.0001
CP, %	9.45^{b}	9.94^{b}	12.64^{ab}	20.54^{a}	0.131	0.0121
EE, %	5.26	9.09	9.56	10.13	1.31	0.058
Ash, %	1.65^{b}	9.34^{a}	10.79^{a}	10.95^{a}	0.09	<0.0001
ADF, %	36.83^{a}	34.66^{ab}	40.98^{a}	23.45^{b}	3.74	0.0136
NDF, %	30.01^{b}	56.24^{a}	46.14^{ab}	49.84^{ab}	5.29	0.0181
GE (cal/g)	3888^{a}	3572^{b}	3439^{b}	3532^{b}	64.9	0.0008

Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)

The post-ensiling characteristics of *I. zollingeriana* showed a vital gap between the nutritive value of the fresh material and the silage quality (Table 4). The silage pH was found to be the most crucial diagnostic parameter, which was relatively high (6.64 in Period 1 and 6.66 in Period 2) but then dropped to 5.56 only in Period 3 (p<0.0001). Such pH values are relatively high in relation to the preservation requirement for leguminous species, which should be around 4.5 (McDonald et al., 1991). Hence, the use of 5% molasses was ineffective against such a strong buffering property of the plant during the first two periods of cutting. The unusually high content of CP in fresh material resulted in an increased buffering effect due to a significant number of organic acids, basic amino acids, and ammonia obtained from the quick action of plant proteases on proteins, thus neutralizing the lactic acid produced by lactobacilli (Jayanegara et al., 2020; Mashudi et al., 2023).

Table 4. Chemical profile of *I. zollingeriana* post-ensiling (silage) across four coppicing periods.

Parameters	Coppicing Period				SEM	p-value
	1	2	3	4		
DM, %	78.92^{a}	57.50^{b}	41.41^{c}	37.62^{c}	0.04	<0.0001
CP, %	7.03	11.79	10.59	10.50	1.32	0.1437
EE, %	2.23^{b}	7.20^{a}	3.40^{b}	9.50^{a}	0.70	0.0002
Ash, %	6.64^{c}	11.83^{ab}	12.07^{a}	10.35^{b}	0.41	<0.0001
ADF, %	21.79^{b}	26.54^{ab}	30.49^{a}	21.70^{a}	1.34	0.0021
NDF, %	25.03	23.84	24.96	28.65	1.84	0.3248
GE (cal/g)	3789^{a}	3594^{bc}	3453^{c}	3644^{ab}	37.8	<0.0001
pH	6.64^{a}	6.66^{a}	5.56^{c}	5.60^{b}	0.06	<0.0001
Temp.	30.73^{a}	30.23^{b}	30.27^{b}	30.28^{b}	0.04	<0.0001

Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)

The effects of partial acidification were observed through protein loss. In period 4, fresh protein content was recorded as 20.54%, while post-ensiled protein content remained stable at 10.50%. The protein content was reduced by about 50% of its original nitrogen content. This extensive proteolysis took place through a series of steps, where plant proteases within the cell hydrolyzed proteins into peptides and amino acids following rupture of the cells, and the high pH facilitated the conversion of these compounds to volatile ammonia by clostridial deaminases (Saez-Plaza et al., 2013; Azzahra et al., 2025). Despite this limitation, NDF collapsed dramatically from fresh peaks of 56.24% to post-ensiling values of 23.84–28.65% (p=0.3248 for the post-ensiling comparison, reflecting uniform acid hydrolysis across periods). This beneficial hemicellulose hydrolysis by fermentation-derived organic acids effectively pre-digested the structural cell wall, enhancing potential ruminal colonization. ADF also remained low (21.70–30.49%), reflecting the highly digestible cell wall profile maintained through intensive coppicing, which ruminants can access more readily.

3.3 Chemical Profile of G. sepium Pre- and Post- Ensiling

Gliricidia sepium exhibited a nutritional trajectory under repeated coppicing that was markedly distinct from the other two species, particularly in its enhanced fermentation outcomes (Table 5). Fresh DM declined from 40.90% in Period 1 to 20.00% in Period 4 (p=0.0011), reflecting physiological rejuvenation under repeated coppicing as apical dominance was broken and non-structural carbohydrates were mobilized from root reserves to generate succulent vegetative regrowths (Figueiredo et al., 2025; Nadir et al., 2024). CP increased dramatically from 8.14% in Period 1 to 19.10% in Period 4 (p<0.0001), the most decisive CP progression observed among all species. Repeated coppicing suppressed nutrient translocation into dense, nitrogen-poor woody stems, concentrating photosynthetic enzymes such as Rubisco in foliar tissues harvested at each cutting (Azzahra et al., 2025; Silva et al., 2025). This consistent trajectory of increasing CP under defoliation management confirms *G. sepium*'s responsiveness to harvest intensity as a management lever for forage quality improvement.

Table 5. Chemical profile of *G. sepium* pre-ensiling (fresh) across four coppicing periods.

Parameters	Coppicing Period				SEM	p-value
	1	2	3	4		
DM, %	40.90^{a}	24.20^{b}	21.70^{b}	20.00^{b}	2.65	0.0011
CP, %	8.14^{b}	7.82^{b}	10.63^{b}	19.10^{a}	0.08	<0.0001
EE, %	4.47^{b}	9.49^{a}	8.98^{a}	10.32^{a}	0.77	0.0011
Ash, %	1.32^{b}	6.63^{a}	5.79^{a}	5.52^{a}	0.49	<0.0001
ADF, %	33.47^{ab}	39.09^{a}	40.37^{a}	26.42^{b}	2.60	0.0175
NDF, %	44.82	52.01	64.51	61.74	5.65	0.1194
GE (cal/g)	3704	3834	3787	3691	77.10	0.386

Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)

Ether extract increased very quickly from 4.47% (Period 1) to a plateau value greater than 8.98% (Periods 2–4, p=0.0011) owing to the development of a dominant leaf composition with high levels of chloroplast galactolipids and epicuticular waxes (Abdullah & Suharlina, 2010). Ash levels were found to increase from 1.32% in Period 1 to more than 5.5% in other periods (p<0.0001) as a result of active mineralization due to canopy regrowth (Ernakovich et al., 2014). The levels of NDF were not influenced significantly by coppicing period (p=0.1194) as total wall formation did not change much since the plant continued to form a flexible hemicellulose wall structure (Antari et al., 2023). ADF level decreased to 26.42% in Period 4 (p=0.0175) as expected, as intensive coppicing increasingly inhibited lignin synthesis in tissues, thereby preserving tissues in an ever-young stage, which is highly desirable for animal digestion (Ath-Thifa et al., 2024). The GE levels were not influenced significantly either (p=0.386)

The post-ensiling performance of *G. sepium* demonstrated the most superior fermentation outcomes among all species evaluated, with silage pH achieving 4.30 in Period 3 and 4.35 in Period 4 — the only silage values to breach the critical 4.5 preservation threshold across the entire study (Table 6, p<0.0001). This progressive acidification improvement across coppicing cycles was directly linked to the plant's increasing succulence (declining DM) in later periods, which enhanced substrate availability and LAB colonization efficiency. The 5% molasses additive supplied sufficient exogenous WSC for epiphytic LAB to generate a decisive lactic acid surge that completely overcame the species' buffering capacity

(Luo et al., 2021; Yang et al., 2025). The achievement of pH below 4.5 represents a qualitative inflection point: at this acidity, plant proteases are irreversibly denatured, and putrefactive Clostridia are completely inhibited, creating a biochemically stable environment for long-term nitrogen preservation (McDonald et al., 1991). CP conservation in *G. sepium* silage further validated fermentation superiority. The silage CP values stayed constant at 8.30–9.27% during all periods ($p = 0.8904$), unlike *I. zollingeriana*, which underwent up to 50% loss in CP content. These constant values indicate that the sharp pH drop in Periods 3 and 4 was enough to denature protein-degrading enzymes and suppress activity of Clostridia bacteria until any nitrogen degradation happened (Yang et al., 2025). Despite seeming low compared to fresh *G. sepium* (19.10% in Period 4), these values reflect effective biochemical silage sealing, where nitrogen was not lost as ammonia gas but stored in a stable, ruminally digestible compound (Yang et al., 2025). Post-ensiled ADF reached a maximum value of 44.96% in Period 2 and later reduced to lower levels in Periods 3 and 4 ($p = 0.005$). This trend continued due to the inability of anaerobic LAB to break down the complex lignocellulosic material (Azzahra et al., 2025). GE stayed constant at 3,866–3,924 cal/g in post-ensiled material ($p = 0.422$), showing the high efficiency of energy conservation using a homofermentative fermentation mechanism enhanced by molasses addition (Luo et al., 2021).

Table 6. Chemical profile of *G. sepium* post-ensiling (silage) across four coppicing periods

Parameters	Coppicing Period				SEM	p-value
	1	2	3	4		
DM, %	82.00[a]	33.33[b]	44.45[b]	45.02[b]	2.70	<0.0001
CP, %	8.30	9.04	8.82	9.27	1.08	0.8904
EE, %	3.13[b]	8.88[a]	6.29[a]	8.64[a]	0.62	0.0005
Ash, %	7.05[b]	12.55[a]	12.93[a]	12.84[a]	0.37	<0.0001
ADF, %	28.33[b]	44.96[a]	38.46[ab]	31.24[b]	2.82	0.005
NDF, %	41.60	40.20	47.60	45.97	0.05	0.0576
GE (cal/g)	3915	3924	3871	3866	29	0.4223
pH	6.46[a]	5.73[b]	4.30[d]	4.35[c]	0.0054	<0.0001
Temp.	30.73[a]	30.21[b]	30.20[b]	30.27[b]	0.00112	<0.0001

Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)

3.4 Comparative Fermentation Dynamics and Species Suitability

Three species were distinguished in their fermentation characteristics, which, however, appeared to follow the same mechanism. *Gliricidia sepium* proved to have the most beneficial fermentation profile characterized by a gradual CP accumulation during coppicing, followed by an effective fermentation acidification process; thus, it became the only species that achieved the preservation pH level below 4.5 during fermentation. A lesser buffering load and hence the better action of molasses addition in comparison with *I. zollingeriana*, both having relatively high levels of CP accumulation at the late periods of growth, may point to *G. sepium*'s leaf tissue susceptibility to acidification. The case of *I. zollingeriana* may seem contradictory in terms of its agronomics (as an excellent high-CP fresh feed up to 20.54% of CP by Period 4) and biochemistry (as a difficult silage crop). Its poor fermentation process and fast proteolysis led to a loss of about 50% of CP. In terms of fresh-cut supplementation or wilted feeding for a limited period of time, *I. Zollingeriana* cannot be surpassed. But for long-term preservation as silage, the natural buffering nature requires further management measures beyond just adding 5% molasses, which has been used in this study. *Flemingia macrophylla* fills an important niche that is unique among the three plants. Despite the high tannin levels that prevent acid formation during fermentation, the ability of tannins to buffer protein digestion and act as a protein bypass (thus preventing proteolysis in the rumen) is highly valued, along with the reduction in enteric methane production (Viennasay &

Wanapat, 2020). Due to the accumulation of NDF (>72%) and high buffering capacity that prevents acid production in silages, this plant is not suitable for pure silage preparation but is highly valued as a complementary forage.

4. Conclusion

The process of coppicing thrice over a period of three months had a profound effect on the nutritional and fermentation traits of the tropical legumes examined by lowering lignin concentration in the biomass and enhancing forage digestibility. The legume that stood out amongst the rest in terms of fermentability, nitrogen conservation, lower fiber proportions, and consistent energy levels, when mixed with 5% molasses, was *G. sepium*. The one species that recorded the highest amount of crude protein when subjected to coppicing, but whose fermentation process resulted in low-quality silage and substantial protein loss, was *I. zollingeriana*. *F. macrophylla* did not have great potential in terms of silage, due to its high fiber concentration and inability to be acidified effectively, even though its nutritive value, adaptability, and ability to conserve proteins with tannins make it a great forage crop.

According to the results presented above, it can be said that species utilization should correlate with the goal of production, whereby the use of *I. zollingeriana* for protein enrichment, *G. sepium* for production of silage and long-term storage of forage material, and *F. macrophylla* for degraded land rehabilitation and feed for animals with low requirements should be encouraged. It can also be suggested to use a 90-day interval of coppicing for maximum leaf yields, minimum lignification, and highest forage quality suitable for ensiling. Furthermore, it can be advised to include 5% of molasses to promote lactic acid fermentation, decrease pH, and reduce nitrogen losses. Pre-ensiling wilting of at least 30% of dry matter is also necessary to improve the fermentation process and prevent undesired microbial activity. Farmers can rely on *G. sepium* to produce a chemically stable silage containing 8.82–9.27% preserved crude protein, as a supplemental source of protein for animals, per day, while optimizing annual dry matter harvests. The future direction of further studies is supposed to include improvement of *I. zollingeriana* fermentation properties through higher inclusion of molasses and/or microbial inoculants; measurement of condensed tannin content of *F. macrophylla* silages; determination of in vivo digestibility and effects on livestock performance by silaging *G. sepium*. Limitations of the current study included that no quantifiable tannin analysis was conducted, and animal growth performance data; these findings require further empirical validation before broad application across diverse tropical ecosystems.

5. Competing Interests

The authors declare no conflicts of interest regarding the publication of this paper.

6. Authors' Contributions

All authors contributed significantly to the work reported in this manuscript. Manuel D. Gacutan Jr. conceived and designed the study and performed the data analysis. Richmond M. Joromat executed the data collection. Richmond M. Joromat, Oscar B. Posas, Jade Dhapnee Z. Compendio, and Manuel D. Gacutan Jr. conducted the literature review, developed the data visualization, and provided critical revisions during the manuscript drafting process. All authors read, approved the final manuscript, and agree to be accountable for all aspects of the work.

7. Use of Artificial Intelligence (AI)-Assisted Technology

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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REGULAR ARTICLE

Potential of Superworm Frass (*Zophobas morio* [Fabricius, 1776]) as a Source of Dietary Protein for the Growth Performance of Heritage Chicken (*Gallus gallus domesticus*)

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Abstract: This study was conducted to determine the potential of varying levels of superworm frass as a source of dietary protein in improving the growth performance of heritage chicks. Three dietary treatments were evaluated in the feeding trial. Diet A (Commercial feed, basal diet) served as a negative control, while two (2) levels of superworm frass (5% and 10% supplement) served as components of Diets B and C, respectively. Chemical analysis revealed that superworm frass contained 78.34% DM, 5.03% CP, 0.18% EE, 34.96% NDF, 7.5% ash, and a gross energy content of 2396 cal/g. In terms of voluntary feed intake (VFI), results revealed that Diet C showed a negative linear effect at Day 56 and overall VFI. Moreover, cumulative weight gain (CWG) at Day 14 showed a quadratic tendency and at Day 70 showed a linear tendency, which indicated that when the concentration of superworm frass increased from 5% to 10%, the CWG likewise increased. Body weight (BW) of chicken showed a quadratic tendency at Day 14. Furthermore, ADG of heritage chicken showed a quadratic tendency at Day 14 and a linear tendency at Day 70, respectively. Meanwhile, FCR at Day 70 showed a linear tendency, which indicates that when the concentration of superworm frass increased from 5 to 10%, the FCR of chicken also decreased, which resulted to a better FCR value. Chickens supplemented with superworm frass showed a better FCR value compared to chickens fed with Diet A (control). Superworm frass showed great potential as a dietary supplement for heritage chickens by enhancing weight gain and feed efficiency. These findings suggest that superworm frass may serve as a sustainable and alternative protein source in poultry nutrition, with practical implications for reducing feed costs and promoting the utilization of organic waste materials in livestock production systems. This contributes to improved production efficiency in animal science and supports environmentally sustainable agricultural practices.

Keywords: chicken; frass; growth; heritage; superworm

1. Introduction

Feed cost consists approximately 60-70% of the total production, and most of the ingredients in feed milling industries in the country were imported (Department of Agriculture 2022). In fact, Miraflor (2021) stated that the cost of animal feed ingredients such as corn and soybean had increased and will continue to increase, not only affecting the Philippines but worldwide. Fish meals are also scarce and expensive. The breach between the supply and demand of feed ingredients and protein is a challenge faced by the animal industry. High poultry growth demands an increase in raw feed ingredients, and inflation in feed ingredients will be unprofitable for the livestock and poultry farmers. This phenomenon gives rise to a significant need for an alternative source of dietary protein for animals that contain essential nutrients for maintenance and improvement in the production performance of chicks. A sustainable protein source that can satisfy the growing demand of consumers for poultry products, and at the same time can reduce the cost of production.

Heritage chicken breeds such as the Rhode Island Red have superior adaptation to tropical and low-input systems, allowing them to perform well under hot and humid conditions typical of the Philippines. These breeds also retain higher genetic diversity, which results in a balanced growth rate and feed efficiency, stronger resistance to diseases, and an adaptive immune system (Yadav et al., 2023). Unlike commercial broilers, which are bred for rapid growth but require highly optimized, expensive commercial feeds and controlled environments, heritage chickens exhibit slower but more robust growth trajectories. Furthermore, while native chickens are highly resilient, they often lack the standardized production characteristics found in heritage breeds. Heritage chickens were selected for this study because they represent a middle ground, offering better productivity than native birds while maintaining the hardiness necessary for sustainable or organic production systems. Their inherent resilience and ability to thrive on diverse dietary inputs make them the ideal subjects to evaluate the viability of non-traditional, low-cost feed alternatives.

Insects are now gaining popularity in science and research. It has been identified as a great protein source for animals and humans. Insects are efficient food converters; they consume various substrates, such as organic waste, and convert them into high-protein sources. Insects are naturally part of the poultry diet, especially scavenging birds. Because of their great nutritional content, insects are a viable substitute for expensive protein sources like soybean and fishmeal. They are good sources of macro and micronutrients; they contain a lot of vitamins such as niacin, vitamin B12, thiamine, and riboflavin. Insects are also rich in minerals like calcium, iron, potassium, magnesium, phosphorus, and zinc (Chia et al., 2019). Black soldier fly (BSF), Housefly (HF), and yellow mealworm are some of the examples of insect larvae that have great potential as feed alternatives because of their high protein content (Cullere et al., 2016). Another study highlighted that superworm is protein-rich and has low purine content (Bednářová et al., 2014). Houben et al. (2021) indicated that mealworm frass (insect excreta) is a good substitute for mineral NPK fertilizer for plants; however, there is no significant evidence that indicates whether this mealworm frass can be used for animal consumption.

Very few studies are available regarding the utilization of insect larvae excreta as feed ingredient for poultry and livestock animals; most of the data focus only on the analysis of the frass. Hence, this study was conducted to determine the chemical composition of the frass and assess the feeding potential of superworm frass (*Zophobas morio*) and its effect on the growth performance of heritage chicken to determine if this waste-to-feed approach can support the nutritional needs of resilient, sustainable poultry breeds.

2. Materials and Methods

2.1 Rearing and Harvesting of Frass

Adult insects of superworm (SW) were reared in an insect research facility with provision of space for laying and hatching of larvae. The larvae were given rice bran with fruit and vegetable peelings as their substrate (1:1 ratio). Insect frass was collected from the larvae litter through sieving and was stored in a container. The frass was then dried and added to the concentrate feed based on the inclusion rate for each treatment.

2.2 Experimental Design

A total of 85 heads of day-old heritage chicks were distributed randomly in three dietary treatments laid out in a Randomized Complete Block Design (RCBD) with four batches of hatched chicks. Diet A (Commercial feed, basal diet) served as the negative control, while two (2) levels of superworm frass (5% and 10% supplement) served as components of Diets B and C. The batch and breed (Rhode Island Red & Black Australorp breeds) served as blocking factors.

2.3 Data Analysis

The data were analyzed using Analysis of Variance (ANOVA) in Proc Mixed of Statistical Analysis System (SAS 9.4 M8). Significant differences were determined using Trend analysis and declared significant at $p<0.05$ and trend at $0.05<p<0.10$.

2.4 Proximate Analysis of Superworm Frass

The superworm frass was analyzed in three replicates using conventional methods for dry matter (DM), crude protein (CP), ether extract (EE), and crude ash (Association of Official Analytical Collaboration, 2006). On the other hand, neutral detergent fiber (NDF) was assayed using Van Soest's (1991) method. Gross energy (GE) of the samples was determined using IKA C1 Bomb Calorimeter Parr Instrument (1L, USA).

3. Results and Discussion

3.1 Chemical Composition of Superworm Frass

Table 1 illustrates the chemical composition of superworm frass. Based on the results, the frass contained 78.34% DM, 5.03% CP, 0.18% EE, 34.96% NDF, 7.5 % crude ash, 92.5% OM, and 2,396 cal/g, respectively. The chemical composition results showed lower protein content and crude fat, but it had higher crude ash and crude fiber compared to the previous study of Ravzanaadii et al. (2012), with 18.51% CP, 1.3% EE, 7.29% Ash, and 13.66% Crude fiber, where the nutritional value of *Tenebrio molitor* larvae was analyzed and its potential as a food source. Higher DM, OM, and NDF were obtained, which may be due to the frass being a dry pellet when excreted by the superworm. Lastly, the frass had a high protein content as a waste product, suggesting it could be used as an additional supplement in animal feed.

Table 1. Nutrient composition of superworm frass

Component	Composition
Dry matter (%)	78.34
Crude protein (%)	5.03
Ether extract (%)	0.18
Neutral detergent fiber (%)	34.96
Crude ash (%)	7.5
Organic matter (%)	92.5
Gross energy (cal/g)	2396

3.2 Voluntary Feed Intake

Table 2 shows the VFI of chickens supplemented with superworm frass in their basal diet. Based on the results, VFI on Day 14 ($p>0.1203$), Day 28 ($p>0.1734$), Day 42 ($p>0.3892$), and Day 70 ($p>0.7380$) showed no significant differences across treatments. However, at Day 56, there was a negative linear trend ($p<0.0014$) with the lowest VFI in birds supplemented with Diet C (602.45g), followed by Diet B (684.97g), and Diet A (755.04g). This means that when the concentration of treatments increased from 5% to 10%, it resulted in a lower VFI in chickens. Also, the overall VFI showed a negative linear tendency ($p < 0.0760$), which means that when the concentration of frass increased from 5 to 10%, it resulted in a decreased VFI.

The low VFI of birds may be influenced by the high NDF value of the superworm frass (Table 1). Hence, birds supplemented with frass were physically full and resulted in less amount of feed ingested. Moreover, based on the observations during the experiment, birds had voluntarily consumed the frass. In fact, they were not selective, and the birds uniformly picked up the feed in the feeding trough. This was an indication that superworm frass was palatable. The results were in contrast with the study of Al-Qazzaz et al. (2016), wherein the inclusion of insect meal on laying hens' basal diet had no significant effect on the feed intake of birds. However, Magsalay et al. (2024) revealed that replacing soybean meal with superworm in the diet of grower native chickens resulted in higher feed intake, better growth rate, and feed conversion ratio, and showed better meat quality. Concepcion et al. (2025) justified that supplementing quails with varying levels of superworm meal (5%, 10%, and 15%) showed better feed intake, weight gain, and feed conversion ratio; however, supplementation of superworm meal up to 20% showed a decline in the performance of quails.

Table 2. Voluntary Feed Intake (g) of heritage chicken supplemented with 0%, 5%, and 10% superworm frass

	Diet			SEM	Diets	*P value*	
Day	A	B	C			Linear	Quadratic
14	213.08	195.94	204.41	14.62	0.1203	0.2560	0.0762
28	427.63	405.37	399.08	25.96	0.1734	0.0832	0.5277
42	458.11	436.26	418.89	29.36	0.3892	0.1878	0.9253
56	755.04	684.97	602.45	34.94	0.0043	0.0014	0.8021
70	756.83	715.37	757.77	51.61	0.7380	0.9882	0.4545
Total	2610.69	2437.92	2382.59	68.57	0.0760	0.033	0.4466

3.3 Cumulative Weight Gain

Table 3 shows the bi-weekly CWG of heritage chicken supplemented with superworm frass in their diet as a source of dietary protein. Based on the results, WG at Day 14 ($p>0.0799$) had a quadratic tendency, which indicated that when the concentration of superworm frass increased from 0 to 5%, the WG would decrease. However, if the concentration of superworm frass further increased from 5% to 10%, the WG likewise increased. For the WG at Day 28 ($p>0.7664$), Day 42 ($p>0.6640$), Day 56 ($p>0.2367$), and the overall WG ($p>0.7663$), no significant differences were observed across treatments. However, WG at Day 70 showed a positive linear tendency ($p < 0.0531$), which indicated that when concentrations of superworm frass increased from 5 to 10%, the WG of chicken also increased.

Results were similar to the findings of Pretorius (2011), where broilers supplemented with 10% housefly larvae (HFL) meal and 10% fishmeal showed no significant effect on their performance; a higher inclusion level of HFL (25%) in the diet resulted in better growth performance of broilers than in birds supplemented with 25% fishmeal. Findings of Mbhele et al. (2019) contradicted this and showed that there was` a quadratic trend between the partial substitution of soybean products with 54g/kg BSFL concentration on the overall feed intake and the overall BW of Jumbo quails.

Table 3. Cumulative Weight Gain (g) of heritage chicken supplemented with 0%, 5%, and 10% superworm frass

Day	Diet			SEM	Diets	*P value*	
	A	B	C			Linear	Quadratic
14	119.71	109.66	114.99	9.98	0.0799	0.2341	0.0474
28	212.96	215.53	202.83	13.83	0.7664	0.5941	0.6415
42	248.70	226.95	230.50	17.61	0.6640	0.4926	0.5791
56	354.19	343.02	310.74	16.59	0.2367	0.1135	0.6221
70	322.38	330.28	388.47	27.68	0.0531	0.0277	0.2513
Total	1257.93	1225.43	1247.52	36.03	0.7663	0.8229	0.5052

3.4 Body Weight

Table 4 presents the bi-weekly BW of heritage chicken supplemented with superworm frass. Based on the results, BW at Day 14 had a quadratic tendency ($p>0.0544$), which means that when the concentration of superworm frass increased from 0 to 5%, the BW decreased; however, it increased when frass was given at 10%. At Day 28 ($p>0.7611$), Day 42 ($p>0.5920$), Day 56 ($p>0.2713$), and Day 70 ($p>0.7503$), results were not significant.

Results indicated that better BW will be acquired with higher inclusion level of frass in the chicken diet. Based on the study of Pretorius (2011), better live weight was obtained through supplementation of 25% HFL meal compared to birds supplemented with 10% HFL meal and broilers supplemented with 25% fishmeal.

Table 4. Body Weight (g) of heritage chicken supplemented with 0%, 5%, and 10% superworm frass

Day	Diet			SEM	Diets	*P value*	
	A	B	C			Linear	Quadratic
14	159.60	148.23	153.58	10.79	0.0544	0.1480	0.0375
28	372.58	363.78	356.40	16.47	0.7611	0.4788	0.9706
42	621.25	590.73	586.90	27.05	0.5920	0.3665	0.6766
56	975.45	933.75	897.63	35.94	0.3713	0.1767	0.9516
70	1297.82	1264.00	1286.10	36.09	0.7503	0.7998	0.4929

3.5 Average Daily Gain

Table 5 shows the average daily gain (ADG) of heritage chicken supplemented with varying levels of superworm frass via feed. Based on the results, ADG at Day 14 showed a quadratic tendency ($p>0.0807$). This indicates that when the concentration of superworm frass increased from 0 to 5%, the ADG decreased. However, if the concentration of superworm frass increased to 10%, the ADG of chicken likewise increased. Moreover, ADG at day 70 had a positive linear tendency ($p>0.0531$) between concentration and ADG of chicken. This means that as the concentrations of superworm frass increased from 5 to 10%, the ADG of chicken also increased. Lastly, the Day 28 ($p>0.7672$), Day 42 ($p>0.6649$), Day 56 ($p>0.2358$), and the overall ADG ($p>0.6370$) of heritage chicken were not significant across treatments.

The results of chickens supplemented with superworm frass showed that better ADG was possible through supplementing chickens with higher than 10% concentration of superworm frass on their basal diet. The study of Benzertiha et al. (2019) mentioned that lower percent inclusion of *Z. morio* meal at

0.2% and 0.3% in broiler chicken basal diet already resulted in a visible improvement in the growth performance of broilers. Kierończyk et al. (2018) also reported that replacing soybean oil with *Z. morio* meal at a 5% level showed better performance than those chickens supplemented with only soybean oil. In contrast, Pretorius (2011) reported that supplementing broilers with 25% HFL meal resulted in broilers having excellent ADG compared with the birds supplemented with 25% fishmeal, which makes it a good replacement for a commercial fishmeal protein source.

Table 5. Average Daily Gain (g) of heritage chicken supplemented with 0%, 5%, and 10% superworm frass

Day	Diet			SEM	Diets	*P value*	
	A	B	C			Linear	Quadratic
14	8.54	7.83	8.21	0.71	0.807	0.2366	0.0477
28	7.60	7.69	7.24	0.49	0.7672	0.5955	0.6416
42	5.92	5.40	5.49	0.41	0.6649	0.4937	0.5792
56	6.32	6.12	5.54	0.29	0.2358	0.1130	0.6216
70	4.60	4.71	5.54	0.39	0.0531	0.0277	0.2511
Total	6.60	6.35	6.41	0.22	0.6370	0.4934	0.5310

3.6 Feed Conversion Ratio

Feed conversion ratio (FCR) is the rate that measures the efficiency of the animal to convert feed into the desired output, and a lower FCR value is desirable. Table 6 presents the FCR of chickens supplemented with varying levels of superworm frass in their diet as a source of dietary protein. Based on the results, FCR at Day 14, 28, 42, 56, and the overall FCR had no significant difference in all treatments. However, FCR at Day 70 had a linear tendency ($p>0.0792$), which indicated that when concentrations of superworm frass increased from 5 to 10%, the FCR of chicken also decreased, which resulted to a better FCR value. Despite no significant difference between treatments, FCR at Day 28 showed that Diet B had the lowest FCR (1.89) compared to that of Diet C (2.00) and Diet A (2.01). However, from Day 42 to Day 70, Diet C showed the best FCR value among treatments.

The results showed better FCR across treatments at Day 14. According to Hollemans et al. (2018), beneficial effects of nutrition on the growth performance of birds are only observed during the first two weeks and will disintegrate later on as the chick matures. The results were similar to the study of Asimaki et al. (2020), where fishmeal was replaced in the diet of sea bream fingerlings with 5% and 10% *Z. morio* larvae meal. In their study, the growth rate, FCR, BW, and feed intake were not significantly affected by the treatments. However, despite this, Diet C showed a better FCR compared to Diet A throughout the experiment. Low feed intake and improved WG contributed to a better FCR value compared with Diet A and Diet B.

Table 6. Feed Conversion Ratio of heritage chicken supplemented with 0%, 5%, and 10% superworm frass

Day	Diet			SEM	Diets	*P value*	
	A	B	C			Linear	Quadratic
14	1.78	1.82	1.78	0.01	0.9212	0.9826	0.6979
28	2.01	1.89	2.00	0.14	0.7741	0.9458	0.4942
42	1.85	1.92	1.83	0.09	0.7731	0.8915	0.4991
56	2.13	1.99	1.95	0.09	0.4022	0.2114	0.6928
70	2.39	2.18	1.95	0.16	0.0792	0.0303	0.9359
Total	2.03	1.96	1.90	0.09	0.5920	0.3260	0.9637

4. Conclusion

Superworm frass showed great potential as a feed ingredient due to its chemical composition. Supplementing heritage chicken with superworm frass had a quadratic effect, with 10% frass supplementation resulting in a better FCR ~~for heritage chicken~~. These findings highlighted the potential of superworm frass as a functional and sustainable ingredient in animal feed.

5. Recommendations

It is recommended that similar studies be conducted with a higher inclusion level of superworm frass to maximize the results of this experiment. Further investigation should also be conducted to understand the effect of chitin present in insect frass on the animal's digestion and absorption, and assess the physiological effects of superworm frass with particular attention to organ function and metabolic health.

6. Competing Interests

The author(s) declare that there is no conflict of interest regarding the publication of this paper.

7. Authors' Contributions

All authors contributed significantly to the work reported in this manuscript. Dr. Manuel Gacutan Jr. designed the study and provided substantial feedback during the manuscript drafting process. Rocelyn Tam-is conducted the data analysis and was responsible for data collection. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

8. Use of Artificial Intelligence (AI)-Assisted Technology

During the preparation of this work, the authors used Grammarly to improve the content of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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REGULAR ARTICLE

Phenotypic, Genetic, and Production Characteristics of Joloano and Paraoakan Native Chicken Breeds of the Philippines: A Review

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Abstract: The Joloano and Paraoakan native chicken genetic populations represent the two types of fighting native chicken breeds found in the Philippines, based on their distinct physical appearances, genetic makeup, and cultural value. The present study is a review paper that collates scientific knowledge pertaining to the two native chicken genetic populations in the Philippines. These two genetic types of chickens, Joloano, which comes from the Sulu Archipelago, and Paraoakan, which comes from Palawan Island, have been proven to have a different lineage than other Philippine native chickens since the microsatellite markers showed that neither Joloano nor Paraoakan belongs to the same lineage as other native chickens such as Labuyo and red junglefowl. In terms of phenotype, Paraoakan is recognized as the biggest chicken among the genetic types of Philippine native chickens due to their strong body and reddish-black feathers, whereas Joloano is known for its vertical stance, deep orange feathers, and muscular body for fighting purposes. Both chicken genetic groups demonstrate moderate to high genetic heterozygosity, suggesting sufficient genetic variability for conservation and selective breeding purposes. In terms of production, Paraoakan shows a competitive net income advantage over other native genetic groups in bio-economic modeling, while Joloano has shown growth responsiveness to locally available feed resources. Conservation efforts remain critical, as both chicken genetic groups face threats from genetic dilution through unregulated crossbreeding with exotic strains. This review identifies priority areas for selection, in situ and ex situ conservation, and genetic improvement programs.

Keywords: fighting-type native chicken; genetic diversity; phenotypic characterization

1. Introduction

The Philippines can be considered one of the world's biodiversity hotspots for animal genetic resources, housing a wide range of native poultry varieties that have evolved through interactions within their specific environmental and cultural settings (Bondoc, 1998). Among the identified genetic stocks of native Philippine chickens, such as Darag, Camarines, Bolinao, Boholano, Banaba, Zampen, Labuyo, Paraoakan, and Joloano, Joloano and Paraoakan represent fighting breeds of great biological and sociocultural importance (Lambio et al., 2000; Santiago et al., 2022). The conservation of these breeds is situated within the broader global agenda for Animal Genetic Resources (AnGR). The FAO's Global Plan of Action for Animal Genetic Resources and the Nagoya Protocol on Access and Benefit-Sharing provide the international framework within which national conservation efforts, including those for Philippine native chickens, must be understood (Food and Agriculture Organization, 2007). Globally, FAO's State of the World's Animal Genetic Resources reports indicate that approximately 20% of livestock breeds are at risk of extinction, with the replacement of commercial breeds accelerating genetic erosion in developing countries (FAO, 2012). The Philippines is not immune to this trend, as unregulated crossbreeding with exotic breeds and the intensification of commercial poultry production continue to threaten the genetic integrity of native chicken populations (Bondoc, 1998; PCAARRD, 2016).

Native Philippine chickens (*Gallus gallus domesticus* L.) play an important part in traditional agricultural systems and provide small-scale farmers with additional sources of income, nutrition, and cultural value in the context of cockfights, which form a fundamental part of Filipino culture (Calsi, 2016). As compared to the commercial varieties, native Philippine chickens are considered more adaptable to the tropical climate, stronger, and disease-resistant (Lambio et al., 2000; PCAARRD, 2016). Among the native chicken breeds, fighting types like the Joloano and Paraoakan breeds are distinguished by their aggressive nature and strength. Throughout this review, the term "breed" is used as the preferred designation for Joloano and Paraoakan, consistent with FAO's definition of a breed as a subspecific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species (FAO, 2012). The terms "genetic group," "genotype," and "genetic population" are used interchangeably in some cited literature and are acknowledged here, but "breed" was used consistently in this review for clarity. It is also important to acknowledge that while cockfighting holds deep cultural significance in the Philippines, it is subject to regulatory and ethical scrutiny in many countries. This review presents Joloano and Paraoakan within their cultural context without endorsing the practice, focusing instead on the scientific and conservation value of these breeds.

While the two breeds are known to possess valuable cultural and genetic importance, they have been scientifically documented less comprehensively compared to other local chicken genetic groups found within the Philippines. Literature on the subject matter can be seen to exist in various studies conducted related to breed characterization, regional assessment, genetic assessment, and production testing. However, the lack of a scientific review regarding these two genetic groups limits one's understanding of the current knowledge available.

This review consolidated the available scientific and institutional literature relating to the native Joloano and Paraoakan chickens, concerning the following topics: (1) Taxonomy and geographic origin; (2) Phenotypic characteristics; (3) Genetic data; (4) Production performance; (5) Status in terms of conservation; and (6) Breeding potential. Through this review, it is hoped that important points will emerge for future consideration in conservation programs of these two genetic breeds.

2. Materials and Methods

This review was conducted through a systematic search of peer-reviewed and institutional literature on Joloano and Paraoakan native chickens of the Philippines. The following electronic databases were searched: Google Scholar, CAB Abstracts, Web of Science, Scopus, Philippine e-Journals, and the University of the Philippines Los Baños (UPLB) institutional repository. The search covered publications from 1990 to 2024. Search terms used included "Joloano chicken," "Paraoakan breed," "Philippine native chicken genetics," "*Gallus gallus domesticus* Philippines," "native chicken conservation Philippines," "Philippine gamefowl breeds," and "fighting chicken genetic resources."

Literature was included if it provided primary data or substantive discussion on phenotypic characteristics, genetic diversity, production performance, conservation status, or breeding opportunities specific to Joloano, Paraoakan, or closely related Philippine native chicken populations. Sources were excluded if they were non-peer-reviewed commentaries without empirical data or if they addressed only commercial chicken breeds without native chicken comparison. A total of 20 primary and institutional references met the inclusion criteria and were synthesized in this review.

3. Results and Discussion

3.1 Taxonomic Classification and Geographic Origin

The Joloano and the Paraoakan both fall under the scientific classification of *Gallus gallus domesticus* Linnaeus, 1758, of order Galliformes and family Phasianidae. These poultry types are considered genetic materials of Philippine chickens under the management of the Bureau of Animal Industry (BAI), Department of Agriculture (DA), Republic of the Philippines (Lambio et al., 2000; Bondoc, 1998). The Joloano (also known as "Jolojano" or "Basilan" in some sources) is an indigenous breed originating from the Sulu Archipelago, specifically the island of Basilan, located within the Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), which itself is situated within Mindanao, the southernmost major archipelago of the Philippine Islands (Lambio et al., 2000; Yan, 2020). The geographical origins of the breed, coupled with its centuries-long development through selective breeding among local Muslims owing to its cockfighting qualities, have created the unique genetic makeup of the breed.

On the other hand, the Paraoakan comes from the island of Palawan. Palawan Island is located in the MIMAROPA region of the Philippines. This island is known for having high biodiversity within the country, and it is its geographical isolation that has played an important role in developing unique chicken breeds in this region as well (Bondoc, 1998; Santiago et al., 2022). Both breeds mentioned are considered part of the strategic national genetic resource in the Philippines, as per the PNAD Program managed by the Bureau of Animal Industry (n.d.).

3.2 Phenotypic Characteristics

3.2.1 Joloano Chickens

It should be noted that the Joloano is a medium-sized, sturdy, fighting-type breed recognized by its erect posture that provides instant height gain in cases of cockfighting events (Yan, 2020). The average weight of adult cocks is 2.20 kg, whereas females, or hens, have a weight of 1.50 kg. The bodyweight is small but is supplemented by strong muscles and skeletal structure due to the needs of cockfighting events (Lambio et al., 2000). Regarding plumage, the typical feature among Joloano roosters is deep-orange to reddish-brown-colored feathers on the hackle (neck feathers) and saddle zones, along with a black tail that sometimes contains specks of white feathers (Yan, 2020). However, there are some birds that also have black-breasted or wheaten plumages. Orange-red plumage is the most common among roosters, an attribute that can be attributed to natural and selective breeding from the ancestor red junglefowl (*Gallus gallus*) (Santiago et al., 2022).

The Joloano is also characterized by a pea comb, which is the common comb type found in tropical chickens, since it helps in dissipating about 40 percent of body heat through its thermoregulatory capability in the tropics (Duguma, 2006, cited in Santiago et al., 2022). The wattles are usually red and moderate-sized, while the ear lobes are usually red. The eye iris coloration is orange-red. This is in agreement with that of other native chickens in the Philippines, in which the shanks of this breed of chicken have dark-black colors due to the presence of dermal melanin in the homozygous black extension factor (Santiago et al., 2022). Among the physical characteristics that is very notable about the Joloano is its very upright body position, which gives it a more dominating appearance than other native chicken breeds. The combination of its erect body stance, along with strong muscles in the neck area and legs, makes it a favorite for cockfighters all across Mindanao (Yan, 2020).

3.2.2 Paraoakan Chickens

The Paraoakan is recognized as the largest native chicken breed in the Philippines, distinguished by its robust body conformation, deep chest, and strongly muscled frame (Lopez et al., 2013; Santiago et al., 2022). Adult Paraoakan roosters average 2.91 kg and hens 1.77 kg at 40 weeks of age (Lopez et al., 2013). The breed's imposing physical stature, developed through geographic isolation on the island of Palawan, reflects both its fighting heritage and its dual-purpose potential as a meat-type breed. Its overall appearance is characterized by a large, compact body, strong, long legs, and a commanding presence that distinguishes it from other Philippine native chicken breeds.

The body colors of the Paraoakan cock are mostly reddish-black, whereas those of Paraoakan hens are mainly black (Lopez et al., 2013). It is worth noting that the plumage color of Paraoakan hens features spangling, a type of feathering that involves having feather tips with spots of contrast at the tip (Santiago et al., 2022). Roosters are known to have more monochromatic plumage than hens. In terms of morphology, Paraoakan roosters show the greatest chest circumference (average: 37.00 cm) out of the six genetic populations tested by Santiago et al. (2022). Paraoakan hens showed greater-than-average chest circumferences (30.97 cm), just next to those of Bolinao hens. Nevertheless, the hens of Paraoakan chickens are relatively short in body length compared to those of other chicken breeds studied (Santiago et al., 2022).

Concerning comb shape, the roosters of the Paraoakan breed show more of the rose comb type, with most of them having medium-sized combs, while the majority of hens possess pea combs (Lopez et al., 2013). The wattles are bright red, and the earlobes are red for Paraoakan roosters, while most of the hens possess white earlobes. The predominant shank color for the Paraoakan breed is black. All members of the Paraoakan breed possess white skin color, an attribute of their red junglefowl parent (Santiago et al., 2022). The strong body structure of the Paraoakan, with its strong chest and muscles in the thighs and long, strong legs, makes this type of chicken very effective in battles and simultaneously a good meat breed. This is because the larger body and muscle composition are some of the main features used for cockfighting and meat production in farms (Lopez et al., 2013; Lopez et al., 2014; PCAARRD, 2016).

3.3 Genetic Information and Diversity

3.3.1 Microsatellite (SSR) Genetic Characterization

Some of the important genetic studies that have been undertaken using both Joloano and Paraoakan include the study conducted by Fabella (2014) at the University of the Philippines, Los Banos (UPLB). The genetic diversity of the genetic groups in Philippine native chickens using seven simple sequence repeat (SSR) loci as suggested by FAO was assessed. The groups assessed included Paraoakan, Darag, Camarines, Bolinao, Boholano, Banaba, Joloano, and Labuyo, along with one commercial breed referred to as Layer. Some of the important findings obtained by Fabella (2014) were observed allele number (MNA) ranges from 4.2 to 6.0 in various genetic groups, a range of observed heterozygosity (Ho) between 0.6232 and 0.7950, and values of the inbreeding coefficient (Fis) ranging between 0.01564 and -0.20304. The genetic diversity of Paraoakan was found to be particularly high according to both Ho and Fis values, and the maximum value of MNA was obtained for Boholano. Importantly, cluster analysis revealed that the Labuyo, which was considered to be the common ancestor of most Philippine native chickens, was not the ancestor of Paraoakan or Joloano. Therefore, it can be concluded that the latter breeds could have originated from different ancestors than most Philippine native chickens

The broader study carried out by Santiago et al. (2022) on six genetic groups of Philippine native chickens, such as Bolinao, Boholano, Darag, Camarines, Paraoakan, and Zampen using the same 15 SSR markers as recommended by FAO showed high genetic diversity of Philippine native chickens. The results showed that the mean number of observed alleles for Paraoakan was na = 2.27; effective number of alleles ne = 1.6334; Shannon's Information Index I = 0.5529; observed heterozygosity Ho = 0.2500; and expected heterozygosity He = 0.3572. The genetic distance of Paraoakan to ZamPen (highest distance being 0.5103) is proof of its unique phylogeny among Philippine native chickens.

According to the study by Santiago et al. (2022), PIC analysis indicated that 93% of the fifteen SSR markers examined had PIC higher than 0.50, with a mean PIC of 0.59. It is important to note that PIC values reflect the informativeness of individual SSR markers rather than population-level genetic diversity per se; high PIC values indicate that these markers are highly polymorphic and therefore suitable for discriminating among populations, but should not be conflated with a direct measure of population genetic variation. In addition, the F-statistic approach indicated the presence of a significant amount of genetic differentiation among the populations since the mean value of FST was 0.1729, with a mean gene flow value (Nm) of 1.1956. An FST of 0.1729 indicates moderate-to-high genetic differentiation, consistent with semi-isolated populations that have undergone limited but not complete gene flow — a finding of practical significance for conservation geneticists, as it suggests that Joloano and Paraoakan maintain distinct genetic identities that warrant separate conservation management. Furthermore, the consistent finding that Ho was lower than He across populations in both Fabella (2014) and Santiago et al. (2022) indicates a heterozygote deficiency that may reflect inbreeding within populations, the Wahlund effect (subpopulation structuring within sampled groups), or the presence of null alleles at some loci. These possibilities should be explicitly investigated in future studies using additional markers or pedigree analysis. Based on the UPGMA phylogenetic tree constructed using Nei's (1972) genetic distance approach, Bolinao and Darag were found to be the least genetically distant among the six groups, while Paraoakan was established as a distinctive phylogenetic cluster.

3.4 Mitochondrial DNA and Population Structure

In the first study carried out by Godinez et al. (2021), genetic structure was determined in Philippine village chickens (n=107) using the entire sequences of their mitochondrial DNA (mtDNA) D-loop, which revealed 34 different haplotypes showing high levels of haplotype diversity (Hd= 0.915 +/- 0.011) in Philippine village chickens. These haplotypes have been classified into five haplogroups (A, B, D, E, and H) out of 13 global mtDNA haplogroups. Through the use of phylogenetic analysis and median-joining network, it has been established that haplogroup D was the common maternal haplogroup found among Philippine chicken populations (Godinez et al., 2021). Red junglefowls belonged to the basal nodes of haplogroup D, implying an earlier importation of Philippine RJF ancestors in mainland Southeast Asia. It is noted that there was a presence of the Philippine-Pacific subclade in the study, thus implying that the Philippines could have played an important role in the dissemination of chicken throughout the Pacific islands.

The results of neutrality test statistics (Tajima's D and Fu's Fs) were found to be highly negative among Philippine chickens, signifying a history of expansion of populations in the past. The Bayesian skyline plot (BSP) test statistics showed that there was an expansion of population size linked with expansion events due to the presence of humans in the Philippine Islands. The high amount of genetic variability observed in Philippine chickens as a whole, including Joloano and Paraoakan breeds as part of Philippine chickens' genetic pool, highlights the importance of conserving such genetic diversity (Godinez et al., 2021). It must be noted, however, that Godinez et al. (2021) did not separately analyze Joloano and Paraoakan as distinct populations; their samples represented general Philippine village chickens. Therefore, the applicability of their haplotype diversity findings and phylogeographic inferences to these two specific breeds is limited and indirect. The results from this study should be treated as contextual background rather than breed-specific genetic evidence. Breed-specific mtDNA analyses for Joloano and Paraoakan remain an unaddressed research priority.

3.5 Reproductive Genetics: Semen Characterization

Baseline information on the volume and quality of semen was determined by the experiment conducted by Pamulaklakin et al. (2024), involving 22 roosters of Banaba, Joloano, and Paraoakan native chicken breeds. The experiment is a pioneer study on the reproductive physiology of the roosters of Joloano and Paraoakan native chickens within the context of assisted reproductive technologies (ART). Among the important results are that Banaba showed the highest quantity of semen, secondly Joloano, and lastly Paraoakan. In addition, the highest level of semen pH was observed in Banaba (7.27). It should be noted that most of the semen samples showed a creamy texture (42.6%) and, consequently, thin and creamy (32.9%). The highest sperm concentration was observed in Paraoakan among the three native chicken breeds studied (Pamulaklakin, 2024).

Regarding the storage properties of the semen samples, the semen samples from Banaba and Joloano roosters were highly viable (more than 50% motile spermatozoa) even after extending them in Ringer's solution for six hours. On the other hand, when extended, the semen samples from the Paraoakan roosters showed very poor motility, especially after eight hours of storage time. The temperature of storage significantly influenced the viability of the Paraoakan roosters, as evidenced by a higher percentage of motile and progressively motile spermatozoa observed at lower temperatures (5-10 degrees Celsius) compared to the normal temperatures (22-25 degrees Celsius) (Pamulaklakin, 2024).

3.6 Production Performance

3.6.1 Paraoakan Production Performance

One characteristic that makes Paraoakan chickens stand out is their high level of competitiveness when compared with the rest of the Philippine native chickens. Phenotype studies conducted on Paraoakan chickens in Palawan proved that Paraoakan is the most heavily built strain of the native chickens in the Philippines. At 40 weeks of age, males have an average body weight of 2.91 kg, while females weigh an average of 1.77 kg (Lopez et al., 2013).

A study conducted on the production performance analysis of three Philippine native chicken genotypic types, namely, Paraoakan, Bolinao, and Banaba, through the Structural Thinking Experiential Learning Laboratory with Animation (STELLA) showed some valuable comparative information about their production performance. The study found that, although Bolinao-type genotypes produced the highest number of chicks during a particular laying period, Paraoakan and Banaba genotypes yielded more marketable chickens after 18 weeks of raising them (Lopez, 2016). Additionally, the total live weight and gross income from Paraoakan and Banaba genotypes were found to be similar but higher than those from Bolinao genotypes. Notably, the net income generated from the Paraoakan genotype was higher than those generated from Banaba and Bolinao genotypes

Production of native chickens, such as the Paraoakan chicken in Palawan, is done mainly by extensive management systems. Investigations into native chicken production management practices in Palawan revealed that farmers rarely maintain production records, do not allocate nests, and leave eggs and chicks to be hatched and brooded naturally (Lopez et al., 2013). Farmers in Palawan do not use deworming practices, vaccinate their chickens, or perform artificial inseminations; their chickens are produced mainly for domestic consumption and as a secondary source of income. This production system constrains the exploitation of the genetic potential of the Paraoakan chicken. Santiago et al. (2022) determined that Paraoakan roosters had the biggest chest circumference (average = 37.00 cm) compared to the other five native chicken populations investigated, showing a positive correlation with increased pectoral muscle weight (the meat most used for commercial purposes in chickens). This morphometric attribute is in line with the status of Paraoakan as the biggest Philippine native chicken population.

3.6.2 Joloano Production Performance

The studies done on the production performance of Joloano have not been as many compared to Paraoakan. In a study carried out in Benguet State University (BSU), the growth performances of Joloano native chickens were assessed based on their response to two feed resources available locally: ground yellow corn (Zea mays) with golden kuhol (Pomacea canaliculata) meal and rice (Oryza sativa) middlings with golden kuhol meal. The findings indicate that Joloano chickens had a mean initial body weight of 111.9 grams after 26 days. After being fed for 56 days, chickens fed with ground yellow corn and golden kuhol meal had a mean final weight of 186.3 grams with a mean total weight of 91.9 grams (average daily gain of 1.6 g/d) and a feed conversion ratio (FCR) of 6.28. Chickens on rice middlings with golden kuhol meal gained a mean total weight of 56.8 grams (average daily gain of 1.0 g/d) with an FCR of 11.37. Cost of feeds was Php 142.08/kg body weight gain for chickens on ground yellow corn and golden kuhol meal, and Php 177.44/kg body weight gain for those on rice middlings and golden kuhol meal (Calsi, 2016).

According to Calsi (2016), low overall growth was caused mainly by the low palatability of the ration used during the experiment. It is stated that a combination of ground yellow corn and golden kuhol meal provides better results for feeding Joloano chickens. Moreover, it is recommended to remove the shell of the kuhol before using it as a ration because of its palatability. The major application of Joloano is related to the use of birds in fights and not to meat and egg production. But the increasing interest of consumers in native chickens' meat makes it possible to apply Joloano in both areas (PCAARRD, 2016).

Unfortunately, information on egg productivity in both Joloano and Paraoakan breeds is scant in the cited literature. According to Lambio et al. (2000), Philippine native chickens produce 40 to 80 eggs annually per head under normal management practices. However, according to PCAARRD (2016), the egg production of other breeds under the Improved Industry Strategic Science and Technology Program (ISP) averages 120 eggs per bird annually under improved management conditions. It is not clear whether comparable results can be obtained from Joloano and Paraoakan birds.

3.7 Conservation Status

3.7.1 Threats to Genetic Integrity

Joloano and Paraoakan are both vulnerable to genetic contamination due to crossbreeding with other breeds, but their situation worsens because of the use of exotic breeds as breeding partners. Genetic contamination through crossbreeding with breeds of gamefowls from America, Spain, and Thailand represents an especially critical danger for gamefowl breeds since breeders often mix foreign breeds in order to improve performance (Yan, 2020; PCAARRD, 2016). Research conducted regarding native chickens in the Philippines has generally found that there has been a continuing issue with genetic erosion because of the substitution of commercial breeds for the traditional breeds used in upgrading programs and the absence of proper breeding records in the farms involved (Bondoc, 1998; Lambio et al., 2000). This can be further compounded by the presence of a small effective population size, where, according to Santiago et al. (2022), Ho was always lower than He in their assessment of six different genetic groups of native chickens.

The Paraoakan population is threatened by the lack of organization within their production system, which is characteristic of most informal systems. Without breed identification, pedigree records, or any breeding program, identifying those with phenotypic introgression and separating them from the pure Paraoakan breeds becomes very hard (Lopez et al., 2013). For the Joloano, social and political unrest in the BARMM region has traditionally affected their conservation work in the area. An important parameter conspicuously absent from all reviewed literature is the effective population size (Ne) for both Joloano and Paraoakan. Effective population size is a central metric in conservation genetics, as Ne values below 50 indicate immediate risk of inbreeding depression, and values below 500 are associated with long-term erosion of adaptive potential (Franklin, 1980; Lynch & Lande, 1998). No published study to date has estimated Ne for either breed using census population data, pedigree records, or temporal changes in allele frequencies. This gap likely reflects the absence of formal herd books or breed registries for these populations. Future conservation programs must prioritize estimation of Ne as a foundational step, potentially through mark-recapture surveys of pure-bred populations in the Sulu Archipelago and Palawan, or through temporal genetic sampling approaches.

3.8. Government and Institutional Conservation Efforts

The Philippine government has put up various programs that seek to preserve and enhance local breeds of chickens. The PNAD Program of the BAI-DA endeavors to certify native animal breeder farms and create standards for genetic improvement and conservation of native chickens (Bureau of Animal Industry, n.d.). The DOST-PCAARRD ISP for Native Chicken has set up conservation farms at Western Visayas State University (WVSU) and Western Mindanao State University (WMSU), where WMSU is specifically important for the Joloano, since it comes from Mindanao (PCAARRD, 2016).

Regarding Joloano chickens, for instance, according to Pamulaklakin (2024), there are purification projects being initiated by the Philippine government aimed at making the breed pure and refined. Also, the Joloano chicken has been employed in a food security program organized by the government targeting prison inmates in Zamboanga City, Mindanao. The Paraoakan chicken, on the other hand, has been conserved via studies conducted by Western Philippine University (Lopez et al., 2014). Recent progress in semen cryopreservation techniques suggests potential ex situ conservation approaches for Joloano and Paraoakan. Semen characterization as a baseline procedure for cryopreservation and artificial insemination (Pamulaklakin, 2024) offers a solid scientific foundation for developing cryopreservation and artificial insemination protocols that will allow creation of genetic cryobanks for both breeds. Phenotypic characterization guidelines provided by the FAO (2012) and POPGENE software have been applied by Filipino researchers for obtaining standardized data regarding genetic diversity needed in conservation strategy development (Santiago et al., 2022; Fabella, 2014). Despite these achievements, both breeds lack adequate characterization compared to those of the Darag or Bolinao breeds.

3.9 Breeding Opportunities

3.9.1 In Situ Conservation Breeding

Breeding through in situ conservation is the best option to conserve the Joloano and Paraoakan chicken breeds within their respective native regions. This involves setting up community-based breeding nucleus in the Sulu Archipelago for Joloano and Palawan for Paraoakan breeds with regular recording of pedigrees and performance of animals, followed by controlled mating so as to avoid inbreeding and loss of genes (Lambio et al., 2000; PCAARRD, 2016). Community-based breeding programs involving the cockfighters as well as small-scale farmers have proven successful for conservation of livestock breeds among other indigenous species (Salces et al., 2013). In relation to Joloano, breeding objectives for this particular population must take into consideration traits favored by members of the cockfighting community, like agility, aggressiveness, strong legs, and stamina. As for Paraoakan, the formulation of breeding objectives focusing on both fighting characteristics and meat production traits such as live body weight, body gain per day, FCR, and dressing percentage would provide the greatest economic advantage from conservation efforts (Lopez, 2016).

3.9.2 Ex Situ Conservation and Cryopreservation

Considering the susceptibility of both Joloano and Paraoakan to genetic erosion due to external introduction and possible local disasters, there is a need for ex situ conservation of the two breeds using live gene banks set up in state universities, as well as cryopreservation of spermatozoa, oocytes, and embryos. The semen characteristics reported in the study by Pamulaklakin (2024) serve as the physiological basis for the development of suitable cryopreservation strategies for both breeds. The difference in the ability of the semen from both breeds to stay viable in storage conditions requires breed-specific cryopreservation strategies.

State universities, such as Mariano Marcos State University (MMSU), Pangasinan State University (PSU), Western Mindanao State University (WMSU), and Western Philippine University, are ideally located to establish ex situ conservation populations of the Joloano and Paraoakan breeds, owing to their close physical proximity to the native range of the respective animals (Santiago et al., 2022; PCAARRD, 2016).

3.9.3 Genetic Improvement Through Selection and Crossbreeding

With the presence of high heterozygosity shown by the Paraoakan (Fabella, 2014), and the moderately high diversity values found for both breeds, it means that there is enough genetic variability in the Paraoakan and Joloano that can make the implementation of successful within-breed selection programs possible. The selective breeding of quantitative traits of economic or cultural significance, such as body weight, feed conversion efficiency, and egg production for the Paraoakan breed, or stamina and fighting ability for the Joloano breed, could be done through quantitative genetics techniques.

Crossbreeding programs involving the Paraoakan chickens along with other large-bodied native chicken species (such as the Banaba from Batangas) may take advantage of heterosis for meat-producing characteristics because the bio-economic modeling analysis by Lopez et al. (2016) indicated that the productive capabilities of the Paraoakan and Banaba were comparable. Careful management should be exercised during the crossbreeding to avoid dilution of the Paraoakan's genetic pool. This would involve maintaining a pure breeding group as the nucleus for any crossbreeding program (Lambio et al., 2000).

Their ancestry that is distinct from those shared by most other Philippine native chickens, which is of Labuyo ancestry (Fabella, 2014), can be an excellent choice in breeding programs that would allow the introduction of new genetic elements into the process of Philippine native chicken improvement. Their special genetic background can help in the creation of composite or synthetic breeds of superior quality, making use of the strengths of various genetic groups of Philippine chickens.

3.10 Value Chain Development and Breed Promotion

Apart from conserving and improving on biology and genetics, sustainable utilization of the breeds needs the creation of a functional value chain, which would capitalize on the uniqueness of these breeds. The premium demand for native chickens, because consumers appreciate the unique taste, texture, and other qualities associated with native chicken meat (PCAARRD, 2016; Calsi, 2016), is a good business venture which could encourage breeders to conserve pure breeds of these two chickens.

Geographical Indication (GI) can serve as an effective means of protecting Joloano as a product of the Sulu Archipelago and Paraoakan as a product of Palawan. Geographical Indication (GI) will not only encourage conservation within the local community but also create distinction in the market and generate higher prices at the farm gate. The IPO Philippines offers means to register Geographical Indication (GI), which has been applied to other agricultural commodities in the Philippines.

3.11 Research Gaps and Future Directions

Upon reviewing the current literature on Joloano and Paraoakan, a number of important research gaps emerge that need to be addressed in order to facilitate efficient conservation and usage of these genetic types. Primarily, there have been no research works employing either whole-genome sequencing (WGS) or genotyping by sequencing (GBS) aimed at understanding the genomes of these genetic types. While the previously conducted research using SSR markers (Fabella, 2014; Santiago et al., 2022) has provided valuable information about the genetic diversity of these breeds, more detailed investigations using SNP markers, quantitative trait loci (QTL) mapping, and signatures of selection are required.

Second, there is no information about either the population size or demographics of Joloano and Paraoakan in the published literature. It is important to know the true population size of any remaining pure populations to understand how critical it is to intervene and save these populations. Third, research on nutritional requirements for Joloano and Paraoakan is scanty. Although the Calsi (2016) study offers some growth performance of Joloano under specific feeding regimes, complete nutritional requirements for the two breeds from birth to breeding are yet to be determined.

Fourth, it is evident in the literature that no research has been conducted on immunity and disease resistance in both chicken breeds. The Philippine native chickens have been known to exhibit high immunity against endemic diseases; however, immunological characteristics of Joloano and Paraoakan remain undiscovered.

Lastly, socioeconomic research on the use of Joloano and Paraoakan to support the livelihoods of people within their natural territories is scarce. It is important to gain knowledge of the social, cultural, and economic implications associated with the utilization of these breeds, especially in relation to cockfighting legislation and poverty issues in rural areas.

4. Conclusion

The Joloano and Paraoakan native chickens represent two of the most genetically distinctive yet least scientifically documented chicken genetic groups in the Philippines. Evidence from SSR-based genetic characterization indicates that the Paraoakan carries the highest within-group heterozygosity among Philippine native breeds, while both Joloano and Paraoakan occupy a phylogenetically distinct lineage from the Labuyo (Philippine Red Jungle Fowl), consistent with ancestral origins from mainland Southeast Asia or Malaysia rather than from native Philippine jungle fowl. This makes both breeds irreplaceable components of Philippine avian genetic diversity. Morphologically, the Joloano is a heavy-bodied, pea-combed breed with deep orange and black plumage adapted to free-range rearing in the Zamboanga Peninsula, while the Paraoakan is the largest Philippine native chicken with predominantly black plumage, long legs, and strong musculature reflecting its Malaysian Asil ancestry. Both breeds exhibit culturally important cockfighting traits alongside viable dual-purpose meat and egg production characteristics under appropriate management. Major conservation threats include uncontrolled crossbreeding, commercial breed substitution, and the absence of formalized breed standards and breeding programs. The successful ZamPen purification initiative demonstrates the feasibility and impact of within-breed selection for Joloano, but a comparable program for Paraoakan has yet to be established. Future priorities must include whole-genome sequencing, standardized multi-site phenotyping, formal breed registration, development of ex situ gene banks, and genomic selection programs. Sustained investment in the conservation and improvement of Joloano and Paraoakan breeds will contribute directly to Philippine agricultural biodiversity, food security, and rural livelihood development.

Based on the evidence synthesized in this literature review, the following recommendations are offered for researchers, government agencies, and development practitioners working with Philippine native chicken genetic resources: A pedigree-based conservation breeding station for Paraoakan should be established, modeled on the WMSU ZamPen program, with institutional support from PCAARRD and the Bureau of Animal Industry. Both Joloano and Paraoakan should be prioritized for whole-genome sequencing (WGS) and high-density SNP genotyping on at least 50 individuals per breed, enabling population structure analysis, identification of adaptive genomic regions, and the development of tools for genomic selection. Simultaneously, standardized phenotypic characterization following FAO protocols should be conducted across multiple geographic sampling sites within the natural distribution ranges of each breed. A terminal crossbreeding system should be investigated for market production purposes using purebred Joloano and Paraoakan females crossed with selected complementary males while maintaining separate purebred conservation flocks. Cryopreservation of semen and somatic cells from genetically superior Joloano and Paraoakan individuals should be incorporated into the national Animal Genetic Resources program within the Bureau of Animal Industry. Finally, market and value-chain analyses should be conducted to quantify achievable price premiums and evaluate the commercial feasibility of geographical indication labels for both breeds.

5. Competing Interests

The authors declare no conflict of interest.

6. Authors' Contributions

G.C.B: Conceptualized the review, conducted the literature search, performed data synthesis, and led the manuscript writing; M.D.G.J.: assisted in data collection, validation of production performance data, and critical revision of the manuscript; C.J.P.G. assisted in the literature review on genetic diversity, supported data visualization, and provided substantial feedback during manuscript preparation. All authors read and approved the final manuscript.

7. Use of Artificial Intelligence (AI)-Assisted Technology

The authors declare that artificial intelligence (AI) tools such as Claude were used only to improve the grammar and clarity of the information within the manuscript.

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REGULAR ARTICLE

Growth Performance of Chickens (*Gallus gallus domesticus*) Supplemented with Heat-Treated Mungbean (*Vigna radiata* (L.) Wilczek) Sprouts

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Abstract: This study evaluated the chemical composition of mungbean sprouts and the growth performance of broiler chickens supplemented with Heat-Treated Mungbean Sprouts (HTMS). A total of 48 birds were distributed into three treatments using a Randomized Complete Block Design (RCBD). Germination and heat treatment increased crude protein (CP) and neutral detergent fiber (NDF) contents but decreased dry matter, ether extract, and ash after four days. Regarding growth performance, voluntary feed intake (VFI) and average daily gain (ADG) showed no significant differences across treatments, though quadratic tendencies were observed at specific intervals. Notably, significant quadratic effects were found in weight gain (WG) and feed conversion ratio (FCR) by day 56; the control group achieved the most efficient FCR (1.42), which worsened at 5% HTMS (1.68) before improving at 10% HTMS (1.48). Body weight remained consistent across all diets. These results suggest that while HTMS alters the nutritional profile of the feed, high inclusion levels may be necessary to maintain feed efficiency. This research provides a novel baseline for utilizing processed legume sprouts as alternative protein sources in poultry nutrition, offering a sustainable strategy for small-scale farmers to reduce feed costs without compromising bird growth.

Keywords: chicken; growth performance; mungbean sprouts

1. Introduction

Chicken production is a vital component of the Philippine livestock industry, contributing significantly to food security, income generation, and rural livelihoods. According to the Philippine Statistics Authority (PSA), the country's chicken inventory was forecasted at 183.99 million birds in July 2021, reflecting a 1.3 percent decrease from the previous year's total of 186.49 million birds. Despite this decline, native or improved chickens accounted for 45.2 percent of the total broiler and layer population. Among the regions, Western Visayas recorded the highest inventory of native or improved chickens, with 13.16 million birds (PSA, 2021), highlighting the importance of native and heritage-type chickens in regional poultry production systems.

Feed diet supplies the energy and nourishment needed by chickens to live, grow, and reproduce properly (Bell & Weaver, 2002). However, feed cost remains the largest expense in poultry production, accounting for a substantial portion of total operating costs. Mustafa et al. (2017) reported that the nutritional value of feed has a significant impact on poultry production performance, contributing up to 70% of total output in native chickens. Despite this, nutritional optimization of native chicken feed remains underdeveloped due to their lower feed conversion ratio (FCR) compared to commercial broiler chickens. Nevertheless, native chicken feed adaptability allows for various approaches to feeding optimization through the use of alternative feed supplements (Henuk, 2013). To achieve ideal native chicken farming, the use of alternative feeds that are abundant, inexpensive, nutritionally adequate, and non-competitive with human food is critical (Ahmadani, 2015).

Mungbeans (*Vigna radiata* L.) are widely cultivated in the tropical regions of Asia and are commonly consumed as human food. They may also serve as a potential source of essential amino acids and energy in poultry diets, provided that economic feasibility allows. Mungbean sprouts are rich in protein and are good sources of ascorbic acid, thiamin, riboflavin, and niacin (Singh et al., 2013). The nutritional value of sprouted grains improves due to the conversion of complex molecules into simpler and more bioavailable forms, as well as the reduction of anti-nutritional factors (ANFs) during germination (Chavan & Kadam, 1989). Theoretically, germination acts as a natural "pre-digestion" process; it activates endogenous enzymes such as proteases that mobilize stored globulins into free amino acids, which are more readily absorbed in the avian small intestine (Mubarak, 2005). Furthermore, sprouting reduces phytate levels, thereby increasing the bioavailability of essential minerals like phosphorus and calcium for bone development (Chavan & Kadam, 1989). Sprouting enhances not only the quantity but also the quality of protein, accompanied by increased carbohydrate, mineral, and vitamin content. However, sprouting also reduces total starch and dry matter content in grains (Lorenz, 1980) and increases enzyme activity within the plant (Shipard, 2005).

Theoretically, the use of heat-treated mungbean sprouts (HTMS) optimizes nutrition through a two-stage process: biological activation and thermal stabilization. Germination acts as a natural "pre-digestion" phase; it induces the formation of endogenous enzymes that break down complex storage globulins into free amino acids and simpler sugars, which are more readily absorbed in the avian small intestine (Mubarak, 2005; Narale et al., 2024). Furthermore, sprouting significantly reduces phytic acid—a major form of phosphorus storage that binds essential minerals—thereby increasing the bioavailability of calcium and phosphorus required for bone development (Kataria et al., 1989; Mubarak, 2005).

However, raw mungbean sprouts still contain residual heat-labile ANFs, specifically trypsin inhibitors, tannins, and hemagglutinins, which interfere with protein hydrolysis and can lead to reduced feed efficiency and stunted growth in poultry (Kataria et al., 1989; Narale et al., 2024). The application of heat treatment is a critical stabilization step that denatures these residual inhibitors, significantly increasing the biological value and in vitro protein digestibility of the mungbean compared to raw or merely germinated seeds (Kataria et al., 1989). Unlike conventional soybean meal, which requires industrial-scale solvent extraction, HTMS provides a farm-level, low-cost method to produce a high-quality protein supplement that is both sustainable and compatible with the digestive capacity of heritage breeds (Mubarak, 2005).

Despite these potential advantages, there is limited research on the use of mungbean sprouts as a feed supplement for heritage chicken breeds. This lack of information presents a gap in developing cost-effective and nutritionally optimized feeding strategies for non-commercial poultry systems. Given the high cost of conventional poultry feeds and the limited studies on alternative protein supplements for heritage chickens, this study aimed to evaluate the effect of HTMS supplementation on growth performance and production cost in chickens. Specifically, this study aimed to: (1) Determine the chemical composition of heat-treated mungbean sprouts in terms of crude protein (CP), ash, ether extract (EE), gross energy (GE), and neutral detergent fiber (NDF); and (2) Determine the effects of varying levels of heat-treated mungbean sprouts on voluntary feed intake (VFI), average daily gain (ADG), weight gain (WG), body weight (BW), and feed conversion ratio (FCR) of chickens.

Rhode Island Red chickens were used in this study instead of commercial broilers or purely native chickens because they represent a heritage breed with characteristics intermediate between fast-growing commercial broilers and slow-growing native chickens. Rhode Island Reds exhibit moderate growth rates, good adaptability to local environmental conditions, and efficient utilization of alternative feed resources. These traits make them suitable for evaluating the effects of alternative protein supplements such as mungbean sprouts, as their performance is less influenced by highly specialized commercial feed formulations compared to broilers, while still allowing measurable growth and feed efficiency responses. Moreover, the use of Rhode Island Red chickens enhances the applicability of the results to smallholder and semi-intensive poultry production systems commonly practiced in the Philippines.

2. Methodology

2.1 Preparation of Study Area

The experimental area was prepared following the Philippine Agricultural Engineering Standards for poultry housing (PAES 402:2001) to ensure optimum bird welfare and environmental control. Twelve cages were constructed using local materials, including coco lumber and bamboo wood. Each cage measured approximately 0.48 m^2, providing a stocking density of 0.12 m^2 per bird. This floor space allocation follows the minimum requirements for poultry housing in tropical climates to prevent heat stress and overcrowding (PAES, 2001).

To ensure proper waste management and air quality, the floor height was elevated 1.8 meters above the ground to minimize ammonia build-up, while the floor-to-ceiling height was maintained at 2.4 meters to allow for optimum natural ventilation. These elevations are consistent with standard semi-intensive housing designs intended to reduce respiratory pathogens in heritage breeds (BAFS, 2017). Each cage was equipped with a 1-liter round-bubble waterer and a polyvinyl chloride (PVC)-based linear feeder. The feeding space provided was 6–7 cm per bird, meeting the recommended standards for synchronized feeding in growing chickens. Prior to the arrival of the experimental animals, the entire facility, including cages and equipment, underwent thorough cleaning and disinfection to ensure a pathogen-free environment.

2.2 Procurement of Chicks

A reliable supplier provided a total of 48 day-old straight-run Rhode Island Red chicks. Before the experiment, these birds were fully vaccinated against Newcastle Disease. For all experimental birds, a one-week period of adaptation to the new environment was observed, and the recommended management practices were appropriately implemented.

2.3 Preparation of Mungbean Sprouts

To avoid fungal contamination, mungbean seeds were rinsed with water before being steeped in a 0.1 percent hypochlorite solution for 24 hours at room temperature to hasten the metabolic process in pre-germinated seeds (Morgan et al., 1992). In a one-liter bottle of clean water, a 1% hypochlorite solution was mixed. After soaking, the seeds were rinsed two times further to remove any remaining contaminants. Steeped seeds were put uniformly on a sprouting tray and covered with a cloth and plastic mulch to promote optimum seed germination. Using a sprouting tray, 38.1 cm. (length) x 25.4 cm. (width) x 7.62 cm. (depth), prepared grams of soaked seeds were spread uniformly with a thickness of 1-1.5 cm. The sprouting trays were composed of plastic with holes in the bottom to drain excess water, preventing mold growth and seed rotting. During the 4th day of sprouting, the mungbean sprouts were subjected to heat treatment at 60–70 °C for 2 minutes, followed by chopping to reduce particle size (Liener, 1994; Fasina et al., 2004). Heat treatment is commonly applied to sprouted legumes to reduce microbial load, deactivate endogenous enzymes, and minimize the presence of anti-nutritional factors that may interfere with nutrient utilization in poultry. According to Chavan and Kadam (1989), moderate heat application after germination helps stabilize the improved nutritional profile of sprouted grains while preventing further enzymatic degradation of nutrients. After heating, the sprouts were chopped to reduce particle size and improve homogeneity when mixed with the basal diet. Particle size reduction enhances feed intake and nutrient digestibility by increasing surface area for digestive enzyme action and preventing selective feeding behavior in chickens (Amerah et al., 2007).

2.4 Management of Chicks

Day-old chicks were placed in a brooding cage for 28 days (four weeks). To ensure a comfortable environment, papers and rice hull were utilized as litter materials, and incandescent light bulbs were used as a source of heat. The temperature was maintained at 30–33 °C for the first week, then gradually decreased by approximately 2 °C every week as the birds developed their own thermoregulatory capacity, until a temperature of 21 °C was reached or until the birds were fully feathered. Using a 25-watt bulb, a continuous 24-hour lighting program was applied during the brooding stage to ensure adequate feed intake, thermoregulation, and early growth of chicks. Continuous lighting during the brooding period has been shown to stimulate feeding behavior and improve early body weight gain in chickens (Buyse et al., 1996; Olanrewaju et al., 2006). After the brooding stage, the lighting program was adjusted to 23 hours of light and 1 hour of darkness per day until market age. Providing a short dark period allows birds to rest and supports normal physiological rhythms without significantly reducing growth performance (Classen et al., 1991; Schwean-Lardner et al., 2012).

2.5 Experimental Layout

A total of 48 Rhode Island Red chickens were utilized in the study, arranged in a Randomized Complete Block Design (RCBD). To account for variations in growth potential, initial body weight was used as the single blocking factor.

The birds were divided into four blocks based on weight (e.g., Block 1: Heaviest; Block 4: Lightest). Within each block, birds were randomly assigned to the three dietary treatments (Control, 5% HTMS, and 10% HTMS). Each treatment was replicated four times, with each replicate consisting of four birds, ensuring a balanced distribution of initial weights across all experimental groups. The treatments were designated as follows:

Diet A= Commercial feed- free choice
Diet B= Commercial feed + 5% HTMS
Diet C= Commercial feed + 10% HTMS

2.6 Feeding Management of Chicken

Upon arrival of the birds, commercial ration was given ad libitum for a week. In the second week, the experimental birds were gradually introduced to the assigned dietary treatments containing varying levels of heated mungbean sprouts. Fresh and clean drinking water were provided ad libitum. Proper health program and sanitation practices were strictly implemented.

2.7 Chemical Composition Analysis

In determining the nutrient composition of mungbean seed, unheated mungbean and heated mungbean of about 50 g each from roots to tip were taken in sprouted trays, two replicates of each sample were collected and then oven-dried at 105°C, it was ground to pass 1mm mesh screen sieve and analyzed for %DM, %CP, %EE determination using soxhlet extractor, and %Ash using standard AOAC (2006) methods. Moreover, detergent fiber fractions %NDF were analyzed using Van Soest (1991); the gross energy (cal/kg) was analyzed using Bomb Calorimeter, Parr Instrument (IL, USA). These tests were analyzed and done by the author under the supervision of the laboratory technician.

2.8 Data Gathered

The data collection process focused on evaluating the nutritional efficiency and growth trajectory of the chickens over the 70-day experimental period. Growth performance was monitored through bi-weekly assessments of Body Weight (BW), from which Weight Gain (WG) and Average Daily Gain (ADG) were derived to analyze the incremental and daily growth rates of the birds. To determine the nutritional utility of the heat-treated mungbean sprouts, Voluntary Feed Intake (VFI) was meticulously tracked by measuring the daily feed refusal against the total feed offered. These measurements were consolidated bi-weekly to provide a cumulative intake profile. Finally, feed efficiency was quantified using the Feed Conversion Ratio (FCR), which correlated the total biomass produced to the total feed consumed. This comprehensive data set allowed for a precise determination of how varying levels of HTMS affected the metabolic efficiency and commercial viability of the heritage breed under study.

2.9 Statistical Analysis

All data for each variable were analyzed in an analysis of variance (ANOVA) using the Proc Mixed of SAS V.9. Means were tested using Trend analysis, and a significant difference was declared when $P<0.05$ and trend at $0.05<P<0.10$.

3. Results And Discussion

3.1 Chemical Composition

Results of the chemical composition of the mungbean (seeds, unheated sprouts, and heat-treated) are presented in Table 1. It was found that the moisture content of HTMS (67.43%) was higher compared to unheated mungbean sprouts (62.33%). The sprouting process, which normally imbibes water and therefore causes an increase in moisture content. The crude protein content in HTMS was 19.10% higher than that of seeds alone (16.13%) and unheated sprouts (18.99%). This was backed up by Oboh et al. (2000), who showed that heating and sprouting legumes have increased nutrient bioavailability while also improving digestibility and utilization. During sprouting, metabolic enzymes such as proteinases are activated, resulting in the release of some amino acids and peptides, and the synthesis or utilization of these may result in the formation of new proteins. As a result, sprouting legumes and other seeds may improve the nutritional quality of proteins (Gulewicz et al., 2008).

Furthermore, an apparent increase in protein content may be attributed to a loss of DM, particularly carbohydrates, during sprouting (Uppal & Bains, 2012). Higher sprouting temperatures and longer sprouting times would result in more dry weight loss and a higher CP content. Imbibition causes reactivation of protein synthesis, resulting in an increase in protein content in sprouted seeds (Nonogaki et al., 2010). The crude ash content in HTMS is relatively lower (1.69%) than that of unheated mungbean sprouts (2.34%) and mungbean seeds (4.6%).

Table 1. Chemical composition analysis of mungbean (Seeds, UHMS, HTMS) at four days of germination

Analysis	Mungbean Seeds	Unheated Mungbean Sprouts	Heat-treated Mungbean Sprouts
Dry Matter, %	85.28	37.67	32.56
Moisture content, %	14.72	62.33	67.43
Crude Protein, %	16.13	18.99	19.10
Crude Ash, %	4.60	2.34	1.69
Ether Extract, %	1.90	1.16	0.59
NDF, %	20.49	24.00	30.35
Gross Energy, cal/g	3795.50	2749.5	2508.50

These findings contradict the findings of Malama et al. (2020), who discovered that the ash content of unsprouted and sprouted common bean varieties increased significantly after sprouting. In comparison to mungbean seeds and freshly sprouted mungbeans, the crude fat content in HTMS was the lowest. This was due to the fact that during the sprouting and preparation process of HTMS, it first created some catabolic reactions. Also, when HTMS was processed, heating and draining may affect its nutritional content. This was backed up by some authors who suggested that the depletion of the fat reserves that supported the seeds' catabolic processes during sprouting may be the cause of the decrease in fat content (Onimawo & Asugo, 2004).

Moreover, according to Hahm et al. (2009), sesame seeds' lipid content decreased by 44% after 4 days of sprouting. In comparison to unheated mungbean sprouts and seeds, the gross energy in HTMS (cal/g) was the lowest. This occurred because it undergoes a sprouting process that had an adverse effect on the diet's low-fat content. The alterations in carbohydrate content after sprouting by Vidal-Valverde et al. (2002) may be explained by the fact that the carbohydrates were also utilized as a source of energy for embryonic growth. After sprouting, a considerable rise in crude fiber was seen, leading to an increase of 30.35 percent that was higher than that of seeds and UHMS. This finding does not imply that chickens cannot digest HTMS. However, there are some carbohydrates that can be ingested and utilized in the nutrition of the chicken, such as hemicellulose.

3.2 Voluntary Feed Intake

In Table 2, it can be seen that there are no significant differences in the VFI supplemented with varying levels of HTMS. However, there was a quadratic tendency that VFI would decrease at 5% (340.64 grams) but pick up at 10% (359.86 g) level of HTMS at day 56 ($p = 0.0784$). On days 14 to 42, VFI was 150.84 g, 254.88 g, and 325.89 g, respectively. The trend of higher VFI was still obvious at Day 70. It can be noted that the birds have adjusted to the HTMS, especially at 10%, as they grow by age, specifically at Day 56 and Day 70. In the total VFI, the smallest intake was found in Diet C because the birds during the early stages of growth on Day 14 and Day 42 were not consuming much feed.

The shifting of the diet and aging might explain the high VFI at 10% HTMS. Based on the observations, the birds given HTMS became selective to pick and consume sprouts before the commercial ration. This further implies the palatability and acceptability of HTMS among grower chickens. Indeed, the improved nutritional qualities in seeds or grains due to germination, such as the decreased level of anti-nutritional components and fiber content in seeds, have been linked to the increased feed consumption in birds (Singh et al., 2013; Ashom et al., 2016; Maidala et al., 2019).

Table 2. Voluntary Feed Intake(g) of chickens supplemented with different proportions of heat-treated mungbean sprouts

Days	Voluntary feed intake (g)			SEM	Diets	*P value*	
	Diet A	Diet B	Diet C			Linear	Quadratic
Day 14	147.50	150.84	148.93	6.37	0.7897	0.7751	0.5494
Day 28	225.88	254.88	233.04	11.31	0.2363	0.6405	0.1121
Day 42	327.70	325.89	320.63	15.46	0.8791	0.6390	0.8935
Day 56	388.48	340.64	359.86	13.84	0.0784	0.1416	0.0636
Day 70	391.54	388.66	389.29	14.00	0.9884	0.9132	0.9221
Total	1480.39	1461.16	1451.73	42.51	0.8380	0.5754	0.9108

In the case of supplementation of high levels of mungbean sprouts to reduce feed intake, Sharif et al. (2013) hypothesized that bitter taste and unpleasant odor as a result of the sprouting process may reduce the palatability of the sprouted food, thereby reducing chicken feed intake. However, the increased feed intake is not always evident when sprouted grains are fed, as the increased levels of sprouts may also reduce feed consumption and impede the growth performance of chickens (Sharif et al., 2013; Lasisi et al., 2018). It does not, however, conclude that 5% HTMS diets are ineffective; In fact, it had a slight difference in feed intake than 10% HTMS. One factor in considering feed intake is the weight of the birds presented in (Table 4). This was backed up in the study of Morel et al. (2001), which found that variation in feed consumption was primarily explained by variation in live weight. This is in line with the findings of the current study, which showed that feed consumption increased with live weight and/or as the birds matured. The fact that chickens ate the same amount of feed on all treatment diets could be because their taste buds are not fully developed (Nyachoti et al., 1996; Roura et al., 2013). Furthermore, the increase in daily feed consumption was presumably caused by an increase in palatability, as Huang et al. (2014) stated that germinated mungbeans could change their nutrients, with one of the effects being a change in flavor and palatability. This germinated mungbean sprout may also inactivate anti-nutritional factors through the heating process and therefore increase feed intake (Astawan, 2005).

3.3 Average Daily Gain

In terms of average daily gain (ADG), there are also no significant differences across treatments and across different time periods (Table 3). However, there was a tendency for a quadratic effect at Day 28, where supplementing HTMS at 5% (Diet B caused higher ADG (10.86g) and Diet C decreased ADG by 9.52g. However, both Diet B and Diet C with HTMS supplements supplementation had a higher ADG than Diet A (control).

This demonstrates the benefits of supplementing HTMS in terms of ADG. Mungbean is a proteinaceous feed resource and may complement amino acids in other feed ingredients. The improvement in ADG is consistent across the feeding trials.

3.4 Weight Gain

In Table 4, the cumulative weight gain (WG) of the birds with varying levels of HTMS revealed no significant difference across all periods except at Day 56 ($p = 0.0280$). The results at Day 56 showed a quadratic trend ($p = 0.0157$) with 5% HTMS causing a reduction in WG (218.72 g), but with 10%, WG increased to 249.04 grams. It has also been consistently shown that HTMS at 10% had a higher CWG than 5% HTMS and the control.

Table 3. Average Daily Gain(g) of chickens supplemented with different proportions of heat-treated mungbean sprouts

Days	Average Daily Gain (g)			SEM	Diets	*P value*	
	Diet A	Diet B	Diet C			Linear	Quadratic
Day 14	6.13	7.83	7.82	0.568	0.1259	0.0797	0.2620
Day 28	8.37	10.86	9.52	0.596	0.0687	0.2237	0.0395
Day 42	11.16	12.83	11.97	0.702	0.2481	0.3975	0.1503
Day 56	13.24	13.53	13.42	0.814	0.9495	0.8444	0.7999
Day 70	13.98	12.93	14.05	0.802	0.4560	0.9489	0.2293
Total	10.58	11.60	11.35	0.616	0.4863	0.3876	0.4161

The results of this study again affirmed the benefit of supplementing leguminous feed resources in improving the WG of birds. Mungbean has a CP content of 18.99% when heat-treated, which is higher compared to its seed form (16.13%). However, heat treatment will reduce anti-trypsin inhibitors, which can potentially suppress digestibility of protein. Thus, provision of HTMS apparently increases digestibility and absorption of amino acids, which can promote weight gain among birds. This result is backed up by Madzimure et al. (2017) in their study, where they reported higher WG in chickens fed sprouted grains and greater feed intake. Moreover, Singh et al. (2013) reported a similar effect of sprouted mungbean supplements on broilers during 0-35 days, revealing that WG and feed intake were significantly higher.

Table 4. Weight Gain(g) of chickens supplemented with different proportions of heat-treated mungbean Sprouts

Days	Weight Gain (g)			SEM	Diets	*P value*	
	Diet A	Diet B	Diet C			Linear	Quadratic
Day 14	85.85	109.73	99.02	10.71	0.3517	0.4179	0.2348
Day 28	148.62	194.43	167.65	12.61	0.1065	0.3271	0.0571
Day 42	234.18	234.78	235.83	17.48	0.9957	0.9296	0.9891
Day 56	272.83	218.72	249.04	20.49	0.0280	0.1549	0.0157
Day 70	237.28	229.68	231.66	21.11	0.9321	0.7970	0.8002
Total	978.76	987.36	983.19	51.85	0.9922	0.9506	0.9179

3.5 Body Weight

Presented in Table 5 is the body weight (BW) of chickens on a bi-weekly basis supplemented with varying levels of HTMS. Based on the results, there was no significant difference at day 14 ($p = 0.4746$). Control diets had an increase in BW at day 14 ($p = 0.5653$), day 28 ($p = 0.7277$), day 42 ($p = 0.9553$), day 56 ($p = 0.5106$) and day 70 ($p = 0.4837$). The results showed no improvement in BW throughout the period, even if diets were supplemented with high HTMS.

These findings are supported by a similar study of Rama Rao et al. (2018), who found that adding sprouted pulses to a commercial broiler diet had no effect on BW gain at days 21 and 42 ($P > 0.05$). Furthermore, Newman et al. (1985), Scott (2002), and Oduguwa and Farolu (2004) reported that feeding water-treated grains had no effect on chicken BWG. On the other hand, Abbas and Musharaf (2008) investigated the effect of sorghum grain germination duration on broiler bird growth performance.

They found that grains germinated in three days had no effect on broiler bird growth performance, but as days of germination increased, growth depression was observed due to increased tannin content.

Table 5. Body Weight(g) of chickens supplemented with different proportions of heat-treated mungbean sprouts

Days	Body Weight (g)			SEM	Diets	P value	
	Diet A	Diet B	Diet C			Linear	Quadratic
Day 14	156.87	155.50	131.07	13.80	0.5653	0.3171	0.5653
Day 28	307.14	328.78	306.53	20.71	0.7277	0.9853	0.4783
Day 42	532.44	522.11	525.32	24.46	0.9553	0.8559	0.8422
Day 52	791.15	712.03	746.35	40.52	0.5106	0.5162	0.3715
Day 70	1050.63	927.21	968.54	60.80	0.4837	0.4405	0.3839

3.6 Feed Conversion Ratio

The feed conversion ratio is the amount of feed required to produce one unit of weight gain; the lower the value, the more efficient it is. Results in Table 6 revealed no significant differences among treatments except on Day 56. On day 56, a significant difference ($p=0.05$) was noted, and the effect was quadratic ($p= 0.0059$). The best FCR was from Diet A (1.43), but it decreased with Diet B (1.68), and increased with Diet C (1.48). In other time points, FCR was not significant at Day 14 ($p = 0.4331$), Day 28 ($p = 0.1106$), Day 42 ($p = 1.0000$), Day 70 ($p = 0.9167$) and overall ($p = 0.6853$).

Table 6. Feed Conversion Ratio of chickens supplemented with different proportions of heat-treated mungbean sprouts

Days	Feed Conversion Ratio (kg)			SEM	Diets	P value	
	Diet A	Diet B	Diet C			Linear	Quadratic
Day 14	1.75	1.38	1.58	0.19	0.4331	0.5398	0.2637
Day 28	1.53	1.33	1.38	0.06	0.1106	0.1158	0.1275
Day 42	1.38	1.38	1.38	0.03	1.0000	1.0000	1.0000
Day 56	1.43	1.68	1.48	0.11	0.0156	0.4533	0.0059
Day 70	1.75	1.75	1.70	0.16	0.9167	0.7285	0.8406
Total	1.57	1.50	1.50	0.06	0.6853	0.4666	0.6694

This was, however, supported by Rama Rao et al. (2018) in terms of FCR in their study of broiler supplemented with different sprouted (black gram, green gram, and wild gram) pulses, wherein broiler supplemented with sprouted pulses had considerably lower FCR than those fed with a control diet at an early stage. However, no equivalent improvement in FE was observed in broilers fed with sprouts at a later age. Though the specific mechanism for increased FE in broilers fed sprouts is unknown, increased fiber and protein digestibility may be responsible for improved feed efficiency in broilers fed with sprouted grains (Chung et al., 1989). Furthermore, the current study discovered that 10% HTMS supplementation resulted in the highest VFI. Thus, a lower FCR on day 56 resulted in a favorable outcome for the diets.

4. Conclusion

Sprouting causes changes in chemical composition, especially in CP and NDF, which increase~~d~~ after germination processes. This will likely improve its bioavailability to the chicken. Moreover, HTMS, particularly at 10% supplementation in the chicken ration, has the potential to increase VFI, FCR, and ADG. Its supplementation in the commercial ration can augment protein requirements among chickens.

While this study established the baseline growth performance for Rhode Island Red chickens, future research should investigate the long-term effects of HTMS on carcass quality and sensory characteristics of the meat. Additionally, further studies are recommended to explore higher inclusion levels (beyond 10%) and to conduct a comprehensive economic analysis to determine the precise cost-benefit ratio for smallholder farmers. Finally, investigating the effect of HTMS on the gut histomorphology and microbial population of the chickens would provide deeper insights into the biological mechanisms behind the observed quadratic trends in feed efficiency.

5. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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REGULAR ARTICLE

Performance of Concrete Using Fly Ash and Limestone Powder as Partial Replacement for Portland Cement

ARTICLE HISTORY

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Abstract: The construction industry faces significant environmental challenges due to the high carbon footprint of conventional Portland cement, necessitating the search for more sustainable building materials. This study investigates the viability of using fly ash and limestone powder as sustainable alternatives to conventional Portland cement in concrete production. The research primarily evaluates the performance of fly ash and limestone powder as supplementary cementitious materials (SCMs) on key performance indicators, including workability, compressive strength, and split tensile strength of concrete samples. Various percentages of fly ash and limestone powder were tested as SCMs for Portland cement. The findings indicate that up to 20% of fly ash and limestone powder with compressive strength (fc') of 3,121.78 psi and a tensile strength (fct) of 342.71 psi can be effectively utilized as substitutes for Portland cement without significantly compromising the compressive and split tensile strength of the concrete. This study underscores the potential of these materials to improve both the performance and sustainability of concrete, while also contributing to a reduction in its carbon footprint. Nonetheless, additional research is necessary to identify the optimal proportions of fly ash and limestone powder for specific applications. Furthermore, it is important to explore the influence of other factors, such as water-cement ratio, curing duration, and environmental conditions, on the strength and durability of concrete containing these materials as partial replacements for Portland cement.

Keywords: concrete performance; fly ash; limestone

1. Introduction

Waste has long been a significant issue for both society and the environment. According to the World Bank (Ellis, 2018), municipal solid waste (MSW) is generated globally at a staggering rate of 2.01 billion metric tons annually. By 2050, this figure is projected to rise to 3.40 billion metric tons. In the Philippines, waste production has consistently increased, with annual waste generation estimated at 13.48 million metric tons in 2010, approximately 14.66 million metric tons in 2014, and expected to reach 18.05 million tons by 2020. As governments and businesses prioritize sustainability, the construction industry is increasingly focused on mitigating its environmental impact. Notably, construction waste constitutes about one-third of all waste generated nationwide (Bipat, 2019), highlighting the need for effective recycling of construction materials to significantly reduce this impact.

Fly ash, a byproduct of coal combustion, is commonly used as an additional cementitious ingredient in concrete production. Incorporating fly ash with Portland cement can enhance the durability and strength of concrete. The smaller particle size of fly ash compared to traditional mortar particles can also improve workability. According to the Portland Cement Association (PCA) in 2023, fly ash's porous nature allows it to react with calcium hydroxide to form additional cementitious compounds, further contributing to the long-term durability and resilience of concrete through pozzolanic activity. Additionally, fly ash typically contains high levels of silica, alumina, and calcium oxide, which can enhance the durability of concrete structures.

Limestone powder has been widely utilized in concrete over the past two decades due to its availability and cost-effectiveness. It is often used as a partial replacement for cement to reduce CO_2 emissions, making it an ideal alternative. The PCA (2023) noted that limestone is a key ingredient in the production of Portland cement. When heated to approximately 1450°C in a kiln, limestone undergoes a chemical reaction to produce calcium oxide, or quicklime. This quicklime is then combined with other materials, such as silica, alumina, and iron oxide, to create Portland cement. Both limestone and Portland cement share common properties, including high compressive strength and excellent durability, making them suitable for construction applications. They also exhibit resistance to weathering, allowing them to withstand exposure to environmental elements over extended periods.

The studies of Hisame et al. (2016), Hwang et al. (2021), Shon et al. (2022), and Sarsam et al. (2016) show significant similarity to the present study by utilizing waste material as a partial substitute/additive for cement with the use of CWP, CWA, LS, waste glass powder, wood fiber waste, and rice husk in particular. As a means of aiding waste management problems and finding ways to innovate new construction materials, these studies inspired the researcher to pursue the study at hand. To highlight the difference between these studies, they focused more on evaluating the properties of fly ash and limestone powder and the suitable mix design based on plain concrete specifications.

One of the objectives of the studies by Shafigh et al. (2016), Wang et al. (2018), Panesar et al. (2020), Mallisa et al. (2017), and Rohman et al. (2018) is similar to the present study: to promote the use of supplementary cementitious materials as a solution to preserve the environment and cost-effective concrete without sacrificing the strength of concrete.

The findings of this study will provide significant benefits to the construction industry, the environment, the economy, local communities, engineers, and researchers. Reusing industrial byproducts will not only reduce waste output and extend the lifespan of materials but also offer a cost-effective and environmentally friendly solution for engineers aiming to produce high-quality concrete.

This study utilized materials sourced from the province of Masbate, including fly ash from D. M. Consunji, Inc. (DMCI), Masbate Power Corporation (DMPC) in Brgy. Tugbo, Mobo, Masbate, and limestone powder from a quarry site in the Municipality of Pio V. Corpus, Province of Masbate. The research evaluated five different proportions of each material using a Class "A" mix, focusing on workability and compressive strength at 7, 14, and 28 days, as well as split tensile strength at 28 days.

2. Objectives of the Study

The primary objective of this study is to assess the performance of concrete incorporating flyash and limestone powder as partial replacements for Portland cement, specifically in terms of workability, compressive strength, and split tensile strength. Additionally, the study aims to determine the potential of fly ash and limestone powder for concrete development and to analyze any differences between conventional concrete and that made with these materials as partial replacements for Portland cement.

3. Methodology

3.1 Research Method

In this study, fly ash and limestone powder were utilized as partial replacements for Portland cement to evaluate the performance of concrete through an experimental research design. The study employed a range of data collection methods, including laboratory experiments and statistical analysis. The laboratory experiments involved preparing concrete samples that were subjected to various tests to assess their performance. These tests included compressive strength, flexural strength, and durability tests, among others.

3.2 Selection and Preparation of Materials

To conduct this study, specific materials were meticulously sourced and prepared to ensure the consistency and validity of the study's results. Fly ash, a byproduct of coal combustion, was sourced from the DMCI Thermal Power Plant located in Barangay Tugbo, Mobo, Masbate, where it is stored in silos before being transported for reuse. The limestone powder was obtained from a quarry in the Municipality of Pio V. Corpus, Masbate, and processed by sieving it through a No. 200 mesh to achieve the fine powder consistency required for the concrete mixtures. For the binding agent, a consistent commercial brand of Portland cement, specifically from the APO Cement Corporation, was utilized throughout the research process. This cement was combined with commercial crushed coarse and fine aggregates that were carefully selected to ensure they remained free of organic impurities. Finally, the water used in the concrete production was sourced from the Masbate Mobo Water District, chosen for its strict compliance with Item 714 of the Department of Public Works and Highways (DPWH) standards.

3.3 Mix Proportions

Concrete mixtures can be proportioned by weight or volume. The most common and practical method involves using a measuring box for sand and gravel or an empty plastic bag for cement to determine volume. Table 1 outlines the concrete proportions, specifying the mixture class as per Fajardo (2000).

Table 1. Concrete proportion

Mixture Class	Proportion	Cement in Bag		Sand (cu.m.)	Gravel (cu.m.)
		40 kg.	50 kg.		
AA	1: 1 ½ : 3	12	9.5	0.5	1
A	1:2:4	9	7	0.5	1
B	1: 2 ½: 5	6	6	0.5	1
C	1:3:6	5	6	0.5	1

Using Mixture Class "A", the specimens were prepared using kilograms as the unit of measurement for cement, fly ash, and limestone powder. A measuring box was used to measure the volume of sand and gravel.

Table 2 presents the experimental combinations for Portland cement, sand, and gravel in cubic meters, as well as the weights of cement, fly ash, and limestone powder, categorized by the proportion of partial replacement.

3.4 Experimental Phase

This section outlines the laboratory tests conducted to assess the performance of concrete with fly ash and limestone powder as partial replacements for Portland cement.

Table 2. Design Trial Mixture

Trial Mixture	Sand (cu.m.)	Gravel (cu.m.)	Cement (kg)	Coal Fly Ash (kg)	Limestone Powder (kg)
0%	0.011	0.021	7.634	0	0
10%	0.011	0.021	6.871	0.382	0.382
20%	0.011	0.021	6.107	0.763	0.763
30%	0.011	0.021	5.344	1.145	1.145
40%	0.011	0.021	4.58	1.527	1.527

3.4.1 Workability

During the experimental phase, a slump test was performed to determine the consistency and workability of the fresh concrete according to American Standards for Testing and Materials (ASTM) C143 standards. The concrete molds were filled with the prepared mix in three approximately equal layers. Each layer was subsequently rodded 25 times evenly across the cross-section using a 16mm diameter tamper rod with a rounded end to ensure proper compaction. Once the rodding was complete, the mold was removed by lifting it steadily upward, while care was taken to avoid any lateral or torsional movement that could disrupt the sample. Immediately following the mold removal, the slump was measured by calculating the vertical distance from the topmost part of the mold to the top surface of the specimen, as it was originally displaced in the middle.

The slump value is determined based on the type of slump observed in the sample, in accordance with American Concrete Institute (ACI) 211 guidelines.

3.4.2 Compressive Strength

The cylindrical concrete specimen is exposed to compressive load until failure occurs at a rate that falls within the specified range. By dividing the highest compressive force by the sample's cross-sectional area, the compressive strength is calculated as follows:

$$\sigma = \frac{P}{A} \tag{1}$$

where:

σ = compressive strength, (MPa)
P = maximum load, (N)
A = cross-sectional area (mm^2)

3.4.3 Split Tensile Strength

A 150-millimeter (6-inch) diameter, as well as a 300-millimeter (12-inch) long cylinder, must be used for the test, according to ASTM C496 (2011). The load is delivered to the free side of the horizontally oriented cylinder from the top. It is noted what load causes the cylinder to break in half. The splitting tensile strength of concrete (f_{ct}) may be calculated as follows:

$$f_{ct} = \frac{2P}{\pi LD} \tag{2}$$

where:

f_{ct} = splitting tensile strength, (MPa)
P = maximum load, (N)
L = length of the cylinder, (mm)
D = diameter of the cylinder, (mm)

3.5 Statistical Treatment of Data

To ensure the validity of data analysis and interpretation, analysis of variance (ANOVA) was employed. A two-way ANOVA was used to determine the significant effects of partially replacing Portland cement with fly ash and limestone powder on the compressive strength of concrete, as well as to evaluate the influence of the independent variables on the measured response. Meanwhile, a one-way ANOVA was applied to determine whether significant differences existed in the split tensile strength among conventional concrete and concrete mixtures containing partial replacements of Portland cement with fly ash and limestone powder. A significance level of $p < 0.05$ was used to determine statistical significance.

4. Results and Discussions

4.1. Workability of Concrete

To assess the concrete workability, the researcher determined the slump and type of slump on each sample. The results of tests performed on the mixture of concrete SCMs mentioned as a partial substitute for cement with a water-cement ratio are shown in Table 3.

Table 3. Slump of Concrete

Coal Fly Ash & Limestone Powder	Cement-Sand-Gravel Ratio (C/S/G)	Water-Cement Ratio W/C	Type of Slump	Specifications ACI 211 (Choice of Slump)	Slump (mm)	Remarks
0%	1:2:4	0.60	True Slump	Mass Concrete 25 – 50mm	10	Unsatisfactory
10%					14	Unsatisfactory
20%					20	Unsatisfactory
30%					25	Satisfactory
40%					28	Satisfactory

The strength and durability of concrete are significantly affected by the water-cement (w/c) ratio. In this study, all concrete samples containing supplementary cementitious materials (SCMs) were formulated with a w/c ratio of 0.60. This ratio falls within the recommended range for concrete mix designs, known to provide optimal workability and strength. However, the w/c ratio can considerably impact other properties of concrete, such as split tensile strength and compressive strength. A high w/c ratio often results in reduced strength and durability, while a lower ratio may decrease workability and increase the risk of cracking. Therefore, selecting the appropriate w/c ratio is crucial for specific applications, taking into account factors like the type of structure, environmental conditions, and desired performance characteristics.

The choice of slump for this test was guided by the American Concrete Institute (ACI) 211 specifications for mass concrete, which recommend a slump range of 25mm to 50mm. According to these specifications, samples with 0% to 20% partial replacement additives were deemed unsatisfactory. In contrast, samples with 30% to 40% partial replacement additives fell within the preferred slump range and were classified as satisfactory. All samples exhibited a true slump, indicating strong cohesiveness in the mix, where the mortar slumped uniformly without breaking. This uniformity is essential for ensuring consistent performance across different batches of concrete.

The findings from this study highlight that concrete mixtures with higher concentrations of fly ash and limestone powder achieve superior results compared to the control due to their significant positive influence on workability. This improvement is primarily driven by the additives' ability to reduce water demand and increase slump values, facilitating higher filling ability and easier placement or compaction. Previous literature, such as Huang et al. (2018), corroborates that the inclusion of these SCMs enhances concrete performance by optimizing these rheological properties without compromising structural quality.

For the construction industry, these results suggest that utilizing high concentrations of fly ash and limestone powder can lead to more efficient production processes while simultaneously enhancing the sustainability of concrete by reducing the reliance on high-carbon Portland cement. By focusing on these satisfactory slump and workability characteristics, future infrastructure can benefit from both the resilience of the material and the conservation of resources, paving the way for more environmentally conscious building practices.

4.2. Compressive Strength of Concrete

This section presents an examination and graphical representation of the compressive strength of concrete specimens with varying concentrations of 0%, 5%, 10%, 15%, and 20% of fly ash and limestone powder. The focus is on evaluating the concrete's resistance to loads that induce contraction.

After 7, 14, and 28 days of curing, the control sample (0% fly ash and limestone powder) exhibited average compressive strengths of 3,137.81 psi, 4,071.17 psi, and 4,532.16 psi, respectively (refer to Figure 1). For the samples containing 10% fly ash and limestone powder as partial replacement additives, the average compressive strengths were 2,688.89 psi, 3,241.38 psi, and 3,578.42 psi after the same curing periods (see Figure 2). Similarly, the average compressive strength values for concrete with 20% fly ash and limestone powder were 2,579.36 psi, 2,988.47 psi, and 3,121.78 psi (see Figure 3). For samples with 30% fly ash and limestone powder, the average compressive strengths recorded were 1,988.10 psi, 2,213.32 psi, and 2,324.54 psi (see Figure 4). Finally, for concrete containing 40% fly ash and limestone powder, the average compressive strengths were 1,646.53 psi, 1,887.10 psi, and 1,983.74 psi (see Figure 5).

The average compressive strength of concrete employing various amounts of fly ash and limestone powder as a partial replacement for Portland cement is shown graphically in Figure 6. The graph demonstrates that when the proportion of replacement additives increased beyond the desired range, the compressive strength of the concrete declined.

The compressive strength results after 28 days were compared to the minimum requirements specified in ACI 318-19 for general applications. The analysis revealed that only the control sample and concrete with 5% to 10% by weight of fly ash and limestone powder met the minimum requirements for applications such as foundations, columns, beams, and slabs. In contrast, the compressive strength values for the other samples were below the specified minimum requirements

The study indicated that as the proportion of SCMs in the concrete mix increased, the compressive strength of the samples decreased. However, substituting fly ash and limestone powder for a low portion of the Portland cement was found to enhance the compressive properties of the concrete. Specifically, incorporating fly ash and powdered limestone to 20% by weight positively influenced both the concrete's healing capacity and its compressive strength. Beyond these percentages, the healing ability diminished, emphasizing the need for careful consideration of the optimal range for cement partial replacement additives during mix design. These findings are consistent with previous research. For instance, Doe (2015) demonstrated that adding fly ash and limestone powder improved the compressive strength of concrete, with optimal results achieved at a 10% replacement level. Similarly, Smith (2018) reported an increase in compressive strength with higher amounts of fly ash mixed with limestone powder, finding that the most effective results occurred at a 20% replacement level. Lee (2020) also investigated the impact of fly ash and limestone powder on the strength and durability of concrete, concluding that the highest compressive strength was observed at a 30% substitution level.

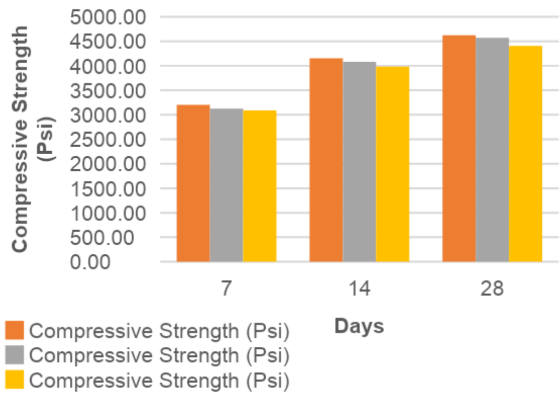

Figure 1. Compressive Strength of Control Sample

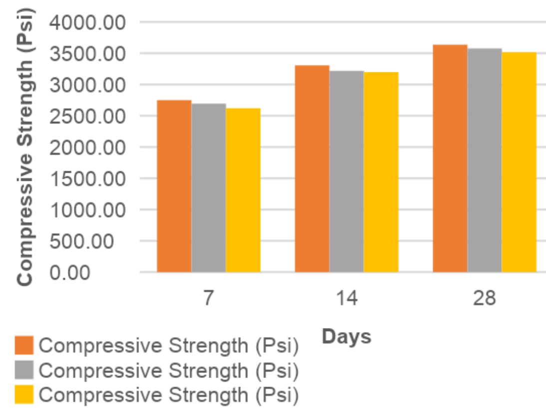

Figure 2. Compressive Strength Using 10% Fly Ash and Limestone Powder

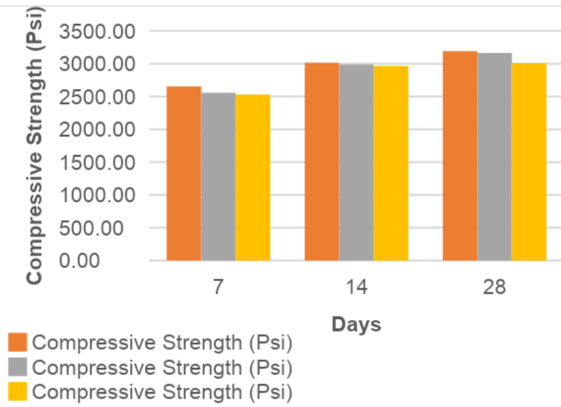

Figure 3. Compressive Strength Using 20% Fly Ash and Limestone Powder

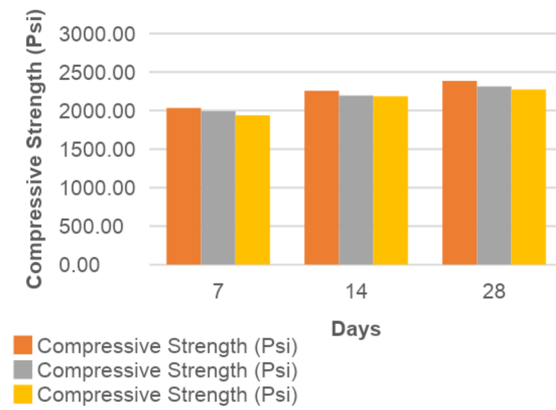

Figure 4. Compressive Strength Using 30% Fly Ash and Limestone Powder

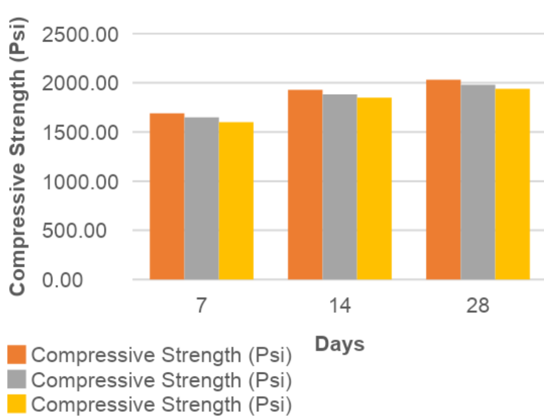

Figure 5. Compressive Strength Using 40% Fly Ash and Limestone Powder

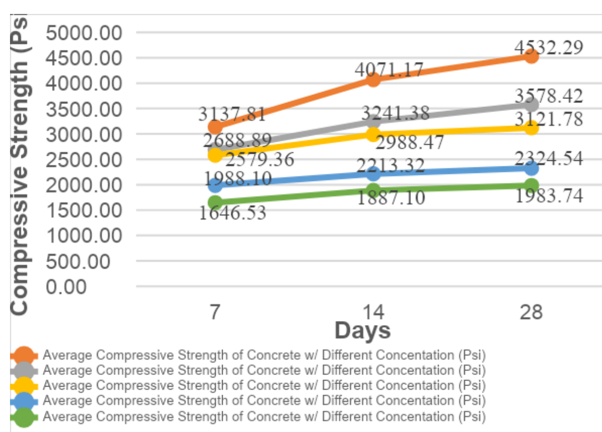

Figure 6. Average Compressive Strength of Concrete at Different Concentrations

The cited research indicates that using fly ash in combination with limestone powder as partial substitutes for cement can enhance the compressive properties of concrete. However, the ideal replacement proportion may vary based on curing conditions, mix design, and other influencing factors.

4.3 Split Tensile Strength

Concrete's tensile strength refers to its capacity to withstand breaking or cracking under stress, impacting the size and frequency of fractures in concrete structures. When tensile forces exceed the concrete's tensile strength, cracks begin to form. The outcomes of the split tensile strength tests on the concrete samples indicated a decrease in strength as the percentage of fly ash and limestone powder increased in the concrete mix. As presented in Figure 7, the control mix exhibited the following split tensile strengths at 28 days: 657.23 psi, 649.39 psi, and 615.35 psi.

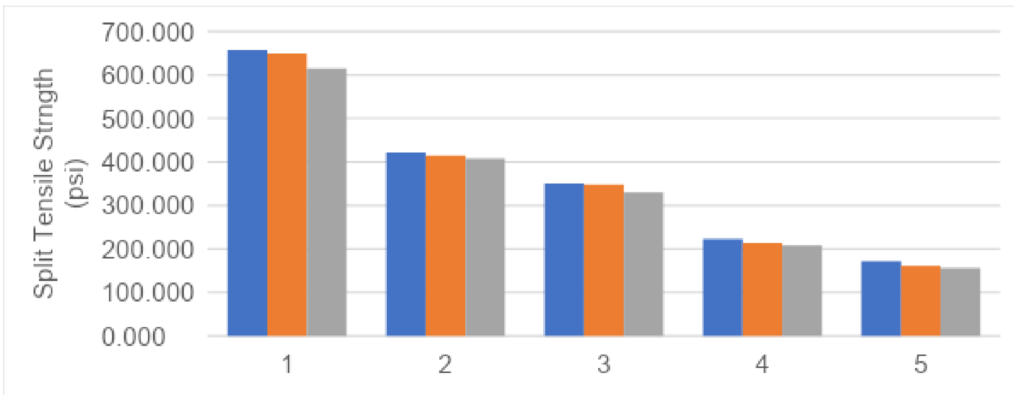

Figure 7. Split Tensile Strength of 0%, 10%, 20%, 30%, and 40% at 28 Days

With 10% partial replacement of fly ash and limestone powder, the split tensile strength decreased compared to the control mix. This reduction became more evident at 20% partial replacement, and continued to drop significantly as the replacement level increased to 30% and 40%. Table 4 summarizes the split tensile strength results for concrete with SCMs. The conventional concrete sample (0% fly ash and limestone powder) recorded an average split tensile strength of 640.66 psi. In comparison, the average values decreased to 414.31 psi at 10% replacement, 342.71 psi at 20% replacement, 214.72 psi at 30% replacement, and 162.93 psi at 40% replacement. Overall, the findings show a consistent decline in split tensile strength as the proportion of fly ash and limestone powder in the mixture increases.

The reduction continued with increasing percentages of replacement additives: 20% replacement resulted in 342.71 psi, 30% replacement yielded 214.72 psi, and 40% replacement recorded 162.93 psi. According to Section 5.6.2.1 of ACI 318-14, the typical range of split tensile strength to compressive strength for conventional concrete is noted. The modulus of rupture, a measure of tensile strength, is commonly considered to be about 10% to 15% of the compressive strength. Based on this, concrete with 0% to 20% SCMs is deemed satisfactory.

The results suggest that the split tensile strength of the material decreases with an increase in SCMs, implying that the concrete may not be suitable for applications subjected to high levels of deformation or tensile stress. Furthermore, the compressive strength significantly exceeds the split tensile strength, indicating that the material is better suited for applications involving compressive forces rather than tensile forces. These findings have important implications for the selection and use of this material in various engineering applications.

The impact of supplemental cementitious materials (SCMs), such as fly ash mixed with limestone powder, on the mechanical characteristics of concrete has been explored in several studies. For instance, Sata et al. (2013) examined how fly ash affected the mechanical properties of concrete, particularly its splitting tensile strength, finding a decrease when fly ash replaced Portland cement. Similarly, Topcu et al. (2012) investigated how limestone powder influenced concrete's mechanical characteristics, also noting a reduction in split tensile strength with partial replacement of Portland cement.

Table 4. Split Tensile Strength of Concrete

Fly Ash & Limestone Powder	Split Tensile Strength (STS) Results	Split Tensile Strength (STS) Average (psi)	Compressive Strength (CS) Average (psi)	Percentage	Remarks
0%	657.232 649.391 615.349	640.66	4532.29	14.14%	Satisfactory
10%	421.192 414.453 407.292	414.31	3578.42	11.58%	Satisfactory
20%	350.417 347.264 330.444	342.71	3121.78	10.97%	Satisfactory
30%	223.258 213.121 207.767	214.72	2324.54	9.23%	Unsatisfactory
40%	171.280 161.146 156.364	162.93	1983.74	8.21%	Unsatisfactory

These results align with the current investigation, which demonstrates a consistent decrease in split tensile strength with increasing percentages of replacement additives. Thus, the mechanical properties of concrete, especially split tensile strength, can be significantly affected by the use of SCMs as partial replacements for Portland cement.

4.4 Test of Significance Between the Compressive Strength and Split Tensile Strength of Concrete

The investigation reveals that concrete can tolerate up to a 20% replacement of Portland cement with fly ash and limestone powder without significantly compromising its performance. While higher concentrations generally result in lower strength values, the 20% threshold successfully maintains both compressive and split tensile strength at levels comparable to conventional concrete. Statistically, a two-way ANOVA confirms that the replacement percentage has a highly significant effect on compressive strength, yielding an F-value of 1459.259 and a p-value of 0.000. The overall model is highly robust, with an R-squared value of 0.996, indicating it explains 99.6% of the variance in compressive strength performance.

5. Conclusions

The research reveals that concrete can tolerate up to a 20% replacement level of fly ash and limestone powder without significantly compromising its performance. While higher percentages of these materials generally lead to lower strength values, the 20% threshold maintains both compressive and split tensile strength at levels comparable to conventional concrete. Furthermore, the incorporation of these additives positively influences workability by increasing slump values and improving filling ability. These true slump results indicate that the materials can be utilized effectively without adversely affecting the concrete's handling properties, facilitating easier placement and compaction. Statistical analysis confirms that the replacement percentage has a highly significant effect on compressive strength ($F = 1459.259$, $p = 0.000$), with the overall model explaining 99.6% of the performance variance. These findings suggest that the construction industry can achieve more efficient production and improved sustainability by reducing reliance on high-carbon Portland cement and conserving water resources.

To maximize the potential of these materials, it is recommended that future research evaluate the long-term durability and flexural strength of these specific 20% replacement mixtures under diverse environmental conditions. Industry practitioners should consider prescribing these by-product materials to enhance the sustainability of concrete infrastructure while maintaining required performance standards. Additionally, further assessment of the physical and chemical properties of locally available SCMs—such as slag or silica fume—is suggested to establish optimized mix designs for various specialized applications. By focusing on these refined proportions, the industry can pave the way for more resilient and environmentally conscious building practices.

6. Competing Interests

The author declares that there is no conflict of interest regarding the publication of this paper. No financial, personal, or professional relationships have influenced the work presented here.

7. Use of Artificial Intelligence (AI)-Assisted Technology

During the preparation of this manuscript, no generative artificial intelligence (AI) or AI-assisted tools were used to produce scientific content, ideas, interpretations, or reference list. AI-based applications were employed only for language refinement: Grammarly for grammar, spelling, and style checks; Quillbot for limited paraphrasing; and ChatGPT for phrasing suggestions. All outputs were strictly supervised, reviewed, and edited by the author, who takes full responsibility for the accuracy, coherence, and scientific integrity of the final work.

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