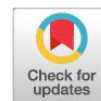


Research Article



The Utilization of Brem Waste in Feed on Growth Performance, Internal, and External Quality of Laying Hens Eggs

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Abstract | This study evaluated the effects of dietary inclusion of brem waste, a byproduct of fermented glutinous rice on growth performance and egg quality in laying hens. Ninety-six Lohmann Brown hens (26 weeks old) were randomly assigned to three dietary treatments, particularly P0 (100% commercial layer feed), P1 (95% layer feed + 5% brem waste), and P2 (90% layer feed + 10% brem waste) for 8 weeks. Each treatment consisted of four replicates of 8 hens housed in battery cages with *ad libitum* access to feed and water. Growth performance, feed intake, feed conversion ratio (FCR), and internal and external egg quality parameters, including Haugh unit (HU), yolk color, yolk index, albumen index, egg weight, egg shape index, shell weight, and shell thickness were carefully measured. Results indicated that supplementation with 10% brem waste significantly increased feed intake and improved egg quality as indicated by higher HU values compared to the control (P0) ($P < 0.001$). No significant differences were observed among treatments for body weight gain, FCR, egg weight, egg shape index, shell quality, yolk characteristics, or albumen index, implies that 10% inclusion did not adversely affect growth performance and egg quality. This study highlights the potential of brem waste as a cost-effective and sustainable feed ingredient for poultry, in a maximum inclusion of 10% (P2) contributing to waste valorization and feed cost reduction in commercial egg production systems. Further investigation into nutrient digestibility and long-term impacts is warranted.

Keywords | Brem waste; Laying hens; Egg quality; Growth performance; Lohmann brown

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INTRODUCTION

Laying hen production represents a vital and rapidly expanding sector in animal protein supply, addressing the growing nutritional demands of human populations. Eggs are highly valued for their rich nutrient profile (Zhao *et al.*, 2021) and serve as an accessible, cost-effective source of high-quality animal protein (Sari *et al.*, 2012). As a key livestock product, eggs significantly contribute to improving dietary protein adequacy at the community level (Mine, 2002). On the other hand, feed accounting for

approximately 70% to 80% of total production costs, and is a critical determinant of productivity in poultry farming. Therefore, optimizing feed formulation and exploring alternative feed resources are essential to enhancing production efficiency and economic sustainability. In this context, the valorisation of nutrient-rich, non-toxic agricultural by products as feed supplements presents a promising strategy to reduce reliance on conventional commercial feeds while maintaining or improving poultry performance. Moreover, advancements in feed technology aimed at protecting nutrients during gastrointestinal transit

warrant further investigation to maximize feed utilization (Dewanti *et al.*, 2024).

Brem waste, a byproduct derived from the traditional production of brem, a fermented *Oryza sativa var. glutinosa* snack native to Java, Indonesia presents a promising but underexplored feed resource. Brem production typically yields about 75% of edible products, leaving approximately 25% as residual waste. Study on chemical characterization of brem waste reveals substantial nutritional value, with crude protein content of 17.13%, moisture at 18.63%, ash at 0.97%, fat at 0.83%, and crude fiber at 5.54%. Additionally, its fresh aroma may enhance feed palatability, an important factor influencing voluntary feed intake in poultry. Despite its evident nutritive potential, utilization of brem waste has been largely restricted to ruminant diets, with minimal application in poultry feeding strategies. Notably, there exists a significant knowledge gap concerning the effects of dietary brem waste on laying hen performance metrics, including growth, egg production, and qualitative egg traits which are critical parameters for commercial viability and consumer acceptance (Lv *et al.*, 2022).

The Lohman Brown laying hen, developed by Lankabel *et al.* (2022), is a globally recognized breed prized for its consistent growth performance, high egg yield, and adaptability to diverse management systems (Habashy *et al.*, 2023). Investigating the integration of brem waste into the diet of Lohman Brown hens could yield valuable insights into sustainable feed resource management by linking local waste valorization with enhanced poultry productivity. This study aims to systematically evaluate the impact of graded levels of brem waste supplementation on the growth performance, egg production, and internal and external egg quality of Lohman Brown laying hens. The findings are expected to inform feed formulation practices, reduce feed costs, promote environmental sustainability by valorizing agro-industrial residues, and ultimately support the advancement of sustainable poultry production systems in Indonesia and comparable agro-ecological regions.

MATERIALS AND METHODS

BREM WASTE PREPARATION

Brem waste was collected as a byproduct from traditional brem production in the Wonogiri region of Indonesia. Brem is a fermented food produced by subjecting steamed glutinous rice to natural microbial fermentation for six days, yielding tape as the intermediate product. Subsequently, the tape is mechanically pressed to separate liquid fractions and solid residues, with the latter constituting brem waste (Figure 1). This waste was thoroughly sun-dried under controlled conditions until reaching consistent moisture content to prevent spoilage. The dried material was then milled using a mechanical grinder into a fine powder suitable for feed

inclusion. The basal commercial layer feed, sourced from PT. Japfa Comfeed Indonesia, served as the control diet. Nutrient profiles for the basal feed and experimental diet formulations incorporating brem waste are comprehensively documented in Tables 1 and 2, respectively.

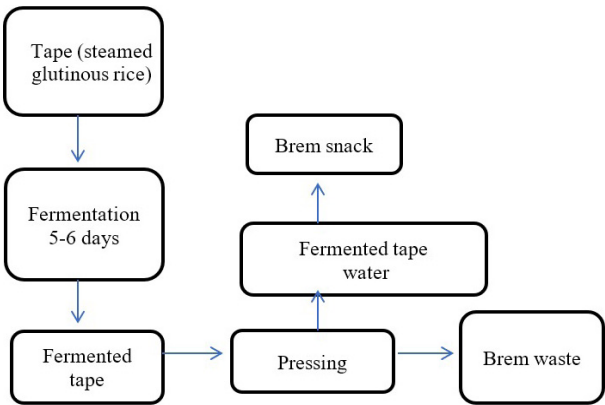


Figure 1: Production process of brem, a traditional fermented product made from glutinous rice.

Table 1: Nutrient content of commercial layer feed and brem waste.

Nutrient	Feed	
	Commercial layer feed ²	Brem waste ³
ME ¹ (kcal/kg)	2804.6	3456.17
Crude protein (%)	17	17.13
Crude fibre (%)	8	5.54
Crude fat (%)	3	0.83
Ash (%)	15	0.97
Calcium (%)	3.6	1.8
Available phosphor (%)	0.4	0.75

¹ME, Metabolizable energy (3951 + (54.4 x crude fat) – (88.7 x crude fiber), (40 x Ash) (Sibbald *et al.*, 1980). ²The Commercial feed was purchased from PT. Japfa Comfeed Indonesia. ³Laboratorium Uji Teknologi Pangan dan Hasil Pertanian, Universitas Gadjah Mada, Indonesia

Table 2: Percentage and nutrient content of feed.

Nutrient content	Treatment (%)		
	P0	P1	P2
Commercial layer feed	100%	95%	90%
Brem waste	-	5%	10%
Sum	100%	100%	100%
ME (Kcal/kg)	2804.6	2837.17	2869.75
Crude protein (%)	17	17	17.01
Crude fiber (%)	8	7.87	7.75
Crude fat (%)	3	2.89	2.78
Ash (%)	15	14.29	13.59
Calcium (%)	3.6	3.51	3.42
Available phosphor (%)	0.4	0.41	0.43

Control (P0, 100% commercial layer feed), P1 (95% commercial layer feed + 5% brem waste), and P2 (90% commercial layer feed + 10% brem waste)

ANIMALS, HOUSING, AND MANAGEMENT

The experimental cohort comprised ninety-six Lohmann Brown laying hens, 26 weeks old, with uniform initial body weights averaging 1.9 ± 0.05 kg. Hens were previously vaccinated according to a standardized pullet vaccination program including CORYZA (week 14), ND-IB-EDS K (week 15), AI PLUS (week 16), a second CORYZA dose (week 18), and ND KILL (week 20). To support health and resilience, vitamin supplementation was administered via feed during housing transitions and pre/post vaccination periods. Birds were individually housed in battery cages constructed of galvanized iron wire, dimensioned at $32 \times 19 \times 35$ cm (length $\times$ width $\times$ height), allowing precise individual feed intake and egg production measurement. Cage sterilization was regularly performed using Rodalon® disinfectant (PT Pyridam Farma Tbk, Indonesia) to maintain hygiene and minimize disease risks. Feeders composed of split 5-inch pipes with individual partitions ensured exclusive access per bird, minimizing feed competition. Drinking water was provided via nipple drinkers, each servicing two adjacent hens to optimize water use efficiency. Lighting conditions were standardized using a 40-watt incandescent bulb, operated daily from 1700 to 2200 hours, simulating dusk to extend productive activity.

EXPERIMENTAL DESIGN AND DIETARY TREATMENT

A completely randomized design (CRD) with three dietary treatments was utilized to robustly evaluate the effects of brew waste inclusion on laying hens. All experimental procedures were carried out in line with international animal ethical standard. Specifically, the dietary treatments included control (P0, 100% commercial layer feed), P1 (95% commercial layer feed + 5% brew waste), and P2 (90% commercial layer feed + 10% brew waste). Each treatment was replicated four times, with 8 hens per replicate (total $n=32$ per treatment). A 7-day acclimation phase preceded the 60-day experimental period, initiating at 26 weeks of age. Water and feed were provided *ad libitum* throughout, permitting *ad libitatem* consumption patterns. Daily egg collection and individual feed intake monitoring allowed precise performance assessment. Diet formulations were isocaloric and isonitrogenous across treatments to isolate the effect of brew waste supplementation.

GROWTH PERFORMANCE

Growth performance monitoring included daily feed intake per bird (g), individual egg weight (g), Hen-Day Average (HDA, %), and feed conversion ratio (FCR). HDA was calculated as the percentage ratio of eggs laid per number of hens present daily. Meanwhile, the FCR was computed as feed intake divided by the product of egg number and average egg weight, expressing feed efficiency. All weighing procedures employed precision digital scales

with appropriate resolution: 0.01 g sensitivity for feed and eggs (capacity 500 g) and 1 g sensitivity for live weight (capacity 5 kg), ensuring accuracy and reproducibility of data.

EGG QUALITY

Egg quality evaluation were conducted at 28 and 32 weeks of age, corresponding with critical stages in the laying cycle when production exceeded 20%. Individual eggs were carefully coded to ensure sample traceability throughout the analysis. External egg quality parameters included the egg shape index, calculated as the ratio of egg width to egg length using a vernier caliper with 0.01 mm accuracy, following the methodology of Mehmet *et al* (2012). Eggshell weight was determined by manually separating the shell from the egg contents, washing and sun-drying the shells to a constant weight prior to precise measurement.

For internal quality, the yolk index was assessed by measuring the yolk height and diameter on eggs cracked onto a flat glass surface, using a vernier caliper according to standardized protocols (Roberts, 2004; Yuwanta, 2010). Yolk color was evaluated visually using the Roche yolk color fan (Hoffmann-La Roche Ltd., Basel, Switzerland), providing a semi-quantitative score aligned with industry standards (Tang *et al.*, 2015). The albumen index was determined by the ratio of the thick albumen height to its diameter, measured with a depth micrometer for height and vernier caliper for diameter, reflecting albumen quality. Yolk weight was obtained by separating the yolk and albumen using an egg yolk separator and subsequently weighing the yolk with a precision scale.

Egg freshness and overall quality were further quantified by calculating the Haugh unit (HU), an objective indicator that correlates albumen height and egg weight. The HU was calculated using the formula:

$$HU \text{ value} = 100 \text{ Log } (H + 7,57 - 1,7 W^{0,37})$$

Where H represents albumen height in millimeters and W is egg weight in grams (Tang *et al.*, 2015; Banibugari *et al.*, 2024). All measurements were performed under standardized laboratory conditions using calibrated instruments to ensure accuracy, reproducibility, and minimize measurement bias.

STATISTICAL ANALYSIS

Data collected from growth performance and egg quality measurements were subjected to statistical analysis using analysis of variance (ANOVA) appropriate for a completely randomized design (CRD). Prior to analysis, data were checked for normality and homogeneity of variance to satisfy ANOVA assumptions. When

significant differences were detected ($P<0.05$), means were compared using Duncan's multiple range test to identify specific pairwise treatment differences. Statistical analyses were conducted using standard software packages with a significance threshold set at $\alpha=0.05$. Results are presented as means $\pm$ standard error of the mean (SEM) to reflect the precision and variability within treatments. This rigorous analytical approach ensured reliable interpretation of the effects of dietary brem waste inclusion levels on laying hen performance and egg quality parameters.

RESULTS AND DISCUSSION

The present investigation rigorously evaluated the impact of dietary inclusion of brem waste on both growth performance and egg quality parameters in Lohmann Brown laying hens and results were presented in Table 3. This study revealed that supplementing diets with brem waste at incremental levels of 5% and 10% resulted in a statistically significant enhancement of feed intake, ranging from 118.56 to 119.32 g per bird per day. This elevation in voluntary feed consumption is plausibly linked to the complex array of aromatic and volatile compounds generated during the traditional fermentation process inherent to brem production, which contributes to improved palatability. Given that sensory attributes such as olfactory and gustatory cues play a pivotal role in modulating feed acceptance and appetite in poultry, these findings are congruent with existing literature emphasizing the critical influence of feed aroma and taste on voluntary intake and subsequent production efficiency (Azizi *et al.*, 2018; Banibugari *et al.*, 2024; Barido *et al.*, 2024). Notably, despite this increased feed consumption, intake remained firmly within physiological norms established for laying hens aged between 20 and 80 weeks (NRC, 1994), indicating that brem waste inclusion did not adversely affect satiety mechanisms, intestinal health, or digestive function.

In parallel, key production indices, including HDA, average egg weight, and FCR, were maintained across treatment groups without significant deviation from control values (Table 3). The HDA values ranged from 94.03% to 95.69%, reflecting consistent and optimal reproductive performance. These results reinforce the concept that breeding uniformity and standardized management practices preserve egg production stability in the face of dietary manipulation (Habashy *et al.*, 2023; Lee *et al.*, 2023). Concurrently, egg weights between 59.81 and 59.82 g closely align with industry benchmarks for the Lohmann Brown strain (USDA, 2000; Azizi *et al.*, 2018), indicating that essential nutrients, particularly metabolizable energy and indispensable amino acids were sufficiently supplied despite partial substitution of conventional feed ingredients

with brem waste. The FCR remained efficiently stable at 2.11 to 2.14, comparable with well-documented feed utilization parameters for commercial laying hens (Milenia *et al.*, 2022). This sustained feed efficiency may be partially explained by the fermentative nature of brem waste, which potentially enhances nutrient bioavailability, reduces anti-nutritional factors, and promotes digestive enzyme efficacy, thereby optimizing pasture lipid and protein assimilation (Guo *et al.*, 2021).

Table 3: Performance and external and internal egg quality of laying hens fed brem waste.

Treatments	P0	P1	P2	SEM	P value
Feed intake (g/bird/d)	118.56 ^b	119.23 ^a	119.32 ^a	0.11	0.013
Egg weight (g/egg)	59.82	59.81	59.82	0.009	0.061
Feed conversion ratio	2.11	2.13	2.14	0.01	0.941
Egg index	79.00	78.25	76.25	0.73	0.079
Hen day average (HDA)	94.03	95.69	94.43	0.48	0.740
Shell weight	6.03	5.62	5.96	0.09	0.250
Yolk index	0.44	0.46	0.49	0.01	0.219
Yolk color	8.25	7.86	7.92	0.12	0.018
Albumen index	0.11	0.14	0.14	0.01	0.332
Yolk weight (g/egg)	14.03	12.61	13.36	0.28	0.435
Haugh unit (HU)	95.02 ^b	95.06 ^b	95.90 ^a	0.14	0.383

^{a-b} Mean with different superscript indicates significant effect within the same row, influenced by feeding treatment ($p<0.05$). Control (P0, 100% commercial layer feed), P1 (95% commercial layer feed + 5% brem waste), and P2 (90% commercial layer feed + 10% brem waste).

Investigation of external egg quality parameters revealed that supplementation with brem waste had no discernible impact on egg shape index ranging from 76% to 79% eggshell weight (maintained at 5 to 6 g), or gross shell morphology (Table 3). These findings suggest preserved eggshell biomineralization processes, which are dependent on adequate dietary provision and intestinal absorption of calcium, phosphorus, magnesium, and trace minerals, as well as efficient transport and deposition mechanisms within the shell gland (Rodehutscord *et al.*, 2023). The maintenance of eggshell quality ensures structural integrity is important for mechanical protection during handling and maximizes shelf-life stability. However, the absence of direct measurements related to mineral bioavailability and transporter protein expression constitutes a limitation in fully elucidating the subtle physiological impacts of brem waste on shell formation.

Internal egg quality parameters which comprise of yolk index (0.44–0.49), albumen index (0.11–0.14), and yolk weight (12.61–14.03 g) were similarly unaffected by dietary

treatments and remain within established physiological norms (Yuwanta, 2010). Preservation of albumen quality is indicative of sufficient protein digestion and utilization, facilitating appropriate synthesis of key structural proteins such as ovomucin, which governs albumen viscosity and gel strength crucial for egg freshness (Tang *et al.*, 2015). The stable yolk index further reflects maintenance of the osmotic equilibrium between yolk and albumen, preventing yolk flattening or membrane rupture that would compromise egg integrity during storage.

Contrastingly, yolk coloration was notably paler (7.86–8.25) compared to consumer-preferred standards, a phenomenon attributable to the paucity of carotenoids and xanthophyll pigments in the brem waste substrate. Given the critical importance of these pigments including lutein, zeaxanthin, and β -carotene in yolk pigmentation as well as their antioxidative properties that benefit both poultry and human health, this finding underscores an essential area for nutritional optimization via targeted supplementation with pigment-rich feed ingredients (Minieri *et al.*, 2016; Eggersdorfer, 2018).

Of particular interest, eggs from brem waste-supplemented hens demonstrated significantly elevated HU values, ranging between 95.02 and 95.90 (Table 3), which categorize these eggs as AA quality according to standardized freshness criteria. HU is a robust and objective indicator of albumen height and protein structural integrity, strongly correlating with egg freshness and consumer acceptability (Jones, 2006). The improvement in HU suggests that brem waste contains bioactive constituents or induces physiological enhancements that improve function of the magnum the oviductal region responsible for albumen secretion and may augment protein synthesis and secretion efficiency. This superior albumen quality surpasses comparative data reported for similar commercial strains, highlighting a beneficial effect of brem waste inclusion that warrants further biochemical and molecular investigations (Abbasabadi *et al.*, 2024; Lu *et al.*, 2023).

The collective enhancement in feed intake and preservation of egg quality attributes observed herein may be mechanistically underpinned by the nutrient-rich profile of brem waste combined with its fermented matrix, which has been shown to facilitate improved digestibility and beneficial modulation of the gut microbiota, thereby supporting systemic metabolic homeostasis and health in poultry (Azizi *et al.*, 2018; Banibugari *et al.*, 2024). Eventually, this study propose a principal strength in its comprehensive evaluation, integrating both productive performance metrics and detailed internal and external egg quality assessments under rigorously controlled experimental conditions using a widely utilized commercial laying strain. Nonetheless, limitations include the absence

of direct quantification of nutrient digestibility coefficients, gut microbial population dynamics, and evaluation of long-term hen health and welfare metrics, which collectively constrain full mechanistic elucidation of the observed phenomena. Future research should incorporate multi-omics methodologies encompassing metagenomics, metabolomics, and transcriptomics to dissect the complex interactions between brem waste feeding, gastrointestinal physiology, and systemic metabolic regulation. Parallel investigations into pigment-rich additive supplementation are imperative to rectify yolk pigmentation deficits and align product quality with consumer expectations.

CONCLUSION AND RECOMMENDATIONS

The incorporation of brem waste at inclusion levels up to 10% in the diets of Lohmann Brown laying hens effectively enhances feed intake without compromising growth performance, reproductive efficiency, or feed conversion. Importantly, brem waste supplementation maintains external egg quality parameters such as eggshell integrity and egg shape, while preserving internal egg quality metrics including yolk index, albumen index, and yolk weight. The significant improvement observed in Haugh unit values indicates enhanced albumen freshness and protein integrity, suggesting that brem waste may confer functional benefits beyond mere nutrient replacement. However, the paler yolk color highlights the need for targeted pigment supplementation in future diet formulations. Overall, these findings substantiate the potential of brem waste as a sustainable, nutritionally viable, and economically attractive alternative feed ingredient, contributing to circular agricultural systems and reducing feed costs in commercial poultry production.

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NOVELTY STATEMENT

This study represents the first comprehensive investigation into the application of brem waste, a fermented glutinous rice byproduct as a partial feed ingredient in diets for laying hens (Lohmann Brown), demonstrating its ability to sustain both production performance and egg quality parameters. Notably, the research uncovered a significant

enhancement in albumen quality, reflected by increased Haugh unit values, indicating functional benefits of brew waste supplementation beyond basic nutritional replacement. Furthermore, the findings highlight the role of fermentation-derived agro-industrial byproducts in improving feed palatability and stimulating voluntary feed intake, thereby offering an innovative and sustainable strategy for cost-effective poultry nutrition. While brew waste inclusion was associated with paler yolk coloration, this observation opens a unique avenue for future research focused on targeted pigment enrichment to reconcile local feed resource utilization with consumer quality expectations. Collectively, this work advances the concept of circular economy in animal production by valorizing traditional food processing residues and provides novel insights into sustainable waste-to-feed conversion pathways within commercial poultry systems.

AUTHOR'S CONTRIBUTION

All authors discussed the results and contributed to the final manuscript. RD: Conceptualization, supervision, funding acquisition, data curation, investigation, writing-original draft, review and editing. R, HS: Data curation, methodology, investigation. FHB, MC: Supervision, data curation writing-original draft, review and editing.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

This manuscript was prepared with assistance from advanced AI language models to enhance readability. AI contributions were limited to linguistic refinements and stylistic improvements while preserving all original scientific content, data, methodology, results, and authorial intent. The authors assume full responsibility for the accuracy, integrity, and ethical compliance of the document, in accordance with prevailing journal policies on AI use.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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