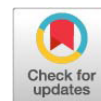


Research Article



Effects of Alternating Probiotic and Phytobiotic Combinations on Production Efficiency and Egg Quality in Mojosari Laying Ducks

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Abstract | This study evaluated the effects of dietary probiotic and phytobiotic combinations on production performance and egg quality in Mojosari laying ducks aged 40 to 44 weeks. A completely randomized design (CRD) was applied with four treatments: T0 (control), T1 (0.25%), T2 (0.50%), and T3 (0.75%), each replicated five times with ten ducks per replicate ($n = 200$). Two alternating formulations were applied: A yellow blend (turmeric and ginger) and a green blend (sambiloto, moringa, and papaya leaves), both combined with fermentative microbes. Key performance indicators as well as internal and external egg quality traits were evaluated. Supplementation significantly influenced several parameters, notably shell thickness at the middle, albumen weight, yolk volume, feed intake, hen-day production, and feed conversion ratio ($p < 0.05$). The 0.50% (T2) supplementation exhibited the overall best performance, increasing albumen weight (+5.6%), feed intake (+1.9%), and egg production (+5.9%), while improving feed efficiency through a 6.7% reduction in FCR compared with the control (T0). Multivariate analysis confirmed strong positive associations among egg mass, egg volume, albumen weight, and shell weight, while feed efficiency showed a negative correlation with productivity. In conclusion, alternating probiotic–phytobiotic supplementation at 0.50% enhanced production efficiency without compromising egg quality. This approach offers a sustainable, natural strategy to improve productivity in laying ducks while reducing reliance on synthetic additives.

Keywords | Egg quality, Laying ducks, Phytobiotics, Probiotics, Production performance, Mojosari duck

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INTRODUCTION

Improving the productivity and egg quality of poultry, particularly in laying ducks such as the Mojosari ducks breed, remains a key priority in modern animal husbandry. Mojosari duck is Indonesia's premier egg-laying native breed, notable for its distinctive colored eggs, prolific performance, and resilience in local farming systems (Hidanah *et al.*, 2018). In response to increasing consumer demand for high-quality, antibiotic-free poultry

products, there has been a growing interest in natural, alternative nutritional strategies. One promising approach is the dietary use of combined phytobiotics and probiotics, collectively referred to as synbiotics (Kismiati *et al.*, 2022). These combinations are believed to provide synergistic benefits by enhancing gut health, improving feed efficiency, and supporting overall production and egg quality.

Phytobiotics are plant-derived bioactive compounds, including those found in turmeric (*Curcuma xanthorrhiza*,

Curcuma domestica), ginger (*Zingiber officinale*), sambiloto (*Andrographis paniculata*), moringa (*Moringa oleifera*), and papaya leaves (*Carica papaya*), which exhibit anti-inflammatory, antimicrobial, and immunomodulatory properties. Meanwhile, probiotics such as *Lactobacillus*, *Bifidobacterium*, and *Actinomycetes* are known to enhance the balance of gut microbiota and improve nutrient absorption. Previous studies have shown that the combined application of phytobiotics and probiotics can positively influence production performance, intestinal morphology, and the reduction of pathogenic bacteria in poultry (Jasim *et al.*, 2024; Ren *et al.*, 2019; Wishna-Kadawarage *et al.*, 2024, 2025) and also improved the laying ducks egg weight (Nuningtyas *et al.*, 2024). However, increasing concerns over antibiotic residues, the development of antimicrobial resistance, environmental sustainability, and shifting consumer preference toward natural and safe food sources have motivated the poultry industry to reduce the use of synthetic additives and antibiotics in feed.

Accordingly, natural feed additives such as synbiotics offer a strategic alternative that promotes animal productivity while aligning with public health and sustainability goals. However, physiological responses to these combinations are highly dependent on the dosage, type of active compounds, as well as the duration and pattern of administration. Some reports indicate that excessive levels of phytobiotics or probiotics may lead to adverse effects such as metabolic disturbances or reduced feed palatability (Rao *et al.*, 2020; Roshanravan *et al.*, 2024). Therefore, careful formulation and optimized delivery are essential to achieve the desired benefits without compromising physiological balance in ducks.

This study aimed to evaluate the effects of dietary supplementation with a weekly alternating combination of phytobiotics and probiotics in Mojosari laying ducks. The feed additive consisted of yellow and green formulation. Specifically, the study assessed the impact of different inclusion levels on performance parameters, feed efficiency, and both internal and external egg quality traits. In addition, multivariate analyses such as Principal Component Analysis (PCA) and correlation mapping were employed to identify individual variability and potential trade-offs between productivity and egg quality traits. The findings are expected to provide a scientific basis for developing functional feed strategies that support sustainable productivity in the laying duck industry.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN AND ANIMAL MANAGEMENT

This study employed a completely randomized design (CRD) with four dietary treatments with five replicates per

treatment. Each experimental unit consisted of ten female Mojosari ducks aged 40–44 weeks, yielding a total of 200 birds. The experiment was conducted under controlled environmental conditions, with standard management practices for housing, feeding, and health maintained throughout the study period. The ducks were reared in an open-house system with an ambient temperature ranging between 26–29 °C and relative humidity of 70–75%, while lighting was provided for 16 hours per day to support optimal laying activity. All experimental procedures were conducted following ethical standards. Ethical approval for this study was granted by the Research Ethics Committee of Brawijaya University (Approval No. 060-KEP-UB-2023). The study did not involve any invasive procedures or modifications in animal handling.

DIETARY TREATMENTS

Basal diet was shown in the Table 1. The feed ingredients were formulated according the nutrient requirements of laying ducks. The feeding treatments were provided continuously for 8 weeks. The experimental treatments were formulated by supplementing the basal diet with different inclusion levels of a phytobiotic–probiotic combination, administered weekly in alternating color-coded formulations:

- T0 (Control): Basal diet without additive
- T1: Basal diet + 0.25% phytobiotic–probiotic combination
- T2: Basal diet + 0.50% phytobiotic–probiotic combination
- T3: Basal diet + 0.75% phytobiotic–probiotic combination

Table 1: Nutrient composition of basal diet.

Nutrient	Content
Moisture (max)	14.00%
Ash (max)	14.00%
Crude protein (min)	18.00%
Crude fat (min)	3.00%
Crude fiber (max)	10.00%
Calcium	2.90–4.25%
Phosphorus	0.45%
Lysine (min)	0.90%
Methionine (min)	0.40%

Max: maximum; min: minimum

The feed additive consisted of two alternating phytobiotic–probiotic formulations. The yellow formulation contained turmeric (*Curcuma xanthorrhiza* and *Curcuma domestica*) and ginger (*Zingiber officinale*), combined with a consortium of functional microbes including *Actinomycetes*, lactic acid bacteria, and photosynthetic bacteria, along with fermentation agents such as *Rhizopus* spp. and *Aspergillus* spp. The green formulation comprised sambiloto (*Andrographis paniculata*), moringa (*Moringa oleifera*), and papaya leaves (*Carica papaya*), utilizing the same microbial

consortium as the yellow formulation. These additives were incorporated into the feed according to the designated treatment percentages.

MEASURED PARAMETERS

To assess the effects of dietary treatments on laying performance and egg quality, both internal and external egg characteristics were measured, along with key production metrics. Egg quality evaluations were conducted using standardized protocols to ensure accuracy and repeatability. External quality parameters included shell integrity, egg mass, and density-related indices, while internal quality focused on yolk and albumen properties. Performance indicators such as feed intake, egg production rate, and feed efficiency were also recorded throughout the study. The parameters observed in this study were grouped into three categories, namely external egg quality, internal egg quality, and production performance. The description and measurement methods of each parameter are summarized in Table 2.

Haugh Unit (HU): Calculated based on albumen height and egg weight using the standard Haugh equation:

$$HU = 100 \times \log_{10}(H - 1.7W^{0.37} + 7.6)$$

Where H is the albumen height (mm) and W is the egg weight (g) (Erek and Matur, 2024).

STATISTICAL ANALYSIS

All data were analyzed using R Studio software version 4.4.3. Analysis of variance (ANOVA) was performed in accordance with the Complete Random Design (CRD) structure to determine the effect of dietary treatments on all variables using ANOVA function. When significant differences were detected ($p < 0.05$), means were separated using Duncan's Multiple Range Test (DMRT) function from the agricolae package. Prior to hypothesis testing, model assumptions were evaluated by assessing the normality of residuals using the Shapiro-Wilk test (shapiro test function).

Table 2: Description and measurement methods of egg quality and production performance parameters in Mojosari laying ducks.

Category	Parameter	Code	Measurement description	Unit
External egg quality	Specific Gravity	SG	Measured using either a salt solution gradient method or a digital densimeter.	g/cm ³
	Shell Weight	SW	Recorded after drying the shell at a standardized temperature using a precision digital scale.	g
	Shell Percentage	SP	Calculated as the ratio of shell weight to total egg weight, expressed as a percentage.	%
	Egg Volume	EV	Determined by water displacement using a graduated cylinder.	cm ³
	Shell Thickness (Top)	STT	Measured at the apical end of the egg using a digital micrometer.	mm
	Shell Thickness (Middle)	STM	Measured at the equator of the egg.	mm
	Shell Thickness (Bottom)	STB	Measured at the basal end of the egg; the mean of STT, STM, and STB represented the average shell thickness.	mm
Internal egg quality	Yolk Weight	YW	Measured with a digital scale after separation from albumen and shell.	g
	Albumen Weight	AW	Recorded using a precision scale after separation from yolk and shell.	g
	Yolk Volume	YV	Measured by transferring the yolk into a graduated cylinder.	cm ³
	Albumen Volume	AV	Determined using the same volumetric technique.	cm ³
	Yolk Height	YH	Measured with a digital caliper or micrometer on a flat surface.	mm
	Albumen Height	AH	Measured with a micrometer at the thickest point of the thick albumen.	mm
	Haugh Unit	HU	Calculated using the formula $HU = 100 \times \log_{10}(h - 1.7w^{0.37} + 7.6)$, where h is albumen height (mm) and w is egg weight (g).	unit
Production performance	Feed Intake	FI	Recorded daily on a per-bird basis throughout the experimental period.	g/hen/day
	Hen-Day Production	HDP	Calculated as the ratio of the number of eggs produced to the number of laying hens per day, expressed as a percentage.	%
	Feed Conversion Ratio	FCR	Determined as the amount of feed consumed per unit of egg mass produced.	g feed/g egg
	Egg Mass	EM	Computed as (hen-day egg production $\times$ average egg weight)/100.	g/hen/day

and testing for homogeneity with Levene's test from the car package. Pearson correlation analysis was conducted to evaluate the relationships among egg quality traits. Furthermore, Principal Component Analysis (PCA) was performed to explore multivariate patterns and treatment proximities based on the observed variables used prcomp function. The PCA loadings, scree plot, and biplot visualizations were produced using the factoextra package and correlation plot produced using ggally package.

RESULTS

Table 3 presents the effects of probiotic and phytobiotic combination supplementation on various parameters related to production efficiency and egg quality in Mojosari laying ducks. The treatments consisted of a control group (T0) and three supplementation levels: T1 (0.25%), T2 (0.50%), and T3 (0.75%). Parameters evaluated include external and internal egg quality traits as well as key performance indicators. The data are expressed as mean $\pm$ standard deviation, and significant differences among treatments.

The dietary inclusion of combined probiotics and

phytobiotics at various levels exerted a differential effect on several egg quality and performance parameters in Mojosari ducks. Statistical analysis revealed significant differences ($p < 0.05$) in shell thickness at the middle (STM), albumen weight (AW), yolk volume (YV), feed intake (FI), hen-day egg production (HDP), and feed conversion ratio (FCR). Notably, shell thickness middle (STM) was significantly lower in groups T1 (0.27 ± 0.05 mm) and T2 (0.27 ± 0.05 mm) compared to the control T0 (0.32 ± 0.1 mm), while AW was significantly higher in T2 (40.70 ± 3.75 g) than in the other treatments. Yolk volume was highest in T0 (24.63 ± 1.91 cm³) and lowest in T1, with T2 and T3 showing intermediate values, indicating a non-linear response to the treatment levels. Feed intake was significantly elevated in T2 (179.34 ± 1.66 g/hen/day), suggesting improved palatability or nutrient utilization, whereas T3 recorded the lowest intake (174.49 ± 7.14 g/hen/day). Hen-day egg production significantly increased in all treated groups (T1–T3) compared to the control, suggesting a positive impact of the additive combination on laying performance. Additionally, FCR was significantly improved (i.e., reduced) in T1–T3, with the most efficient conversion observed in T2, highlighting its potential to optimize productivity. Although several traits (STM, FI, HDP)

Table 3: Effect of using probiotic and phytobiotic combinations on production efficiency and egg quality in Mojosari laying ducks.

Trait	Units	T0 (Mean $\pm$ SD)	T1 (Mean $\pm$ SD)	T2 (Mean $\pm$ SD)	T3 (Mean $\pm$ SD)	P (ANOVA)	P (Levene)	P (Shapiro)
SG	g/cm ³	1.08 $\pm$ 0.01	1.08 $\pm$ 0.02	1.07 $\pm$ 0.01	1.08 $\pm$ 0.01	0.28	0.34	0.05
SW	g	10.21 $\pm$ 0.76	9.93 $\pm$ 1.06	10.07 $\pm$ 0.59	10.12 $\pm$ 0.65	0.69	0.27	0.18
SP	%	13.52 $\pm$ 0.89	13.42 $\pm$ 0.91	13.28 $\pm$ 1.15	13.59 $\pm$ 0.90	0.73	0.51	0.01
EV	cm ³	70.60 $\pm$ 4.28	68.75 $\pm$ 4.98	71.25 $\pm$ 5.82	69.33 $\pm$ 5.45	0.14	0.50	0.04
STT	mm	0.34 $\pm$ 0.1	0.31 $\pm$ 0.05	0.32 $\pm$ 0.10	0.33 $\pm$ 0.08	0.68	0.05	0.28
STM	mm	0.32 $\pm$ 0.1 ^a	0.27 $\pm$ 0.05 ^b	0.27 $\pm$ 0.05 ^b	0.32 $\pm$ 0.08 ^{ab}	0.00	0.25	0.00
STB	mm	0.32 $\pm$ 0.09	0.31 $\pm$ 0.08	0.32 $\pm$ 0.08	0.31 $\pm$ 0.08	0.95	0.99	0.03
YW	g	24.92 $\pm$ 1.52	24.03 $\pm$ 1.76	24.39 $\pm$ 1.47	24.84 $\pm$ 1.86	0.20	0.75	0.61
AW	g	38.54 $\pm$ 3.35 ^b	38.57 $\pm$ 2.78 ^b	40.70 $\pm$ 3.75 ^a	38.4 $\pm$ 3.94 ^b	0.02	0.39	0.34
YV	cm ³	24.63 $\pm$ 1.91 ^a	23.13 $\pm$ 1.79 ^b	23.63 $\pm$ 1.56 ^{ab}	24.13 $\pm$ 2.03 ^{ab}	0.03	0.82	0.42
AV	cm ³	37.39 $\pm$ 4.35	37.90 $\pm$ 2.73	39.55 $\pm$ 3.63	37.68 $\pm$ 4	0.09	0.39	0.81
YH	mm	18.87 $\pm$ 1.08	18.76 $\pm$ 0.9	18.91 $\pm$ 0.81	18.65 $\pm$ 0.76	0.74	0.69	0.60
AH	mm	5.59 $\pm$ 0.79	5.26 $\pm$ 0.92	5.28 $\pm$ 1.20	4.85 $\pm$ 1.06	0.10	0.94	0.05
HU		0.34 $\pm$ 0.1	0.31 $\pm$ 0.05	0.32 $\pm$ 0.10	0.33 $\pm$ 0.08	0.11	0.63	0.00
FI	g/hen/day	176.00 $\pm$ 6.07 ^{ab}	176.37 $\pm$ 4.23 ^{ab}	179.34 $\pm$ 1.6 ^{6a}	174.49 $\pm$ 7.1 ^{4b}	0.03	0.02	0.00
HDP	%	88.86 $\pm$ 9.72 ^b	95.14 $\pm$ 7.00 ^a	94.14 $\pm$ 5.52 ^a	94.86 $\pm$ 5.61 ^a	0.01	0.02	0.00
FCR		2.70 $\pm$ 0.30 ^a	2.54 $\pm$ 0.2 ^b	2.52 $\pm$ 0.20 ^b	2.48 $\pm$ 0.26 ^b	0.01	0.24	0.47
EM	g/hen/day	74.66 $\pm$ 3.98	73.40 $\pm$ 3.98	75.90 $\pm$ 5.18	74.66 $\pm$ 4.83	0.13	0.39	0.02

Note: Superscript letters on the rows indicate significant difference ($p < 0.05$) between the means. SG: Specific Gravity, SW: Shell Weight, SP: Shell Percentage, EV: Egg Volume, STT: Shell Thickness Top, STM: Shell Thickness Middle, and STB: Shell Thickness Bottom. Internal egg quality parameters consist of YW: Yolk Weight, AW: Albumen Weight, YV: Yolk Volume, AV: Albumen Volume, YH: Yolk Height, AH: Albumen Height, and HU: Haugh Unit. Performance parameters assessed include FI: Feed Intake, HDP: Hen-Day Production, FCR: Feed Conversion Ratio, and EM: Egg Mass.

showed significant treatment effects, their ANOVA assumptions were not fully met, particularly for normality and homogeneity ($p < 0.05$). These violations imply that some degree of bias is possible in the reported p-values, even though the overall mean patterns remain biologically consistent. This limitation should be acknowledged in the interpretation of the findings.

Conversely, no statistically significant differences ($p > 0.05$) were observed among treatments in parameters such as specific gravity (SG), shell weight (SW), egg volume (EV), yolk width (YW), albumen volume (AV), yolk height (YH), albumen height (AH), Haugh unit (HU), and egg mass (EM). These findings indicate that while the supplementation influenced certain productive and economic traits, the structural and compositional aspects of the eggs remained largely unaffected.

Multivariate analysis using Principal Component Analysis (PCA) and Pearson correlation was conducted to investigate the interrelationships among the measured traits and to identify the major contributors to variation. The scree plot indicated that the first two principal components (PC1 and PC2) accounted for 66.3% of the total variation, with PC1 explaining 41.8% and PC2 contributing 24.5% (Figure 1).

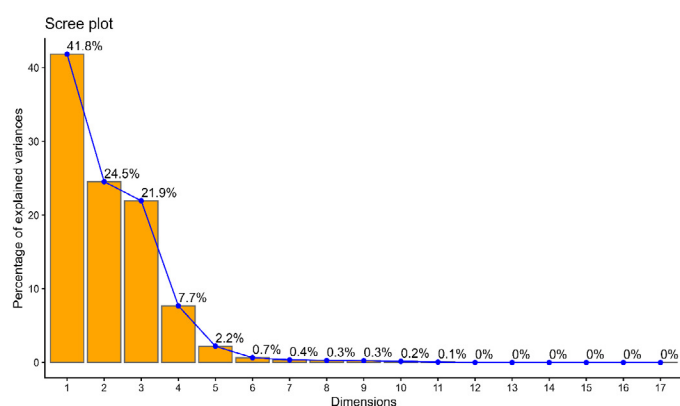


Figure 1: Scree plot showing the percentage of explained variance for each principal component. PC1 and PC2 accounted for the largest proportion of variation and were therefore selected for biplot interpretation.

The biplot on Figure 2 revealed that egg mass (EM), egg volume (EV), albumen width (AW), and shell weight (SW) were closely associated, forming a distinct cluster with similar vector directions, suggesting strong positive intercorrelations among these productivity traits. In contrast, Haugh unit (HU) exhibited an opposite vector orientation, indicating a potential trade-off or negative relationship with the mentioned variables. Feed intake (FI) and feed conversion ratio (FCR) clustered closely, while PC1 was largely driven by HU and PC2 by HDP and FI, capturing distinct gradients related to internal egg quality and production–feeding dynamics. Thus, while

HDP and FI showed clear treatment effects consistent with their strong contributions in PC2, HU demonstrated the opposite pattern, exhibiting no significant treatment effect in ANOVA yet dominating PC1 due to large individual variation independent of the dietary treatments. Biologically, this trend indicates that HU does not respond to the phytobiotic–probiotic supplementation in the same way as production traits, because HU is primarily determined by albumen protein deposition rather than utilization of energy for laying performance. Ducks receiving phytobiotic–probiotic supplementation may prioritize nutrient partitioning toward yolk development and egg mass rather than albumen thickening, leading to improved EM and EV without a corresponding increase in HU. Although the feed treatment was effective in elevating productivity, the formulation did not specifically target metabolic pathways associated with albumen protein synthesis, resulting in high individual variability in HU that is not directly driven by the feed additive levels.

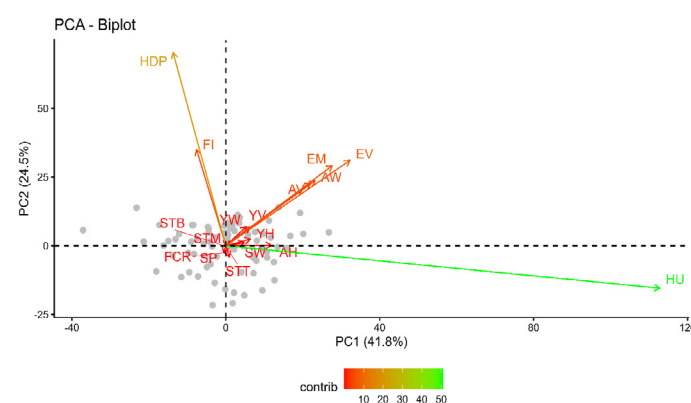


Figure 2: Principal Component Analysis (PCA) biplot based on the first two principal components (Dim1 and Dim2), illustrating the distribution of individuals (grey dots) and the contribution of each variable (arrows) to the observed variation. Longer arrows indicate greater influence of variables, and direction reflects their correlation with the principal components.

The individual factor map showed partial but observable separation among the treatment groups (T0–T3), with group T3 demonstrating the widest distribution along PC1 and PC2 (Figure 3). Interestingly, although T3 exhibited broader coverage in the biplot space, the directionality of its distribution was more centralized toward the positively correlated trait cluster (EM, EV, AW), indicating that the T3 treatment may have enhanced uniformity across productive traits. This pattern suggests a favorable treatment effect in terms of aligning individual performance within a more consistent physiological range. The PCA findings complemented the Pearson correlation results by integrating pairwise relationships into broader multivariate patterns. Traits with strong positive correlations ($r > 0.84$), such as EV, EM, AW, and SW, were projected along the

same PCA axis, confirming their shared contribution to overall productivity. In contrast, HU and AH showed a negative correlation trend, forming a separate internal quality dimension. This coherence between the two analyses reinforces the conclusion that productivity and egg quality traits are biologically related yet regulated by distinct physiological mechanisms.

Pearson correlation analysis on Figure 4 supported these findings, showing strong positive correlations between key production traits, including AV–AW ($r = 0.944, p < 0.001$), EV–EM ($r = 0.845, p < 0.001$), and AW–EM ($r = 0.855, p < 0.001$). FCR was negatively correlated with EV, AW, and EM ($p < 0.001$), underscoring the negative relationship between feed efficiency and production output. HU showed only moderate correlation with albumen height ($r = 0.602$), aligning with its role as a quality indicator rather than a production trait. These results demonstrate that the probiotic–phytobiotic treatments, particularly in T3, not only improved production traits but also contributed to greater trait uniformity, as evidenced by the tighter trait clustering and reduced multidimensional spread.

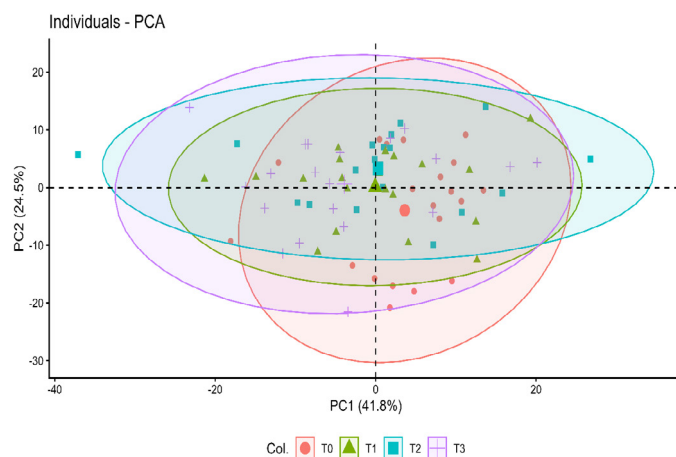


Figure 3: Principal Component Analysis (PCA) plot of individuals based on the first two dimensions (Dim1 and Dim2), which explain 41.8% and 24.5% of the total variation, respectively. Individuals are grouped by categories (P0, P1, P2, P3) and represented with different colors and shapes. Ellipses represent the 95% confidence interval for each group, showing the spread and overlap of individuals within and among groups.

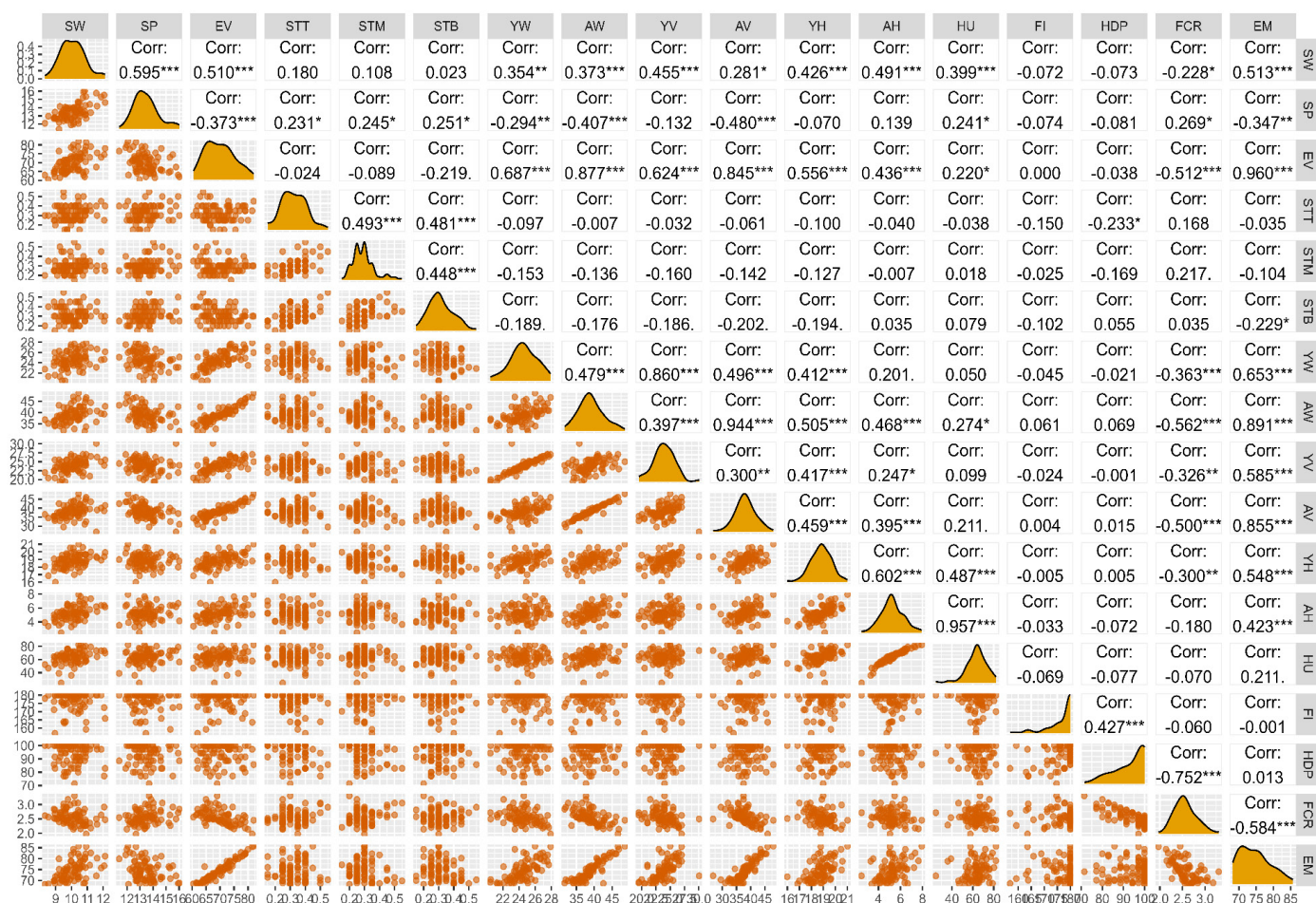


Figure 4: Scatterplot matrix showing pairwise correlation coefficients above the diagonal, variable distributions along the main diagonal, and data dispersion patterns below the diagonal. A single asterisk (*) indicates a significant relationship at the 0.05 level; double asterisks (**) indicate highly significant relationships at the 0.01 level; and triple asterisks (***) indicate very highly significant relationships at the 0.001 level.

The dietary inclusion of combined probiotics and phytobiotics at varying concentrations elicited differential responses across key egg production and quality parameters in Mojosari ducks. The experimental design employed a unique weekly alternation of two color-coded phytobiotic–probiotic formulations: a yellow formulation (containing turmeric *Curcuma xanthorrhiza*, *Curcuma domestica*, and ginger *Zingiber officinale*) and a green formulation (comprising sambiloto *Andrographis paniculata*, moringa *Moringa oleifera*, and papaya leaves *Carica papaya*). The improvement in overall performance is consistent with the known roles of phytobiotics and probiotics in supporting gut health and nutrient utilization, although the present study focused on production responses rather than underlying physiological mechanisms (Gadde *et al.*, 2018; Alagawany *et al.*, 2022).

These physiological mechanisms may partially explain the dose-dependent responses observed in this study, although additional factors such as individual variability and baseline health status of the ducks may also contribute. However, higher dosages may yield diminishing returns or unintended interactions, as supported by prior studies suggesting the need for optimized inclusion rates to balance efficacy and safety (Jasim *et al.*, 2024; Ren *et al.*, 2019; Roshanravan *et al.*, 2024; Wishna-Kadawarage *et al.*, 2025). This alternation might have contributed to reducing microbial habituation, potentially supporting microbial diversity and resilience, which could influence enzymatic adaptation and immune-related pathways, although these mechanisms were not directly measured in this study. The absence of immune, enzyme, or intestinal morphology measurements constitutes a limitation of the present work, and future research incorporating histological, microbiome, or metabolomic analyses is needed to verify the putative biological mechanisms. The observed improvement in average egg weight (AW) at the 0.50% level (T2) suggests that this dosage achieved an optimal synergy between phytochemical stimulation and microbial activity. Additionally, the concurrent enhancements in feed intake (FI) and feed conversion ratio (FCR) further support the hypothesis of improved digestive and metabolic efficiency under balanced bioactive provision. Therefore, T2 appears to represent a nutritionally effective and economically feasible dose for application in laying duck production systems.

Yolk volume (YV) exhibited a non-linear response to the dietary treatments, being highest in the control group (T0) and lowest in the low-dose combination group (T1). Such patterns likely reflect biological adjustments in nutrient partitioning rather than detrimental effects, as internal

egg quality traits remained within acceptable commercial ranges across treatments. Despite the modulations observed in yolk volume and shell thickness, other internal egg quality parameters including yolk weight (YW), albumen volume (AV), yolk height (YH), albumen height (AH), Haugh unit (HU), shell weight (SW), egg volume (EV), and embryo mass (EM) remained statistically unaffected. This suggests that the additives did not impair the physiological processes responsible for albumen and yolk formation. From an industry perspective, this stability is important because nutritional interventions should not compromise basic egg quality traits. This stability suggests that the phytobiotics–probiotics, even when administered at low levels, maintained compatibility with essential reproductive and metabolic processes involved in egg formation. Prior findings have emphasized that synbiotic supplementation can support albumen quality, yolk pigmentation, and cholesterol regulation without compromising egg structural integrity (Saleh *et al.*, 2024; Salem and El-Dayem, 2024; Youssef *et al.*, 2013). Moreover, the ability of probiotics to maintain intestinal health and enhance nutrient absorption may underpin the observed resilience in internal quality traits (Youssef *et al.*, 2013). This indicates that although the additives influenced certain performance indicators and specific traits like YV or STM, they did not compromise the overall biological function of the egg. These findings may have practical relevance for commercial application, as they suggest that phytobiotic–probiotic combinations can be used without obvious negative impacts on key egg quality traits under the tested conditions.

PCA successfully grouped EV, EM, AW, and SW into a cohesive productivity cluster, while HU was positioned in the opposite vector direction, reflecting a potential trade-off between production output and internal quality. More specifically, treatments that promoted higher egg mass and volume tended to be associated with lower HU scores, although HU values remained within acceptable commercial quality thresholds (>72).

Quantitatively, this means that increases in productivity do not necessarily translate into thicker albumen, and high-producing ducks may allocate nutrients more toward yolk and total egg mass rather than albumen deposition (Onbaşlar and Özkaya, 2011). Thus, the trade-off is biologically relevant but economically manageable as long as HU stays in the premium/grade-A range for consumer standards. This pattern aligns with previous findings that PCA can synthesize multiple egg quality traits into interpretable composite indices, as demonstrated in Chinese native duck breeds where 65.32% of variation was explained by PCA components (Bing-Xue *et al.*, 2013). The greater variability observed in T3 further indicates

that excessively high inclusion rates may generate inconsistent physiological responses, reinforcing the need for precise dosing. Correlation analysis further supports this, showing strong positive relationships between productivity traits (e.g., AW–AV, EM–AW) and a negative association between FCR and EV, EM, and AW, confirming enhanced feed efficiency is tightly coupled with performance, in agreement with probiotic and phytobiotic effects reported in prior studies (Li *et al.*, 2011; Natsir *et al.*, 2019). Additionally, the moderate correlation between HU and AH ($r = 0.602$) indicates that HU may act as a semi-independent internal quality marker, not necessarily aligned with productivity gains, supporting findings that highlight differential responses of internal egg quality to dietary interventions (Özek *et al.*, 2012; Rao *et al.*, 2020; Sandi *et al.*, 2022a). Overall, these results illustrate how PCA can be a useful tool for detecting potential patterns of individual variation and trade-offs in performance and quality traits, though the biological interpretation should be made. This multivariate approach is especially valuable in interpreting biologically heterogeneous responses that may be masked in univariate comparisons.

The current study found that the 0.50% inclusion level of combined phytobiotics and probiotics in Mojosari laying ducks yielded the most favorable outcomes across feed efficiency, productivity, and internal egg quality. This aligns with previous reports where such combinations improved feed conversion ratios and nutrient utilization through gut microbiota modulation and enzymatic enhancement (Kismiati *et al.*, 2022; Rao *et al.*, 2020; Sandi *et al.*, 2022b). Although HU did not show significant treatment effects, productivity-related traits consistently improved at the 0.50% level, supporting the conclusion that moderate supplementation is preferable to higher doses. Practically, this dose has potential for adoption by feed manufacturers and duck farmers because it increases egg mass and feed efficiency without compromising commercial egg quality standards. Collectively, these findings suggest that moderate-level supplementation strikes a functional balance between phytochemical stimulation and microbial synergy, making it a promising strategy to optimize both physiological and economic performance in laying ducks.

CONCLUSION

This study demonstrates that the weekly alternating supplementation of combined phytobiotics and probiotics can positively influence production performance and feed efficiency in Mojosari laying ducks. The 0.50% inclusion level was identified as the most effective dosage, leading to improvements in average egg weight, feed conversion ratio, and overall productivity, while maintaining the stability of both internal and external egg quality traits.

Although reductions in yolk volume and shell thickness were observed at certain levels, these changes did not compromise the structural integrity or biological function of the eggs. The overall pattern indicates that key production traits such as egg mass, egg volume, albumen width, and shell weight were strongly and positively associated, forming a coherent performance cluster. These traits showed an negative relationship with Haugh unit, suggesting a potential trade-off between productivity and internal egg quality. Among all treatments, T2 was most closely aligned with the productive trait cluster and showed improved uniformity in individual responses, indicating its potential as a balanced supplementation strategy for enhancing production while maintaining physiological consistency. Overall, these findings support the use of phytobiotic–probiotic combinations as a functional nutritional strategy to enhance sustainable productivity in laying ducks without adverse effects on egg quality. This study provides a scientific basis for the development of natural feed additives in modern duck farming systems.

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NOVELTY STATEMENT

This study introduces a novel alternating supplementation strategy combining probiotics with two different phyto-biotic formulations derived from local herbs (turmeric, ginger, sambiloto, moringa, and papaya leaves) in Mojosari laying ducks. Unlike previous studies that used single or fixed combinations, this approach applies alternating herbal and microbial blends to enhance productivity. The research also integrates production performance, egg quality evaluation, and multivariate analysis, demonstrating that 0.50% supplementation improves feed efficiency and egg production, offering a sustainable natural alternative to synthetic feed additives.

AUTHOR CONTRIBUTIONS

Yuli Frita Nuningtyas and Pornsin Keanthao: Conceptualization, manuscript writing, and critical revision. Muhammad Halim Natsir, Heli Tistiana, and Eko Widodo: Investigation, supervision and funding acquisition. Rizal Rinaldi: Statistical analysis and data interpretation.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

STATEMENT OF CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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