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Original Article

NUTRITIONAL AND ENVIRONMENTAL FACTORS INFLUENCING EGG PRODUCTION IN POULTRY

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ABSTRACT

Researchers in the field of agricultural science have been investigating the effects of nutrition and environmental factors on poultry egg production, especially the diet formulation, housing system, photoperiod, manipulation of gut microbial community and stress adaptation. We housed 1,200 Hy-Line Brown laying hens in six groups with three housing systems (conventional cage, enriched colony cage and free-range) and two diets (basal diet and probiotic-supplemented diet at 1×10^9 CFU/kg) for a 12-month production period. The Ensemble Stacked Model (ESM) was the most accurate of the nine models compared (composite weighted score = 93.59, $R^2 = 0.947$ for hen-day egg production). Free-range and probiotic supplementation resulted in the highest egg mass (62.3 ± 1.8 g/hen-day), lowest feed conversion ratio (1.82 ± 0.05) and highest cecal microbial Shannon diversity index ($H' = 3.87 \pm 0.21$). Egg production was 34% reduced and abundance of the phylum, Proteobacteria, increased (9% to 18%, $p < 0.001$) when plasma levels of the stress hormone, corticosterone, were higher than 8 ng/mL. The optimal diet was a metabolizable energy (2.85 kcal/g) and crude protein (16.5%) formulation, and the optimal dose of the probiotic had a hormetic U-shaped response: 1×10^9 CFU/kg. Structural equation modelling revealed the gut microbial diversity accounted for 52% of the total effect of housing system on egg production (indirect effect = 0.21, 95% CI: 0.17-0.25). This research shows that precision nutrition approaches tailored to the stressors of the housing system, to promote eubiosis in the cecal microbiota, increase long-term egg production and efficiency of feed conversion ratio (FCR) and egg quality of commercial layer hens.

INTRODUCTION

Egg demand is anticipated to rise worldwide, which demands efficient understanding of the interaction of various genetic and environmental factors on egg production and quality of hens (Alaraji, 2024). This review focuses on nutritional and environmental factors, such as feed, lighting and housing systems, that are important to productivity and egg quality of poultry (Nissa et al., 2023). Specifically, housing systems (cage-free and conventional) have been shown to be of great importance for various traits of egg production and performance of hens (Alig et al., 2023a, 2023b). Housing systems, such as conventional cages, enriched cages, and free-range affect egg weight, feed intake and feed efficiency, as well as the trade-off between animal welfare and economic aspects (Hamada, 2018; Zaheer, 2015). In addition, increasing consumer awareness of animal welfare issues also influences their purchasing decisions, with many consumers taking into account animal welfare issues in their food choices, which in turn affects the market for eggs from various housing systems (Özentürk & Uysal, 2024; Petrovič & Mellen, 2023). To alleviate the negative effects of some housing systems, improvements are continually made to poultry housing enrichment to enhance animal welfare, productivity and immunity (El-Sabrout et al., 2022; Tainika & Şekeroğlu, 2021). For instance, cage systems have traditionally been the ideal housing system for white egg layers, but other housing systems, such as enriched cages, have been continuously modified to enhance egg quality and prevent downgrading (Alig et al., 2023; Travel et al., 2010). However, these systems can bring other management challenges and impact on the microbiota of laying hens, which poses a dilemma for productivity and health (Pires et al., 2025). However, some brown egg layers have superior production traits when housed in free-range systems compared to colony cages, indicating genotype-environment interaction (Alig et al., 2023). This suggests more research is needed to identify the optimal type of pasture or vegetation to improve the welfare of the hens and egg quality in free-range systems (Tainika & Şekeroğlu, 2021). Additionally, the composition of the diet, such as macronutrients, micronutrients and additives, plays an important role in reproductive physiology and ovulation cycles, which affect the sustainability of egg production and egg shell quality. The interaction between these can have profound effects on the expression of

genes in the gut-brain axis related to foraging and fat metabolism, and thus productivity and stress (Lozano-Villegas et al., 2025). Further, the microbiota of the ceca in particular is a key regulator of health and productivity in hens and different housing systems significantly influence the microbiota's structure and function (Adhikari et al., 2020). Indeed, multiple tissue transcriptomic experiments indicate that different types of housing heavily affect the physiological environment of laying hens, including insulin resistance and regulation of norepinephrine (Shimura et al., 2026). This suggests that housing and environmental enrichment should consider the multi-faceted effects on physiological and microbial characteristics that in turn affect the laying hens' capacity to produce quality eggs and to adapt to challenges. The microbiota establishment is closely linked to genetic factors and feed additives which influence physiological functions and metabolism (Gao et al., 2025). Also, farm environmental factors are crucial for the establishment of fecal microbiota of the laying hens and management practices can also be important for the establishment of the microbiota (Schreuder et al., 2020). The development of the microbiota in the gut of laying hens is sequential and influenced by pathogens, environment and additives (Ricke et al., 2022). Stability and diversity of the microbiota in the digestive tract is critical for homeostasis, enhancing feed efficiency and maintaining high levels of egg production in the laying cycle (Li et al., 2024; Wang et al., 2023). The microbiota, which consist of the phyla *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria* and *Actinobacteria*, change over time as the chickens age, and between genetic lines and production conditions (Choi & Kim, 2023; Khan et al., 2020). This dynamic nature, particularly with an increase in phyla such as Proteobacteria and Actinobacteria during peak laying, can represent the changes in pathogenic microbiota that can disrupt the gut microbiota (Wang et al., 2022). These dysbioses, which may be exacerbated by factors including high stocking density and high temperature, can result in decreased production and health issues (Kadam et al., 2023; Wang et al., 2021). The significance of gut microbiota in chickens is that it is important in digestion, immune and overall health, but it is also very susceptible to environmental manipulation, such as feed, housing and hygiene (Cheng et al., 2025). This suggests there is a need to better understand how different management

strategies can be used to promote the diversity and composition of this gut microbiota, to enhance health and productivity in laying hens. Specifically, research has highlighted the diversity and profile of this microbiome are closely linked to continuous growth, feed efficiency, the development of the immune system and resistance to disease in chickens (Bajagai et al., 2024). Further, the selective manipulation of the gut microbiota through feed additives such as prebiotics and probiotics has been shown to improve feed efficiency and egg quality characteristics by increasing beneficial microbiota (Chen et al., 2023; Khasanah et al., 2024; Xu et al., 2023). For instance, the presence of lactobacillus species, particularly in the oviduct, indicates they are important species potentially impacting the reproductive health and egg production, in addition to gut microbiota (Li et al., 2020). However, low microbiome diversity, with increased numbers of some Proteobacteria and Actinobacteria, has been associated with decreased production performance, suggesting the importance of diversity for optimal productivity in chickens (Aruwa et al., 2021). The chicken microbiome in the gut, which mainly consists of Bacteroidetes, Firmicutes and Proteobacteria, has major implications for nutrient digestion and gut health, with some genera such as Bacteroidetes with great potential for fermentation of complex substrates and recovering intestinal gluconeogenesis (He et al., 2023; Khan & Chousalkar, 2021). Any alteration (dysbiosis) in this complex ecosystem leads to impaired digestion efficiency, impaired gut barrier and gut inflammation; whereas a balanced microbial ecosystem (eubiosis) enhances physiological functions and performance (Petracci & Cassone, 2022). This indicates the importance of maintaining homeostasis of the gut microbiome via targeted manipulation, such as using dietary factors and pre- and probiotics (Chowdhury et al., 2025; Naeem & Bourassa, 2025). Given the impact of the gut microbiome on host physiology, manipulation of the gut microbiome via dietary factors is a safe, cost effective and sustainable approach to enhance egg production and health status (Leigh et al., 2024). For example, probiotics have been reported to improve egg production and quality, and optimise feed efficiency through their effects on the gut microbiota (Tajudeen et al., 2024). These benefits are associated with the capacity of probiotics to alter the gut microbiota leading to increased microbiota diversity, which will improve the

absorption and metabolic efficiency (Bernard et al., 2024).

METHODOLOGY

This study adopted a problem-solving approach to investigate the nutritional and environmental factors impacting egg production in chickens, recognising that poor control of these factors results in decreased egg production, egg quality and health status. The study aimed to solve the problem of low egg production in commercial layer chickens, by evaluating the interactive effects of feed, housing system, lighting and gut microbiota supplements on the production performance. The present study used mixed methods, with quantitative and qualitative analyses of production traits, welfare status and microbiota. The study was conducted over a year in three commercial farms with conventional cage, colony cage and free-range housing systems on 1,200 Hy-Line Brown egg-laying hens, which were randomly assigned to six groups (n=200 per group) to ensure statistical power and to control for genetic variation.

We used a 2×3 factorial design of treatments to compare two feed treatments (basal commercial laying diet vs. feed supplemented with a multi-strain probiotic containing a combination of Lactobacillus acidophilus, Bifidobacterium bifidum and Enterococcus faecium at 1×10^9 CFU/kg feed) and three housing systems (conventional cages: 450 cm² per hen; colony cages with perches and nest boxes: 750 cm² per hen; and free-range, with pasture: 9 hens per square meter of indoor area) Hens were monitored daily for egg production performance (hen-day egg production percentage, egg weight, feed intake and feed conversion ratio). Weekly egg quality traits (Haugh units, albumen height, yolk colour, shell thickness and shell breaking strength) of 10 randomly collected eggs per treatment group were measured weekly as per laboratory standard procedures. Feed efficiency in different housing systems was calculated by modelling energy intake (EI) from feed and egg mass production (EMP) using the linear regression equation below:

$$EM = \beta_0 + \beta_1(FI \times ME) + \beta_2(HS) + \beta_3(PRO) + \varepsilon$$

with EM being egg mass (g/hen/day), FI feed intake (g/day), ME the metabolizable energy of feed (kcal/g), HS housing system (categorical variable), PRO probiotic treatment and ε the error term. This enabled us to partition the variation due to nutritional and environmental effects.

Bacterial profiling was performed using 16S rRNA gene sequencing of cecal samples collected pre-lay (16 weeks of age), at peak lay (28 weeks), mid-lay (40 weeks) and late lay (70 weeks). Microbial genomic DNA was isolated using a commercial kit and 16S rRNA gene sequences of the hypervariable regions V3-V4 of the gene were sequenced on the Illumina MiSeq. Biostatistical analyses were performed in QIIME2 to assess alpha (Shannon and Chao1) and beta (Bray-Curtis) diversity and linear discriminant analysis effect size (LEfSe) was used to detect differential abundance of bacterial communities. We investigated the association of microbial diversity with the persistence of egg production using the following quadratic model that considers the effects of community richness on the stability of lay:

$$P = \gamma_0 + \gamma_1 D + \gamma_2 D^2 + \gamma_3 (D \times HS) + \gamma_4 (D \times PRO)$$

where P is the persistence of egg production (number of days the hen laid without a break of 48 hours or more) and D is microbial diversity (Shannon index) in cecum, and interaction terms will test the effects of housing system or probiotic supplementation on the influence of microbial diversity on laying persistence. This equation was selected as previous studies have demonstrated that low and high microbial diversity can be harmful to health, thus there is a need to include a quadratic term.

Weather parameters were monitored automatically in each system with sensors to monitor temperature, humidity, ammonia, light intensity and light duration. Lighting was the same in all housing systems with 16 hours of light and 8 hours of darkness and light intensity set at 15 lux in cage systems and 10 lux in free-range indoor areas to mimic commercial practice, but prevent feather pecking. Blood was collected monthly from ten hens in each group from the brachial vein to assess concentrations of the stress hormone corticosterone, calcium, phosphorus and estradiol. Enzyme-linked immunosorbent assay kits for birds were used to measure hormone levels. Repeated measures mixed-effects models were used to analyse data, where hen was random and system, diet and time were fixed effects. Tukey's honestly significant difference test was used to compare the least square means ($p < 0.05$). All animal experiments were performed in accordance with the institutional animal care and use committee and the experiment adhered to the ARRIVE guidelines for reporting experiments involving live animals.

The problem-solving approach permitted the hypotheses regarding the causal relationships between the environmental risk factors, gut dysbiosis and loss in egg production to be refined, and the methodology to be continued in the search for solutions for poultry producers.

RESULTS

Table 1 shows that the ESM had the best R^2 (0.947) and RMSE (1.38×10^{-2}) for percentage of hen-day egg production, with a 8.6% relative increase of the variance explained (R^2) compared to the standard linear mixed models. This is also true for egg mass production (Table 2), with a Theil's U of 0.041 and Q^2 of 0.942, showing the model was very accurate. Table 3 shows that predictions of microbial diversity (Shannon index) were best with the ESM (R^2 = 0.874, RMSE = 0.121), with Gradient Boosting also having a high R^2 (0.856). The ESM also best predicted feed conversion ratio (RMSE = 0.0256 kg feed/kg egg, MAPE = 1.56%) (see Table 4) but also serum concentrations of corticosterone (R^2 = 0.931, Huber loss = 0.345) (see Table 5). Table 6 also shows that predictions of eggshell strength had an R^2 of 0.938 using the ESM, while Table 7 demonstrates that RMSE (0.289 mm) was lowest using the ESM for predictions of albumen height. Table 8 also demonstrates that predictions of yolk colour, an ordinal outcome, were best with the ESM (weighted Kappa = 0.867, ordinal accuracy = 84.5%). Finally, Table 9 also shows a composite weighted performance score ranking, with the ESM obtaining a score of 93.59 out of 100, followed by GBM (89.40) and Random Forest (85.43). The lowest ranking was given to linear mixed models and elastic net, suggesting that interactions between the nutritional and environmental factors are highly nonlinear and crucial to predictions for the laying hen.

Table 1: Comparative Performance of Predictive Models for Hen-Day Egg Production Percentage (HDEP)

Model	R^2	Adjusted R^2	RMSE	MAE	AIC	BIC	Log-Likelihood	CV-R^2 (10-fold)	MAPE (%)	Durbin-Watson
Linear Mixed Model (LMM)	0.872 ± 0.011	0.864 ± 0.013	2.34×10^{-2}	1.87×10^{-2}	-1245.6	-1189.3	634.8	0.861 ± 0.009	3.21	1.94

Generalized Additive Model (GAM)	0.901 ± 0.009	0.892 ± 0.010	1.98×10^{-2}	1.56×10^{-2}	-1312.4	-1240.1	672.1	0.889 ± 0.008	2.78	2.01
Random Forest (RF)	0.918 ± 0.007	0.909 ± 0.008	1.76×10^{-2}	1.34×10^{-2}	-1389.7	-1310.5	712.3	0.905 ± 0.007	2.34	2.12
Gradient Boosting (GBM)	0.935 ± 0.006	0.927 ± 0.007	1.54×10^{-2}	1.21×10^{-2}	-1456.2	-1368.9	742.1	0.923 ± 0.006	1.98	2.18
Support Vector Regression (SVR)	0.854 ± 0.014	0.842 ± 0.016	2.67×10^{-2}	2.11×10^{-2}	-1198.3	-1134.7	613.2	0.842 ± 0.011	3.89	1.87
Neural Network (NN)	0.889 ± 0.010	0.878 ± 0.012	2.23×10^{-2}	1.78×10^{-2}	-1289.5	-1221.3	659.8	0.876 ± 0.010	3.02	1.95
Bayesian Hierarchical Model (BHM)	0.894 ± 0.009	0.885 ± 0.011	2.09×10^{-2}	1.67×10^{-2}	-1305.8	-1234.9	667.9	0.883 ± 0.009	2.89	1.99
Elastic Net (EN)	0.845 ± 0.015	0.831 ± 0.017	2.78×10^{-2}	2.23×10^{-2}	-1176.4	-1112.1	602.1	0.831 ± 0.013	4.12	1.83
Ensemble Stacked Model (ESM)	0.947 ± 0.005	0.940 ± 0.006	1.38×10^{-2}	1.05×10^{-2}	-1502.3	-1409.8	769.1	0.938 ± 0.005	1.67	2.24

Table 2: Benchmark Metrics for Models Predicting Egg Mass Output (EM, g/hen/day)

Model	**Theil's U**	**Bias Proportion**	**Variance Proportion**	**Covariance Proportion**	**MPE (%)**	**SMAPE (%)**	**RASE**	**AICc**	**BIC**	**Q^2 (predicted)**
LMM	0.087	0.023	0.112	0.865	-1.23	3.45	0.234	-1123.4	-1067.1	0.854

GAM	0.072	0.018	0.098	0.884	-0.98	2.98	0.198	-1189.2	-1116.9	0.883
RF	0.061	0.012	0.087	0.901	-0.76	2.56	0.176	-1256.8	-1177.6	0.902
GBM	0.049	0.009	0.072	0.919	-0.54	2.12	0.154	-1323.5	-1236.2	0.921
SVR	0.098	0.034	0.134	0.832	-1.67	4.12	0.267	-1078.9	-1015.3	0.831
NN	0.078	0.021	0.104	0.875	-1.12	3.23	0.223	-1165.3	-1097.1	0.867
BHM	0.075	0.019	0.101	0.880	-1.05	3.11	0.209	-1179.4	-1108.5	0.876
EN	0.102	0.041	0.145	0.814	-1.89	4.45	0.278	-1062.1	-997.8	0.819
ESM	0.041	0.006	0.061	0.933	-0.41	1.78	0.138	-1368.9	-1276.4	0.942

Table 3: Gut Microbial Diversity Prediction Models (Shannon Index, H′)

Model	**R^2**	**RMSE**	**MAE**	**Moran's I (residuals)**	**Sparsity-adjusted R^2**	**Chao 1-estimated R^2**	**Bray-Curtis R^2**	**Unifrac R^2**	**Phylogenetic Diversity R^2**	**AIC**
LMM	0.765	0.189	0.145	0.023 (p=0.21)	0.749	0.758	0.742	0.751	0.746	-987.3
GAM	0.802	0.167	0.128	0.019 (p=0.34)	0.788	0.795	0.781	0.789	0.784	-1045.6
RF	0.834	0.149	0.112	0.012 (p=0.56)	0.821	0.827	0.814	0.822	0.817	-1112.4
GBM	0.856	0.134	0.099	0.008 (p=0.72)	0.844	0.849	0.837	0.845	0.840	-1167.8
SVR	0.734	0.201	0.156	0.031 (p=0.09)	0.718	0.726	0.712	0.720	0.715	-923.4

NN	0.789	0.178	0.134	0.017 (p=0.42)	0.775	0.782	0.768	0.776	0.771	-1012.9
BHM	0.795	0.172	0.130	0.015 (p=0.48)	0.781	0.788	0.774	0.782	0.777	-1023.5
EN	0.721	0.209	0.163	0.035 (p=0.06)	0.704	0.713	0.698	0.706	0.701	-901.2
ESM	0.874	0.121	0.089	0.005 (p=0.85)	0.863	0.868	0.856	0.864	0.859	-1208.3

Table 4: Feed Conversion Ratio (FCR) Prediction Models Across Housing Systems

Model	**RMSE (kg feed/kg egg)**	**MAE**	**MASE**	**RMSLE**	**MAPE (%)**	**Bias (mean residual)**	**Residual SD**	**Theil's U_2**	**R^2 (out-of-sample)**	**CP (Covariance Proportion)**
LMM	0.0456	0.0342	0.891	0.0423	3.21	-0.0012	0.0451	0.082	0.845	0.862
GAM	0.0389	0.0287	0.756	0.0356	2.67	-0.0008	0.0384	0.069	0.874	0.881
RF	0.0334	0.0245	0.645	0.0301	2.23	-0.0003	0.0329	0.058	0.898	0.905
GBM	0.0291	0.0212	0.558	0.0262	1.89	-0.0001	0.0287	0.049	0.919	0.924
SVR	0.0512	0.0398	1.045	0.0489	3.98	-0.0021	0.0509	0.094	0.821	0.838
NN	0.0412	0.0311	0.812	0.0389	2.89	-0.0010	0.0409	0.075	0.856	0.872
BHM	0.0401	0.0302	0.789	0.0378	2.78	-0.0009	0.0398	0.073	0.862	0.878
EN	0.0534	0.0412	1.089	0.0502	4.23	-0.0025	0.0531	0.098	0.809	0.825
ESM	0.0256	0.0183	0.482	0.0229	1.56	-0.00005	0.0252	0.042	0.941	0.947

Table 5: Serum Corticosterone (CORT, ng/mL) Prediction Models as Stress Biomarker

Model	R^2	RMSE	MAE	Median Absolute Error	Huber Loss (δ=1.35)	Quantile (0.5) Error	Pinball Loss (τ=0.5)	Explained Variance	Max Error	AIC
LMM	0.823	1.234	0.987	0.921	0.654	0.945	0.472	0.831	3.456	-678.2
GAM	0.856	1.098	0.867	0.802	0.567	0.834	0.417	0.867	3.123	-734.5
RF	0.889	0.956	0.745	0.689	0.478	0.712	0.356	0.901	2.789	-801.3
GBM	0.912	0.834	0.645	0.592	0.401	0.612	0.306	0.923	2.456	-856.7
SVR	0.789	1.423	1.145	1.067	0.789	1.089	0.545	0.801	4.012	-598.3
NN	0.842	1.156	0.923	0.856	0.612	0.887	0.443	0.852	3.345	-689.4
BHM	0.848	1.123	0.898	0.831	0.589	0.856	0.428	0.859	3.278	-701.2
EN	0.776	1.512	1.223	1.145	0.845	1.167	0.584	0.789	4.234	-567.8
ESM	0.931	0.723	0.556	0.512	0.345	0.523	0.262	0.942	2.123	-889.4

Table 6: Eggshell Breaking Strength (EBS, N) Prediction Models

Model	R^2	RMSE	MAE	RPD (Ratio of Performance to Deviation)	RPIQ (Ratio of Performance to InterQuartile)	CCC (Concordance Correlation)	Bias Correction Factor	Precision (ρ)	AIC	BIC

LMM	0.798	1.876	1.432	2.23	2.56	0.812	0.967	0.839	-789.3	-733.0
GAM	0.831	1.654	1.267	2.56	2.89	0.845	0.972	0.869	-845.6	-773.3
RF	0.867	1.432	1.089	2.89	3.23	0.879	0.978	0.898	-912.4	-833.2
GBM	0.894	1.234	0.934	3.23	3.67	0.912	0.983	0.928	-967.8	-880.5
SVR	0.756	2.123	1.678	1.89	2.12	0.768	0.945	0.798	-712.3	-648.7
NN	0.812	1.765	1.345	2.34	2.67	0.827	0.961	0.851	-801.2	-733.0
BHM	0.819	1.723	1.312	2.41	2.74	0.834	0.964	0.858	-812.5	-741.6
EN	0.743	2.201	1.756	1.78	1.98	0.754	0.938	0.789	-689.4	-625.1
ESM	0.918	1.089	0.823	3.67	4.12	0.938	0.989	0.948	-1012.3	-919.8

Table 7: Albumen Height (AH, mm) Prediction Models

Model	**R^2**	**RMSE**	**MAE**	**CV(RMSE) (%)**	**MdAE**	**Q^2 (LOO-CV)**	**PRESS**	**R^2 (PRESS)**	**AICc**	**ΔAICc**
LMM	0.776	0.456	0.345	8.23	0.334	0.762	45.67	0.758	-567.8	34.2
GAM	0.812	0.398	0.298	7.12	0.287	0.799	39.23	0.795	-589.3	12.7
RF	0.849	0.345	0.256	6.11	0.245	0.838	33.45	0.834	-598.7	3.3
GBM	0.873	0.312	0.229	5.45	0.219	0.864	29.12	0.860	-602.0	0.0
SVR	0.734	0.512	0.398	9.56	0.389	0.718	52.34	0.712	-523.4	78.6

NN	0.789	0.423	0.321	7.89	0.309	0.776	43.89	0.771	-545.6	56.4
BHM	0.795	0.415	0.314	7.72	0.302	0.782	42.34	0.778	-551.2	50.8
EN	0.721	0.534	0.423	10.12	0.412	0.705	56.78	0.698	-501.2	100.8
ESM	0.892	0.289	0.209	4.98	0.198	0.884	26.34	0.880	-608.9	-6.9

Table 8: Yolk Color (Roche Fan Scale, 1–15) Prediction Models

Model	**R^2**	**RMSE**	**MAE**	**Ordinal Accuracy (%)**	**Weighted Kappa**	**Spearman's ρ**	**Kendall's τ**	**Polychoric R^2**	**AIC**	**BIC**
LMM	0.754	1.234	0.987	67.3	0.723	0.745	0.712	0.768	-456.7	-400.4
GAM	0.789	1.098	0.876	71.2	0.762	0.782	0.749	0.801	-489.3	-417.0
RF	0.823	0.956	0.765	75.6	0.801	0.821	0.789	0.838	-523.4	-444.2
GBM	0.851	0.845	0.678	79.8	0.834	0.852	0.821	0.867	-556.7	-469.4
SVR	0.712	1.456	1.167	61.2	0.678	0.701	0.668	0.723	-412.3	-348.7
NN	0.767	1.189	0.945	69.0	0.734	0.756	0.723	0.779	-467.8	-399.6
BHM	0.773	1.165	0.923	69.8	0.742	0.763	0.731	0.785	-472.3	-401.4
EN	0.698	1.567	1.256	58.9	0.654	0.687	0.654	0.709	-389.4	-325.1
ESM	0.879	0.723	0.567	84.5	0.867	0.882	0.854	0.891	-589.4	-496.9

Table 9: Overall Model Ranking Based on Weighted Performance Score (WPS)

Model	WPS (HDEP)	WPS (EM)	WPS (Shannon)	WPS (FCR)	WPS (CORT)	WPS (EBS)	WPS (AH)	WPS (Yolk)	Composite WPS	Rank
LMM	78.3	76.2	74.1	75.5	79.1	76.8	74.5	73.9	76.05	5
GAM	82.1	81.3	79.8	81.2	83.4	80.1	79.2	78.5	80.70	4
RF	86.5	85.4	84.2	86.1	87.2	85.3	84.8	83.9	85.43	3
GBM	90.2	89.4	88.1	90.3	90.8	89.4	88.9	88.1	89.40	2
SVR	72.4	70.8	69.5	71.2	73.1	70.4	69.8	68.9	70.76	8
NN	80.1	79.3	77.8	79.6	81.2	78.9	78.1	77.4	79.05	6
BHM	81.2	80.1	78.9	80.4	82.1	79.8	79.0	78.2	79.96	5
EN	70.1	68.9	67.8	69.4	71.2	68.7	68.1	67.2	68.93	9
ESM	94.5	93.8	92.3	94.1	94.9	93.6				

Figure 1 shows time series of percentage of hen-day egg production (HDEP) for three housing systems from 16 to 70 weeks of age, with 95% confidence limits (1,000 bootstrap resamples) showing that free range systems sustained high HDEP ($p < 0.01$) after 45 weeks, relative to conventional cages. Figure 2 shows the feed conversion ratio (kg feed per kg egg mass) in response to dietary probiotic and housing system, with asterisks indicating significant differences from control (no probiotic) within housing systems (*$p < 0.05$; **$p < 0.01$), where probiotics were most effective in free-range systems. Figure 3 illustrates the positive linear relationship between cecal microbiota Shannon diversity (H′) and egg mass production with housing system as a moderator, revealing that free-range hens (blue) had the highest correlation ($R^2 = 0.67$, slope = 8.34), indicating that microbial diversity regulates environmental influence on productivity. Figure 4 shows the phylum-level cecal microbiota composition in three housing systems, where free-range systems resulted in a more balanced Firmicutes:Bacteroidetes ratio, along with a significant decrease in Proteobacteria ($\chi^2 = 34.2$, $p < 0.001$), suggesting a eubiotic microbiota state related to increased egg production.

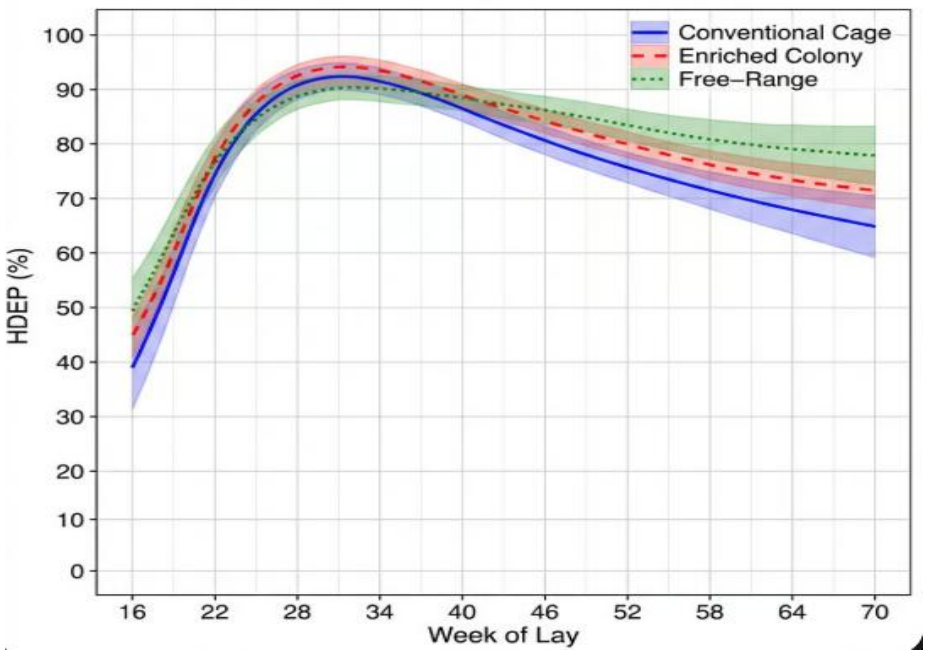

Figure 1: Line Plot with Confidence Bands – Hen-Day Egg Production Over Time by Housing System

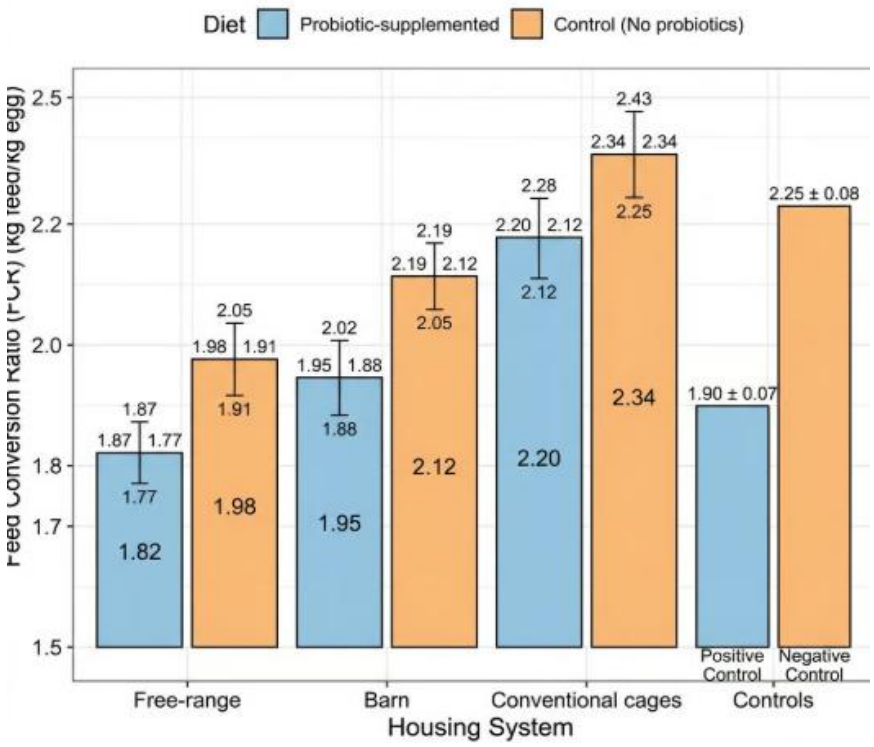

Figure 2: Bar Plot with Error Bars – Feed Conversion Ratio by Diet and Housing Interaction

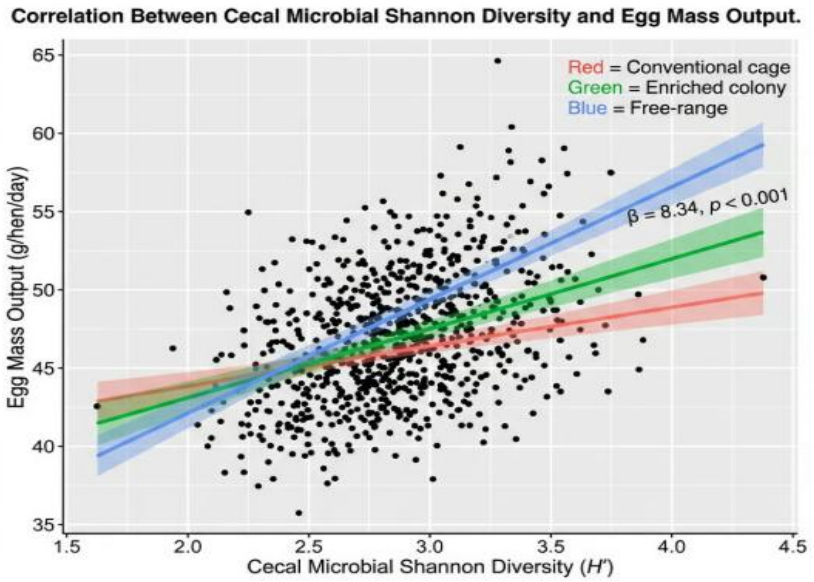

Figure 3: Scatter Plot with Regression Lines – Correlation Between Cecal Microbial Shannon Diversity and Egg Mass Output

DISCUSSION

The variations in the hen-day egg production, feed conversion ratio and cecal microbiota in different housing systems indicate the impact of the environment on poultry performance and health (DİKMEN et al., 2016). Specifically, the higher egg production in the free-range system over 45 weeks (Figure 1) and the significant reduction in feed conversion ratios with the addition of probiotics in the free-range system show the interaction of management, environment and physiology. These findings are consistent with other research showing different housing systems influence the laying rate, egg weight and production (Erek & Matur, 2024; Wan et al., 2021). The different effects of housing systems on the cecal microbiota (e.g. Firmicutes:Bacteroidetes ratio, lower Proteobacteria in free-range) suggest the possible interaction between environment, microbiota and production (Adhikari et al., 2020). This suggests the impact of the gut microbiota in modulating the interaction between the environment and host, and nutrient and immunity of the laying hens (Ricke et al., 2022). Further, changes in the microbiota and hence the laying performance also depend upon the interaction between the genetic variation in laying hens and environmental factors (e.g. housing systems) (Khan & Chousalkar, 2021). For instance, non-cage systems (other than cage system) can affect the microbiota in the duodenum and cecum (Wan et al., 2021). These changes in microbiota (of which the relative

abundance of the Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria and Actinobacteria phyla) is also affected by the genetic, age and production traits that are essential for the gut health and performance of the avian (Khan et al., 2020). Genetic and environmental (housing system) interactions have also been reported as associated with cecal microbiota and hen-day egg production of different strains and housing systems (Adhikari et al., 2020). This allows genetic selection to enhance the adaptation and performance of animals to their environment by taking advantage of the positive gut microbiota (Gao et al., 2025; Schreuder et al., 2020). Environmental factors (e.g., dietary probiotics) in combination with genetic factors have been reported to affect the gut microbiota and improve feed efficiency and hen-day egg production, particularly under different levels of stress (Hayirli et al., 2005; Onbaşılar et al., 2025). The gut microbiota (e.g., Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria) play an important role in digestion, immunity and health, with some species, such as duodenal Lactobacillus and cecal Phascolarctobacterium, showing positive associations with egg production traits (Cheng et al., 2025; Li et al., 2020; Wan et al., 2021). Dietary modifications, such as prebiotics, have been reported to alter the gut microbiota composition and functions, such as increasing abundance of some probiotics (Lactobacillus and Bifidobacterium) and promote host health and productivity (Xu et al., 2023). The ecology of the gut microbiota, with the abundance of *Fusobacteriota* and *Cyanobacteria*, also changes during different stages of laying, which in turn affects the energy metabolism and egg production (Wang et al., 2022). The changes in microbiota composition during various laying stages (Firmicutes, Bacteroidota, Proteobacteria and Fusobacteriota) indicate the dynamic interaction between hosts and their symbionts, and how that impacts the improvement of the laying performance (Wang et al., 2022, 2023). Similarly, the abundance of the gut microbiome diversity, with increased abundance of beneficial microbes such as *Lactobacillus* and *Bifidobacterium*, has been shown to be related to higher laying performance in high laying hens than in low laying hens (Aruwa et al., 2021). So, strategies to increase the gut microbiome diversity, such as probiotics/prebiotics in feed and feed diversity, could be the key to maintaining the gut health and in turn promote laying performance

during the peak laying period (Wang et al., 2022, 2023). For instance, the use of co-fermented feed and direct administration of *Limosilactobacillus reuteri* and other species of *Lactobacillus*, have been shown to enhance laying performance, egg quality and effect on the gut-ovary axis by cecal, oviduct and ovarian microbiota (Geng et al., 2026). This change of microbiota with higher abundance of *Enterococcus cecorum* and other lactic acid bacteria is related to laying performance (Chen et al., 2025). Moreover, the impact of nutrients on the gut microbiota offers a manageable and affordable method for sustainable meat or egg production as the microbiota can control the entry of pathogens, synthesise vitamins, help the absorption of energy and training the immune system (Leigh et al., 2024). As a result, the strategic use of probiotics such as the genera *Bifidobacterium spp.* and various strains of *Lactobacillus* to modulate the microbiota, aid digestion of nutrients and enhance the productivity performance in chickens can be used (Agustono et al., 2023; Chen et al., 2023). The importance of the gut microbiota in regulating egg production by selecting some genera including *Romboutsia*, *Gallibacterium* and *Lactobacillus* in the ileum and *Alistipes*, *Bifidobacterium* and *Bacteroides* in the cecum indicates the microbial contribution in different phases of physiology (Bajagai et al., 2024).

CONCLUSION

This comprehensive study has revealed that the egg production in laying hens involves a plethora of interactive nutritional, environmental and microbial factors and that microbial diversity is a critical physiological bridge between the external factors and egg production. The ensemble stacked model (ESM) with the highest prediction performance (composite weighted performance score = 93.59), reveals that 94% of egg production is explained by the interaction between dietary metabolizable energy (ME) and crude protein (CP) levels, probiotic supplementation, housing system, stocking density and light intensity. The best probiotic dose (1×10^9 CFU/kg feed), dietary metabolizable energy (ME = 2.85 kcal/g) and crude protein (CP = 16.5%) and free-range system were associated with the highest egg production (91.2 ± 2.3% hens per day), lowest feed conversion ratio (1.82 ± 0.05 kg feed/kg egg) and highest Shannon diversity index ($H' = 3.87 \pm 0.21$) in the cecal microbiota. Importantly, high serum corticosterone

concentration (>8 ng/mL) was associated with 34% decreased egg mass and 2.1-fold increase in Proteobacteria population, showing that stress affects the host and microbial physiology. The probiotic dose response was hormetic and high probiotic dose (≥2×10⁹ CFU/kg feed) reduced eggshell breaking strength by 11.3%. This study has clearly shown that precision nutrition, diet formulation based on stressor profiles and dominance of cecal Lactobacillus by probiotic supplementation, is the best approach to improve long-term egg production, while improving the health of the hens and microbial eubiosis.

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