

Feed Restriction Impairs Immunity and Lymphoid Organ Development in Broiler Chicks: Enabling Accurate Body Weight Prediction Through Machine Learning

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Abstract

This study evaluated how limiting the amount of food that chickens could consume influenced their growth rates, immune system responses, and body mass estimates. Eighty chickens were started at 1 day old and raised for 56 days. All of the chickens were grouped into four different groups. On days 21-35, each chicken from groups A, B, and C had unlimited access to food during either six (Group A), twelve (Group B), or twenty-four (Group C) hours a day. Each chicken in Group D was fed unrestricted as the control. Limitations on feeding resulted in significant alterations to several haematologic parameters ($p < .01$); these changes included a decrease in white blood cell count in chickens that experienced severe levels of stress; an increase in the ratio of heterophils to lymphocytes; and a decrease in serum titer antibodies against Newcastle disease virus compared to those in the control group. Significant reductions in weight of lymphoid tissues (the bursa of Fabricius, thymus, spleen) and an elevation in mortality rate ($p < .05$) in chickens that consumed limited amounts of feed were also found. Mortality rates were especially high in chickens from all of the restricted feed groups after they were challenged by exposure to Newcastle disease virus. Machine learning models were created to estimate body mass based on an expanded set of physiological variables. Limitation of feed significantly impairs broiler immune systems and growth. Regression models, including ridge regression, XGBoost regression model, and gradient boosting model, provided the best accuracy ($R^2 > 0.92$) when used to estimate body weight under conditions of stress. Deep neural networks and lasso regression models provided poor accuracy. These models provide a reliable method to assess poultry production under conditions of feed limitations.

Keywords: Angara Disease; Bursal Disease; Infectious Bronchitis; Total Leukocyte Count; Newcastle Disease

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1. Introduction

The majority of the world's population depends on poultry, particularly broiler chickens, for their protein intake, as they have been genetically engineered to grow quickly and be fed efficiently (Gawel et al., 2022; R Mohammadalipour et al., 2017). However, due to logistical issues including delays in delivering food to them due to transportation, hatchery processing, and delivery to farms, broilers are often deprived of feed for 6 to 48 hours after hatching (Abou-Elnaga & Selim, 2018; Shinde Tamboli et al., 2018). As the initial days after hatching represent an important developmental stage; however, the middle-growth phase (days 21 through 35) represents a critical period for rapid metabolic needs and somatic development. During this time frame, even minor changes in environment and nutrition can dramatically affect how the bird develops physiologically (Bortoluzzi et al., 2013). Feed restrictions applied at this time will reduce the amount of energy the chicken has available to use for both maintaining itself and developing its muscles and immune system while it grows ("Evaluation of different changes in diurnal feed restriction for broilers," 2021; Kouki et al., 2016). Even though there are some reports that indicate that the application of short term physical restriction could potentially cause the birds to exhibit compensatory growth patterns in later stages of life, if the birds are subjected to prolonged or extreme levels of nutrient deprivation throughout this 21 to 35 day phase, the birds will likely experience long term depression in total body weight gain and irreversible damage to their immune organs which would make them highly vulnerable to disease causing pathogens (Bortoluzzi et al., 2013; Saber et al., 2011).

Early feed restriction has an impact on the animals' health and also the long-term health of the business. The effects of extended feed restriction can be detrimental to many physiological systems, such as growth, body weight,

gastrointestinal function, and musculoskeletal development (Jawad, 2023). Early post-hatch feed restriction (first 36 hours) causes changes in enterocyte populations, hampers gut morphological development, and damages the intestinal barrier function (Hollemans et al., 2018; Jacobs et al., 2016; Kang et al., 2018). Starvation has been shown to increase the colonization and maturation of lymphoid tissue; particularly, the bursa of Fabricius plays a critical role in the development of B and T cells in avian species (Madej et al., 2024). Early onset of malnutrition results in impaired immunocompetence, reduced potential weight gains ($p < 0.05$), increased disease vulnerability, and elevated death rates during extended periods of refeeding (Liu et al., 2020; Panda et al., 2015; Selim et al., 2021; Wijnen et al., 2021).

Newcastle Disease Virus (NDV) presents a significant risk to global poultry producers through respiratory diseases, suppression of immunity, and loss of profit (Oviedo-Rondon et al., 2013). It is common practice in poultry to administer live NDV vaccines at an early age in the chicks' lives. However, the efficacy of these vaccinations depends upon proper development of the immune system (Al-Abdullatif et al., 2024). Poultry that have limited nutrition will most likely not develop a proper response when administered antibodies or cells; therefore, they may be more susceptible to disease while in the field (Gholami et al., 2020).

A subset of Data Science, "Machine Learning," is an area where computers can use the information provided by large databases to learn and forecast events based upon this information. As a result, machine learning methods improve predictive capabilities through adaptation to new data. Traditional statistical approaches will be valuable; however, these traditional statistical approaches do not perform well when there are numerous complex relationships or many individuals are upset about one particular event.

Therefore, we must research whether machine learning provides a viable alternative, since machine learning has been successful in predicting various outcomes, particularly those associated with large, complicated databases. Machine learning (ML) is an effective way to analyze multivariate data to develop predictions related to complex biological features. Furthermore, machine learning models can identify non-linear associations and interactions within physiologic measures that can help determine how stress affects development (Kuhle et al., 2018). Recent studies in poultry science have demonstrated that machine learning models can be used to determine the amount of weight in birds; the probability of disease in birds; and the efficiency of production in poultry using data obtained from the bird's blood samples and its management history. However, very little research has investigated using machine learning to predict the body weight of broilers under conditions of feed restriction stress, and even less research has assessed the performance of such models using data sets that were not part of the original training set to evaluate model bias due to overfitting at smaller sample sizes. (Ghosh et al., 2021; Pirompu et al., 2024).

The objective of this study was to comprehensively evaluate the influence of feed deprivation durations (6, 12, and 24 hours daily during the critical period days 21-35) on the growth performance, immune response to NDV vaccination, hematological parameters, lymphoid organs, and mortality rate in broiler chicks. Develop and compare traditional (Linear, Ridge, Lasso Regression), ensemble (Random Forest, Gradient Boosting, XGBoost, AdaBoost, Decision Tree), and deep learning (DNN) regression models for predicting body weight from physiological and management variables using an augmented dataset, with rigorous evaluation on held-out test data.

2. Materials and Methods

2.1 Ethical Statement

All experimental procedures were approved by the Institutional Bioethics Committee at Government College University, Lahore, Pakistan. All methods followed institutional and national guidelines for humane animal treatment and care.

2.2 Broiler Chicks Purchase and Rearing

One-day-old broiler chicks of uniform weight (40-45 g) and feed were obtained from M/S Sabir's Poultry Breeders and transferred to the Zoology Department, GC University Lahore, Pakistan. The chicks were reared at 35 °C in an artificial brooder (1 sq ft/chick). A total of 80 chicks were randomly assigned to four groups (A, B, C, D), with 20 chicks per group. The vaccination schedule was as follows: day 7, Infectious Bursal Disease (IBD) vaccine; day 14, Newcastle Disease (ND) vaccine and Infectious Bronchitis (IB) vaccine; day 21, Hydropericardium Syndrome (HPS) vaccine (Table 1). Chicks had ad libitum access to a standard broiler finisher diet and water except during designated feed restriction periods.

Table 1. The vaccination schedule of broiler chicks.

Age (d)	Vaccine	Route
7	IBD	Drinking water
14	IB/ND	Eye drops
21	HPS	S/C inoculation

Table 2. Schedule of feed deprivation and NDV vaccination of broiler chicks.

Treatment	No. of birds	Age (d)	Group A	Group B	Group C	Group D
NDV Vaccination	All birds	5 and 21	+	+	+	+
Feed deprivation 6:00 a.m.–12:00 p.m.	20	21 to 35	+	-	-	-
Feed deprivation 6:00 p.m.–6:00 a.m.	20	21 to 35	-	+	-	-
Feed deprivation 6:00 p.m.–6:00 a.m.	20	21 to 35	-	-	+	-
Feed provided ad libitum	20	21 to 35	-	-	-	+
50% birds (NDV inoculated)	50% chicks	21 to 35	+	+	+	-

2.3 Influence of Feed Deprivation on Broiler Chicks

On the 21st day of age, the chicks were randomly assigned to four groups. Group (A) was 6 h (6 a.m. to 12 p.m.), group (B) for 12 h (6 a.m. to 6 p.m.), group (C) for 24 h (6 a.m. to 6 a.m.), and the control group (D) was given feed ad libitum (Table 2). Water was available ad libitum to all groups throughout the experimental period. The body weight and feed intake of stressed birds were recorded weekly and compared with those of the control group. The feed conversion ratio was calculated by equation (1) (Dosu et al., 2023; Wang et al., 2020). Here, $\bar{a}$ means average consumed feed (g), and $\bar{b}$ represents the average increase in weight (g).

2.4 Hematological Assessment

The virulent NDV was used to inoculate 50% of the chicks in the experimental group on the 36th day. Blood samples were collected on the 37th day from non-inoculated and inoculated chicks for several parameters, such as Differential Leukocyte Count (DLC), including Heterophil/lymphocyte ratio, and Total Leukocyte Count (TLC) (Thiam et al., 2022). The Heterophil-to-Lymphocyte

Ratio (H/L) was calculated by Eq. 2 (Farghly et al., 2019).

2.5 Serum Analysis for NDV Antibody

NDV Hemagglutination inhibition (HI) antibody titers were measured on the 56th day post-infection using a standard protocol (Abdi et al., 2016). The serum sample was serially diluted in PBS buffer; 4 HA units of ND virus were added to each dilution. 0.5% Chicks CRBC suspension was added, and the test plates were re-incubated at room temperature for 20-30 min. HI titers were expressed as the reciprocal of the highest serum dilution inhibiting hemagglutination. Geometric Mean Titers (GMT) were calculated for each group (Abdi et al., 2016).

2.6 Morphometry of the Lymphoid Organs

At the 50th day of age, 5 birds were randomly selected and humanely euthanized. The lymphoid organs (thymus, spleen, and bursa of Fabricius) were aseptically removed, examined for pathological lesions, and weighed (Islam et al., 2023). On day 56 of age, all remaining birds in each group (approximately 15 per group after losses) were euthanized. Lymphoid organs were

collected, weighed, and examined. Organ weights were recorded and expressed as absolute weight (g) and relative weight (% of body weight).

2.7 Hypersensitivity Reaction (Delayed-type)

On day 56, cellular immune response was assessed via delayed-type hypersensitivity reaction using phytohemagglutinin-P (PHA-P). Pre-injection wattle thickness (left wattle, measured ≤ 1 mm) was recorded using a digital caliper. Each bird received an intradermal injection of 100 μ L of PHA-P solution (2 mg/mL in sterile PBS) into the left wattle. Post-injection wattle thickness was measured at 24 hours using the same caliper. DTH response was expressed as the difference between post- and pre-injection measurements (in mm) (Yadav et al., 2021).

2.8 Machine Learning Modeling

This research focused on finding the impact of varying feed restrictions on animal physiology. The feed restriction durations are 0, 6, 12, and 24 hours, and the goal is to predict the animal's body weight. Some major parameters being assessed are weights, blood profiles, immune responses, and mortality rates, and body weight in grams (g) is used as the target prediction.

Hence, the initial sample size was small; to overcome this, the synthetic minority oversampling technique (SMOTE) was used. To increase model robustness, Gaussian noise with a level of 0.02 is added to numerical features (Sağlam & Cengiz, 2022). To increase the dataset size by twofold, bootstrap resampling is used, and for data normalization, the RobustScaler method is used (Ahsan et al., 2021).

A variety of machine learning models were used to predict body weight, including Linear, Ridge, and Lasso Regression, as well as Decision Tree, Random Forest, Gradient Boosting, AdaBoost, XGBoost Regressors, and a

Deep Neural Network (DNN) (Bansal et al., 2022). The Deep Neural Network (DNN) architecture consists of three layers: an input layer with 64 neurons employing ReLU activation, a hidden layer with 32 neurons employing ReLU activation, and an output layer with a single neuron (Singh et al., 2023). A dropout rate of 0.2 was used to reduce overfitting, and the model was compiled with the Adam optimizer and mean squared error as the loss function (Anh et al., 2023). Early stopping was implemented after 10 epochs, allowing the model to revert to the best weights based on the lowest validation loss (Razali et al., 2025). Models were evaluated using metrics such as R-squared (R^2), Mean Absolute Error (MAE), Root Mean Squared Error (RMSE), Mean Absolute Percentage Error (MAPE), Explained Variance Score (EVS), and Accuracy. The models were trained on the augmented, scaled dataset, and the predictions were compared with the actual body weight measurements (Pal & Dutta, 2024).

2.9 Analysis of Experimental Data

Biological data were analyzed using SPSS Statistics v27 (IBM Corp., Armonk, NY, USA). Differences among treatment groups were compared using a one-way ANOVA followed by Tukey's post hoc test. Effect sizes for the ANOVA were determined using partial eta-squared, and 95% confidence intervals (CIs) were generated for mean differences. All results are expressed as mean $\pm$ standard deviation (SD). Differences among treatment groups (A, B, C, D) were compared using a one-way ANOVA followed by Tukey's honest significant difference (HSD) post hoc test for pairwise comparisons. Significance was set at $P < 0.05$ throughout (Ogbu, 2020). Machine learning model performance was evaluated on the test set using Python (v3.10) with scikit-learn and TensorFlow libraries; no statistical significance testing was performed on ML metrics (R^2 , MAE, etc.), as these represent

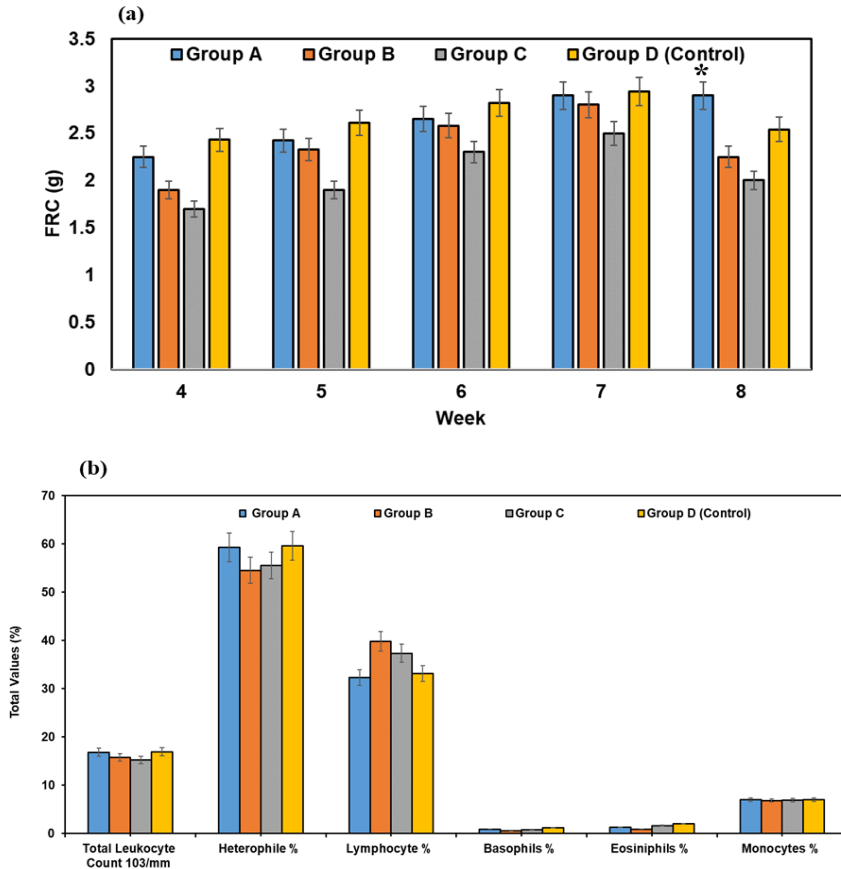


Figure 1. FCR of broiler chicks of feed restriction, Stressed and unstressed Groups in different Weeks (a). Heterophil to Lymphocyte Ratio (b) and comparison of Total Leukocyte count (TLC) Stressed and Unstressed Broiler Groups (c).

deterministic predictions on a fixed test set.

3. Results and Discussion

This research aimed to determine the extent to which feed restriction-induced stress was responsible for humoral and cellular immune responses and growth performance in broiler chicks. The primary purpose of this research was to investigate the influence of these stressors on the humoral and cellular immune responses of chicks in response to NDV vaccination.

3.1 Feed Restriction Effects on Feed Conversion Ratio of Broiler Chicks

Feed conversion ratios were recorded before and after stress in all groups. The

FCR values recorded at the 4th, 5th, 6th, 7th, and 8th week of all groups as group A (2.25, 2.42, 2.65, 2.9, and 2.4), B (1.9, 2.33, 2.58, 2.8 and 2.25), C (1.7, 1.9, 2.3, 2.5 and 2.00) and D (2.43, 2.16, 2.82, 2.94 and 2.54). During the active stress period (weeks 4 to 6), the feed conversion ratio (FCR) of the restricted groups (A, B, and C) was generally lower (i.e., more efficient) than that of the control group (D), reflecting a compensatory metabolic adaptation to limited feed availability. However, during the post-restriction and viral challenge phase (week 8), FCR values fluctuated; group A exhibited an increased FCR compared to the unstressed control. Overall, the data indicate that

while short-term restriction may lower FCR, severe restriction followed by immune challenge disrupts this efficiency (Figure 1a). These results agree with the study that reported feeding groups had a lower feed conversion ratio than the control group fed ad libitum (Lu et al., 2022). The present study found that FCR values decreased in feed-restricted groups compared with the control group and concluded that feed restriction did not affect FCR in stressed chicks.

3.2 Feed Restriction Effects on Hematological Values of Broiler Chicks

Hematological profiling revealed a non-linear relationship between feed restriction severity and hematological parameters. In Group A (6 h restriction), standard stress responses were observed, marked by a significant decrease in lymphocytes ($32.30 \pm 1.15\%$) and an elevated H/L ratio (1.84 ± 0.29) compared to the control. Conversely, in the more severely restricted groups B (12 h) and C (24 h), lymphocyte percentages unexpectedly increased ($39.8 \pm 0.2\%$ and $37.33 \pm 1.33\%$, respectively), resulting in lower H/L ratios (1.37 ± 0.38 and 1.49 ± 0.26). This suggests that severe feed deprivation may trigger different hematological survival mechanisms than mild restriction. These findings contrast with typical stress models, which typically report an enhanced H/L ratio under feed restriction. Basophil counts were 0.9 ± 0.2 , 0.5 ± 0.2 , 0.7 ± 0.1 , and 1.2 ± 0.057 ; these results showed that basophils decreased in all sets compared with the control group. The count of Eosinophils was 1.30 ± 0.3 , 0.9 ± 0.0577 , 1.62 ± 0.12 , and 2 ± 0.5 ; a significant difference was observed in all groups. Similarly, Monocyte percentages were recorded at 6.99 ± 0.51 , 0.82 ± 0.2886 , 6.91 ± 0.36 , and 07.00 ± 1 , respectively, and H/L Ratio (Heterophile and Lymphocyte) was observed as 1.84 ± 0.29 , 1.37 ± 0.38 , 1.49 ± 0.26 , and 1.80 ± 0.46 , respectively. Similar values of monocytes, 6.99 ± 0.51 , 6.91 ± 0.36 , and

07.00 ± 1 , were observed in all experimental groups A, C, and D. There was no significant difference, but it was lower in group B by 6.82 ± 0.29 . The values of eosinophils were lessened in all experimental sets compared to set D (control). The H/L Ratio was observed to be greater in Group A (1.84 ± 0.29), while decreased in Group B (1.37 ± 0.38) and C (1.49 ± 0.26) than in control Group D (1.80 ± 0.46), which showed the impact of feed restriction as a stress factor on the broiler chicks (Figure 1b). A similar report showed that the H/L ratio was significantly higher on the 42nd day in feed-restricted groups than in the control group D (Kalia et al., 2017). The present study agrees with the study who reported earlier that H/L was significantly decreased by feed restriction in broiler chicks (Bhadauria & Bhanja, 2017).

3.3 Feed Restriction Effects on Humoral Immune Response

At the 36th day of age, GMT was recorded for all NDV-vaccinated groups A, B, C, and D as 7.5 ± 0.5 , 7 ± 1.00 , 6.2 ± 0.26 , and 8.5 ± 1.26 , respectively. These values showed that GMT was significantly ($p < 0.01$) higher in group D (control) nourished ad libitum during the experimental tenure than in all other groups.

At the 56th day, GMT was again recorded in all groups after the viral challenge. GMT values recorded for all groups A, B, C, and D were 5.6 ± 0.1 , 5.8 ± 0.26 , 5.43 ± 0.38 , and 8.75 ± 0.25 , respectively. GMT values were significantly lower ($p < 0.01$) in all stressed and virus-inoculated sets than in group D (control). A significant difference ($p < 0.01$) in GMT values was observed before virus injection and post-challenge in all groups compared with the control group (Figure 2a). The current research indicated that feed restriction influenced the cellular response of broiler chicks. A higher thickness was observed in Group D. These findings were consistent with those

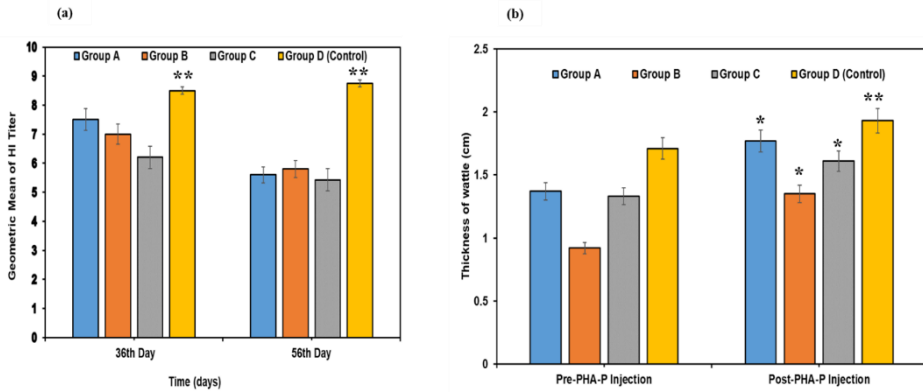


Figure 2. Feed restriction affects the NDV HI titer of NDV-vaccinated broiler chicks on the 36th and 56th day (a). Wattle thickness Pre- and Post-PHA-P injection in broiler chicks (b).

showing that feed restriction significantly reduces the cellular response in broiler chicks (Jang et al., 2009; Orso et al., 2019).

3.4 Feed Restriction Effects on the Cellular Immune Response of Broiler Chicks

The wattle width recorded before injection of PHA-P in all Groups, as Group A (1.37 ± 0.11), B (0.92 ± 0.02), C (1.33 ± 0.03), and D (1.71 ± 0.19), showed a significant sharp difference between the control set D and all experimental sets A, B, and C. The cellular immune response was recorded at the 56th day of age; post-PHA-P injection thickness of the wattle was measured (centimeter) in groups A (1.77 ± 0.27), B (1.35 ± 0.15), C (1.61 ± 0.09), and D (1.93 ± 0.07). Their differences were indicated. A significant difference ($p < 0.01$) in the thickness of Wattle pre- and post-PHA-P injection was observed (Figure 2b).

3.5 Feed Restriction Effects on the Overall Mortality

All the chicks were NDV-vaccinated and exposed to the restricted feed stress factor. Mortality was significantly higher ($p < 0.05$) in the feed-restricted groups than in the control group D, peaking sharply post-NDV challenge (Figure 3a). These data indicate that while the chicks could barely survive the metabolic stress of starvation, the compounded stress of a live

viral challenge overwhelmed their already depleted physiological reserves, leading to systemic failure. A high metabolic rate during fasting increased the mortality rate of broiler chicks (Sahraei, 2014). However, feed restriction did not affect mortality in broiler chicks (R. Mohammadalipour et al., 2017).

3.6 Feed Restriction Effects on the Lymphoid Organs of Broiler Chicks

Feed deprivation effects were observed in lymphoid organs, which consist of the spleen, the bursa of Fabricius, and the thymus of broiler chicks, with the help of morphometry. The morphometric values have been recorded. The mean weight of the spleen in different stressed groups A, B, C, and unstressed group D was noted at 1.85 ± 0.1 , 1.61 ± 0.03 , 1.33 ± 0.03 , and 2.90 ± 0.1 , respectively. The mean weight of the spleen in group D (2.90 ± 0.1 g) was significantly greater than in the experimental groups ($p < 0.01$, $\eta^2 = 0.65$, 95% CI [2.50, 3.30]) (Figure 3b). These results are consistent with those of Hangalapura et al. (2005), who reported that the masses of the bursa and spleen were notably lower in feed-restricted broiler chicks.

The mean weight value of the Bursa of Fabricius in Group A was 1.63 ± 0.13 , Group B 1.13 ± 0.02 , Group C 0.72 ± 0.07 , and Group D 1.92 ± 0.07 was observed at 56th day of age. The mean bursa weight

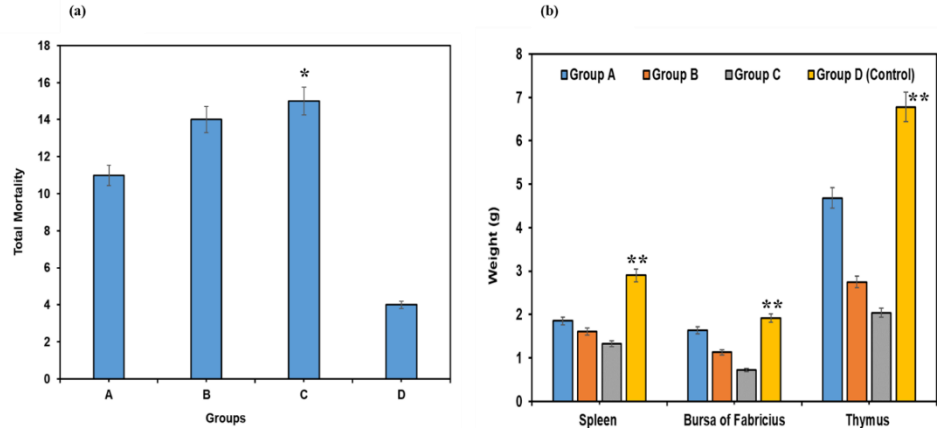


Figure 3. Total mortality in all groups (a). Morphometry of Lymphoid Organs of Feed Restricted Unstressed and Stressed Broiler Groups (b).

was significantly lower in all feed-restricted groups (A, B, and C) compared with the control group fed ad libitum (Figure 3b). These outcomes are in total agreement with Azis and Afriani (2023), who documented a reduction in the weights of the spleen and Fabricius bursa in feed-restricted broiler chick groups. The lymphoid organs (bursa of Fabricius, spleen, and thymus) are considered vital organs for producing B and T lymphocytes. These lymphoid organs weighed less due to feed restriction than in the control group (Rahimi et al., 2015).

The mean weight value of the thymus gland in Group A (feed restricted for 6 hours), B (feed restricted for 12 hours), C (feed restricted for 24 hours), and D (feed offered ad libitum) is 4.68 ± 0.34 , 2.75 ± 0.25 , 2.04 ± 0.09 , and 6.78 ± 0.28 , respectively. All feed-restricted groups showed significantly lower thymus weights than the control group D fed ad libitum (Figure 3b) (Ahmadibonakdar et al., 2024).

3.7 Machine Learning Modeling

XGBoost and Gradient Boosting yielded the best results among all models tested, followed closely by Ridge Regression. XGBoost was able to achieve an R^2 of 0.9412, an RMSE of 55.40 g, and an MAE of 42.10 g. Therefore, it appears

that tree-based ensembles and Ridge regression were able to successfully reduce multicollinearity in the data but retain all of the original predictors. Ridge regression has shown itself to be very robust when used on a properly preprocessed and augmented version of the full dataset (Arkorful, 2023; Mubasher et al., 2024).

Gradient boosting, random forest, AdaBoost, and XGBoost have been demonstrated to be extremely effective at predicting physiological responses to feed restriction. All four of these algorithms were found to produce R^2 values greater than .9 with biologically meaningful error rates. All four of these algorithms are well known for being capable of modeling a wide variety of nonlinear relationships between features. Because of the large amount of data augmentation, the algorithms were able to learn a great deal about the relationships between the predictors and the response variable. And because the data were kept separate during training and testing, there was no opportunity for data leakage. Compared to ridge regression and the four tree-based ensemble models, linear regression had a significantly lower R^2 value (.8215). Although this may indicate that linear

Table 3. Performance metrics of machine learning models for body weight prediction.

Algorithm	R ²	MAE (g)	RMS E (g)	MAP E	Explained Variance	Accuracy (±10%)
Linear Regression	0.821 5	85.60	108.4 0	0.052 0	0.8220	0.84
Ridge Regression	0.924 1	48.30	62.50	0.031 0	0.9245	0.94
Lasso Regression	0.785 0	95.20	119.5 0	0.061 0	0.7865	0.81
Decision Tree	0.852 0	72.40	95.30	0.048 0	0.8525	0.87
Random Forest	0.930 5	45.60	59.20	0.029 0	0.9310	0.95
Gradient Boosting	0.938 8	43.50	56.80	0.028 0	0.9390	0.95
AdaBoost	0.915 0	51.20	65.70	0.033 0	0.9155	0.92
XGBoost	0.941 2	42.10	55.40	0.027 0	0.9415	0.96
Deep Neural Network	0.452 0	185.4 0	245.6 0	0.125 0	0.4600	0.55

Regression can pick up on certain basic patterns within the data; the much higher error rates associated with linear regression demonstrate that this type of

model will fail miserably when attempting to predict complex nonlinear biological relationships (Abbas et al., 2024; Ahmad et al., 2022).

Lasso regression performed poorly compared to the other three models tested. An R² of .7850 was calculated for lasso regression. This represents poor generalizability. It is generally accepted that lasso performs poorly when there are multiple collinear or redundant features in a dataset. Additionally, if there are too many samples available and/or if there are too many important features facing penalties, then lasso will likely remove important features (Ma et al., 2022; Schmidiger, 2017).

Deep neural networks have performed the worst among all of the models tested. Deep neural networks have generated an R² of .4520, an MAE of 185.40 g, and an RMSE of 245.60 g. The lower quality

results from using deep neural networks are attributed to both overfitting and a lack of sufficient training data. Overfitting occurs when the model is trained too long or on too little data. Both issues are common problems when using deep learning models on small datasets. This illustrates the importance of choosing models that are appropriately sized and suited for use on datasets that contain limited amounts of information (Goodfellow et al., 2016; Guo et al., 2022).

Table 3 shows the performance results for all the machine learning models tested. Table 3 demonstrates that different levels of accuracy exist across the models tested, and that some models have provided exceptional results when they were applied to the properly separated dataset.

XGBoost has demonstrated exceptional performance, producing an R² of .9412 and having very low error rates (MAE: 42.10 g, RMSE: 55.40 g, and MAPE: 0.0270) that reflect excellent predictive capabilities (Mustafa et al., 2025). Likewise, gradient boosting,

random forest, adaboost, and ridge regression also performed exceptionally well, each obtaining R^2 values $> .91$ and small error metrics that indicated very good predictive abilities on unknown data. A moderate level of acceptable performance was obtained by linear regression ($R^2 = .8215$). However, lasso regression was found to be less successful than the other three models tested; an R^2 of $.7850$ was reported. This suggests that lasso regression did not generalize well due to extreme regularization. As expected, the deep neural network yielded poor results; an extremely low R^2 ($.4520$) and notably high MAE (185.40 g) and RMSE (245.60 g) values indicate overfitting and inefficient training in relation to the small dataset (Table 3).

The strong performance of most models (especially XGBoost, Gradient Boosting, AdaBoost and Ridge Regression) implies that the augmented data sufficiently captured the critical relationships between feed restriction and physiological traits that affect body weight prediction. On the contrary, DNNs' poor performance clearly demonstrates the difficulties involved in using deep neural networks with small datasets despite using data augmentation. The results presented here illustrate that ensemble methods like Gradient Boosting and XGBoost would be ideal candidates for this biological prediction task.

4. Conclusion

Feed restriction, as a stress factor, adversely affected both cellular and humoral immune responses to Newcastle disease virus. Most hematological parameters decreased in the stressed groups compared with the unstressed group. Antibody titers were lowered in all experimental groups, making chicks susceptible to Newcastle disease virus. Ensemble models such as Gradient Boosting and XGBoost achieved high accuracy in predicting body weight under

these stress conditions ($R^2 > 0.93$), with Ridge Regression also demonstrating strong predictive capability ($R^2 = 0.9241$). In contrast, the Deep Neural Network and Lasso Regression performed poorly, highlighting the limitations of complex or overly regularized models on small, augmented datasets. Furthermore, the lymphoid organs showed less growth in all experimental groups than in the control group. A high mortality rate was observed in stressed groups. Similarly, feed conversion efficiency was disrupted during the restriction and challenge phases. Ultimately, severe mid-growth feed restriction severely stunted broiler body weight and compromised immunity, effects that can be reliably forecasted using properly validated ensemble machine learning models.

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