Research Square

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Haematology and Serum Biochemistry of Broiler Strain Under Daily Gap-Feeding Strategy

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Abstract

This study investigated the effect of strain, daily gap-feeding and their interactions on haematological and serum biochemical profiles of broiler strains. The strains were: Arbor acre, Ross-308 and Cobb-500 broilers, while the gap-feeding schedule levels were: [thrice daily schedule at 8:00 AM, 12:00 PM, and 4:00 PM (1-1-1)], once daily (morning schedule) at 8:00 AM (1-0-0), once daily (evening schedule) at 4:00 PM (0-0-1)]. A total of 459-day-old chicks, with 153 birds per strain, were assigned to a 3 × 3 factorial experiment in a Completely Randomized Design. Each factor-combination level was replicated three times with 17 birds per pen. At 6 weeks, two birds per replicate were sampled, and analyzed for full blood count; and serum biochemical parameters in two separate sets of sample bottles for the hematological and serum biochemistry analysis. Strain influenced WBC, heterophils, lymphocyes, total cholesterol, triglycerides, AST, urea, total protein, conjugated and total bilirubin concentrations ($P < 0.023–0.048$). while gap-feeding influenced WBC, lymphocytes, AST, ALT, urea, creatinine, total protein, conjugated and total bilirubin ($P < 0.011–0.048$). Interaction of strain by Gap-feeding schedule was significant on haemoglobin, WBC, monocytes, total cholesterol and creatinine ($P < 0.020–0.043$). Findings suggested that late evening gap-feeding schedule (0-0-1) could be most effective feeding strategy of the three GF schedules for optimizing haematological and biochemical stability; and also minimizing health risks.

Highlights

Strain significantly influenced WBC, heterophils, lymphocyes, total cholesterol, triglycerides, AST, urea, total protein, conjugated and total bilirubin concentrations.

Gap-feeding influenced WBC, lymphocyte, AST, ALT, urea, creatinine, total protein, conjugated and total bilirubin concentrations.

Interaction of strain by Gap-feeding was important for haemoglobin, WBC, monocytes, total cholesterol and creatinine concentrations.

The late evening gap-feeding schedule (0-0-1) enhanced biochemical stability and reduced potential health risks across all broiler strains.

Findings suggest that strategic feeding time can be used as a management tool to improve physiological health and metabolic status in broiler chickens.

Introduction

Broiler production plays a very important role in the supply of protein for human consumption. A healthy labor force is a result of adequate protein intake. The importance of broiler production in Nigeria cannot be overemphasized. In a recent comparison study of nutrient content of rabbit meat with other farm animals, Broiler chicken was reported to possess the highest protein content of 21–50% as against rabbit and beef (20–22 and 18% protein respectively (FAO, 2008; Mohammed et al., 2017 & Kassali et al., 2022). Due to fast growth and short commercial maturity interval, broiler farming has developed much faster than indigenous chicken farming around the world. Broiler chickens are typically raised in unfertile areas with heightened stocking concentrations resulting in various health issues (Bessei, 2006; Kim et al., 2025). There is a need to satisfy the nutritional requirements of broilers since feed is an integral part of the food chain and plays an important role in the overall growth and development of broiler, and the quality and quantity of livestock products such as milk, meat and egg (Guerre, 2016). Blood biochemical analysis aids disease detection and health monitoring in poultry, as certain metabolic disorders may not show visible clinical signs (Zalesakova et al., 2025). Variations in blood components can result from factors such as nutritional status, age and management (Shaat et al., 2025). Protein levels provide crucial information regarding dehydration, immune diseases and inflammatory responses (El-Katcha et al., 2024). Serum proteins, primarily synthesized in the liver, serve many functions, including blood volume maintenance, hormone transport and immune response (Rezende et al., 2017). Lipoproteins like LDL and HDL play roles in cholesterol transport and serum cholesterol reduction, respectively (Nurul, 2016). Haematological parameters are essential for diagnosing health issue and monitoring feed toxicity in chickens (Etim et al., 2024). Blood parameters can serve as baseline data for assessing the health and improving breeding strategies for poultry (Zalesakova et al., 2025). Differences in haematological values have been reported between male and female chickens, males generally having higher mean values (Gambo et al., 2021). Serum biochemical and haematological parameters serve as essential, reliable indicators of avian health status, identifying physiological stress and diagnosing diseases. Liver enzymes,

particularly **alanine aminotransferase (ALT)** and **aspartate aminotransferase (AST)**, are critical diagnostic biomarkers for poultry, as elevated levels directly signal liver damage, toxicosis, or specific viral/bacterial (Barde et al., 2022).

Intermittent feeding and lighting have been used to restrict feed consumption and improve feed efficiency, which can lower production costs, reduce excessive abdominal fat deposition and lower the incidence of metabolic problems (Sahraei, 2014; Farghly & Makled, 2015). Feed restriction program is one of the main techniques for increasing production efficiency in broilers (Akinsola et al., 2019), controlling certain problems (sudden death syndrome and ascites) and tempering the growth rate of broilers (Adeyemi et al., 2015). However, the impact of feed restriction on physiological parameters can vary based on sex, strain, and feeding intensity (Zhan et al., 2007; Mohammadalipour et al., 2017). Chicken meat is unique in that its price is comparatively low, easy to partition into smaller parts and no religious restriction against its consumption (Nathaniel et al., 2022). Significant genotype x environment interaction effects on egg production among four strains of commercial layers had been reported in earlier study (Hakan and Turker, 2021).

Gap feeding is a management strategy used to control growth rate, reduce fat deposition and improved feed efficiency, reduce feed cost and mortality (Zubair and Leeson, 1996). Thus, this study evaluated the effects of strain and daily-gap feeding on the haematological and serum biochemical parameters of three broiler strains (Cobb 500, Ross 308, and Arbor Acre) in the humid tropics.

Material and Methods

Experimental Site

The research was carried out at the Poultry Unit of Teaching and Research Farm of the Department of Animal Production and Health, Federal University Oye Ekiti, Ikole Campus, Ekiti state, Nigeria which lies between latitude 7.4729^0N and longitude 5.3031^0E (Mindat.org, 2024). With an average annual temperature of 24.84^OC and humidity levels ranging between 19.66^OC and 22.61^OC (Weather and Climate, 2024).

Source of Experimental Birds

The experimental birds (Arbor acre, Cobb-500, and Ross-308) were purchased from Midho farms; Adebimpe village, Idi Ayunre Ibadan, Oyo state, Nigeria.

Experimental Design, Birds and Management

A total of 153-day-old chicks per strain were employed for the study, and was conducted for 56 days. On arrival, the day-old broiler chicks were unboxed, checked for deformity, counted and brooding commenced. The chicks were brooded for twenty-one days on strain basis. Thereafter, they were randomly assigned to the Strain by gap-feeding (GF) schedule replicate pens in a completely randomized manner. The experimental design adopted was a 3x3 Factorial treatment with Strain as Factor 1 (Arbor Acre, Ross-308 and Cobb-500) and Gap-feeding as Factor 2 (3-times daily, Morning and Evening feeding schedules), each factor-combination replicated 3 times making a total of 27 pens and 459 birds in all. Each replicate of factor-combination was composed of 17 chicks. Birds were fed in replicates at the pen level. Routine vaccination and medication were strictly adhered to according to prescription and specification.

Gap-Feeding Schedule and management

Chicks were fed *ad-libitum* in the first week, and then on percent body weight basis at 10.0, 9.0, 8.8, 7.7 and 7.4% of previous week's body weight; at 2, 3, 4, 5 and 6 weeks of age respectively (Jesuyon et al., 2026). The three Gap-feeding scheduled evaluated in study were as presented in Fig. 1. Each feeding schedule offered similar amount of feed daily, based on body physiological development, among treatments. Therefore, the observed effects were due to gap-feeding schedule and not due to differences in feed offered. From the 8th day (week 2) onward, each replicate received feed based on previous week's body weight in order to offer feed based

on body physiological and genetic ability. On daily basis, new feed was added to feed refusal and mixed together, but at the end of the week, final refusal was measured.

Statistical Model

The model for the study was as follows;

$Y_{ijk} = \mu + S_i + GF_j + SGF_{ij} + \varepsilon_{ijk}$

Where,

i = Levels of strain = 1–3

j = Levels of daily-gap feeding = 1–3

Y_{ij} = Observation of a trait in replicate k, of strain i, daily-gap feeding j.

μ = Intercept or overall constant

S_i = Effect of broiler strain i (i = Ross-308, Arbor acre and Cobb-500)

GFj = Effect of daily-gap feeding j (j = 1-1-1, 1-0-0, 0-0-1)

SGFij = Interaction effect of strain i, and gap-feeding j.

ε_{ijk} = Random error component committed haphardly ~ NID (0, δ^2) in strain i, gap-feeding j, replicate k.

Data collection

At the 6th week of the experiment, two birds were randomly selected from each replicate for blood sampling. Blood was collected between 07.00–10.00 hours on the collection day, about > 12 hours after the last feeding event. Samples were collected from the wing-web vein for haematological and serum biochemical analyses. Two (2) mls of blood was dispensed in well-labelled bijou bottles containing specks of ethylene diamine tetra-acetic acid (EDTA) for haematology analysis, while sterile bottles were used for collecting blood samples for serum biochemical analysis.

Haematological parameters

An aliquot of blood was diluted 1:200 with 0.4% formaldehyde and 3% triodium citrate to determine the number of RBCs in a Neubauer counting chamber (hemocytometer). The white blood cell (WBC, $X10^9$/L), red blood cell (RBC, $x10^{12}$/L), haemoglobin (Hb, g/L), and packed cell volume or hematocrit (PCV, %) were determined according to the method of Dacie and Lewis (1991). The values obtained for RBC, Hb and PCV were used to calculate mean corpuscular volume (MCV, fL) and mean corpuscular haemoglobin (MCH, pg) and mean corpuscular haemoglobin concentration (MCHC, g/L). The differential WBC counts (%) were obtained by making a differential smear stained with Wright's stain, and the percentage count taken for lymphocytes, monocytes, basophils, and neutrophils. The hematocrit value was determined by centrifuging the blood in a capillary or micro hematocrit tube at 12,000 rpm for five minutes. The haemoglobin concentration was obtained using the cyanmethaemoglobin method.

Serum biochemical parameters

Blood samples for serum analyses were transferred to a Vacutainer for serum preparation. Centrifugation was done at 3000 rpm for 15 min for serum separation. Then, the serum was transferred to an Eppendorf tube for storage at − 20°C. All samples were processed at the laboratory of the Departments of Animal Production and Health, Federal University, Oye-Ekiti. Serum samples were tested using an automatic biochemical analyser (Humalyzer-3000, USA) to estimate urea, triglyceride, total cholesterol, albumin, total protein (TP), alanine aminotransferase (ALT), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and aspartate transaminase (AST).

Statistical Analysis

Data were generated from the two blood samples randomly taken per replicate, and tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test prior to analysis. With assumptions satisfied, replicate data were arranged in the long format involving the two factors of strain, gap-feeding and subjected to Factorial ANOVA analytical procedures of SAS version 9.3 (SAS, 2010). Treatment means were separated using Tukey's Honestly Significant difference (HSD) at $P < 0.05$.

Results and Discussion

Effect of Single Factors of Strain and Gap-feeding on Broiler Haematology

Table 1 reveals the effects of the single factors, namely, strain and Gap-feeding, on the haematological parameters of broiler birds. There was significant ($P < 0.05$) effect of strain on WBC, NEU and LYMP. Arbor acre recorded highest Neut (65.75%) value, while Cobb strain recorded highest WBC (62.54%) value, but Ross strain produced the highest lymphocyte value (39.06%) respectively. Strain had no ($P > 0.05$) effect on RBC, Hb, HCT, Mon, Eos and Basophils.

The Gap-feeding regime showed significant ($P < 0.05$) effects on white blood cells and lymphocytes. The 3-times daily gap-feeding schedule (1-1-1) exposed a high lymphocyte (32.38%), while night feeding schedule (0-0-1) recorded highest WBC and lymphocytes (125.32 $x10^9$/L, 34.35%). The morning feeding regime (1-0-0) showed mid-values in WBC (117.72 $x10^9$/L) but lowest lymphocyte values (31.97%) in above mentioned parameters.

Effect of Single Factors of Strain and Gap-feeding on Serum Biochemistry

Table 2 revealed that strain and gap-feeding schedule had similar effect ($P > 0.05$) on HDL, LDL and albumin values. The values of Total cholesterol and Aspartate Aminotransferase were highest (4.90 mmol/L, 341.81µ/L) in Arbor acre, Triglyceride was highest (1.32 mmol/L) in Ross strain, while Aspartate Aminotransferase and Urea, Total protein, Conjugated Bilirubin and Total Bilirubin were highest (324.65, 6.48, 81.35, 2.29 and 3.97) in Cobb strain respectively.

Gap-feeding on the other hand revealed that 3-times daily feeding recorded significant ($P < 0.05$) highest ALT, Urea and creatinine (24.71, 6.05, 5.92). Morning feeding (1-0-0) revealed medium-level values on AST ALT, creatinine and total protein, but the evening feeding (0-0-1) revealed highest AST, TP, conjugated bilirubin and total bilirubin (330.79, 60.53, 2.14 and 3.46) respectively.

Table 1
Effect of Strain and Gap-feeding Schedule on the Haematological Indices of Broiler Chicken

PARAMETERS	***STRAIN***				***GAP-FEEDING***				***P-values***	
	AA	***RS***	***CB***	***SEM***	***1-1-1***	***1-0-0***	***0-0-1***	***SEM***	***STRAIN***	***G-F***
RBC ($\times 10^{12}$/L)	2.34	2.15	2.42	0.08	2.23	2.24	2.44	2.95	0.070	0.450
Hb (g/dl)	9.23	8.60	9.43	0.72	9.17	8.37	9.73	0.80	0.557	0.262
HCT (%)	27.70	25.80	28.30	0.75	27.50	25.10	29.20	1.19	0.557	0.262
WBC ($\times 10^{9}$/L)	119.25^{b}	113.72^{c}	125.12^{a}	2.05	115.05^{c}	117.72^{b}	125.32^{a}	1.90	0.026	0.038
HETERO (%)	65.75^{a}	52.79^{c}	62.54^{b}	2.38	60.92	60.94	59.22	2.85	0.024	0.905
LYMP (%)	28.17^{c}	39.06^{a}	31.47^{b}	2.18	32.38^{a}	31.97^{ab}	34.35^{a}	2.00	0.044	0.042
Hetero/Lymph Ratio	2.33	1.35	1.98	1.09	1.88	1.91	1.72	2.42	0.348	0.426
MONO (%)	2.76	4.18	2.68	0.70	3.03	3.48	3.11	0.80	0.628	0.777
EOS (%)	3.33	3.52	3.57	0.42	3.51	3.49	3.41	0.52	0.811	0.963
BASO (%)	0.33	0.25	0.31	0.20	0.18	0.22	0.23	0.28	0.686	0.698

NOTES: RBC = red blood cells, Hb= haemoglobin, HCT=haematocrit concentration, WBC= white blood cells, NEUT= neutrophils, HETERO=heterophils, MONO= monocytes,

EOS= eosinophils, BASO= basophils, AA= Arbor acre strain, RS= Ross-308 strain, CB = Cob-500 strain, Gap-feeding schedules: 1-1-1 = 3-times daily gap-feeding, 1-0-0 =

morning daily feeding, and 0-0-1 = evening daily feeding. ^{a,b,c,} Means with different superscripts in a row differ significantly ($p < 0.05$); SEM: Standard error of the

means, G-F = Gap-feeding schedule, *P-values* = significance level

Table 2
Effect of Strain and Gap-feeding Schedule on Serum Biochemistry of Broiler Chicken

PARAMETERS	*STRAIN*				GAP-FEEDING				*P-VALUES*	
	AA	*RS*	*CB*	*SEM*	*1-1-1*	*1-0-0*	*0-0-1*	*SEM*	*STRAIN*	*GF*
Total Cholesterol (mmol/L)	4.90^{a}	4.32^{b}	4.20^{b}	0.20	4.62	4.50	4.30	0.27	0.023	0.381
Triglycerides (mmol/L)	0.60^{c}	1.32^{a}	1.00^{b}	0.21	0.90	0.87	1.15	0.31	0.048	0.662
High-Density Lipoprotein (mmol/L)	2.80	2.32	2.38	0.26	2.57	2.40	2.53	0.30	0.239	0.830
Low Density Lipoprotein (mmol/L)	1.83	1.42	1.35	0.30	1.63	1.72	1.25	0.26	0.316	0.349
AST (U/L)	341.81^{a}	266.33^{b}	324.65^{a}	22.84	284.70^{b}	327.31^{a}	330.79^{a}	32.50	0.036	0.046
ALT (U/L)	22.38	22.67	22.68	0.32	24.71^{a}	23.46^{a}	19.55^{b}	1.72	0.990	0.048
Urea (mmol/L)	3.83^{c}	4.90^{b}	6.48^{a}	0.77	6.05^{a}	5.63^{b}	3.53^{c}	1.00	0.028	0.027
Creatinine (µmol/L)	4.19	4.62	4.78	0.31	5.92^{a}	5.21^{a}	2.46^{b}	0.65	0.807	0.011
Total Protein (g/L)	37.15^{b}	46.28^{b}	81.35^{a}	13.47	44.70^{b}	59.55^{a}	60.53^{a}	10.90	0.048	0.042
Albumin (g/L)	24.68	24.70	29.43	2.20	26.73	25.08	27.00	2.45	0.296	0.322
Conjugated. Bilirubin (µmol/L)	0.72^{b}	1.74^{a}	2.29^{a}	1.10	1.30^{b}	1.32^{b}	2.14^{a}	0.42	0.047	0.035
TOTAL Bilirubin (µmol/L)	2.13^{b}	3.28^{ab}	3.97^{a}	1.00	3.02^{a}	2.90^{c}	3.46^{a}	0.49	0.044	0.041

NOTES: AST= aspartate transaminase, ALT= alanine aminotransferase, AA= Arbor acre, RS= Ross-500, CB = Cob-500, Gap-feeding schedule: 1-1-1 = 3-times

daily gap-feeding, 1-0-0 = morning daily feeding, and 0-0-1 = evening daily feeding, SEM= Standard error of the means, ^{a,b,c,} Means with different superscripts in a row differ

significantly ($p < 0.05$).

The Interaction Effect of Strain by Gap-feeding on the Haematology of Broiler

Table 3 shows the significant ($P < 0.05$) influences of strain and Gap-feeding on haemoglobulin, white blood cells, and monocytes. This implied a genotype x environment effect on the parameters of study. AA x 3-Times daily feeding schedule revealed highest WBC (16.6 x10^{9}/L), while RS x morning (1-0-0) feeding produced the highest monocytes (4.28%); but Cobb x evening (0-0-1) feeding yielded the highest Hb values (10.75 g/dL, $P < 0.05$). All other interacting levels revealed mid-values to the above.

Interaction Effect of Strain by Gap-feeding on Serum Biochemistry of Broiler

Table 4 revealed a significant interaction effect ($P < 0.04$) of strain by Gap-feeding on total cholesterol (TC) and creatinine concentration in the serum. The Arbor Acre x three times (1-1-1) feeding exposed the highest TC (5.40 mmol/L); while RS x 1-0-0 feeding schedule recorded highest creatinine (8.01µmol/L) values. All other interacting factor-levels reported below these values.

Table 3
Effect of Interaction of Strain and Gap-feeding Schedule on the Haematology of Broiler

Parameter	STRAIN									SEM	*P-value*
	ARBOR ACRE			ROSS-308			COBB-500				
Gap-feeding	1-1-1	1-0-0	0-0-1	1-1-1	1-0-0	0-0-1	1-1-1	1-0-0	0-0-1		
Red Blood Cell ($\times10^{12}$/L)	5.0	4.02	5.10	4.15	3.90	6.30	3.76	4.00	4.87	0.56	0.052
Haemoglobin (g/dL)	9.35^{b}	8.60^{c}	9.75^{ab}	9.00^{bc}	8.10^{d}	8.70^{bc}	9.15^{b}	8.40^{c}	10.75^{a}	0.72	0.043
Hematocrit (%)	28.10	25.80	29.30	27.00	24.30	26.10	27.50	25.20	32.30	0.81	0.859
White Blood Cell ($\times10^{9}$/L)	14.60^{b}	9.60^{d}	9.37^{d}	16.60^{a}	13.30^{b}	9.50^{d}	10.20^{d}	12.40^{c}	5.55^{e}	1.92	0.038
Heterophils (%)	63.35	69.15	64.75	52.80	52.97	52.60	66.63	60.70	60.30	5.95	0.861
Lymphocytes (%)	30.45	25.02	29.02	39.45	37.60	40.12	27.60	33.27	33.90	4.85	0.051
Hetero/Lymph Ratio	2.08	2.76	2.23	1.34	1.41	1.31	2.41	1.82	1.78	0.98	0.436
Monocytes (%)	2.60^{b}	3.13^{ab}	2.55^{b}	3.98^{a}	4.28^{a}	3.57^{a}	2.28^{b}	3.03^{ab}	2.73^{b}	0.72	0.041
Eosinophils (%)	3.60	2.70	3.68	3.05	4.28	3.23	3.88	3.50	3.33	0.52	0.184
Basophils (%)	0.33	0.32	0.34	0.08	0.07	0.06	0.25	0.27	0.26	0.46	0.464

^{a,b,c,d,} Means with different superscripts in a row differ significantly ($p < 0.05$); SEM: Standard error of mean

Table 4
Effect of Interaction of Strain and Gap-feeding Schedule on Serum Biochemistry of Broiler

STRAIN	ARBOR ACRE			ROSS-308			COBB-500				
GAP-FEEDING	1-1-1	1-0-0	0-0-1	1-1-1	1-0-0	0-0-1	1-1-1	1-0-0	0-0-1	SEM	*P-value*
Total Cholesterol (mmol/L)	5.40^{a}	5.20^{a}	4.10^{ab}	3.85^{b}	4.45^{ab}	4.65^{ab}	4.60^{ab}	3.85^{b}	4.15^{ab}	0.20	0.020
Triglyceride (mmol/L)	0.60	0.55	0.65	0.75	0.95	2.25	1.35	1.10	0.55	0.27	0.139
High-Density Lipoprotein (mmol/L)	2.95	3.15	2.30	2.25	2.00	2.70	2.50	2.05	2.60	0.29	0.261
Low-Density Lipoprotein (mmol/L)	2.20	1.80	1.50	1.25	2.05	0.95	1.45	1.30	1.30	0.42	0.525
Aspartate Aminotransferase (U/L)	327.08	323.69	404.68	197.15	304.52	297.33	329.87	353.72	290.35	52.00	0.697
Alanine aminotransferase (U/L)	27.94	21.14	18.07	20.47	23.28	24.26	25.73	25.98	16.32	2.40	0.134
Urea (mmol/L)	2.15	5.50	3.85	6.40	4.65	3.65	9.60	6.75	3.10	1.90	0.323
Creatinine (µmol/L)	7.11^{ab}	3.36^{bc}	2.10^{d}	3.64^{bc}	8.01^{a}	2.21^{d}	7.02^{ab}	4.25^{b}	3.09^{c}	0.76	0.043
Total protein (g/L)	34.05	39.90	37.50	49.90	42.10	46.85	50.15	96.65	97.25	10.20	0.619
Albumin (g/L)	22.60	27.60	23.85	25.90	23.15	25.05	31.70	24.50	32.10	3.45	0.588
Conjugated Bilirubin (µmol/L)	0.64	0.89	0.65	1.64	1.46	2.13	1.62	1.61	3.64	0.68	0.609
Total Bilirubin (µmol/L)	1.57	2.53	2.28	3.04	3.33	3.46	4.44	2.84	4.63	0.70	0.625

^{a,b,c,} Means with different superscripts across a given row differ significantly ($p < 0.05$); SEM: Standard error of mean.

Discussion

Effect of Strain on Haematological Parameters

The similarity in erythrocytic indices indicate that the three commercial strains maintained comparable oxygen-carrying capacity and erythropoietic activity, suggesting that genetic differences among the strains did not compromise normal haematological function.

The higher WBC count, and the slightly high variability in heterophil and lymphocyte percentages observed in Cobb 500 could indicate an activation of the immune system in normal broiler (Scanes, 2015), while these indices were lower in the Ross broiler except lymphocytes that was very high. The higher lymphocytes in Ross might indicate chronic antigenic stimulation or stress due to regular handling. The high heterophil-lymphocyte ratio greater than 0.8 (1.35–2.33) among strains might be due to high stress through experimental stress, which could affect performance. However, all measured values were within normal physiological limits, and likely reflecting normal biological variation than evidence of impaired health or disease. The present findings partially agree with Essien et al. (2023), who reported genotype-related differences in selected haematological indices. From production perspective, Arbor Acre, Ross 308 and Cobb 500 maintained satisfactory haematological levels under the experimental conditions. The minor differences in immune-related indices observed did not suggest adverse physiological consequences. Consequently, strain selection

for commercial production could still be based primarily on production objectives, while recognizing that broiler strains are capable of maintaining normal blood health under appropriately managed gap-feeding programmes.

Effect of strain on serum Biochemistry

The high total serum cholesterol concentration of 4.9 mmol/L in Arbor Acre (AA) agrees with previous reports that this strain has a greater tendency for lipid deposition (hyperlipidemic) than Ross 308 and Cobb 500 (Al-Marzooqi et al., 2019) and this could cause poor carcass quality. The high AST concentration of 341.81 U/L meant possible muscle damage in AA. Aspartate transaminase (AST) probably leaked from damaged muscle tissue fibres, due to regular handling, capture myopathy and traumer (Lee and Choi, 2022), but urea, total protein, conjugated and total bilirubin were lowest in AA. Ross strain with mid-values of AST, urea, conjugated and total bilirubin, but the very high triglycerides could signal insulin resistance, strain and aging effect (Zhuang et al., 2019). The highest concentrations of urea, total protein, conjugated bilirubin, and total bilirubin observed in Cobb chickens should not necessarily be interpreted as normal physiological conditions. Rather, these elevations may indicate a strain-dependent metabolic response, reflecting possible genetic differences in protein metabolism, hepatic processing, renal excretory capacity, and adaptation to the imposed gap-feeding schedule. Previous studies have demonstrated that broiler strain can influence serum biochemical characteristics, supporting the possibility that genetic background contributes to variation in metabolic responses among chicken populations (Anjola et al., 2024; Bonnefous et al., 2024). Similarly, genetic relationships between blood biochemical traits have been reported in broiler lines, indicating that some biochemical indicators may have potential relevance as markers of genetically associated physiological variation (Badmus et al., 2024).

The observed pattern may therefore represent an early subclinical indication of hepato-renal stress and/or dehydration, particularly if the concentrations exceeded established physiological reference ranges. Elevated urea may reflect altered protein catabolism or impaired renal handling, whereas increased bilirubin concentrations may suggest altered hepatic processing or excretory function; however, these interpretations should be made cautiously and in conjunction with other biochemical and clinical indicators. The apparent differential response among strains further suggests that genetic background may influence the capacity of chickens to maintain metabolic homeostasis under intermittent feed restriction. This interpretation is consistent with evidence that breed and feeding restriction can differentially affect biochemical metabolism in chickens (Zhang et al., 2023), while nutritional and environmental stressors are also known to disrupt normal metabolic and physiological functions in poultry (Mangan and Siwek, 2024; Ghaleghafi et al., 2025). Consequently, these biochemical traits may have potential implications for identifying strains with greater metabolic resilience or susceptibility to nutritional and management-related stress, thereby warranting early management intervention.

The variations in AST activity among strains further demonstrated differences in metabolic activity. The levels of ALT, creatinine and bilirubin values which were within the normal physiological ranges for broiler meant no evidence of impaired liver or kidney function in evaluated strains. Above findings also suggest genetic differences in nutrient partitioning and metabolic efficiency among evaluated strains. The observed biochemical differences could also reflect normal genetic variation in metabolism rather than pathological changes. The above strain-dependent responses indicate that genetic background should be considered when optimizing feeding schedules for improved physiological performance.

Effect of Gap-Feeding on Haematological status

The high effect of gap-feeding (GF) schedule on the white blood cell (WBC) count and lymphocyte percentage could indicate some form of immune-related responses due to the 0-0-1 (evening feeding) feeding schedule, thus indicating that the timing of feed provision influenced immune-related blood indices. Since WBCs and lymphocytes play central roles in innate and adaptive immune responses, the relatively higher values observed under the evening feeding schedule may reflect enhanced immune competence and improved physiological adaptation to intermittent feeding rather than pathological changes, as all values remained within normal physiological limits. This finding partially agree with reports that feeding management can influence immune-related haematological parameters by altering nutrient availability and physiological stress responses. However, finding differ with the observations of Akinsola et al. (2019) and Ibigbami et al. (2024), who reported no significant effect of feed restriction on lymphocyte count in broiler chickens. This may be attributed to differences in feeding schedules, restriction intensity, environmental conditions and genetic background of the birds used in respective studies.

The similar effects of GF on RBC, haemoglobin, haematocrit, heterophils, H/L ratio, monocytes, eosinophils and basophils concentrations suggest that the gap-feeding programmes did not adversely affect erythropoiesis or overall haematological

homeostasis. This indicated that intermittent feeding can be implemented without compromising blood health when birds are managed under appropriate nutritional and environmental conditions. From a practical standpoint, the 0-0-1 gap-feeding schedule appears to support a more favourable immune profile while maintaining normal haematological status. Therefore, strategic evening feeding may serve as an effective management practice for improving physiological resilience without negatively affecting the health of broiler. The similarity in values of RBC, Hb and HCT observed suggests that daily gap-feeding may have minimal strain-related variation in erythrocyte production. Erythrocyte production may also be associated with variation in genetics, age, growth rate, nutritional and environmental factors.

Effect of Gap-feeding on Serum Biochemistry

The influence of GF on aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine and total protein concentrations decreased with decreasing gap-feeding intensity (1-1-1, 1-0-0, 0-0-1); whereas conjugated and total bilirubin increased. This indicated that gap-feeding primarily influenced protein metabolism and some metabolic activity, while the similarity among GF levels for total cholesterol, triglyceride, HDL, LDL and albumin may have indicated that lipid metabolism and overall liver function remained relatively stable among levels. Birds subjected to the 0-0-1 (evening feeding) schedule exhibited lower creatinine, urea and ALT concentrations than those fed three times daily (1-1-1) or once in the morning (1-0-0), and this may suggest reduced protein catabolism and improved metabolic efficiency, while the lower ALT activity indicated that the evening feeding schedule did not impose additional metabolic burden on the liver. These observations agree with previous studies reporting that appropriately timed feeding can improve metabolic adaptation and reduce physiological stress in broiler chickens (Konpechr & Sohsuebngam, 2020; Oluyemi et al., 2020). Although, AST activity was higher under the evening feeding schedule, the values remained within acceptable physiological limits and were not accompanied by corresponding increases in ALT or creatinine that could indicate liver or kidney dysfunction. Therefore, the observed differences most likely reflected normal metabolic adaptation to feeding time rather than tissue damage.

Interaction effect of strain by daily-gap feeding on haematology

The significant interaction between strain and gap-feeding schedule on haemoglobin (Hb), white blood cell (WBC), and monocyte counts demonstrates that the haematological response of broiler chickens to feeding regimen was influenced by the interaction between genetic background and nutritional environment. This interpretation is consistent with the findings of Adewole et al. (2021), who reported strain-related differences in haematological indices, particularly WBC and heterophil-to-lymphocyte ratio, under feed restriction. Similarly, Zubair and Leeson (1991) demonstrated that genotype, feeding regimen, and their interactions could influence physiological and immune-related responses in meat chickens, highlighting the complexity of genotype–environment relationships. Thus, the present findings suggest that physiological responses to gap feeding may differ among strains in traits associated with oxygen transport and immune function. Although significant strain × feeding interactions were observed, the haematological indices remained within normal physiological ranges for healthy broiler chickens, suggesting that none of the feeding schedules adversely compromised the general health status of the birds. This interpretation is also supported by Ahemen et al. (2024), who reported significant strain-related effects on selected haematological parameters while concluding that the observed values remained within normal reference ranges for healthy broilers. Thus, the main physiological response of broiler to strain by gap-feeding was manifest on haemoglobin, WBC and monocytes. The superior Hb values of Cob-500 by 0-1-1 schedule suggested improved oxygen-carrying capacity; the higher WBC and monocyte counts observed on Ross by 1-1-1 feeding schedule indicated enhanced immunological responsiveness rather than pathological alterations because values remained within established physiological limits. White blood cells and their differential components are essential for innate and adaptive immune defence; therefore, moderate increases within the normal range may reflect improved physiological adjustment to feeding management rather than disease challenge. The similarity of neutrophils, eosinophils and basophils counts further confirm that gap-feeding schedule did not induce adverse haematological disturbances. Above findings demonstrate that all three commercial broiler strains maintained normal haematological status under evaluated gap-feeding programme. Result suggests that the 0-0-1 daily gap-feeding schedule can be adopted in commercial broiler production to ensure health and welfare of birds in relation to low WBC and moncytes' concentrations.

Interaction effect of strain by gap-feeding on serum biochemistry

The important effect of strain by gap-feeding on total cholesterol and creatinine demonstrated that metabolic responses in broiler could depend on both genetic background and feeding regime. The similarity of other serum biochemical parameters indicated that liver function, protein metabolism and lipid metabolism were largely maintained irrespective of strain or feeding schedule, and that gap-feeding did not adversely affect physiological welfare of the birds. Both TCL and creatinine decreased in AA and Cob-500 with

with decreased intensity of GF schedule (1-1-1, 1-0-0, 0-0-1), but Ross increased TCL while Creatinine was highest on the afternoon feeding schedule.

Elevated total cholesterol observed in Arbor Acre × gap-feeding combinations (AAx1-1-1, and AAx1-0-0) suggested greater genetic predisposition for lipid deposition. This observation agrees with reports that Arbor Acre broilers generally exhibit higher serum cholesterol concentrations than Ross 308 and Cobb 500 due to inherent differences in lipid metabolism (Al-Marzooqi et al., 2019). Conversely, Ross 308 and Cobb 500 maintained comparatively lower cholesterol concentrations for greater efficiency in lipid metabolism under gap-feeding schedule suggested basal genetic similarity on energy partitioning when exposed to different GF feeding schedules.

Similarly, the high value observed for creatinine pointed to the variability in muscle metabolism and renal function among strains under the GF schedule. Although Ross 308 recorded comparatively higher creatinine values under the morning feeding schedule, all values remained within acceptable physiological limits, indicating normal renal function. The low creatinine concentrations recorded under the evening feeding schedule may reflect reduced muscle protein catabolism and improved metabolic adjustment during gap-periods, supporting previous reports that appropriately timed feeding can reduce metabolic stress in broiler chickens (Konpechr & Sohsuebngam, 2020; Oluyemi et al., 2020).

Results suggest that the 0-0-1 (evening) gap-feeding schedule promoted more favourable metabolic profile by maintaining relatively lower creatinine concentrations and moderating serum cholesterol across the evaluated strains; and suggested that strategic evening feeding may reduce metabolic stress without compromising normal physiological function, thereby providing poultry producers with a practical feeding strategy for improving flock health and high productive performance. Thus, strategic gap-feeding can be implemented without compromising the metabolic health, while the 0-0-1 feeding schedule consistently produced favourable biochemical profile by reducing indicators of protein catabolism while maintaining normal liver and kidney function. These findings therefore provided evidence that evening gap-feeding may be a strategic feeding practice to improve metabolic stability and physiological resilience in commercial broiler production.

Conclusion

Ross-308 strain produced the least WBC and highest triglycerides, while three-times daily Gap-Feeding schedule revealed the lowest WBC and AST values but interaction suggested the strain x evening gap-feeding with lowest WBC, monocytes, total cholesterol and creatinine as best option for broiler performance.

Abbreviations

AA
Arbor acre
ALB
Albumin
ALT
Alanine Aminotransferase
AST
Aspartate Aminotransferase
BASO
Basophils
CREAT
Creatinine
CB
Cobb-500
EOS
Eosinophils
GF
Gap-feeding

Hb
Hemoglobin
HCT
hematocret
HDL
High Density Lipoprotein
LDL
Low Density Lipoprotein
LYMP
Lymphocytes
MCV
Mean Corpuscular Volume
MCHC
Mean Corpuscular and Haemoglobin Concentration
MONO
Monocytes
HETER
Heterophils
PVC
Packed Cell Volume
RBC
Red Blood Cell Count
RS
Ross-308
TC
Total Cholesterol
TP
Total Protein
WBC
White Blood Cell Count

Declarations

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Author Contributions:

K. L.A. and O.M.A.J. conceived and designed the research and methodology. O.C.D. and A.M.A conducted the experiment, performed the literature search, and drafted the original manuscript. K.L.A. and O.M.A.J. supervised the research. O.Y.O, O.G.G., O.A.A., and K.K.S. assisted with the literature review and editing of the original draft. K.K.S.and K.L.A performed the statistical analyses and data interpretation. O.M.A.J., A.M.A., and K.L.A. edited and finalized the manuscript. All authors read and approved the final version of the manuscript before submission for publication.

Conflicts of Interest

We declare that no conflict of interest.

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Ethics Considerations: This research was conducted with the approval of the Animal Welfare and Ethics Committee of the Department of Animal Production and Health, Federal University Oye-Ekiti (approval number: APH/R-015/12/03/26), and adhered to the ethical guidelines for the use of animals in research. This study involved no clinical trial; hence, clinical trial registration is not applicable.

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Figures

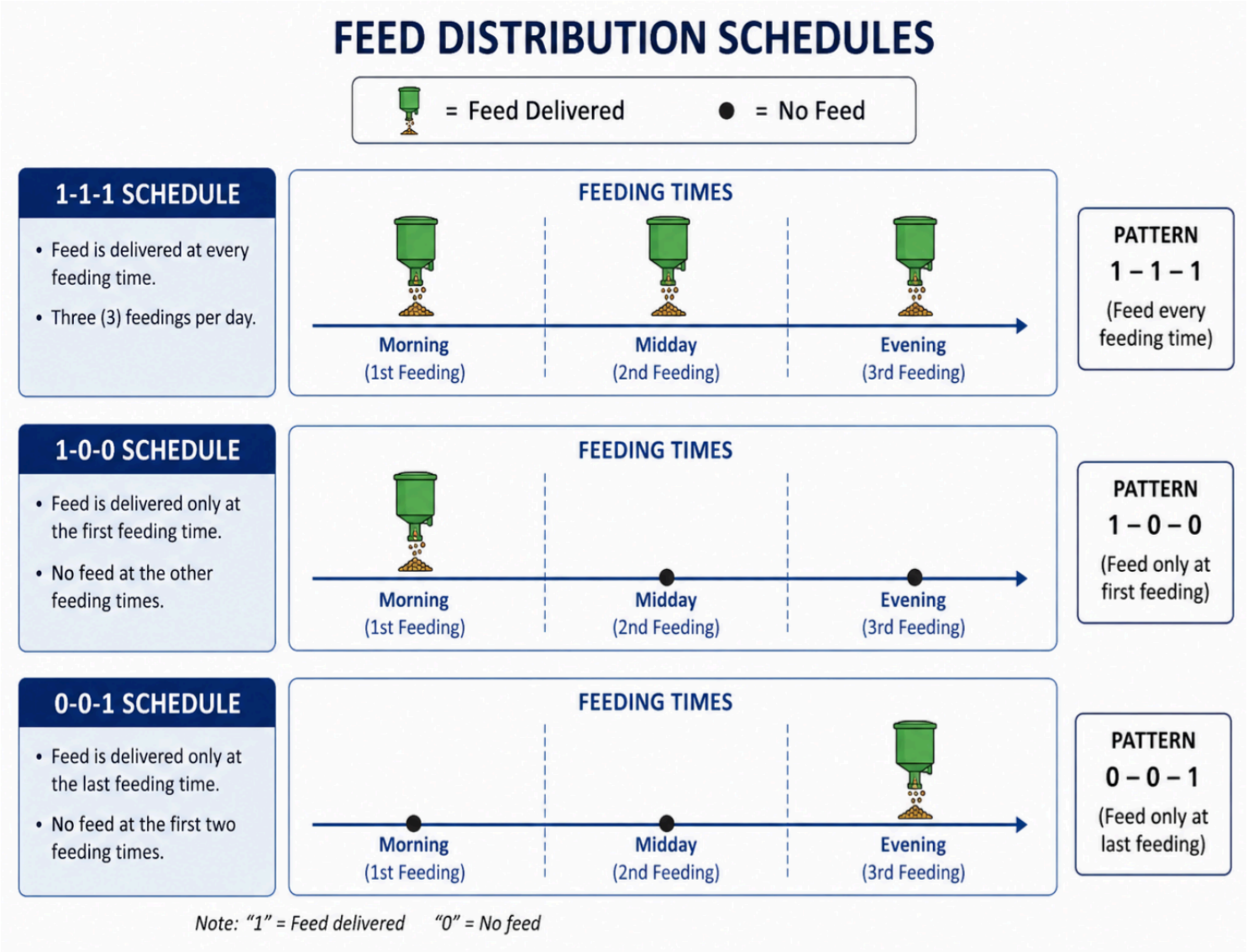


Figure 1

Schematic diagram of the three daily Gap-feeding schedules evaluated