

## Article

# Estimation of Nitrogen and Phosphorus Excretion in Different Broiler Chicken Strains and Sexes

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## Abstract

Broiler production efficiency depends on the capacity of different strains to utilize nutrients and minimize environmental losses. This study aimed to quantify the excretion and digestibility of dry matter, nitrogen, and phosphorus in Cobb 500, Ross 308, and Hubbard Flex strains at different ages under Southwest Piauí conditions. A total of 300 broilers of both sexes were distributed in a 3 × 2 factorial design (3 strains × 2 sexes) with five replicates, totaling 30 experimental units. The six-week trial included adaptation periods, excreta collection, and feed intake control each week. Dry matter, nitrogen, and phosphorus were analyzed in diets and excreta to calculate intake, excretion, and retention coefficients (DMR, NR, PR). Interactions between sex and strain were observed after 25 days for some variables. Males excreted 10.21% more phosphorus than females, and strain effects were mainly observed for phosphorus excretion, with Hubbard exhibiting lower excretion in the 32–35-day phase, Cobb presenting the highest, and Ross demonstrating intermediate excretion. Variations between sexes were observed within strains across ages. These results demonstrate that nutrient utilization is influenced by both strain and sex, highlighting the need for tailored nutritional strategies to improve feed efficiency and reduce environmental impact in broiler production.



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**Keywords:** broiler strains; nitrogen excretion; phosphorus excretion; dry matter retention; phosphorus retention; nitrogen balance; feed intake; sex effect; growth phase

## 1. Introduction

Broiler production, as well as other agribusiness sectors, contributes significantly to the generation of residues with polluting potential. Among the main elements excreted by

poultry, nitrogen (N) and phosphorus (P) stand out, as they can promote eutrophication of aquatic ecosystems and soil contamination, in addition to being involved in the emission of greenhouse-related compounds.

N and P are nutrients supplied in broiler diets through both plant- and animal-based ingredients, as well as inorganic sources. Understanding their utilization by the organism is essential for developing nutritional strategies aimed at reducing their excretion [1]. N, derived mainly from proteins, when not fully utilized, is excreted as uric acid. Under certain conditions, this compound is degraded by microorganisms, releasing ammonia and contributing to environmental issues and animal welfare problems [2]. P, in turn, has a specific limitation: a large proportion of the P present in plant ingredients is bound to phytate, a form with low availability to birds. As a result, excretion of this nutrient increases and deficiencies may occur, especially when practices to enhance its bioavailability are not adopted [3].

To minimize nutrient losses, nutritionists have explored strategies such as lowering dietary crude protein levels with the inclusion of synthetic amino acids based on the ideal protein concept, using highly digestible ingredients, and supplementing with additives that improve nutrient absorption, such as enzymes. However, the feasibility of these strategies depends directly on cost-effectiveness and the biological limitations of each genetic line [4].

Advances in broiler genetic improvement have established the sector as a producer of high-quality animal protein. Enhanced feed efficiency and increased weight gain have led to shifts in growth curves and nutritional requirements of the lines, thereby increasing heterogeneity among them [5]. Consequently, fast-growing birds tend to consume more feed, which increases nutrient intake and ultimately leads to greater nutrient excretion. In addition, factors such as feed conversion and body composition directly affect how nutrients are utilized. In other words, birds with better feed efficiency make more effective use of nutrients and consequently excrete less, while others tend to deposit more protein than fat, which alters the N and P balance [6].

Despite progress in genetics and nutrition, information regarding nutrient excretion among different broiler lines remains limited. This lack of knowledge hinders the adoption of sustainable and line-specific management practices. Therefore, the present study aimed to quantify the excretion and retention of dry matter, P, and N in Cobb 500, Ross 308, and Hubbard Flex broilers at different ages.

## 2. Materials and Methods

The experiment was conducted at the Poultry Sector of the Colégio Técnico de Bom Jesus, Professora Cinobelina Elvas Campus (CPCE), Federal University of Piauí (UFPI), located in Bom Jesus—PI, Brazil (09°04'26" S, 44°21'32" W; mean altitude 277 m). According to the Köppen classification, the region has a tropical climate with a dry winter season (Aw) [7].

The study was conducted following the guidelines of the Brazilian National Council for Animal Experimentation Control (CONCEA) and approved by the Ethics Committee on Animal Experimentation of the Federal University of Piauí (protocol number 056/2014).

The broiler strains were selected based on their commercial relevance, similar target market weights, and distinct growth profiles. A total of 300 broiler chickens from the commercial lines Cobb 500, Ross 308, and Hubbard Flex, of both sexes, hatched from fertile eggs of breeder hens aged 42, 37, and 38 weeks, respectively. Eggs were obtained from breeders in the states of Ceará, Goiás, and Minas Gerais, incubated at the COAVE cooperative (COAVE Ltda., Teresina, PI, Brazil), and chicks were housed approximately 15 h after hatching. On day 1, chicks were weighed and allocated to metabolic cages (1 × 1 × 0.5 m) equipped with trough feeders, cup drinkers, and excreta collection trays.

Cages were placed in a covered experimental poultry house with ceramic roof tiles, 3 m ceiling height, and movable side curtains to control environmental conditions. Heating was provided with 150 W incandescent lamps (C-Light Ltda., Feira de Santana, BA, Brazil) during the first days. Temperature and relative humidity were monitored daily with a thermo-hygrometer TOMATE® (Model PD003, TOMATE, São Paulo, Brazil).

The experimental design was completely randomized in a 3 × 2 factorial arrangement (lines × sexes), with five replicates and ten birds per experimental unit, totaling 30 units. The lighting program consisted of 23 h of light (natural + artificial) from day 1 to 7, and 20 h from day 8 to 42. The experimental period lasted six weeks.

Total excreta collection was performed, with three days of adaptation and four days of collection per week (days 4–7, 11–14, 18–21, 25–28, 32–35, and 39–42). Each collection period was marked at the beginning and end by including 1% ferric oxide (LANXESS Ltda, São Paulo, SP, Brazil) in the diet. Excreta were collected twice daily (12 h intervals), weighed, labeled, and stored at −18 °C. At the end of each collection, samples were thawed, homogenized, pre-dried (−40 °C for 72 h) in a freeze dryer L101 (Model 1L101, Liobras, São Carlos, SP, Brazil), and ground in an IKA micromill (Model A11 - IKA, Guangzhou, Guangdong, China). Feed and excreta samples were analyzed for phosphorus and nitrogen content. Phosphorus concentration was determined by colorimetric analysis using the molybdenum blue method at the Laboratory of Animal Nutrition, UNESP/Jaboticabal, Brazil [8]. Nitrogen content was determined by distillation using the Kjeldahl method at the Laboratory of Animal Nutrition, CPCE–UFPI, Bom Jesus, Brazil, following the procedures described in [8].

A single basal diet was provided to all strains to minimize dietary confounding effects and to allow the evaluation of intrinsic differences in nutrient utilization among genotypes and sexes under identical nutritional conditions. The nutritional composition presented in Table 1 was calculated based on ingredient composition and nutritional values reported in the Brazilian Tables for Poultry and Swine [9] and were offered in three phases (1–7, 8–21, and 22–42 days). Feed intake was calculated as the difference between the amounts supplied and the leftovers. Feed samples were stored for further analysis.

Based on feed intake, excreta production, and dry matter, nitrogen and phosphorus contents and retention coefficients (R) for each nutrient were calculated according to Sakomura and Rostagno [10].

$$R (\%) = \frac{\text{Nutrient intake} - \text{Nutrient excreted}}{\text{Nutrient intake}} \times 100$$

Data were tested for normality (Cramer–von Mises) and homogeneity of variances (Levene). When assumptions were met, data were subjected to analysis of variance (ANOVA) using the following statistical model:  $Y_{ij}(k) = \mu + L_i + S_j + L \times S_{ij} + e_{ij}(k)$ , where  $Y_{ij}(k)$  = evaluated variables of birds of line  $i$  and sex  $j$ ;  $\mu$  = overall mean;  $L_i$  = effect of line  $i$ ;  $S_j$  = effect of sex  $j$ ;  $L \times S_{ij}$  = effect of the interaction between lines and sex; and  $e_{ij}(k)$  = experimental error. When the interaction between strain and sex was significant, additional planned simple-effect analyses were conducted to better interpret the interaction. In these cases, data were stratified by one factor, and comparisons within each level were performed. Means were compared using Duncan's multiple range test at a significance level of 5%. Analyses were performed using SAS 9.0 (2002) software (SAS Institute Inc., Cary, NC, USA) with the "GLM" procedure.

**Table 1.** Ingredient and nutrient composition of the initial (1 to 7 days of age), starter (8 to 21 days of age), and grower/finisher (22–42 days of age) diets.

Ingredients (%)	1 to 7	8 to 21	22 to 42
Corn	48.738	54.589	58.803
Soybean meal	43.826	37.994	33.214
Soybean oil	3.439	3.718	4.772
Dicalcium phosphate	1.880	1.518	1.198
Limestone	0.779	0.814	0.732
Salt	0.452	0.482	0.452
Premix <sup>1 2 3</sup>	0.400	0.400	0.400
DL-Methionine (99%)	0.306	0.290	0.256
L-Lysine HCl (78.5%)	0.136	0.158	0.152
L-Threonine (99%)	0.044	0.037	0.021
Total	100.00	100.00	100.00
Nutritional Composition (%)			
ME (kcal/kg)	2.960	3.050	3.175
Crude Protein	24.00	22.00	20.25
Calcium	0.920	0.841	0.711
Available Phosphorus	0.470	0.401	0.332
Sodium	0.220	0.210	0.198
Digestible Lysine	1.324	1.217	1.096
Digestible Methionine	0.615	0.579	0.525
Digestible Methionine + Cystine	0.953	0.876	0.800
Digestible Threonine	0.861	0.791	0.712
Digestible Tryptophan	0.283	0.249	0.223
Digestible Valine	1.024	0.936	0.855
Crude Fiber	3.437	2.958	2.778
Total Phosphorus Analyzed	0.991	0.916	0.808
Total Nitrogen Analyzed	3.775	3.678	3.549

<sup>1</sup> (1 to 7): Folic acid—200.00 mg; Biotin—10.00 mg; Hydroxyquinoline chloride—7500.00 mg; Zn—17.50 g; Vitamin A—1,680,000.00 IU; Vitamin B1—436.50 mg; Vitamin B12—2400.00 µg; Vitamin B2—1200.00 mg; Vitamin B6—624.00 mg; Vitamin D3—400,000.00 IU; Vitamin E—3500.00 IU; Vitamin K3—360.00 mg; Niacin—8399.00 mg; Nicarbazine—25.00 g; Pantothenic acid—3120.00 mg; Choline—78.10 g; Se—75.00 mg; Fe—11.25 g; Mn—18.74 g; Cu—1997.00 mg; I—187.00 mg. <sup>2</sup> (8 to 21): Folic acid—199.00 mg; Biotin—10.00 mg; Hydroxyquinoline chloride—7500.00 mg; Zn—17.50 g; Vitamin A—1,680,000.00 IU; Vitamin B1—436.50 mg; Vitamin B12—2400.00 µg; Vitamin B2—1200.00 mg; Vitamin B6—624.00 mg; Vitamin D3—400,000.00 IU; Vitamin E—3500.00 IU; Vitamin K3—360.00 mg; Niacin—8400.00 mg; Monensin—25.00 g; Pantothenic acid—3119.00 mg; Choline—80.71 g; Se—75.00 mg; Fe—11.25 g; Mn—18.74 g; Cu—1996.00 mg; I—187.47 mg. <sup>3</sup> (Grower/Finisher): Folic acid—162.50 mg; Hydroxyquinoline chloride—7500.00 mg; Zn—17.50 g; Vitamin A—1,400,062.50 IU; Vitamin B1—388.00 mg; Vitamin B12—2000.00 µg; Vitamin B2—1000.00 mg; Vitamin B6—520.00 mg; Vitamin D3—360,012.00 IU; Vitamin E—2500.00 IU; Vitamin K3—300.00 mg; Niacin—7000.00 mg; Salinomycin—16.50 g; Pantothenic acid—2600.00 mg; Choline—71.59 g; Se—75.00 mg; Fe—11.25 g; Mn—18.74 g; Cu—1996.00 mg; I—187.47 mg.

### 3. Results

In the pre-starter phase (4 to 7 days), no interaction ( $p > 0.05$ ) between strain and sex, nor isolated effects of these factors, were detected for any of the evaluated variables (Table 2). From 11 to 14 days, an interaction occurred only for dry matter excretion (DME) (Table 3), with Cobb 500 males showing the highest excretion, followed by Ross 308 and, at an intermediate level, Hubbard Flex. A strain effect was also observed for phosphorus excretion (PE), with Cobb 500 birds presenting higher values.

**Table 2.** Means of DMI, dry matter intake; PI, phosphorus intake; NI, nitrogen intake; DME, dry matter excretion; PE, phosphorus excretion; NE, nitrogen excretion; DMR, dry matter retention coefficient; PR, phosphorus retention coefficient; NR, nitrogen retention coefficient of different broiler strains, in males and females, from 4 to 7 days of age.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
DMI (g/bird)	Male	77.34	75.66	76.38	76.46	0.879	0.7068	0.0898	0.8494
	Female	74.46	73.60	71.86	73.30				
	Mean	75.90	74.63	74.12					
PI (g/bird)	Male	0.55	0.54	0.54	0.54	0.006	0.7122	0.0916	0.8468
	Female	0.53	0.52	0.51	0.52				
	Mean	0.54	0.53	0.53					
NI (g/bird)	Male	2.94	2.87	2.90	2.90	0.034	0.6913	0.0937	0.8428
	Female	2.83	2.79	2.73	2.78				
	Mean	2.88	2.83	2.81					
DME (g/bird)	Male	24.98	22.96	23.60	23.84	0.537	0.1384	0.1896	0.8469
	Female	24.36	20.82	21.80	22.32				
	Mean	24.67	21.89	22.70					
PE (g/bird)	Male	0.36	0.34	0.34	0.34	0.006	0.0956	0.3194	0.6953
	Female	0.36	0.32	0.31	0.33				
	Mean	0.36	0.33	0.32					
NE (g/bird)	Male	1.34	1.28	1.23	1.28	0.032	0.1626	0.0962	0.6153
	Female	1.30	1.10	1.15	1.18				
	Mean	1.32	1.19	1.19					
DMR (%)	Male	67.66	69.50	69.26	68.80	0.637	0.1294	0.5381	0.6622
	Female	67.24	71.84	69.70	69.59				
	Mean	67.45	70.67	69.48					
PR (%)	Male	52.84	54.25	54.86	53.99	0.938	0.1216	0.4899	0.3508
	Female	51.18	59.06	55.52	55.25				
	Mean	52.01	56.66	55.19					
NR (%)	Male	54.29	54.98	57.42	56.29	0.716	0.1616	0.5977	0.8102
	Female	54.09	57.06	57.86	56.56				
	Mean	54.19	55.91	57.64					

SEM: standard error of the mean.

Between 18 and 21 days, no interactions or significant effects of strain and sex were observed for any variable (Table 4). From 25 to 28 days, however, there was an interaction between strain and sex for dry matter intake (DMI), phosphorus intake (PI), nitrogen intake (NI), nitrogen excretion (NE), phosphorus retention coefficient (PR), and nitrogen retention coefficient (NR) (Table 5). In Ross 308, females showed higher values of DMI, PI, and NI compared with males. For NE, Cobb 500 females excreted more than Hubbard and similarly to Ross. Regarding PR, Cobb 500 males retained more than females, while females had lower values than the other strains. For NR, Ross 308 females showed higher retention than males. Although no interaction was detected for DME and PE, there was a strain effect, with Cobb 500 showing higher excretion.

**Table 3.** Means of DMI, dry matter intake; PI, phosphorus intake; NI, nitrogen intake; DME, dry matter excretion; PE, phosphorus excretion; NE, nitrogen excretion; DMR, dry matter retention coefficient; PR, phosphorus retention coefficient; NR, nitrogen retention coefficient of different broiler strains, in males and females, from 11 to 14 days of age.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
DMI (g/bird)	Male	145.46	133.68	140.36	139.83	2.175	0.6197	0.1634	0.1180
	Female	133.04	140.42	128.10	133.85				
	Mean	139.25	137.05	134.23					
PI (g/bird)	Male	0.91	0.84	0.84	0.88	0.014	0.6163	0.1613	0.1172
	Female	0.83	0.88	0.80	0.84				
	Mean	0.87	0.86	0.84					
NI (g/bird)	Male	5.38	4.94	5.19	5.17	0.081	0.6179	0.1631	0.1191
	Female	4.92	5.19	4.73	4.95				
	Mean	5.15	5.07	4.96					
DME (g/bird)	Male	53.70 Aa	47.62 Ab	50.18 Aab	50.50	0.664	0.0760	0.7708	0.0455
	Female	50.88 Aa	51.68 Aa	47.90 Aa	50.15				
	Mean	52.29	49.65	49.04					
PE (g/bird)	Male	0.75	0.65	0.69	0.70	0.010	0.0290	0.1157	0.2542
	Female	0.69	0.67	0.66	0.67				
	Mean	0.72 a	0.66 b	0.68 b					
NE (g/bird)	Male	2.83	2.43	2.66	2.66	0.046	0.0860	0.1838	0.1475
	Female	2.61	2.55	2.41	2.53				
	Mean	2.71	2.49	2.55					
DMR (%)	Male	63.06	64.28	64.16	63.83	0.418	0.4588	0.0985	0.9893
	Female	61.68	62.94	62.54	62.38				
	Mean	62.37	63.61	63.35					
PR (%)	Male	43.22	45.95	45.65	44.94	0.659	0.0791	0.8194	0.4760
	Female	42.97	47.56	43.41	44.65				
	Mean	43.09	46.7	44.53					
NR (%)	Male	46.01	50.54	48.71	48.42	0.814	0.1276	0.9617	0.9348
	Female	46.82	50.66	48.02	48.53				
	Mean	46.41	50.60	48.41					

Means followed by the same letters, lowercase in the row and uppercase in the column, do not differ according to Duncan's test at 5% probability ( $p > 0.05$ ). SEM: standard error mean.

From 32 to 35 days, no isolated effects of strain or sex were observed, but there was interaction for DMI, PI, NI, DME, NE, PR, and NR (Table 6). In Ross 308, females again showed higher DMI, PI, and NI. For DME and NE, Cobb 500 females excreted more than Hubbard and similarly to Ross. In PR, differences were found only among females, with Hubbard Flex showing higher retention than Cobb 500, while Ross 308 was similar. For NR, Cobb 500 males retained more than females.

In the final phase (39 to 42 days), no strain effect was observed for any variable (Table 7). However, a sex effect was detected for PE, with males excreting more than females in all strains. An interaction was found only for DME, with lower excretion in Hubbard Flex females and higher values in Ross 308 females.

**Table 4.** Means of DMI, dry matter intake; PI, phosphorus intake; NI, nitrogen intake; DME, dry matter excretion; PE, phosphorus excretion; NE, nitrogen excretion; DMR, dry matter retention; PR, phosphorus retention; NR, nitrogen retention of different broiler strains, males and females, during the period from 18 to 21 days of age.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
DMI (g/bird)	Male	232.9	239.66	234.30	235.83	2.417	0.4167	0.6309	0.3425
	Female	246.1	237.84	230.16	237.47				
	Mean	239.56	238.75	232.23					
PI (g/bird)	Male	1.46	1.50	1.47	1.48	0.015	0.4191	0.6307	0.3397
	Female	1.55	1.49	1.45	1.49				
	Mean	1.51	1.50	1.46					
NI (g/bird)	Male	8.62	8.86	8.51	8.72	0.089	0.4188	0.6311	0.3475
	Female	9.10	8.80	8.66	9.78				
	Mean	8.86	8.83	8.59					
DME (g/bird)	Male	78.06	71.64	75.42	75.04	1.573	0.0942	0.6140	0.3650
	Female	80.20	72.56	67.74	73.50				
	Mean	79.13	72.10	71.58					
PE (g/bird)	Male	1.18	1.12	1.12	1.14	0.025	0.0920	0.4033	0.5292
	Female	1.22	1.07	1.03	1.10				
	Mean	1.20	1.09	1.07					
NE (g/bird)	Male	3.89	3.92	4.12	3.98	0.074	0.6519	0.5726	0.1841
	Female	4.17	3.81	3.69	3.87				
	Mean	4.03	3.86	3.90					
DMR (%)	Male	68.84	70.14	67.74	68.90	0.339	0.4237	0.2839	0.0764
	Female	68.74	69.50	70.58	69.60				
	Mean	68.79	69.82	69.16					
PR (%)	Male	48.22	48.84	47.27	48.11	0.637	0.5465	0.1461	0.4441
	Female	48.06	50.84	51.13	50.02				
	Mean	48.14	49.86	49.20					
NR (%)	Male	54.79	56.82	54.43	55.05	0.536	0.3140	0.5597	0.4802
	Female	53.68	56.69	56.60	55.66				
	Mean	54.23	56.25	55.64					

SEM: standard error of the mean.

**Table 5.** Means of DMI, dry matter intake; PI, phosphorus intake; NI, nitrogen intake; DME, dry matter excretion; PE, phosphorus excretion; NE, nitrogen excretion; DMR, dry matter retention; PR, phosphorus retention; NR, nitrogen retention of different broiler strains, in males and females, during the period from 25 to 28 days of age.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
DMI (g/bird)	Male	420.82 Aa	382.00 Ba	419.86 Aa	406.77	5.650	0.574	0.385	0.004
	Female	416.94 Aa	439.00 Aa	392.76 Aa	416.23				
	Mean	418.67	410.72	406.31					

Table 5. Cont.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
PI (g/bird)	Male	2.33 Aa	2.12 Ba	2.33 Aa	2.25	0.032	0.573	0.383	0.004
	Female	2.31 Aa	2.43 Aa	2.17 Aa	2.31				
	Mean	2.32	2.27	2.25					
NI (g/bird)	Male	15.57 Aa	14.15 Ba	15.43 Aa	15.05	0.209	0.572	0.383	0.004
	Female	15.42 Aa	16.24 Aa	14.53 Aa	15.40				
	Mean	15.49	15.19	15.03					
DME (g/bird)	Male	91.48	84.08	83.00	86.18	1.630	0.014	0.683	0.669
	Female	92.40	84.48	78.02	84.96				
	Mean	91.94a	84.28 b	80.51 b					
PE (g/bird)	Male	1.27	1.23	1.14	1.21	0.030	0.005	0.252	0.126
	Female	1.49	1.19	1.14	1.24				
	Mean	1.35 a	1.21 b	1.14 b					
NE (g/bird)	Male	4.13 Aa	4.26 Aa	4.21 Aa	4.20	0.108	0.070	0.749	0.028
	Female	4.85 Aa	3.83 Aab	3.73 Ab	4.11				
	Mean	4.45	4.07	3.97					
DMR (%)	Male	78.90	77.96	80.20	79.02	0.446	0.198	0.594	0.147
	Female	77.56	80.76	80.12	79.48				
	Mean	78.23	79.36	80.16					
PR (%)	Male	64.51 Aa	60.97 Aa	66.96 Aa	64.14	1.124	0.023	0.264	0.004
	Female	54.41 Bb	67.25 Aa	64.70 Aa	62.67				
	Mean	60.01	64.11	65.83					
NR (%)	Male	72.76 Aa	69.85 Ba	72.83 Aa	71.74	0.675	0.523	0.124	0.048
	Female	70.89 Aa	76.11 Aa	74.31 Aa	74.06				
	Mean	71.96	72.63	73.57					

Means followed by the same letters, lowercase in the row and uppercase in the column, do not differ according to Duncan's test at 5% probability ( $p > 0.05$ ). SEM: standard error mean.

**Table 6.** Means of DMI, dry matter intake; PI, phosphorus intake; NI, nitrogen intake; DME, dry matter excretion; PE, phosphorus excretion; NE, nitrogen excretion; DMR, dry matter retention; PR, phosphorus retention; NR, nitrogen retention of different broiler strains, in males and females, during the period from 32 to 35 days of age.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
DMI (g/bird)	Male	503.14 Aa	454.80 Ba	503.08 Aa	487.01	6.473	0.383	0.563	0.007
	Female	499.32 Aa	513.84 Aa	467.43 Aa	497.40				
	Mean	501.44	484.32	489.71					
PI (g/bird)	Male	2.79 Aa	2.52 Ba	2.79 Aa	2.70	0.036	0.383	0.562	0.007
	Female	2.77 Aa	2.85 Aa	2.59 Aa	2.76				
	Mean	2.78	2.68	2.71					
NI (g/bird)	Male	17.10 Aa	15.46 Ba	17.10 Aa	16.55	0.220	0.385	0.564	0.007
	Female	16.97 Aa	17.47 Aa	15.89 Aa	16.91				
	Mean	17.04	16.46	16.64					

Table 6. Cont.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
DME (g/bird)	Male	96.98 Aa	93.18 Aa	102.52 Aa	97.56	2.409	0.114	0.300	0.006
	Female	113.68 Aa	104.66 Aab	87.00 Ab	101.78				
	Mean	105.33	98.92	94.76					
PE (g/bird)	Male	1.41	1.44	1.37	1.41	0.020	0.022	0.956	0.194
	Female	1.50	1.44	1.29	1.40				
	Mean	1.44 a	1.44 a	1.33 b					
NE (g/bird)	Male	4.96 Aa	5.03 Aa	5.33 Aa	5.11	0.113	0.252	0.552	0.011
	Female	5.71 Aa	5.41 Aab	4.54 Ab	5.19				
	Mean	5.30	5.22	4.93					
DMR (%)	Male	80.74	79.48	79.66	79.96	0.282	0.539	0.607	0.059
	Female	78.57	79.64	80.82	79.75				
	Mean	79.77	79.56	80.24					
PR (%)	Male	64.32 Aa	59.52 Aa	63.30 Aa	63.12	0.607	0.123	0.634	0.002
	Female	59.53 Ab	64.30 Aab	64.81 Aa	62.41				
	Mean	62.19	61.91	64.10					
NR (%)	Male	70.99 Aa	67.45 Aa	68.87 Aa	69.10	0.518	0.416	0.585	0.018
	Female	66.29 Ba	68.96 Aa	70.50 Aa	68.75				
	Mean	68.90	68.20	69.68					

Means followed by the same letters, lowercase in the row and uppercase in the column, do not differ according to Duncan's test at 5% probability ( $p > 0.05$ ). SEM: standard error of the mean.

**Table 7.** Means of DMI, dry matter intake; PI, phosphorus intake; NI, nitrogen intake; DME, dry matter excretion; PE, phosphorus excretion; NE, nitrogen excretion; DMR, dry matter retention; PR, phosphorus retention; NR, nitrogen retention of different broiler strains, in males and females, during the period from 39 to 42 days of age.

Variable	Sex (S)	Lines (L)			Mean	SEM	Probability		
		Cobb	Ross	Hubbard			L	S	L × S
DMI (g/bird)	Male	371.72	362.54	383.24	372.50	3.982	0.8087	0.3649	0.2068
	Female	371.78	368.22	365.30	365.10				
	Mean	371.75	365.38	368.27					
PI (g/bird)	Male	2.06	2.01	2.12	2.06	0.022	0.8060	0.3671	0.2080
	Female	2.06	2.04	1.97	2.02				
	Mean	2.06	2.02	2.04					
NI (g/bird)	Male	12.64	12.32	13.02	12.66	0.136	0.8050	0.3665	0.2087
	Female	12.64	12.52	12.08	12.41				
	Mean	12.64	12.42	12.55					
DME (g/bird)	Male	133.24 Aa	117.30 Aa	131.42 Aa	127.32	2.498	0.6594	0.2691	0.0393
	Female	122.16 Aab	129.62 Aa	114.42 Ab	122.06				
	Mean	127.70	123.46	122.92					
PE (g/bird)	Male	2.14	2.00	1.99	2.05 A	0.044	0.3869	0.0372	0.8804
	Female	1.92	1.88	1.79	1.86 B				
	Mean	2.03	1.94	1.89					

Table 7. Cont.

Variable	Sex (S)	Lines (L)			Probability				
		Cobb	Ross	Hubbard	Mean	SEM	L	S	L × S
NE (g/bird)	Male	7.56	7.05	7.34	7.32	0.140	0.7629	0.6770	0.4402
	Female	7.21	7.47	6.89	7.19				
	Mean	7.36	7.28	7.12					
DMR (%)	Male	64.18	67.58	65.80	65.85	0.560	0.6845	0.5454	0.1009
	Female	67.02	64.80	67.76	66.85				
	Mean	65.60	66.19	66.78					
PR (%)	Male	30.08	32.75	33.73	32.31	0.901	0.5110	0.1453	0.8822
	Female	32.22	34.93	35.83	34.99				
	Mean	32.38	33.96	34.78					
NR (%)	Male	42.53	43.57	43.84	43.42	0.923	0.9420	0.7869	0.9823
	Female	42.49	42.89	42.85	42.73				
	Mean	42.51	43.23	43.34					

Means followed by the same letters, lowercase in the row and uppercase in the column, do not differ according to Duncan's test at 5% probability ( $p > 0.05$ ). SEM: standard error of the mean.

#### 4. Discussion

In the pre-initial phase, from 4 to 7 days of age, no significant effects were observed between strains and sex, nor was any significant interaction detected for any of the variables evaluated. This homogeneity among the results may be related to the fact that young animals still do not have a fully functional gastrointestinal tract and rely on nutrients from the residual yolk. According to Noy and Sklan [11] and Uni et al. [12], during the first week post-hatch, chicks still use the yolk as their main source of energy and protein, going through a period of rapid gastrointestinal development, which reduces the direct impact of dietary variations or genetic differences on nutrient intake and digestibility, thereby homogenizing physiological responses even among different genotypes.

Between 11 and 14 days, Cobb 500 chicks exhibiting higher dry matter and phosphorus excretion, resulting in lower retention. Ross 308 showed better utilization, and Hubbard remained in an intermediate position. According to Richards-Rios et al. [13], in a study investigating ileal microbiota development in three broiler strains, Cobb 500, Hubbard JA87, and Ross 308, it was observed that Ross 308 chicks develop their microbiota earlier than the others, with a sequential increase in species such as *Candidatus* *Arthromitus* and *Lactobacillus*, whereas Cobb 500 showed a delay, with *Enterobacteriaceae* predominating up to 14 days. Bacterial colonization occurs later in the other strains, followed by Hubbard and lastly Cobb 500. Although gut microbiota development is influenced by diet composition [14], all strains in the present study received the same diet, allowing differences in microbiota maturation to be interpreted primarily as strain-related rather than diet-driven. This difference directly impacts the maturation of the intestinal epithelium and compromises mineral absorption, such as phosphorus, which corroborates the results obtained in the present study, where Cobb 500 exhibited higher PE and lower PR.

The period from 25 to 28 days showed the greatest heterogeneity among the factors, with interaction effects observed for DMI, PI, NI, NE, PR, and NR. For DMI, PI, and NI, differences were detected only in the Ross 308 strain, with females consuming more than males. This pattern contrasts with most reports in the literature, which generally describe higher feed intake in males. However, sex-related differences in nutrient intake and utilization are known to depend on the interaction between growth phase and genetic background

and may not be consistently expressed across all strains, as interactions between genotype and sex have been shown to significantly affect nutrient utilization in broilers [15].

Male and female broilers also differ in crude protein requirements and nutrient utilization patterns, with males having higher protein requirements and differing transporter expression, which can influence intake and retention patterns [16]. Additionally, growth and body composition differ by sex, with female broilers tending to plateau in growth earlier and deposit more fat than males, which may further affect nutrient partitioning and utilization [17]. Patterns of nutrient partitioning also differ between sexes, with males depositing more body protein and females storing more fat and energy, which could contribute to differences in nitrogen and phosphorus utilization [18]. Therefore, the higher intake observed in Ross 308 females appears to reflect strain-specific growth characteristics that allow sex-dependent differences in nutrient utilization to be expressed during this growth phase [15,17].

Regarding NE, Cobb 500 females exhibited higher excretion compared to Hubbard and were similar to Ross 308, a pattern observed only in females. According to Wecke and Liebert [18], unlike males, which show higher protein retention, females tend to direct part of consumed nitrogen toward lipid deposition, reducing retention efficiency and increasing excretion. Additionally, Cobb exhibits a faster growth curve than other strains, which can intensify nitrogen losses in females, leading to lower efficiency compared to males [19].

For PR, a significant difference was observed between males and females of Cobb 500, with higher retention attributed to males. Coupled with higher PE in this strain, the impact on retention was more evident in females. According to Wecke and Liebert [18], males tend to have higher protein and mineral deposition from the third week, maintaining constant lipid deposition, while females increase fat mass.

Influenced by higher NI, Ross 308 females exhibited higher NR compared to males. This response may be associated with strain-specific growth characteristics and sex-dependent differences in nutrient partitioning, allowing females to utilize dietary nitrogen more efficiently during this growth phase.

The behavior of DMI, PI, and NI remained in the subsequent phase, 32 to 35 days, reinforcing the environmental and growth curve influence on nutrient intake in Ross 308 broilers. The same applied to NE and DME, with Cobb 500 females showing higher excretion and Hubbard Flex females showing lower excretion.

Adding the effect of PR, Hubbard Flex females stood out with higher mineral retention, resulting from slightly lower intake than males and lower PE. Overall, Hubbard Flex broilers exhibited greater nutrient utilization efficiency, especially in females, who showed lower excretion. According to Sakomura et al. [19], Ross 308 has better mineral absorption and protein deposition efficiency than Cobb 500, despite the latter's precocity. This aligns with the present study, where Cobb 500 had higher nutrient excretion rates.

Although studies on phosphorus retention in Hubbard Flex are scarce, this strain is considered intermediate, with traits favoring higher mineral deposition in later growth phases. Ross 308 females may have been influenced by factors increasing intake, as noted in this and the previous phase, which may have benefited Hubbard Flex females compared to Ross 308. Finally, Cobb 500 females had lower NR than males, consistent with females directing part of nutrients, particularly nitrogen, toward fat deposition from the third week.

In the final phase, from 39 to 42 days, results were mostly uniform among strains, with DME being the only variable showing interaction. During this period, Hubbard Flex females stood out with lower dry matter excretion compared to others, in contrast to Ross 308, which had the highest values. This behavior may relate to higher previous intake in Ross 308 females, possibly influenced by environmental factors and physiological sex

traits, such as prioritizing body maintenance and lipid deposition while reducing protein deposition [18].

Supporting this hypothesis, DME was influenced by NE, which was numerically higher in Ross 308 females. Thus, higher intake combined with stabilized growth in the final phase may have contributed to increased excretion observed in this study. Conversely, Hubbard Flex females, with traits favoring nutrient utilization efficiency and a more gradual growth profile, maintained lower DME.

The only main variable showing significant variation was PE, with females generally excreting less than males. This may relate to higher male intake at older ages, leading to greater phosphorus intake when bone formation is nearly complete, resulting in higher phosphorus excretion. As noted by Muñoz et al. [20] and Leeson and Summers [21], up to approximately 20 days, feed intake is similar between sexes, but males subsequently consume larger amounts, reaching values around 10% higher than females.

In this final phase, evaluated parameters across the three strains showed greater homogeneity. According to Macari et al. [22], regarding maturity, protein growth mainly varies with age rather than genotypic or phenotypic traits. Macari et al. [22] also report deposition peaks around 42 days, stabilizing briefly and decreasing near 60 days. Additionally, Demuner et al. [23], applying Gompertz growth models to Ross 308, Cobb 500, and Hubbard Flex, observed the inflection point corresponding to peak protein deposition between 37 and 40 days for males and 38 and 41 days for females.

Thus, regardless of strain, by around 42 days, the animals reached peak protein deposition and consolidated bone formation, which may explain the reduced differences in nutrient retention between strains observed in this final phase.

## 5. Conclusions

Significant differences were observed in the excretion and retention of dry matter, nitrogen, and phosphorus among the evaluated broiler strains, becoming more pronounced from the third week of life as strain-specific growth characteristics began to influence nutrient utilization. Overall, Cobb 500 showed higher nutrient excretion and lower retention coefficients, particularly in females, whereas Hubbard Flex, especially females, exhibited greater nutrient utilization efficiency, with lower losses and higher retention. Ross 308 presented intermediate responses.

These results highlight the importance of considering both strain and sex in diet formulation, as accounting for such differences may improve nutrient efficiency and contribute to reduced nitrogen and phosphorus excretion, supporting more sustainable poultry production and environmental management.

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## Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
CONCEA	Council for Animal Experimentation Control
DME	Dry matter excretion
DMI	Dry matter intake
DMR	Dry matter retention coefficient
N	Nitrogen
NE	Nitrogen excretion
NI	Nitrogen intake
NR	Nitrogen retention coefficient
P	Phosphorus
PE	Phosphorus excretion
PR	Phosphorus retention coefficient
PI	Phosphorus intake
SEM	Standard error of the mean

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