Differences in broiler bone, gut, and tissue mineral parameters, as influenced by broilers grouped based on bodyweight.

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Abstract

Context: Variation in bodyweight is an undesirable feature in broiler production. Compositional differences between high and low bodyweight (BW) chicks in bone parameters and tissue mineral concentrations may provide insight into underlying causes of variation in bodyweight.

Aims: This study aimed to investigate differences in bone measurements, tissue mineral concentrations, and gut parameters of Ross 308 male broiler chicks with identical diet and environmental conditions but with distinct BW on day 21.

Methods: A 3-week growth study was carried out involving 40 male, day-old chicks from the Ross 308 line. Chicks were reared in a deep litter house with a controlled environment and the same commercial diet. On D21 BW data collected from chicks were used as a criterion to rank them into high and low BW groups (n = 11/group). Retrospective bodyweight measurements were compared between groups. Birds were selected for assessing bone parameters, liver mineral profile, gut pH, gizzard NDF and ADF contents.

Key results: Retrospective BW measurements among the high and low BW groups showed a consistent difference in bodyweight between the two groups in early life. Tibial concentration of Mn and Sr were significantly higher ($p<0.05$) in the low weight (LW) group relative to the high weight (HW) group. Concentrations of Mn, Cd and Cs in the liver tissue showed significant differences, with the LW group having higher concentration of these trace elements. The LW chicks had lower gizzard digesta pH, higher gizzard NDF and a statistical tendency for higher ADF concentrations compared to the HW group.

Conclusions and Implications: In summary, broilers ranked based on day 21 BW showed differences in tibial bone, gut, and tissue mineral parameters. The LW group had lower gizzard pH and higher gizzard fiber content compared to the HW group, which may be attributed to factors

such as behavioral activities relating to more litter consumption among the LW group compared to the HW group.

Keywords: Broilers, Bone strength, Minerals, Animal production

Introduction

Bodyweight (BW) uniformity is an important consideration in broiler production (Molenaar, et al., 2008; Jin et al., 2019) as poor BW gain in young broilers is a critical factor underpinning morbidity, mortality, and welfare (Noy and Pinchasov, 1993; Noy and Sklan, 1997 and Chen, et al., 2013). The growth of contemporary meat-type chickens has been improved over decades through genetic selection, improved husbandry, nutritional and health management (Zuidhof et al., 2014). However, significant variation in BW has been reported (Chen, et al., 2013; Neto, et al., 2013; Vasdal, 2019; Lundberg, et al., 2021) which may lead to carcass devaluation and economic loss at the end of broiler production (Lundberg, et al., 2021).

Different strategies and feeding managements to improve the growth performance and uniformity of broilers have been investigated and excellent reviews are available (Ipek, et al., 2009; Islam, et al., 2012; Wang, et al., 2015; Zuidhof, et al., 2017; Gous, 2018, and Jha, et al., 2019). However, such dietary and management strategies have investigated the physiological and genomic responses of treatments on groups of broiler chicks without accounting for their individual differences and responses. The heterogenicity of the experimental test population may limit the success of nutritional interventions and hinder an understanding of the underlying mechanism.

Many factors affect broiler growth in early life including genetics, age of parent breeders, environmental conditions, nutrition, and management practices which consequently may contribute to variation in bodyweight of broilers (Lundberg, et al., 2021). In a previous study, some of these factors such as diet, sex, breeder flock age and environmental conditions were controlled

to understand the inherent variation that arises in a flock of Ross 308 male chicks (Elvis-Chikwem et al., 2020). In that study under highly controlled experimental conditions, a uniformity score of 56% was noted which was considerably lower than the recommended uniformity score of 80-85% in broilers (Toudic, 2007).

Mineral metabolism is a critical factor in broiler growth performance, as some minerals play major roles as catalyst in many enzyme and hormone systems (Suttle, 2010). Minerals support growth, skeletal integrity, and other physiological processes; with mineral homeostasis regulated by the balance between tissue mineral storage and excretion. Tissue mineral concentration has been a tool reported by researchers (Sunder, et al., 2006: Wang, et al., 2007 and Yair and Uni, et al., 2011) to assess bioavailability, physiological mineral utilization, and storage, especially in the bone and liver tissues. The rapid absorption of minerals especially Ca in hatchlings (Skinner and Waldroup 1995; and Ravindran and Abdollahi, 2021) to meet the developing skeletal need, makes it a dynamic and potentially valuable biomarker important to ascertain the mineral status of chicks' post hatch.

Gut pH has been used to assess gut health functionality and the solubility of minerals such as Cu and Ca (Shafey, et al., 1991; Maenz et al., 1999; Dibner and Buttin, 2002; Loddi et al., 2004; Ferket, 2004; Pang and Applegate, 2007 and Morgan et al., 2014). A healthy gut is vital in enhancing the productivity of broiler chicks and facilitates nutrient uptake by absorptive cells, greater nutrient digestibility, and its bioavailability (Lewis et al., 2003; Niewold, 2007). This study aimed at understanding the differences in selected bone parameters, liver mineral profile and gut parameters of low BW and high BW chicks in the early weeks of life.

Materials and methods

Ethical Approval and location of the experiment:

The current study was conducted at the poultry research unit of the Nottingham Trent University, Brackenhurst campus and the laboratory analysis was carried out at the University of Nottingham, Sutton Bonington Campus. All experimental protocols used in the animal study were approved by Nottingham Trent University's Animal Ethics Review Committee (code: ARE192024), and the University of Nottingham's Animal Welfare and Ethical Review Body (Approval reference number: 255)

Birds, Housing and Diets:

A total of 40-day-old male Ross 308 broiler chickens were used in this experiment. Chicks were individually weighed then randomly allocated to one of four deep litter pens, each containing 10 chicks. Each pen was equipped with feeders and nipple drinkers until day 21, the lighting protocol started on 23hr on D1 with darkness increasing by 1hr/day until 6hrs of darkness was established. The chicks were fed a common commercial starter mash diet from D1 till D14 and were fed a grower diet until 21 days of age *ad libitum*. The starter and grower diets used in the present study are presented in table 1A and B below, water was also provided *ad libitum* via nipple drinkers. The temperature of the deep litter house was set to 31°C on D0 and gradually decreased over the course of a 21-day period to 22 degrees, while pine shavings were used as litter substrate. Birds were individually weighed each week up to day 21.

Table 1A: Ingredient composition and calculated nutrient composition of commercial starter diet (Fed from D0-D14)

Ingredients	%
Wheat	40.77
Soyabean meal	29.00
Oats	15.00
Maize	10.00
Limestone flour	0.88
Dicalcium phosphate (18%)	1.17
Soya oil	0.80
Salt	0.23
Sodium bicarbonate	0.10
Liquid lysine 50(T)	0.61
Methionine H-A liquid	0.51
Soya oil spray	0.40
Vitamin premix[1]	0.35
L-Threonine	0.15
Ronozyme liquid 35.7%[2]	0.03
Total	100
Calculated components	
Metabolisable energy MJ/Kg	12.56
Dry matter	87.72
Moisture	12.28
Crude protein	21.81
Crude Ash	5.12
Crude fat	3.74
Crude fiber	3.92
Total Calcium (g/kg)	0.78
Available Phosphorus (g/kg)	0.58

[1] Vitamin/mineral premix supplied per kg diet: Selenium: 0.25mg, Iron: 50mg, Manganese: 120mg, Molybdenum: 1mg, Vitamin A: 12,00iu, Vitamin D: 2500iu, HyD: 2500iu, Vitamin E: 100iu, Vitamin K: 5mg, Vitamin B1: 3mg, Vitamin B2: 8mg, Vitamin B6: 6mg, Vitamin B12: 30ug, Iodine: 2mg, Folic: 2mg, Nicotinic: 70mg, Cal-D-Pant: 18mg, Biotin: 0.3mg, Choline: 250mg, Copper: 20mg, Zinc: 100mg

[2] Ronozyme WX: 100mg, Ronozyme HiPhos: 100mg, Ronozyme ProAct: 200mg, Maxiban: 625mg, CRINA poultry plus: 300mg, Aresto: 25mg.

Table 1B: Ingredient composition and calculated nutrient composition of commercial grower diet (Fed from D15-D21)

Composition	Quantity	Units
Wheat	46.50	%
Soya bean meal (Dehulled)	34.50	%
Rapeseed (Whole)	6.60	%
Maize distillers' grain	4.76	%
Soya bean oil	3.45	%
Calcium Carbonate	0.85	%
Mono Dicalcium Phosphate	1.85	%
Sodium Chloride	0.30	%
Lysine-HCL	0.39	%
Methionine-DL	0.30	%
Premix	0.50	%
Total	100	%
Analytical Constituents		Units
Crude fat	5.02	%
Crude protein	21.27	%
Crude fibre	2.78	%
Crude ash	4.74	%
Methionine	0.65	%

Sodium	0.13	%
Lysine	1.32	%
Total phosphate	0.44	%
Calcium	0.64	%
Vitamin A	8	MIU/T
Vitamin D3	5	MIU/T

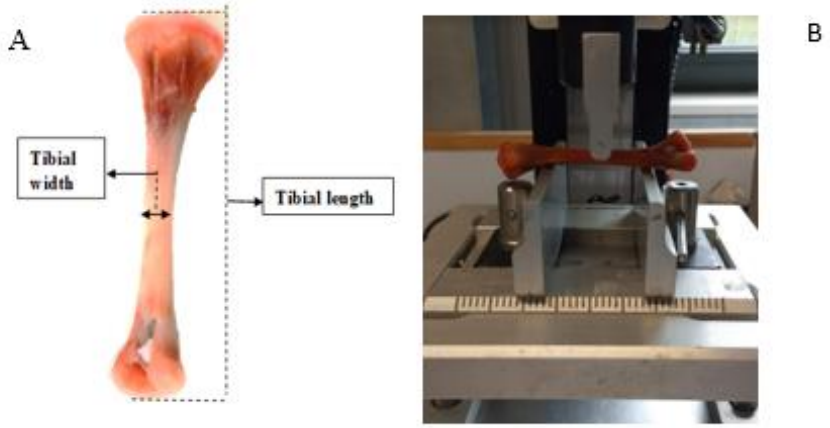
Birds grouping and sample collection.

On day 21, all chicks were weighed individually and then the heaviest (n = 11) and lightest (n = 11) chicks from across all pens were selected for euthanasia as high bodyweight (HW) and low bodyweight (LW) chicks respectively. Birds were euthanized by cervical dislocation and immediately after euthanasia, the crop, gizzard, and small intestine were dissected from each individual chick of each BW group. A digital pH piercing probe (Apera instruments PH60S spear pH tester) was inserted directly into the digesta in the crop lumen, proximal gizzard, and distal ileum of the sampled birds. The pH was measured and recorded in triplicate for each chick, and liver, gizzard, and tibial bones were collected for further analysis and stored at -80^0C.

Bone morphometric measurements and breaking analysis:

Tibial weights were recorded using a precision balance (Ohaus Spu6001) and the tibial length and width were measured using a digital caliper as shown in Figure 1. Bone breaking strength was analyzed using a texture analyzer TA. XT plus 100 (Stable Microsystems, Guildford, UK) with a 50 kg load cell set up and 3 point–bend fixture (Alkhtib, et al., 2020) which generated the maximum force (N) value for each sample.

Figure 1. Tibial length and width measurement and bone breaking analysis using the texture analyzer.



(A) Tibia bone showing points of morphometric measurements for width, and length. (B) Bone breaking test using a texture analyser TA. XT plus 100 (Stable Microsystems, Guildford, UK)). The tibia bone was placed horizontally on two support holders and submitted to a vertical force from above.

Crude ash and mineral analysis

The legs collected and stored at -80°C were allowed to thaw at room temperature and were subsequently defleshed to extract the tibial bones. The extracted tibias of each bird were collected for estimation of percentage ash determination on a fat-free, dry basis. Tibias were defatted by soaking in petroleum ether for two hours, and then allowed to dry in a fume cupboard to expel petroleum ether residues. The defatted tibial bones were oven-dried at 105°C for 24hrs to achieve a constant weight and ashed at 600°C overnight to determine the tibial ash concentration. Tibial bone ash was acid digested using the following hot plate method for sample preparation. A maximum of 0.2g of each sample was digested with 10ml of hydrogen peroxide and heated for 2 hours at 95^0C in the fume cupboard. A solution of 50ml MilliQ water was added to each tube after digestion and 8ml taken from the top into 8ml tubes. Digested samples were diluted to 1/10 and

mineral concentration analyzed using an ICP-MS (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Bremen, Germany).

Liver sample digestion and mineral analysis:

The liver mineral concentration was determined using an ICP-MS method. The liver samples were freeze dried using a freeze drier (Thermo Savant SuperModulyo) at the temperature setting of -45^{0}C for one week prior to digestion. Approximately 0.2g of the freeze-dried samples were weighed into the digestion vessels and the weight recorded. Each of the samples were digested using 3ml of nitric acid, 3ml MilliQ water and 2ml of hydrogen peroxide in the fume cupboard. The digestion tubes containing the samples were positioned in the microwave rotor for 45 min to obtain complete digestion. The liquid was decanted into universal containers and digestion tubes rinsed with 7ml of MilliQ water which was decanted back to the labelled universal tubes ready for ICP-MS analysis. Samples were diluted to 1/10 into the ICP tubes and analyzed using the ICP-MS (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Bremen, Germany) for mineral concentrations.

Statistical analysis

Birds were grouped in the basis of 21-day BW. The individual broiler served as the experimental unit. Descriptive statistics of the bodyweight weight data were analyzed using the SPSS software tool version 21. The Shapiro-Wilk test of the graph pad prism 9.0 software was used to assess the normality of data and then the bone mineral profile, liver mineral and gut pH data were analyzed using the student t-test of the GraphPad Prism 9.0 software with body weight as the main factor. Differences were considered statistically significant at 0.05 level of probability.

Results

Growth Performance:

Descriptive statistics of the 39 broiler chicks is shown in table 2 while the histogram graph showing bodyweight distribution of all chicks is presented in figure 2. The experimental chicks had a low body weight from D0 till D21 compared with the suggested breed bodyweight target on D0, D7, D14 and D21. However, their low body weight was not an indication of apparent poor health status as they were all healthy and thrived with only one mortality recorded throughout the experimental period. The bodyweight of chicks was noted on D0 with mean bodyweight of 37.9 (±6.22 g; percentage uniformity), D7 with mean bodyweight of 138 (±20.32), D14 with mean bodyweight of 369 (±49.08) and D21 with mean bodyweight of 887 (±118.16). The association between chick bodyweights on D0 and D7, D14 and D21 are presented in figure 3.

The bodyweight performance and bodyweight changes during the experimental periods between the high and low BW groups are shown in table 3 and figure 4 respectively. The mean bodyweights of the high and low weight chicks on D7 and D21 were (155g versus 122g) and (1020g versus 746g) respectively while the total weight gain from 0-21days were 976g for the high weight chicks and 710g for the low weight chicks.

Table 2: Descriptive statistics of weekly bodyweight performance of 39 male Ross 305 broiler chicks

Age	Mean bodyweight (g)	Min	Max	SD	CV (%)
D0	37.9	29.5	49.6	6.2	16.4
D7	138	100	188	20.3	14.7
D14	369	273	499	49.1	13.3
D21	887	655	1159	118	13.3

D0: Day 0, D7: day 7, D14: day 14, D21: day 21, Min: minimum, Max: maximum, SD: standard deviation, CV: coefficient of variation

Figure 2: Histograms showing bodyweight distribution of 39 Ross 308 male broiler chicks on (A) D0, (B) D 7 (C) D14 and (D) D21, (n= 39)

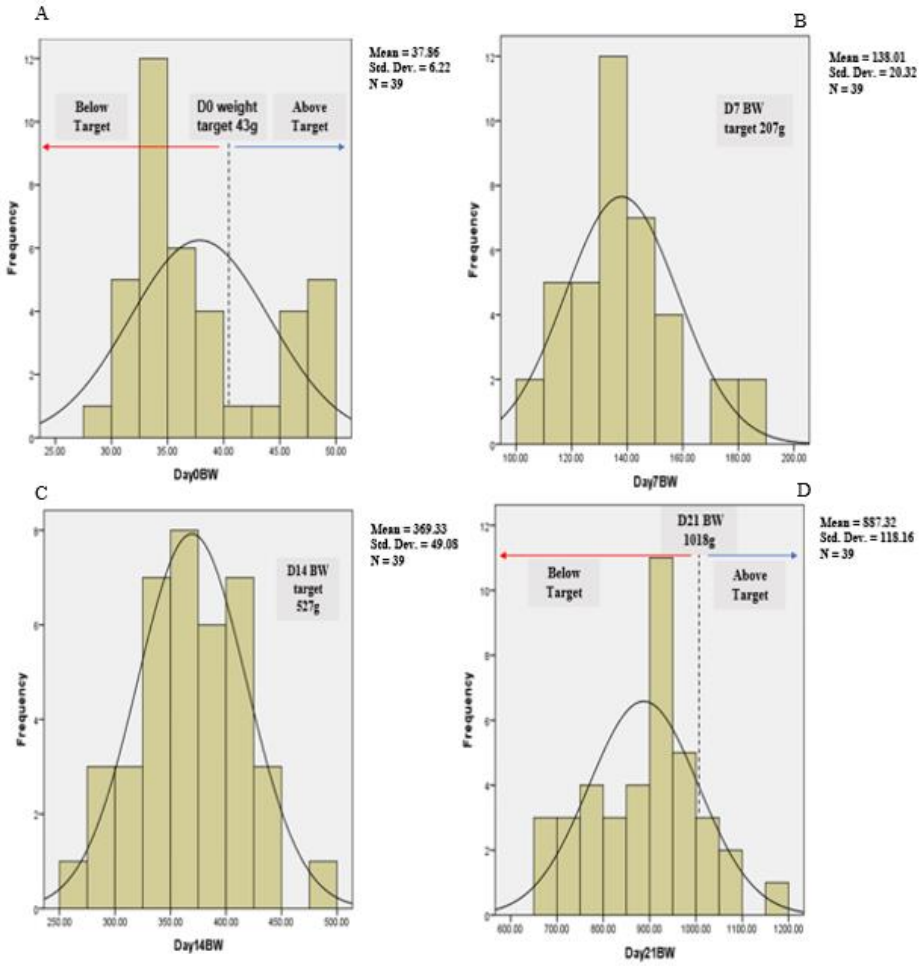
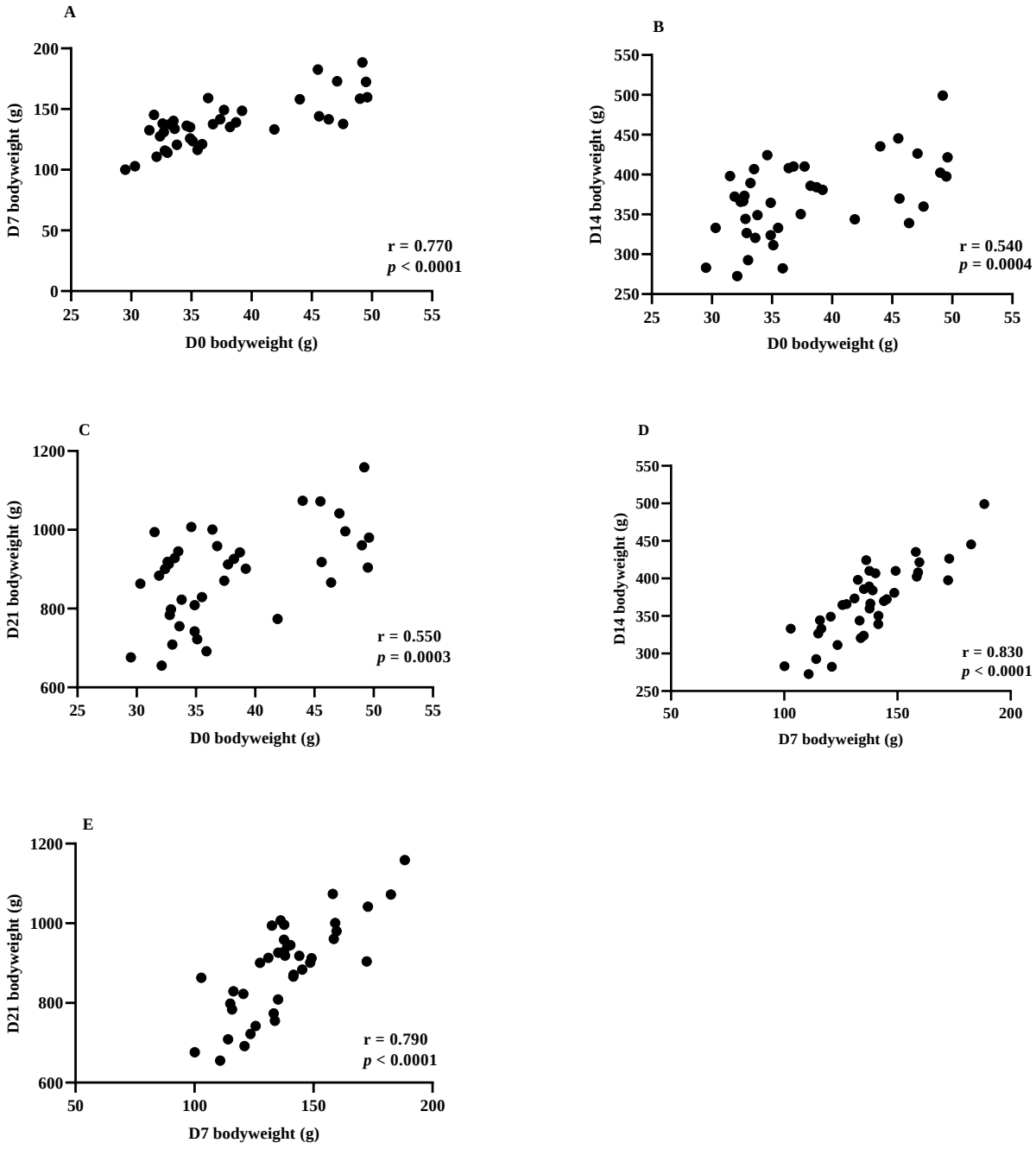


Figure 3: Association between bodyweight performance of the experimental chicks



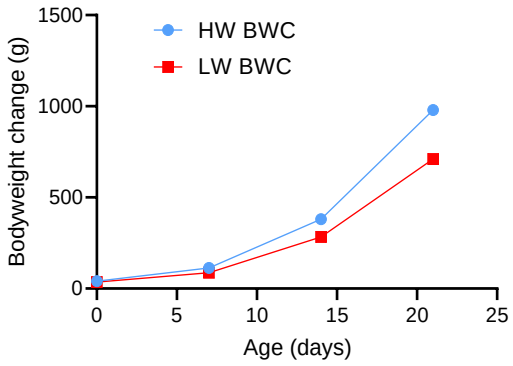
(A) Day 0 and D7 BW, (B) D0 and D 14 (C) D0 and D21, (D) D 7 and D14, (E) D7 and D21, (n=39).

Table 3: Average bodyweights of HW and LW male Ross 308 Chicks at different stages in starter phase

Age (Days)	HW BW (g)	LW BW (g)	SEM	P value
D0	41	35	2.52	0.025
D7	155	122	6.96	.0001
D14	421	318	13.8	<.0001
D21	1021	743	26.19	-
ADWG (0-7)	16	12	0.8	<.0001
ADWG (7-14)	38	28	1.5	<.0001
ADWG (14-21)	85	61	2.5	<.0001
TWG (0-21)	979	711	-	-

HW: High weight; LW: low weight; BW: bodyweight; ADWG: Average daily weight gain; TWG: total weight gain, (n=11/group)

Figure 4: Bodyweight change (g) over age (days)



HW: High weight; BWC: bodyweight change; LW: low weight**, (n=11/group)**

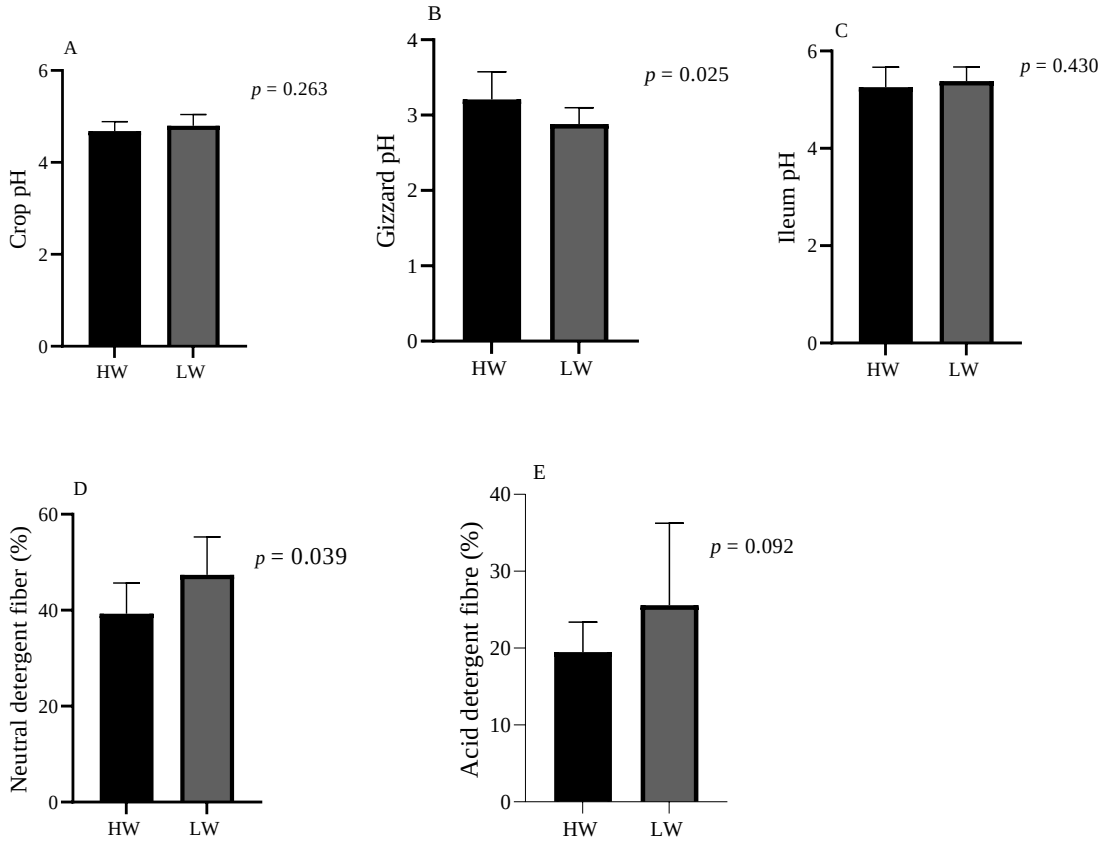
Gastrointestinal pH and gizzard fibre content:

The gastrointestinal pH values of the crop, gizzard and ileum and the gizzard fiber content are presented in figure 5. The result of this study showed a significant difference ($p<0.05$) between the high and low weight chicks in the gizzard digesta pH; the mean gizzard pH of the low and high

bodyweight chicks was 2.88 and 3.21 respectively. The ileal and crop pH was not different between BW groups.

There was also a greater ($p<0.05$) gizzard NDF content of the LW group compared to the HW group. There was a non-significant trend for a greater ADF concentration ($P<01$) in the low BW group when compared with the high BW group.

Figure 5: pH values of (A) crop, (B) gizzard, (C) ileum, (D) gizzard NDF and (E) gizzard ADF contents of 21d broilers in high or low weight group

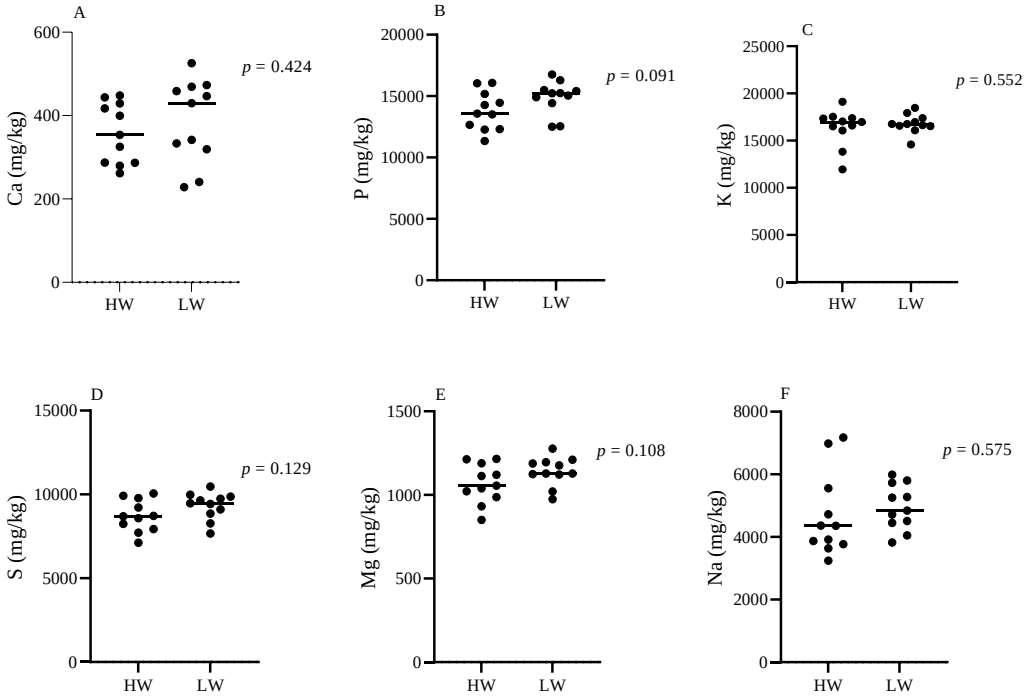


HW: High weight; LW: low weight; NDF: neutral detergent fibre from gizzard digesta; ADF: acid detergent fibre (% of freeze-dried material) from gizzard digesta, (n = 11/group)

Liver mineral profile

The macro and trace liver mineral profile of the high and low BW are presented in figures 6 and table 4. The concentrations of Mn (P = 0.018), Cs and Cd were significantly greater (P<0.05) in the low weight group relative to the high weight group. There was a non-significant tendency for higher liver Co concentrations (P = 0.052) in the HW group when compared to the LW group.

Figure 6: Liver macro mineral concentration of high and low bodyweight broiler chicks on D21



(A) calcium, (B) phosphorus (C) potassium, (D) sulphur, (E) magnesium, (F) sodium, HW: High weight; LW: low weight**,** (n=11/group)

Table 4: Liver trace mineral concentration of high and low bodyweight broiler chicks on D21

Minerals (mg/kg)	HW	LW	SEM	P-value
Zn	102	93	±4.95	0.369
Cu	15.1	14.5	±0.811	0.499
Fe	558	614	±97	0.570
Mn	15.9	18.1	±0.871	0.018*
Sr	0.3	0.3	±0.030	0.413
Cr	0.1	0.2	±0.073	0.419
Mo	2.6	2.4	±0.162	0.224
Pb	0.0	0.0	±0.003	0.433
Cs	0.1	0.11	±0.005	0.004*
Cd	0.05	0.07	±0.010	0.005*
Co	0.11	0.07	±0.021	0.052
Se	2.63	2.82	±0.134	0.176

(Zn) zinc, (Cu) copper, (Fe) iron, (Mn) manganese, (Sr) strontium, (Cr) chromium, (Mo) molybdenum, (Pb) lead, (Cs) caesium, (Cd) cadmium, (Co) cobalt, (Se) selenium, HW: High weight; LW: low weight. * Denotes significant difference at ≤ 0.05**,** (n=11/group)

Bone morphology and breaking strength:

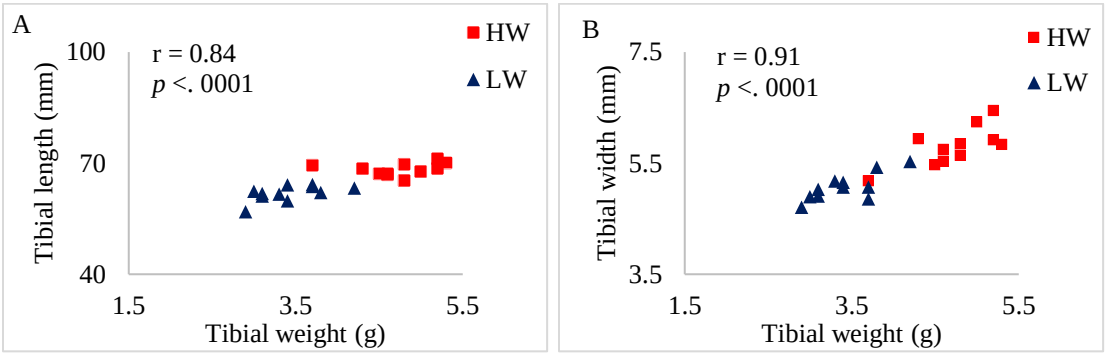
The bone morphometric measurements and breaking strength results are presented in table 5 and the association between the tibial length and weight and width and length are presented in figure 7. The HW chicks had a significantly greater bone breaking strength when compared with the LW group (219N versus 156N; $P<0.05$). Tibial width, length, and weight (g) showed significant differences ($p<0.05$) between the LW and HW chicks. The HW group had higher values of tibial width, length and weight compared to the LW groups.

Table 5: Bone morphometric parameters of high and low bodyweight broiler chicks on D21

Bone parameters	HW	LW	P-value
Bone strength	219	156	<.0001
Tibial weight	4.7	3.4	<.0001
Tibial length	68	62	<.0001
Tibial width	5.8	5.1	<.0001

HW: High weight; LW: low weight, (n=11/group)

Figure 7: Association between tibial length and weight and tibial width and weight



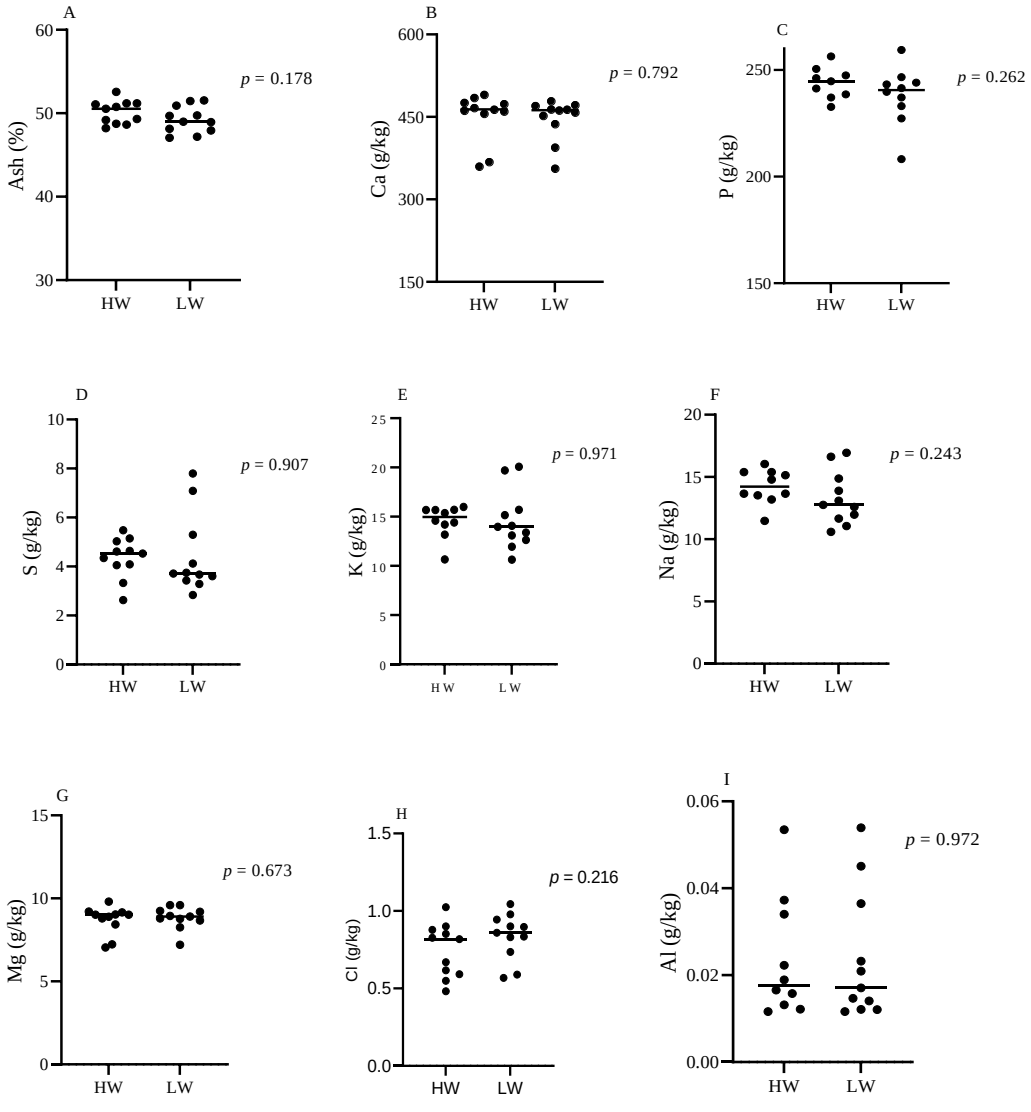
(A) Tibial weight and tibial length, (B) tibial weight and tibial width, HW: High weight; LW: low weight, (n=11/group)

Bone mineral profile:

The tibial ash, and macro mineral concentrations of the high and low weight chicks are presented in figure 8, and the tibial trace mineral concentration are shown in table 6, while the heatmap showing the correlation among mineral concentrations in the tibial of the broiler chicks is shown in figure 9. The LW group showed significantly higher Mn and Sr concentrations when compared with the HW group; Mn concentration of (24.5mg/kg versus 20.3mg/kg, P=0.019) and strontium (293mg/kg versus 266mg/kg, P=0.037). There was no significant difference (P>0.05) in other trace mineral concentrations between the groups, and no significant difference (p>0.05) in the bone ash

and macro mineral concentrations between the two groups. There was a strong positive correlation between bone Ca and P, Na, Mg, Mn, and Cs. There was also a weak negative correlation between bone strength and Mn, Cd and Cs, and positive strong correlation between bone and D7 and D21 BW

Figure 8: Tibial ash and macro mineral concentration of high and low bodyweight broiler chicks on D21



(A) ash content, (B) calcium, (C) phosphorus, (D) sulphur, E) potassium, (F) sodium, (G) magnesium, (H) chlorine, (I) aluminum. HW: High weight; LW: low weight, (n= 11/group)

289 Table 6: Tibial trace mineral concentration of high and low bodyweight broiler chicks on D21

Minerals (mg/kg)	HW	LW	SEM	P-value
Zn	501.10	491.50	±21.700	0.664
Cu	2.99	3.20	±0.315	0.528
Fe	312.40	308.9	±20.580	0.868
Mn	20.31	24.53	±1.653	0.019*
Sr	266.10	292.90	±11.990	0.037*
Cr	0.46	0.41	±0.080	0.503
Mo	0.95	0.92	±0.060	0.602
Pb	0.33	0.28	±0.040	0.298
Cs	0.10	0.12	±0.010	0.109
Cd	0.02	0.02	±0.003	0.163
Co	0.12	0.11	±0.020	0.881
Se	0.14	0.15	±0.010	0.118

290 (Zn) zinc, (Cu) copper, (Fe) iron, (Mn) manganese, (Sr) strontium, (Cr) chromium, (Mo)
291 molybdenum, (Pb) lead, (Cs) caesium, (Cd) cadmium, (Co) cobalt, (Se) selenium, HW: High
292 weight; LW: low weight. * Denotes significant difference at ≤ 0.05, (n=11/group)

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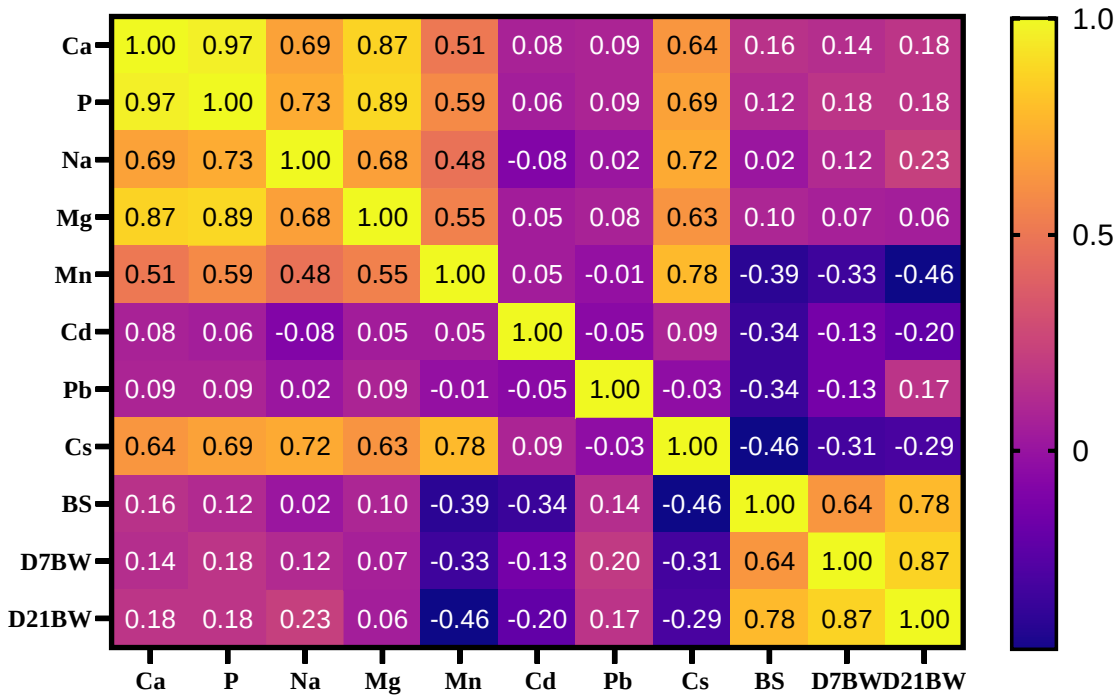
Figure 9: Heatmap showing the correlation (r) among mineral concentrations in the tibial bone of 21-day old broiler chicks.



Correlation (r) between 0.5-1 indicates strong positive correlation while -0.5 – (-1) indicates strong negative correlation, 0 indicates no correlation, (n=11/group), BS: bone strength, D7BW: day 7 bodyweight, D21BW: day 21 bodyweight.

Discussion

This study evaluated bodyweights and relevant metabolic organs and tissues to pinpoint essential physiological differences between low and high BW broiler chicks during the early weeks of life. The retrospective bodyweight performance of the chicks investigated in this study showed higher variations as reflected in the CV values of 13-16% which exceeded the recommended range of 8-10% in broiler chicks (Feddes, et al., 2002; Toudic, 2007). Generally, chicks were observed to exhibit low bodyweights when compared with the Ross 308/Ross 308 FF male broiler performance objectives (2019). Despite the low bodyweights exhibited by the experimental chicks, they

appeared healthy throughout the period of the experiment, with 2.5% mortality. The very low mortality observed in this study suggest that the experimental chicks were not health challenged. While hatch weight is an important consideration to ensure broilers have a strong start to life, the BW after the first week of life is often used in the industry as a measure of early performance and future growth potential. There was a positive strong association between D0 and D7 bodyweights (r = 0.77), and a positive weaker association was also found to exist between D0 and D14 (r = 0.54) and D0 and D21 (r = 0.55). The D7 bodyweight was observed to have a stronger positive association with D14 (r = 0.83) and D21 (r = 0.79) compared to D0 bodyweight. This result also agreed with the findings of (Tona, et al., 2004b) who reported no relationship between day-old chick weight and slaughter weight but indicated that a stronger relationship exists between weight on D7 and slaughter age weights in broiler chicks.

The chicken gut provides a means for acquiring nutrients and energy but is also a route for disease entry which can directly affect overall growth performance. The pH of the digesta contents along the gastrointestinal tract has been reported to influence mineral solubility in the gizzard and its absorption in the small intestine (Lee, et al., 2021). The pH of the gut has a direct influence on Ca solubility and its availability for absorption, thus an acidic gut environment has been shown to promote the dissolution of $CaC0_3$ (Walk et al., 2012). Low gizzard digesta pH has been suggested to increase pepsin activity and the solubility and absorption of mineral salts (Buclaw, 2016). Gizzard digesta pH in broiler chicks has been reported to be influenced by diet type, wholly or coarsely ground grains, and fiber which decreases the pH of the gizzard by 0.2 to 1.2 units (Svihus, et al., 2013, Jiménez-Moreno et al., 2019, Aziz-Aliabadi, et al., 2023). This could be attributed to increased gizzard volume and more retention time which allows more fermentation to produce various acids (Svihus, 2014). In our previous study, it was reported that low BW chicks had

339 proportionally heavier gizzards compared to the high BW group. In the present study, the low BW
340 group had a low gizzard digesta pH compared to the high BW group, which was very interesting,
341 and it may suggest greater gizzard functionality in the low BW group, which may seem more like
342 a compensatory strategy. Alternatively, it could reflect a lower quantity of gizzard contents, and
343 the various acid buffering components such as Ca which would be present.
344 It was speculated that the low gizzard pH observed in the low BW group in the present study could
345 also be linked to the increase intake of fibrous litter substrate compared to the high weight group
346 which influenced acid production in the gut, thus resulting in a lower gizzard digesta pH. During
347 the study some feeding behaviors were observed, for example, the tendency for chicks to habitually
348 consume litter and at necropsy litter was found in the gizzards It was speculated that HW chicks
349 that displayed dominance may have led to greater consumption of litter in the LW group (Estevez,
350 et al., 1997; Bokkers and Koene, 2004).
351 The crop pH obtained in the present study ranged from 4.7-4.8 which fall within the range of 4.5-
352 5.9 reported by other researchers (Svihus, 2014) and lower than the range of 4.8-4.9 in low and
353 high BW broiler starter respectively reported by (Dono, et al., 2014). The digesta pH of the gizzard
354 in a healthy chick was summarized in a report by (Svihus, 2011) and it ranged from 1.9-4.5 with
355 an average of 3.5, the present study reported gizzard digesta pH within this range in both groups.
356 It has been reported that most of the pathogenic microbes have been reported to thrive in pH close
357 to 7 and above (Rahmani, et al., 2005), but in this study, the pH values recorded in both high and
358 low BW chicks for the crop, gizzard, and ileum were below 7 and could suggest healthy gut
359 environment in those gut segments measured in this study across the two groups.
360 Liver mineral concentrations are an important indication of its status, storage, and bioavailability
361 of ingested minerals (Wang, et al., 2007). The liver plays an important role as a storage facility, it

has an important role in the metabolism of minerals and detoxification, it is also specifically the main storage site of copper and fat-soluble vitamins (Zaefarian, et al., 2019). It is the target organ for the accumulation of cadmium which exerts several negative effects such as cellular changes, acceleration of lipid peroxidation, DNA chain breakages and impact on mitochondrial function (Berzina et al., 2007 and Toman et al., 2005, Hu, et al., 2018). In the present study, there was an interestingly higher concentration of cadmium in the LW group. Cadmium is one of the heavy metals which has deleterious effect on growth performance, it has high water solubility and toxicity even at low levels (Tkalec, et al., 2008). In the present study, liver Cd concentration in the LW group exceeded the permissible limit of 0.05ppm in broiler chicken liver (FAO/WHO, 2002; Korish, et al., 2020) compared to the HW group which may have detrimental implications on growth performance and gut functions. Bioaccumulation of cadmium in the liver of chicken has been reported to be associated with poor live weight in chickens (Akyolcu, et al., 2003). Higher liver Cd concentration in the low weight group observed in this study may possibly lead to distinct pathological changes in the liver even in very low concentrations, thus influencing the mitochondrial function and may induce hepatotoxicity in the low weight group compared to the high weight (Casalino et al., 2002; Arnold., 2006; Berzina et al., 2007 and Li, et al., 2013). The significant increase in Mn, and Cs in the low weight group may be attributed to various reasons such as differences in individual intake, nutrient requirement, and its retention in the hepatic tissue. It is noteworthy that Cd and Cs concentrations were significantly higher in the tibial bones of the low BW chicks in our previous study with a similar experimental design (Elvis-Chikwem, et al., 2021). This interesting trend in bioaccumulation of cadmium in both liver and bone of the low BW chicks requires further investigation.

Bone ash and mineral concentrations such as calcium and phosphorus were not significantly different between the BW groups in this study. These constituents are important markers of overall bone composition and integrity. The bone concentrations of Cd, Pb and Cs were not affected by bodyweight differences in the present study, as was observed in previous work, albeit in 7-day old chick (Elvis-Chikwem, et al., 2021), where these heavy metals where higher in the LW group compared to the heavy group.

Mn is an essential trace element which acts as a co factor to many enzymes such as manganese superoxide dismutase, arginase, and pyruvate carboxylase, which aid reactive oxygen scavenging, bone formation and immune response (Shahnazari, et al., 2007). Sr on the other hand has been reported to have a positive effect on bone formation and strength in broiler chicks (Shahnazari, et al., 2007). Both minerals Mn and Sr are associated with bone health performance. The reasons for the higher concentrations of these minerals especially Mn in the tibias of the LW chicks relative to the HW chicks is unclear and may be partly attributed to variation in Mn requirement for individual chicks influenced by bodyweight differences. It has been reported that Mn concentration in the tibial bone has a linear relationship with Mn intake by the chicks and an indication of its bioavailability (Sunders, et al., 2006; Sunders, et al., 2007). This might not be the case in the present study as the high and low BW chicks were fed the same diet without additional dietary supplementation of Mn or Sr. It is also noteworthy to mention that there was no environmental source of Mn and Sr accessible to the chicks in the present study. Taken together, the greater concentration of Mn see in both the bones and the liver of the LW group suggests a more systemic accumulation of this mineral in the lighter chicks.

Conclusion

This study evaluated differences in bone, gut parameters, and liver mineral profile of male Ross 308 broilers with varying BW on D21, which were exposed to the same environmental and management conditions. Chicks with high and low BW showed differences in bone characteristics, liver trace mineral concentrations of Mn, Cd and Cs, gizzard digesta pH and gizzard fiber content. The significant reduction in gizzard digesta pH, and high gizzard fiber content of the LW group relative to the HW group could be linked to fibrous litter consumption of the low BW group, which may be contributing to a shift in acid production in the chicken gut. This study also indicated that broilers with low BW on D21 had higher concentration of Mn in both bone and liver tissues which may have physiological implications hence more research is needed to understand Mn requirements, and tissue retentions as influenced by bodyweights in early life of broiler chicks.

Conflict of Interest

Cormac J. O'Shea is an associate Editor of the Animal Production Science, therefore in other to mitigate this potential conflict of interest, he was blinded from the review process.

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Data availability

The data from this study is available and would be shared upon reasonable request.

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