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Effect of body weight on uniformity, livability, and skeletal development and strength of broiler breeder females

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ABSTRACT
An experiment was conducted with broiler breeder females (up to 30 wks of age) to study the effect of body weight (BW) at 5 wks on BW uniformity, livability, and skeletal development and strength. At 5 wks of age, 160 females were randomly selected within a flock of 1,988, and they were reared without grading. Among these 160 females, the 40 with the lowest BW (Light Non-Graded, LNG) and the 40 with the highest BW (Heavy Non-Graded, HNG) were studied. LNG females, compared to HNG, had a higher BW coefficient of variation (CV) from 10 to 25 wks ($P \leq 0.05$). At 25 wks, mortality of the LNG females was higher (20.0% *vs.* 2.5%, $P = 0.027$); they had shorter tibias ($P \leq 0.05$), and a tendency to lower-tibia breaking strength ($P = 0.085$) and elastic modulus ($P = 0.072$). At 5 wks, their alkaline phosphatase was lower (2781 UI *vs.* 3839 UI, $P = 0.023$), and taking together 5 and 10 wks their osteocalcin was also lower (976 ng *vs.* 1239 ng, $P = 0.029$). Results indicate that without grading in rearing, light females had lower BW uniformity, lower livability, shorter and less resistant tibias than heavy females.

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Broiler breeders; uniformity; grading; skeletal development and strength

Introduction

Correct management of broiler breeders is essential to prevent having uneven flocks, where larger or aggressive females are likely to out-compete smaller or timid pullets, resulting in unequal access to feed and increasing flock body weight (**BW**) variation (Zuidhof et al. 2015). Grading is a management tool used in broiler breeder rearing farms, with the purpose of increasing BW uniformity. The flock is divided into pens (normally 2 or 3) of females with different average BWs (physiological state), so that each pen can be managed in a way that will result in bringing the whole flock back to the standard BW profile and in good, whole flock uniformity at the point of laying.

Uniform flocks are easier to manage, since the birds will respond similarly to management factors such as light stimulation and increasing of the feed ration (Ross Parent Stock Management Handbook. Aviagen 2013b). However, low uniformity may result in broiler breeder pullets being under the advised standard BW, not having received the nutrients required, thus leading to the poor body and skeletal development, which might cause leg health issues.

Bone measurements, such as bone breaking strength (**BS**) and elastic modulus (**EM**), have been used as indicators of bone status in several studies (Rath et al. 2000; Lewis et al. 2009; Shastak et al. 2012; Shim et al. 2012). Bone strength is determined not only by the volume of bone tissue and the micro-architectural organization, but also by the degree of mineralization of the bone matrix (Boivin and Meunier 2002). Nutritional deficiencies may affect bone mineralization and, in turn, bone strength, which enables the skeleton to withstand gravity and additional loading.

Alkaline phosphatase (**ALP**) and osteocalcin (**OC**), which are serological markers of bone formation (Seibel 2005), might provide direct insight into skeletal development, particularly bone growth. These markers are currently used in humans to evaluate bone metabolism, and provide a real-time assessment of bone formation, resorption and turnover. Ekmay et al. (2012) used ALP serological levels to assess broiler breeder growth and bone mineral deposition during transition into sexual maturity; nevertheless, these two serological markers have not been used to date in broiler breeder females to assess their development. ALP is a ubiquitous enzyme attached on the outer cell surface (Stinson and Hamilton 1994). The precise function of the enzyme is as yet unknown, but it obviously plays an important role in osteoid formation and mineralization (Harris 1990). OC is considered a specific marker of osteoblast function (Brown et al. 1984). It is estimated that, directly after its release from the osteoblast, the largest part of the newly synthesized protein is incorporated into the extracellular bone matrix, but a smaller fraction is released into the blood circulation where it can be detected by immunoassays (Bouillon et al. 1992; Monaghan et al. 1993; Parviainen et al. 1994; Chen et al. 1996).

As was explained before, broiler breeder flocks under the standard BW and with low uniformity might lead to poor skeletal development and leg health issues, which cause economic losses to breeder producers. Therefore, it is important to evaluate bone development and strength in the rearing period to prevent these issues.

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The aim of this study is to evaluate whether 5-wk-old broiler breeder females with a lower BW than standard and never graded in rearing are affected in their BW uniformity, livability, and skeletal development and strength. Likewise, the usefulness of two different serological markers is proposed to assess the skeletal development throughout the initial phase of the broiler breeders in the rearing period.

Materials and methods

Birds and facility

All of the animal experimentation procedures used in the experiment were approved by the animal Ethics Committee of the Universitat Autònoma de Barcelona (UAB; Barcelona, Spain) and were in compliance with the European Union guidelines for the care and use of animals in research (European Parliament 2010).

A total of 1,988 Ross 308 broiler-breeder female chicks were placed in an all-in all-out commercial farm during a period from 0 to 30 wks of age. A vaccination programme adapted to the epidemiology of the farm area was followed throughout the rearing period. Management applied was according to the guidelines of the Ross Parent Stock Management Handbook (Aviagen 2013b).

Until 21 wks of age, pen dimensions were 48.50 m long by 6.5 m wide, 315.25 m^2 in total, with 6.30 females/m^2. Feed space was 0.87 females per pan hole and drinking space was 5.58 females per nipple. At 21 wks old, the females could use the whole house and mixed with the males (ratio 10/1). From this moment, stock density was 5.30 females/m^2, feed space was 0.74 females per pan hole and drinking space was 4.74 females per nipple. Throughout the whole trial, feed and drinker space was sufficient for all the birds to reach feed and water at the same time. Wood shavings were utilized as litter material, and litter was topped up weekly to keep it dry.

The house was environmentally controlled and the temperature set-point was modified depending on the birds' age, starting with 32°C at one day old, and decreasing on a regular basis until 20°C at 27 d old; this set-point was kept until the end of the trial. The lighting programme was 23 h of light during the first 4 d and, then, gradually reduced to 8 h until 14 d; from 2 to 20 wks the birds had 8 h of light, and later on light was increased 1 h per wk until 14 h (26 wks). Light intensity was 100–80 lux the first wk, 20 lux from 2 to 20 wks and 60 lux from then on. Management data such as light programme, light intensity and environmental temperature were weekly recorded.

Trial description

From a flock of 1,988 Ross 308 broiler breeder females, 160 birds were randomly selected at 5 wks of age; they were individually identified and never graded-divided during the rearing period, depending on their BW. Within this group of Non-Graded females, two experimental groups of weight were created: the first quarter, which included the 40 lightest females (below the standard BW at 5 wks) and that was named Light Non-Graded (LNG), and the fourth quarter, which included the 40 heaviest females (over the standard BW at 5 wks) and that was named Heavy Non-Graded (HNG).

The females of the flock (1828, 1988 birds minus 160) were graded-divided depending on their BW (Graded Flock). At 5, 12 and 18 wks, they were weighed individually, and each time they were divided into three different BW pens: light (20%), medium (70%) and heavy (10%) females. The pens were managed during the rearing period to bring them back to the standard BW profile, and in good, whole flock uniformity at the point of laying. The 160 Non-Graded females always stayed together and mixed in the Graded Flock (inside the pen of the medium BW), with the aim of studying the birds of the experimental groups (LNG and HNG) in commercial conditions, but without any grading.

Feeding program, diets and feed intake

Feed was produced in a feed mill of CAG (Tarragona, Spain). The different diets were formulated according to Ross 308 Parent Stock Nutrition Specifications (Aviagen 2013a). A five-diet-phase feeding programme was followed: starter I (from 0 to 3 wks of age), starter II (from 4 to 6 wks), grower (from 7 to 15 wks), pre-breeder (from 16 to 24 wks) and breeder (from 25 to 30 wks). Feed form presentation was crumble. The ingredients and nutrient composition are shown in Table 1.

Feed was provided *ad libitum* during the first 2 wks in order to ensure a correct initial development. From this point on, in order to follow the standard BW profile, the total daily feed distributed was weekly calculated by multiplying all of the females present at the moment by the daily metabolizable energy (**AMEn**) allowance per bird and divided by the AMEn of the diet. The average BWs of the three different BW pens of the Graded Flock were taken into account to decide the daily AMEn allowance per bird in each pen. Both the standard BW profile and daily AMEn allowance per female were based on Ross 308 Parent Stock Performance Objectives (Aviagen 2012).

Collected data, sampling and analytical determinations

Feed. Experimental feed samples were collected from the different diets supplied during the rearing and production periods of the trial. These samples were ground and stored at 5°C until their analyses. Diet proximate analyses were performed according to the method of the Association of Official Agricultural Chemists (AOAC) International (2005): dry matter (Method 934.01), crude protein (Method 968.06), crude fibre (Method 962.09), crude fat (Method 2003.05) and ash content (Method 942.05). All analyses were carried out by the Servei de Nutrició i Benestar Animal (SNiBA) laboratory of the UAB.

Body Weight, Uniformity and Mortality. The Graded Flock was monitored weekly by sampling BW and calculating the BW coefficient of variation (CV). The females were randomly caught (minimum 2% of the flock) by using catching frames, and all birds rounded up were weighed in order to eliminate any selective bias. All 160 Non-Graded females were individually weighed every 5 wks, starting when they were 5 wks old and finishing at 30 wks old, and their BW CV calculated.

Tibia Parameters. To perform post-mortem analyses, 3 females from the LNG and 3 females from the HNG were

Table 1. Ingredients (%), metabolizable energy and nutritional composition of the experimental diets.

	Starter I 0 to 3 wks	Starter II 4 to 6 wks	Grower 7 to 15 wks	Pre-breeder 16 to 24 wks	Breeder I 25 to 30 wks
Wheat	35.2	36.2	27.7	33.1	30.8
Soybean meal	25.6	17.8	-	6.2	15.8
Corn	24.5	31.6	30.0	35.0	38.0
Sunflower 28–30%	5.00	6.00	15.76	10.50	4.10
Wheat bran	3.00	1.23	19.70	7.00	–
Dicalcium phosphate	2.33	2.23	1.87	1.53	1.70
Rapeseed meal	–	2.00	1.57	3.00	–
Soybean oil	2.00	1.00	1.20	0.77	2.00
Limestone	0.97	0.90	0.90	1.87	6.57
Premix[1]	0.30	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.27	0.24	0.22	0.20	0.18
Salt	0.23	0.23	0.21	0.23	0.24
L-lysine sulfate 54.6%	0.23	–	0.26	0.12	0.03
Methionine	0.20	0.10	0.11	0.10	0.14
Choline Cl-60%	0.10	0.10	0.10	0.07	0.07
Calcium pidolate	0.05	0.03	0.03	–	–
Threonine	0.05	0.03	0.04	0.01	0.04
AMEn, Kcal/Kg	2810	2801	2550	2701	2800
Dry matter %[2]	89.0	88.6	88.6	88.7	89.4
Crude Protein %[2]	20.7	16.9	13.3	14.4	14.8
dig Lys %	0.95	0.67	0.50	0.52	0.58
dig Met %	0.47	0.35	0.32	0.32	0.35
dig M + C %	0.74	0.60	0.53	0.54	0.56
dig Thre %	0.64	0.54	0.39	0.42	0.47
dig Trp %	0.20	0.17	0.13	0.14	0.14
Crude Fibre %	4.17	4.48	7.99	5.99	3.46
Crude Fat %[2]	4.06	3.05	3.98	3.02	4.13
Ash %[2]	5.87	5.68	5.30	5.92	10.21
Ca %	1.06	1.00	0.91	1.19	2.99
dig *P* %	0.48	0.46	0.42	0.36	0.36

[1]Premix provided per kilogram of diet: vitamin A (retinyl acetate), 10000 UI; vitamin D_3 (cholecalciferol), 1500 UI; 25-hydroxycholecalciferol, 0.0375 mg; vitamin E (DL-α-tocopheryl acetate), 100 UI; vitamin K_3, 10.00 mg; Vitamin B_1- (thiamin), 7.00 mg; vitamin B_2 (riboflavin), 20.00 mg; vitamin B_5 (pantotfemaleic acid), 27.22 mg; vitamin B_6 (pyridoxine hydrochloride), 10.00 mg; vitamin B_{12} (cyanocobalamin), 0.07 mg; vitamin B_3 (nicotinic acid), 87.09 mg; vitamin B_9 (folic acid), 5.00 mg; vitamin B_7 (biotin), 0.60 mg; vitamin C (ascorbyl phosphate), 150 mg; iodine, 2.00 mg; Mn (manganous oxide), 80.00 mg; Mn (manganese chelate), 40.00 mg; Cu (cupric sulfate), 6.00 mg; Cu (copper chelate), 10.00 mg; Zn (zinc oxide), 60 mg; Zn (zinc chelate), 40.00 mg; Se (sodium selenite), 0.20 mg; Se (selenium organic form), 0.20 mg; Fe (ferrous carbonate), 65.00 mg.
[2]Analyzed values.

euthanized every 5 wks (starting at 5 wks and finishing at 30 wks of age).

Right legs were used to dissect tibias, which were utilized to determine tibia length (**TL**), tibia breaking strength (**BS**) (maximum load endurance) and elastic modulus (**EM**). TL was measured every 5 wks by using a caliper (0.03-mm precision and 0.01-mm resolution). BS and EM were determined at 15, 25 and 30 wks of age. The BS value is the load required to reach the point where the bone is no longer resilient and reflects the rigidity of the bone as a whole. The EM value is the slope of the linear region of the stress *vs.* strain-curve and reflects the intrinsic stiffness or rigidity and material properties of the bone (Turner and Burr 1993).

To measure BS and EM, the method of the 3-point flexural bending test was carried out (Fleming et al. 1998). Measurements were made by using a MTS Alliance RT/5 material testing system (MTS Systems Corp.; Eden Prairie, MN 55344, US) (force resolution 0.001 N and distance resolution 0.001 mm). These two studies were carried out by IRTA (Institut de Recerca i Tecnologia Agroalimentaria, Girona, Spain).

Serological Markers. To determine ALP levels, the 14 lightest females of the LNG and the 14 heaviest females of the HNG were blood-sampled at 5 and 10 wks of age. For OC determination, blood samples coming from the same females were used, but only those of the 10 lightest birds of the LNG and those of the 10 heaviest birds of the HNG were used.

Blood samples were spun at 1107 g for 20 min to obtain the serum, which was stored at −20°C until the moment to be processed. To test ALP, a colorimetric method based on the catalytic transformation of p-nitrophenylphosphate as substrate (International Federation of Chemical Chemistry (IFCC) method) on the Beckman Coulter AU analyser (Beckman Coulter, Inc.; Brea, CA 92821, US) was used. In the case of OC, the Rat-MID™ Osteocalcin EIA (ELISA) (Immunodiagnostic Systems Holdings Plc; Didcot Way, NE35 9PD; UK) was the test performed. ALP and OC analyses were carried out by the Servei de Bioquímica Clínica Veterinària (SBCV) of the UAB.

Statistical analysis

All statistical analyses were performed with the R® statistical software (version 3.5.1, R Core Team 2018). A one-way ANOVA was applied to analyse body weight data within each week. The model was $y_{ij} = \mu + EG_i + e_{ij}$, where y_{ij} corresponds to weight of the bird, EG_i is the experimental group (Light Non-Graded and Heavy Non-Graded), and e_{ij} is the error term. Coefficients of variation were compared using the asymptotic test of Feltz and Miller (1996) implemented in the R package cvequality (Version 0.1.3; Marwick and Krishnamoorthy 2019). Accumulated mortality was analysed by contrasting the number of initial birds with the accumulated mortality through a Chi-square Test for Count Data in the two experimental groups: Light Non-Graded and Heavy Non-Graded. Tibia length, Breaking strength and Elastic modulus variables were analysed using a factorial design according to the following model: $y_{ijk} = \mu + EG_i + Wk_j + EG*Wk_{ij} + e_{ijk}$, where y_{ijk} was the observation for each of the 3 variables, EG_i was the experimental group (Light Non-Graded and Heavy Non-Graded), Wk_j was the week effect (5, 10, 15, 20, 25, 30), $EG*Wk_{ij}$ was the interaction effect between experimental group and week, and e_{ijk} was the error. A mixed model was used to analyse Alkaline Phosphatase and Osteocalcin serological levels: $y_{ijkl} = \mu + EG_i + Wk_j + EG*Wk_{ij} + B_l + e_{ijk}$, where y_{ijkl} were the observations, EG_i was as before, Wk_j had 2 week levels (5, 10), $EG*Wk_{ij}$ was the interaction effect, B_l was the random effect of the bird, and e_{ijkl} was the error term. Means were compared through a Tukey test. In all cases, *P*-values lower than 0.05 were considered to be significant.

Results and discussion

Evolution of BW of the LNG and HNG from 5 to 30 wks is shown in Table 2. BW of the LNG was lower and significantly different from that of the HNG until 25 wks of age. However, at 30 wks of age, BWs of the LNG and the HNG females were similar (LNG: 3944 and HNG: 3995 g, $P = 0.571$). Light Non-Graded females

Table 2. Body weight (g) of the Light Non-Graded (LNG) and Heavy Non-Graded (HNG) broiler breeder females.

	Body weight (g)			
Age (wks)	LNG[1]	HNG[2]	SEM ($n = 40$)	*P*-value
5	587	831	6.2	<0.001
10	829	1184	16.9	<0.001
15	1346	1762	24.6	<0.001
20	2035	2438	42.3	<0.001
25	3292	3543	48.0	<0.01
30	3944	3995	59.8	0.571

Note: Non-Graded: 160 females randomly selected at 5 wks of age within the 1988 broiler breeder females kept in a standard breeder farm.
[1]The 40 lightest birds, below the standard body weight at 5 wks, of the Non-Graded.
[2]The 40 heaviest birds, over the standard body weight at 5 wks, of the Non-Graded.

Table 4. Accumulated mortality (%) of the Light Non-Graded (LNG) and Heavy Non-Graded (HNG) broiler breeder females.

	Accumulated mortality (%)		
Age (wks)	LNG[1]	HNG[2]	*P*-value
5	0	0	0
10	7.5	0	0.089
15	12.5	0	0.030
20	15.0	0	0.018
25	20.0	2.5	0.027
30	22.5	7.5	0.115

Note: Non Graded: 160 females randomly selected at 5 wks of age within the 1988 broiler breeder females kept in a standard breeder farm.
[1]The 40 lightest birds, below the standard body weight at 5 wks, of the Non-Graded.
[2]The 40 heaviest birds, over the standard body weight at 5 wks, of the Non-Graded.

were under the standard BW profile (based on Ross 308 Parent Stock Performance Objectives; Aviagen, 2012), from 5 to 25 wks of age, whereas HNG were over the standard all throughout the trial; these results are not shown. Similar results were found in Renema et al.'s (1999) study; they reported that in feed intake controlled broiler breeders, the 20% difference between the standard and low or high bird BW at 21 wks declined at sexual maturity to 5% and 4% for the low and high bird BW, respectively. In the present study, after 21 wks, feed allocation was the same for all of the females, whereas the birds of the mentioned study (Renema et al. 1999) were fed at the level that maintained constant rates of gain in each body-size group.

Evolution of BW CV of the Graded Flock, Non-Graded, LNG and HNG females from 5 to 30 wks of age is shown in Table 3. From 10 to 25 wks, the BW CV of the LNG females was higher and significantly different from that of the HNG. During this period, the BW CV of the LNG females was over 10% (with a maximum of 12.9% at 10 wks), whereas that of the HNG was under 10% throughout all the trial. At 30 wks, the BW CVs of the LNG and HNG females were similar and both, under 10%. According to Uniformity of Female Broiler Breeders (Aviagen 2016), 10% should be the maximum value obtained if management is correct.

From 5 to 20 wks of age, the BW CV of Non-Graded females was higher and significantly different from that of the Graded Flock. During this period, the BW CV of the Non-Graded females was over 10% (with a maximum of 15.5% at 10 wks), whereas that of the Graded Flock was under 10% throughout all the trial, and specifically at 20 wks it was 6.5%. A similar result, 6.2% at 22 wks of age, was obtained by Zuidhof et al. (2015), who studied the effect of broiler breeder feeding management practices, and they reported that the grading treatment resulted in the highest flock BW uniformity.

The present study shows that grading in rearing is essential to obtain a correct uniformity, and that in the absence of grading, the lack of uniformity of the Non-Graded females comes from the LNG; in fact, the HNG kept a correct BW CV (lower than 10%) throughout the trial. After 25 wks of age, the BW CV of the LNG females decreased to a correct value (under 10%) and, at 30 wks, their BW CV was similar to the HNG. These similar BW CVs and BWs of the LNG and HNG females at 30 wks might be explained by more feed availability, more feed space and a longer clean-up time in the production period; hence, the light birds would be able to obtain more feed.

Accumulated mortality (%) of the LNG and HNG is shown in Table 4. Regarding LNG females, during the period from 5 to 15 wks of age, they had their highest mortality (1.25%/wk); after this, between 15 and 20 wks, their mortality decreased to 0.5%/wk. HNG females had no mortality at all from 5 to 20 wks. At 25 wks, the accumulated mortality of LNG was 17.5% higher than that of HNG (20.0% vs. 2.5%, $P = 0.027$). At 30 wks, the LNG females had a higher accumulated mortality (22.5%) than did those of the HNG (7.5%).

The LNG females had their highest mortality between 5 and 15 wks, when the weekly feed increase was lower; their lowest mortality was between 15 and 20 wks, when feed availability was higher, since this is the period of feed stimulation. However, from 5 to 20 wks of age the HNG females had no mortality at all, and were not affected by the period of a lower weekly feed increase.

Table 3. Body weight coefficient of variation (%) of the Graded Flock and Non-Graded broiler breeder females, and of the Light Non-Graded (LNG) and Heavy Non-Graded (HNG) broiler breeder females.

	Coefficient of variation (%)									
Age (wks)	Graded Flock[1]	*n*	Non-Graded [2]	*n*	*P*-value	LNG[3]	*n*	HNG[4]	*n*	*P*-value
5	8.2	40	13.3	160	<0.01	6.2	40	4.9	40	0.129
10	8.4	40	15.5	155	<0.001	12.9	37	8.1	40	<0.01
15	7.0	40	12.3	150	<0.001	11.8	35	7.0	40	<0.01
20	6.5	40	13.0	149	<0.001	12.5	34	8.6	40	0.029
25	7.0	40	8.1	143	0.286	11.0	32	6.7	39	<0.01
30	7.3	40	6.0	137	0.123	7.5	31	7.2	37	0.806

[1]1828 broiler breeder females kept in a standard breeder farm (the initial 1988 minus the 160 females of the Non-Graded).
[2]160 females randomly selected at 5 wks of age within the 1988 broiler breeder females.
[3]The 40 lightest birds, below the standard body weight at 5 wks, of the Non-Graded.
[4]The 40 heaviest birds, over the standard body weight at 5 wks, of the Non-Graded.

Table 5. Tibia length, breaking strength and elastic modulus data according to broiler breeder body weight at 5 wks (LNG: Light Non-Graded and HNG: Heavy Non-Graded) and weeks of age.

Effects	TL[3] (mm)	BS[4] (kgf/mm^2)	EM[5] (Kgf/mm)
Body weight			
LNG[1]	108	64.3	61.5
HNG[2]	120	71.4	68.9
n	18	9	9
SEM	1.0	2.6	2.58
Age (wks)			
5	86^c		
10	102^b		
15	120^a	38.9^b	37.9^b
20	126^a		
25	125^a	80.3^a	75.6^a
30	126^a	80.1^a	78.2^a
n	6	6	6
SEM	1.8	3.2	3.16
P-value			
Body weight	<0.001	0.085	0.072
Age (wks)	<0.001	<0.001	<0.001
Interaction	0.281	0.905	0.438

Note: Non-Graded: 160 females randomly selected at 5 wks of age within the 1988 broiler breeder females kept in a standard breeder farm.
[a-c]Means within a column and within a source with no common superscript differ significantly.
[1]The 40 lightest birds, below the standard body weight at 5 wks, of the Non-Graded.
[2]The 40 heaviest birds, over the standard body weight at 5 wks, of the Non-Graded
[3]Tibia length.
[4]Breaking strength (maximum load endurance).
[5]Elastic modulus (rigidity of the bone).

Table 6. Alkaline Phosphatase and Osteocalcin serological levels according to broiler breeder body weight at 5 wks (LNG: Light Non-Graded and HNG: Heavy Non-Graded) and weeks of age.

Effects	ALP[3] (UI)	OC[4] (ng/ml)
Body weight		
LNG[1]	2290	976
HNG[2]	2949	1238
n	28	20
SEM	108.6	56.6
Age (wks)		
5	3374	1189
10	1944	1025
n	28	20
SEM	108.6	56.6
Body weight/Age (wks)		
LNG/5	2781^b	1032
LNG/10	1798^c	920
HNG/5	3839^a	1346
HNG/10	2059^{bc}	1130
n	14	10
SEM	153.6	79.6
P-value		
Body weight	0.032	0.029
Age (wks)	<0.001	0.050
Interaction	0.023	0.527

Note: Non-Graded: 160 females randomly selected at 5 wks of age within the 1988 broiler breeder females kept in a standard breeder farm.
[a–c]Means within a column and within a source with no common superscript differ significantly.
[1]The 40 lightest birds, below the standard body weight at 5 wks, of the Non-Graded.
[2]The 40 heaviest birds, over the standard body weight at 5 wks, of the Non-Graded.
[3]Alkaline Phosphatase.
[4]Osteocalcin.

In field conditions, results of this study indicate that livability of the LNG females (compared to the HNG) was lower, as the LNG probably consumed below their requirements.

Evolution of TL, BS and EM, according to BW at 5 wks (LNG and HNG) and age (wk), is presented in Table 5. According to BW, the LNG females had shorter tibias, as compared to the HNG (108 mm *vs.* 120 mm, $P < 0.001$). Taking age into account (wk), TL increased between 5 and 15 wks, with weekly differences being significant. Within the period from 15 to 30 wks of age, TL was not significantly different. This study shows that the LNG females had shorter tibias than did the HNG, which confirms that in the rearing period BW and skeletal growth are related, as has been observed by other authors (Robinson et al. 2007).

Regarding BW, BS results of the LNG tibias show a statistical tendency to support less weight before breaking, as compared to the HNG tibias (64.3 and 71.4 kgf/mm^2, $P = 0.085$). According to age (wk), tibia BS at 15 wks was lower than at 25 and 30 wks of age (38.9 *vs.* 80.3 and 80.1 kgf/mm^2; $P < 0.001$).

In the case of EM, and also regarding BW, results of the LNG tibias also show a statistical tendency to be intrinsically less rigid or more elastic, when compared to the HNG tibias (61.5 and 68.9 kgf/mm; $P = 0.072$). According to age (wk), EM at 15 wks was lower than at 25 and 30 wks of age (37.9 *vs.* 75.6 and 78.2 kgf/mm; $P < 0.001$).

Therefore, BS and EM results show that tibias of the LNG females have lower load endurance and are more elastic than are the HNG, thus they are more vulnerable. This could be related to a bone with lower EM being less mineralized, as may also be expected of a rachitic bone (Turner and Burr 1993).

After these results, it might be concluded that the light females in breeder rearing farms will always have shorter tibias and poorer bone mineralization than will the heavy ones. To recover bone development of the light females (to obtain compensatory growth) it might be necessary to grade them early in rearing, since TL reaches its maximum at 15 wks of age and tibia BS and EM between 15 and 25 wks of age. This is in line with the statement that 90% of the skeleton of the broiler breeders is already developed at 11–13 wks (Ross Parent Stock Management Handbook. Aviagen 2013b).

Evolution of ALP and OC serological levels of the LNG and HNG are presented in Table 6. An interaction BW/age (wk) shows that at 5 wks of age the LNG females had a significantly lower ALP level than did the HNG (2781 UI *vs.* 3839 UI, $P = 0.023$), however no differences were observed at 10 wks. Likewise, ALP levels of both LNG and HNG were significantly higher at 5 wks, as compared to 10 wks of age (LNG: 2781 UI *vs.* 1798 UI; HNG: 3839 UI *vs.* 2059 UI; $P = 0.023$). Regarding OC serological levels, related to BW at 5 wks, the LNG had a lower OC level than did the HNG (976 ng/ml *vs.* 1238 ng/ml, $P = 0.029$). If age (wk) is taken into account, results show a tendency of a higher OC level at 5 wks than at 10 wks of age (1189 ng/ml *vs.*1025 ng/ml, $P = 0.050$).

There are no studies which have used ALP or OC to assess skeletal growth in broiler breeders in rearing. However, in chickens, elevated ALP activity has been predominantly related to increased osteoblastic activity and used as a marker for evaluating skeletal health and bone disease, such as skeletal growth, rickets, fracture repair and osteomyelitis (Lumeij and Westerhof 1987). In the present study, regarding skeletal or bone growth, it may be concluded that at 5 wks of age the LNG females had lower ALP serological levels than did the HNG, and taking into account 5 and 10 wks together, the LNG females had also lower OC levels, which also suggests that there is a relation between BW and bone development. Therefore, these two serological markers may be useful as additional information of BW and uniformity, in order to assess if bone development is correct during the first 10 wks of the rearing period of the broiler breeders. Nevertheless, serological levels of ALP and OC are directly related to skeletal or bone development, whereas BW is only an indicator and does not directly relate to skeletal development.

ALP and OC serological levels decrease significantly from 5 to 10 wks of age, which might confirm that when the females reach 10 wks of age the rate of skeletal growth has already become reduced, and which is in turn related to this study result of TL ceasing to increase at 15 wks. From a management point of view, lower ALP and OC serological levels at 10 wks of age suggest that at this age their skeletal development has already become reduced and, therefore, it is worth grading the females early in rearing in order to recover the skeletal frame size of the light birds before their development starts to reduce.

Conclusions

Without grading in rearing, the light females (with BW below standard at 5 wks of age) presented lower BW, uniformity and livability than did the heavy females. Likewise, they had shorter, less resistant and more ductile tibias. Serological markers, ALP and OC, proved to be efficient ways to measure and assess correct bone development of broiler breeder females during the first 10 wks of rearing, the phase of major skeletal development.

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