Feed intake control in grand parent stock hens and impact in their skeletal development.

X. ASENSIO[1,2*], J. PIEDRAFITA[3], A. PUENTE[2] y A.C. BARROETA[1]

[1]Servicio de Nutrición y Bienestar Animal (SNiBA), Departamento de Ciencia Animal y de los Alimentos, Facultad de Veterinaria, Universidad Autónoma de Barcelona, 08193 Bellaterra, España.
[2]Aviagen S.A.U., 08416 Riells del Fai, España.
[3]Departamento de Ciencia Animal y de los Alimentos, Facultad de Veterinaria, Universidad Autónoma de Barcelona, 08193 Bellaterra, España.
***Corresponding author: xasensio@aviagen.com**

During the rearing period of Grand Parent Stock (GPS) of the meat lines it is essential to achieve the correct growth rate. For this, the amount of feed allocated must be controlled and calculated so as to achieve the proper nutrient supply. As a result of volume intake control and of the fact that the quantity of feed given is calculated taking into account the average body weight (BW) of the flock, a variable feed intake among the birds is the result and so pullets with lower BW than the standard profile appear. The aim of this study is to evaluate if the 5 week old pullets with a lower BW than standard are affected in their development and skeletal strength. Likewise, the usefulness of different serological markers is proposed, to assess the skeletal development throughout the initial phase of the GPS rearing period.

A commercial flock of 1,988 GPS (PEN) Ross 308 of the female line was used. During the whole rearing period feed intake was controlled following the recommendations of the genetic company. At 5 weeks of age 160 pullets (CTR) were randomly selected within the PEN and they were individually identified and assigned to 3 experimental groups of weight: Light (L, first quartile of the lightest), Medium (M, 2nd and 3rd quartiles) and Heavy (H, forth quartile of the heaviest). These 160 pullets were not graded or grouped according to weight and they were weighed individually every 5 weeks, starting in week 5 and finishing in week 30 of age. The weeks 5 and 10 of age, and from L and H pullets, blood samples were taken to determine Alkaline Phosphatase (FA) and Osteocalcin (OCN). Likewise, and also every 5 weeks, 3 pullets from each group (L and H) were slaughtered and the extraction of tibias from the right leg was carried out to determine tibia length (TL) and breaking strength (BS). Similarly, the gastrocnemius muscle tendon from the left leg was dissected to carry out histological studies to assess semi-quantitative inflammation and fibrosis.

In relation to weight evolution throughout the rearing period, L pullets maintained an inferior weight, significantly different ($P < 0.05$), from that of the H pullets until week 25 of age. From this point, the differences in BW reduced until their disappearance in week 30 ($P > 0.05$). On the other hand, FA and OCN levels, serological markers of bone formation, were inferior for the L pullets compared to the H pullets in weeks 5 and 10 of age ($P < 0.05$).

Related to tibia development, the L hens in comparison to the H hens, showed shorter tibias at 30 weeks of age ($P < 0.05$) and a tendency for lower BS in weeks 15, 25 and 30 ($P = 0.085$).

Histological studies of the gastrocnemius muscle tendon showed that in weeks 20 and 25, L hens had more incidence of inflammation and fibrosis than H hens.

The results of this study indicate that an insufficient intake of nutrients during the initial phases of development of the breeders could be related to inferior bone development and to poor

tendon structure. Pullets with BW below standard at 5 weeks of age will have shorter and less resistant tibias, along with weak tendons, with low strain resistance and as a result a risk of inflammation, fibrosis and breaking. Furthermore, FA and OCN have proved to be efficient ways to measure and assess correct bone development of breeder pullets during the first 10 weeks of rearing, the phase of major skeletal development.

Key words: Grand Parent Stock; feed intake control; skeletal development; Alkaline Phosphatase; Osteocalcin

Durante el periodo de recría de las reproductoras abuelas (GPS) de las líneas de carne es esencial conseguir un adecuado ritmo de crecimiento. Para ello, la cantidad de pienso suministrada debe ser controlada y calculada para alcanzar el aporte adecuado de nutrientes. Como consecuencia del control del volumen de ingesta y del hecho que la cantidad de alimento suministrado se calcula teniendo en cuenta el peso medio del lote, se provoca un consumo variable de pienso según individuos y por lo tanto aparecen aves que presentan pesos inferiores al perfil estándar. El objetivo de este estudio es evaluar si las pollitas reproductoras que a las 5 semanas de vida presentaban pesos inferiores al estándar se ven afectadas en su desarrollo y fortaleza esquelética. En paralelo, se plantea la utilidad de diferentes marcadores serológicos para evaluar el desarrollo esquelético a lo largo del período de recría de las GPS.

Para ello, se utilizó un lote comercial de 1.988 GPS (PEN) Ross 308 de la línea hembra. Durante toda la recría se controló el volumen de ingesta siguiendo las recomendaciones de la casa de genética. A las 5 semanas de vida se identificaron de forma individual 160 pollitas (CTR) elegidas al azar dentro del PEN y se asignaron a 3 grupos de peso experimentales: Ligeras (L, primer cuartil de aves con menor peso), Intermedias (M, 2º y 3er cuartil) y Pesadas (P, cuarto cuartil de aves con mayor peso). Estas 160 pollitas no fueron seleccionadas o agrupadas según peso y fueron pesadas de forma individual cada 5 semanas, empezando la semana 5 de vida y acabando la semana 30. Las semanas 5 y 10 de vida, y de las aves L y P, se tomaron muestras de sangre para la determinación de Fosfatasa Alcalina (FA) y Osteocalcina (OCN). En paralelo, y también cada 5 semanas, 3 aves de cada grupo (L y P) fueron sacrificadas y se procedió a la extracción de tibias de la extremidad derecha para determinar su longitud y su resistencia a la rotura. Asimismo, se diseccionó el tendón del músculo gastrocnemius de la extremidad izquierda para efectuar estudios histológicos de valoración semi-cuantitativa de inflamación y fibrosis.

En relación a la evolución del peso a lo largo del periodo de recría, las aves L mantuvieron un peso inferior, de forma estadísticamente significativa ($P < 0.05$) al de las P hasta la semana 25 de vida. A partir de aquí, las diferencias se redujeron hasta desaparecer en la semana 30 ($P > 0.05$). Por otro lado, los niveles de FA y OCN, marcadores serológicos de formación ósea, fueron inferiores para las aves L respecto a las P en las semanas 5 y 10 de vida ($P < 0.05$).

En cuanto al desarrollo de las tibias, las pollitas L en comparación con las P presentaron tibias más cortas a las 30 semanas ($P < 0.05$) y una tendencia a menor resistencia a la rotura en las semanas 15, 25 y 30 ($P = 0.085$).

Los estudios histológicos del tendón del músculo gastrocnemius, mostraron que en las semanas 20 y 25 de vida, las aves L presentaban más incidencia de inflamación y fibrosis que las P.

Los resultados de este trabajo indican que una insuficiente ingesta de nutrientes durante las fases iniciales del desarrollo de las pollitas reproductoras podría estar relacionada con un menor desarrollo óseo y una pobre estructura tendinosa. Las aves con pesos por debajo del estándar a la 5ª semana tendrán tibias más cortas y menos resistentes, acompañado de tendones de estructura débil, con poca resistencia a la tensión y en consecuencia con riesgo de inflamación,

fibrosis y rotura. Además, la FA y la OCN han resultado eficaces para medir y evaluar el correcto desarrollo óseo de las pollitas reproductoras durante las primeras 10 semanas de la recría, fase de mayor desarrollo esquelético.

Palabras clave: reproductoras abuelas; control de la ingesta; desarrollo esquelético; Fosfatasa Alcalina; Osteocalcina

Introduction

Genetic selection for broilers has focused on rapid juvenile growth, feed conversion ratio and increased yield of breast meat (Pollock, 1999). These facts are desired and beneficial in broiler production; however, they may compromise reproductive fitness and efficiency in the parent stock broiler breeders (PS) and grand parent stock (GPS) (Siegel & Dunnington, 1985). PS are subjected to feed intake (FI) control during rearing to reduce obesity-related reproductive problems such as lower peak lay, erratic ovulation, defective eggs, low fertility, and reduced hatchability and chick quality (Katanbaf et al., 1989). Reducing FI to levels needed to control BW has intensified competition for feed, resulting in low BW uniformity. Larger or aggressive pullets likely out-compete smaller or timid pullets, resulting in unequal access to feed and increasing flock BW variation (Zuidhof et al., 2015). Therefore, controlled FI, the different capabilities of the hens to get feed and incorrect management can lead to a percentage of PS or GPS which under consume and are not able to attain correct growth. Under consumption in rearing leads to underweight pullets which will not be receiving the nutrients that they require and this can produce Ca, P, protein and vitamin deficiencies.

Rickets, varus & valgus and rupture of the gastrocnemius muscle tendon are currently leg health issues in PS and GPS, and have as a result poor viability due to culls appearance. Poor mineralisation affects bone strength that enables the skeleton to withstand gravity and additional loading (Boivin & Meunier, 2002). Related to gastrocnemius muscle tendon issues, it is worth taking into account that in some investigations, the rupture tendon was associated with several non-infectious factors, such as deficiency states, variation in tensile strength, glucosaminoglycan content or cellular structure of the tendon (Cook et al., 1983ab).

Biochemical markers, which are currently used in humans to evaluate bone metabolism, provide a real-time assessment of bone formation, resorption and turnover. In fact, FA and OCN, which are serological markers of bone formation, may add useful information for assessing fracture risk and for monitoring osteoporosis in women (Christenson, 1997). Conversely, these two markers have never been used in GPS hens to assess bone metabolism. The precise function of FA enzyme is yet unknown, but it obviously plays an important role in osteoid formation and mineralization (Harris, 1990). OCN is considered a specific marker of osteoblast function (Markus J Seibel, 2005).

The aim of this study is to evaluate if the 5 week old pullets with a lower BW than the standard are affected in their development and skeletal strength, in particular in tibia and gastrocnemius muscle tendon status. Likewise, the usefulness of different serological markers is proposed, to assess the skeletal development throughout the initial phase of the GPS rearing period.

Material and methods

Facility, animals and experimental design

The trial was carried out in an all in all out GPS farm (Avícola Sichar) of Aviagen SAU during a period of 30 weeks (25 weeks of rearing and the first 5 weeks of production). A total of 1,988 Ross GPS hens (PEN) from an Aviagen S.A.U. commercial hatchery were kept in a pen of this standard

breeder farm during 21 weeks. Pen dimensions were 48.50 m long by 6.5 m wide, 315.25 m^2 in total and so 6.30 hens/m^2. Feed space was 0.87 hens per pan hole and drinking space was 5.58 hens per nipple. At 21 weeks old these females were released in the whole house and mixed with the other females and the males (ratio 10/1). From this moment, stock density was 5.30 hens/m^2, feed space was 0.74 hens per pan hole and drinking space was 4.74 hens per nipple. The house was environmentally controlled and the temperature set point was modified depending on the hen age, starting with 30ºC at one day old and decreasing on a regular basis until 20ºC at 27 days old; this set point was kept until the end of the trial. Light programme and light intensity followed the standard applied to PS.

At 5 weeks of age 160 pullets were randomly selected within the PEN so as to create the CTR group. They were tagged to be individually identified and assigned to 3 experimental groups of weight: Light (L, first quartile of the lightest), Medium (M, 2nd and 3rd quartiles) and Heavy (H, forth quartile of the heaviest). After 5 weeks and until 21 weeks, the PEN was graded on a regular basis whereas the CTR group was never graded or grouped according to BW.

Diets

Feed was produced in a feed mill of CAG (Reus, Spain). The different diets were formulated to meet the requirements of the GPS hens for each phase of the rearing and production period. PS Ross 308 nutritional specifications 2014 were taken into account.

Collected data, sampling and analytical determinations

The PEN was monitored weekly by sampling BW and calculating the coefficient variation percentage (%CV). Feed and water intake, daily mortality and management data such as light program, light intensity and environmental temperature were recorded. All the pullets of the CTR group were weighed every 5 weeks, starting when they were 5 weeks old and finishing at 30 weeks old. BW and %CV were also recorded.

In weeks 5 and 10 of age, and from the L and H pullets, blood samples were taken to determine Alkaline Phosphatase (FA) and Osteocalcin (OCN). For FA all the L and H pullets were sampled, and to be evaluated a colorimetric method based on catalytic transformation of p-nitrophenylphosphate as substrate (IFCC method) on the Beckman Coulter AU analyser was used. To determine OCN the lightest 10 of the L quartile and the heaviest 10 of the H quartile were sampled, and to be evaluated the Rat-MID™ Osteocalcin EIA (ELISA test) was used.

Likewise, and every 5 weeks, 3 pullets from each group (L and H) were slaughtered and to determine tibia length (TL) the extraction of tibias from the right leg was carried out. TL was measured by using a calliper (0.03 mm precision and 0.01 mm resolution). Tibia breaking strength (BS), maximum load, of these same tibias was measured in weeks 15, 25 and 30 of age, by using a MTS Alliance RT/5 material testing system (force resolution 0.001 N and distance resolution 0.001 mm).

Similarly, the gastrocnemius muscle tendon from the left leg was dissected and histological studies were carried out by the CreSA (Centre de Recerca en Sanitat Animal). Tendons from both L and H hens were analyzed. Samples were fixed in 10% buffered formalin and routinely processed for histopathology. Slides were cut at 3 μm, stained with hematoxylin-eosin and examined in a "blind-fashion" manner. The presence of fibrosis and inflammation were assessed by using a semi-quantitative approach: (0) absence, (1) low amount, (2) moderate amount, (3) large amount. Inflammation, when present, was composed by mononuclear cells (lymphocytes, plasma cells and few macrophages).

Statistical analysis

For statistical analysis R® program (version 3.3.3, 2017) was used. A one way ANOVA was carried out to analyse body weight data. A mixed model was used to analyse FA and OCN; class and week were fixed effects and the bird was a random effect. Factorial ANOVA was carried out to analyse TL and tibia BS; the factors were class and week, without interaction between them. Tukey test was also used to compare means.

In all cases, p-values lower than 0.05 were considered to be significant.

Results and discussion

BW and %CV evolution in both PEN and CTR group are presented in Table 1. During the rearing period the CRT group had higher %CV than the PEN. As was explained before, after 5 weeks of age the pullets of the PEN were graded on a regular basis, so their %CV was correct throughout the trial, however the CTR group was not graded hence its %CV is poorer. This lack of uniformity was mainly due to the L pullets. Therefore, when feed intake amount is controlled, a non existing grading is going to induce a lack of uniformity and thus birds with lower BW than the target; probably due to the fact that they consume under their needs.

BW of the L pullets was lower and significantly different from the H ones until 25 weeks of age. Between 25 and 30 weeks BW of both groups of birds came close and finally was similar and not significantly different ($P > 0.05$). These results could be explained since after 21 weeks there is more feed availability, more feed space and a longer clean-up time, hence the L GPS are able to obtain more feed. Therefore, after 25 weeks BW of the L hens matches that of the H and the %CV of the L decreases to a correct level (the same percentage as the H).

Table 1. Weekly evolution of coefficient variation percentage (%CV) and body weight (BW).

	% CV				BW (g)		
Week	PEN	CTR	L	H	L	H	p - value
5	13.3	11.5	5.1	4.2	587.0^{b}	830.8^{a}	< 0.001
10	9.7	17.7	16.9	9.4	829.3^{b}	1184.0^{a}	< 0.001
15	6.5	13.0	12.9	6.4	1345.5^{b}	1762.4^{a}	< 0.001
20	6.6	13.4	14.2	9.2	2035.0^{b}	2437.9^{a}	< 0.001
25	6.8	11.4	9.7	6.3	3292.4^{b}	3542.7^{a}	< 0.01
30	6.9	5.8	7.3	7.3	3943.9^{a}	3994.5^{a}	ns

PEN (1,988 pullets); CTR group (160 pullets); L (first quartile); H (forth quartile)

Related to FA and OCN levels, as shown in Figures 1 and 2, L GPS had significantly ($P < 0.05$) lower levels. These two markers are considered to be bone formation markers (Markus J Seibel, 2005), and the L GPS having lower levels, it also suggests that there is a relation between BW and bone development. Therefore, these two markers can be useful as additional information of BW and uniformity, in order to assess if skeletal development during the first 10 weeks of the rearing period of GPS and PS is correct. On the other hand, both biochemical markers decrease from week 5 to week 10 (presented in Figures 3 and 4) which could confirm that when they reach 10 weeks the pullets have already reduced their skeletal growth.

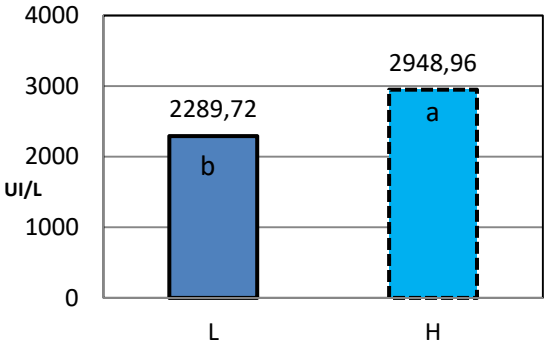


Figure 1. Alkaline Phosphatase (FA) level of the CTR group at 5 and 10 weeks of age according to BW.

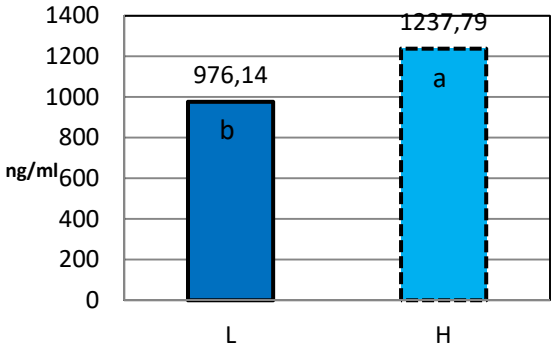


Figure 2. Osteocalcin (OCN) level of the CTR group at 5 and 10 weeks of age according to BW.

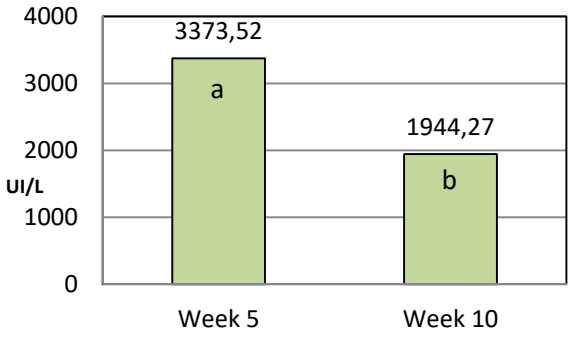


Figure 3. Alkaline Phosphatase (FA) levels of CRT group (L and H) according to week of age.

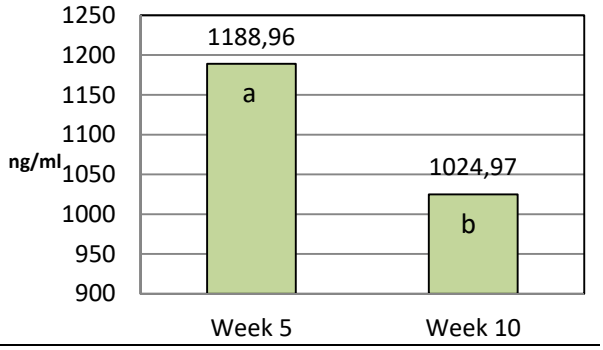


Figure 4. Osteocalcin (OCN) levels of CRT group (L and H) according to week of age.

Tibia length (TL) and tibia breaking strength (BS), maximum load endurance, according to CTR group weight and week are shown in Table 2 and TL differences between both BW groups (L and H) are presented in Figure 5. TL increased and differed significantly ($P < 0.05$) over the weeks until week 15. It is stated that 90 % of the skeleton of the PS is already developed at 11-13 weeks (Ross PS Handbook 2013). Within the period from 20 to 30 weeks, TL was not significantly different. On the other hand, regarding pullet weight, L hens had throughout the whole trial shorter tibias compared to the H (significant difference, $P < 0.05$). This fact also confirms that in the rearing period BW and skeletal growth are related as has been observed by other authors (Robinson et al., 2007). Tibia BS, maximum load endurance, at week 15 was lower and significantly different ($P < 0.05$) from weeks 25 and 30. During the period between 15 and 25 weeks tibias reach their maximum strength. Tibias of the L hens resisted less weight before breaking; the difference with the H hens being close to differing significantly ($P = 0.085$).

Table 2. Tibia length (TL) and breaking strength (BS), maximum load endurance, according to CTR group weight and week

	CTR group		Week of age					
	H	L	5	10	15	20	25	30
TL (mm)	119.52^{a}	108.43^{b}	85.5^{c}	101.61^{b}	119..62^{a}	126.01^{a}	125.28^{a}	125.85^{a}
BS. Maximum load (gf)	71373.3	64284.1			38899.11^{b}		80254.57^{a}	80101.69^{a}

Values within a line with no common superscripts differ significantly ($P < 0.05$).
Tibia BS values of the CTR group are close to differing significantly ($P = 0.085$).

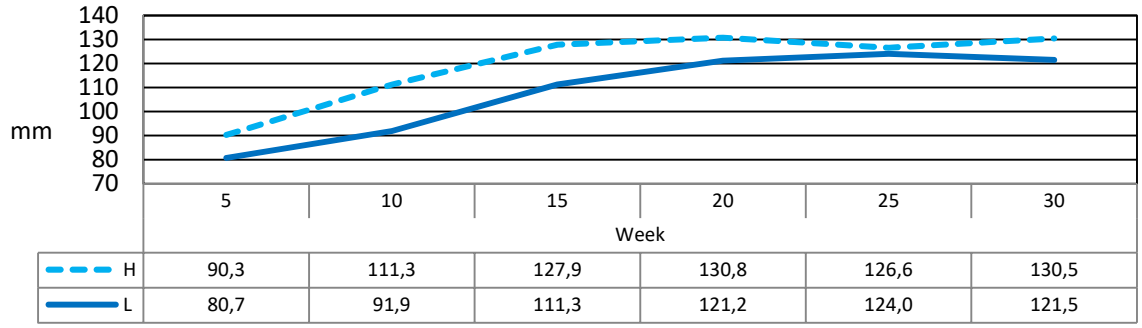
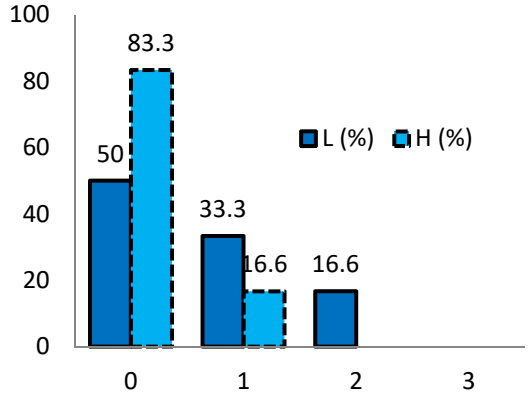


Figure 5. Tibia length (TL) of the L and H pullets of the CTR group according to week.

Histological analyses were carried out so as to assess if there was any structural difference in the gastrocnemius muscle tendon of the L hens in comparison with the H ones. The percentage of the L and H hens in each of inflammation and fibrosis scores (weeks 20 and 25) are presented in the Figures 6 and 7. Results showed that in weeks 20 and 25 of age, L hens had more incidence of inflammation and fibrosis than the H ones. Damaged tissues can result from mechanical injuries, which can lead to fibrosis as a result of chronic inflammation (Wynn, 2007). The origin of damaged tendons of the L hens could come from feed under consumption, and thus from a lack of the necessary nutrients to build a tendon able to endure strain and BW at the onset of production.



Inflammation and fibrosis score. (0) absence, (1) low amount, (2) moderate amount, (3) large amount.

Figure 6. Percentage of L and H hens in each inflammation score (weeks 20 and 25).

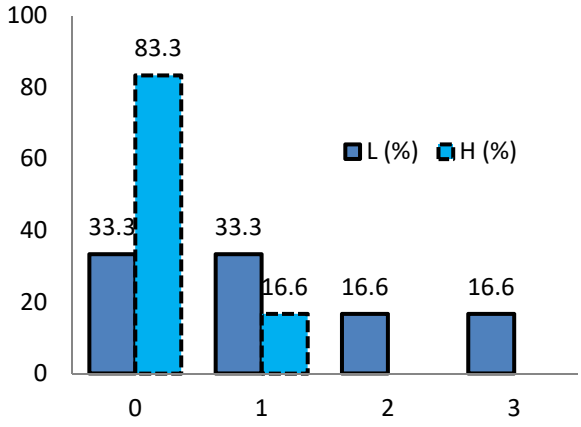


Figure 7. Percentage of L and H hens in each fibrosis score (weeks 20 and 25).

The results of this study show a paradox. L hens, with smaller skeletons and lower BW than the H ones, consume early in egg production (25 weeks) more than they need for their maintenance and production, until the point of nearly matching the BW of the H hens at 30 weeks. Therefore, to have uniform GPS or PS flocks, with a similar skeletal development in rearing period and hence a similar body frame at the beginning of production, is essential in order to feed the birds evenly and thus to provide them with the necessary nutrients in order to accurately satisfy their needs.

It is also relevant that pullets with BW below standard at 5 weeks of age will have shorter and less resistant tibias, along with weak tendons, with low strain resistance and as a result a risk of inflammation, fibrosis and breaking. An insufficient intake of nutrients during the initial phases of development of the GPS or PS could be related to this inferior bone development and poor tendon

structure; and even the L hens recovering BW, are not able to recover their skeletal frame and, as the histological analyses show, to achieve a tendon structure to endure strain.

Besides, FA and OCN have proved to be efficient ways to measure and assess correct bone development of breeder pullets during the first 10 weeks of rearing, the phase of major skeletal development. Therefore, these two serological markers can be useful as additional information of BW so as to evaluate if the skeletal development in the rearing period of the GPS and PS is correct.

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