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ANIMAL PRODUCTION

Correlation and path analysis of assessment methodologies of bone quality from brown egg layers at final of the production cycle

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ABSTRACT. This study aimed to investigate direct and indirect correlations of methodologies of bone quality analysis from brown egg layers, at final of the production cycle. Twelve femurs of Dekalb Brown laying hens, euthanized at 85-week-old, were assessed to evaluate breaking strength (BS), Seedor index (SI), mineral matter (MM), calcium (Ca) and phosphorus (P) contents, besides cortical (CorD), medullar (MedD) and epiphysis (EpiD) diameters. Correlations and path analysis were obtained with the aid of SAS® University ($p \leq 0.05$). The BS directly represented the bone quality and was compared to other methodologies. Greater linear correlations occurred between BS and MM (r = 0.82), MM and Ca (r = 0.72), and BS and Ca (r = 0.70). The MM content displayed the greatest direct effect on the BS (r = 0.53). The Ca content showed a reduced direct effect on the BS (r = 0.18), with indirect effects through MM content (r = 0.44) and EpiD (r = 0.15), however, presented a great total correlation (r = 0.78). Determination of mineral matter content is the main methodology associated with femur breaking strength from brown egg layers at final of the productive cycle. Because of that, this methodology is more reliable to determine bone quality.

Keywords: Direct correlation; indirect correlation; mineral matter; Seedor index; bone-breaking strength.

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Introduction

The intensive production of laying hens in cages, associated with space restriction, may cause bone deformities and deteriorations at final of the laying phase. Moreover, the genetic improvement aiming to increase the number of eggs per caged hen, and the longevity of this animal that extends the production period, may increase the occurrence of hens with bone illnesses (Moraes et al., 2020).

The bone structure of laying hens performed many essential functions such as sustaining, locomotion, mechanical protection of organs, and metabolic reserve of minerals such as phosphorous (P) and calcium (Ca). The hens' bones are composed of three main tissues: cortical, trabecular, and medullar. This last one is responsible for the calcium reserve needed to form the eggshell (Olgun & Aygun, 2016). The cortical and trabecular bone tissues are responsible to maintain the physical integrity of hens' skeleton, however, when they reach sexual maturity, it starts the formation of medullar bone type on the long bones (femur, tibia, and humerus). These bones work like calcium labile reserves that can suffer reabsorptions during the production phase. Moreover, they can provide 40% of the necessary calcium to form the eggshell (Nys & LeRoy, 2018).

Different factors influence bone quality (Rodriguez-Navarro et al., 2018; Wistedt, Ridderstråle, Wall, & Holm, 2019; Reis et al., 2020; Wang et al., 2020), but considering the production cycle longevity, there is a reduction of absorbed calcium by the laying hen, from 60% in the youngers to 40% in the older ones (Saldanha et al., 2009). This fact is associated with a greater intensity of bone mobility during the laying phase, resulting in a greater number of fractures, and illnesses associated with the locomotion system like osteoporosis (Olgun & Aygun, 2016).

Comparing the bone quality analyses that are often assessed, the breaking strength (BS) and Seedor index (SI) stand out because they can represent physical characteristics of bone structure with great accuracy (Faitarone et al., 2012). Conversely, determination technics of mineral matter (MM), calcium (Ca) and phosphorous (P) contents represent the chemical aspects of this structure (Kim, Bloomfield, Sugiyama, &

Ricke, 2012). The breaking strength, mineral matter content, and Seedor index tend to reduce with the age advance of hen, due to the bone mineral mobilization during the production phase. Because of that, it is important to observe the hens' bone quality, mainly at final of the production cycle.

Paz et al. (2008) studied the effect of dietary calcium on bone quality from brown egg layers and reported no correlation between the breaking strength (BS) and ashes content, but a correlation between BS and bone volume, which in turn is determined by the Seedor index (SI). In addition, Paz et al. (2009) reported that the SI methodology displayed the lowest correlation with BS, besides the contents of MM, Ca and P. Moreover, Schreiweis, Orban, Ledur, Moody, and Hester (2004) reported that the mineral density, measured with the aid of bone densitometer, was the only methodology that showed significant correlation with bone quality, body weight, and eggshell quality of the hens, highlighting the importance of the mineral density assessment.

Beyond the different impacts of these methodologies on bone quality, another implication is the logistics to make them feasible, added to their reliability degrees. Direct measurements, although easier, require equipment with semi-analytic scales (such as balances and pachymeter). In addition, methods that depend on morphometric evaluations (SI, CorD, MedD, and EpiD) are subject to variations according to the equipment management. As reported by Molino et al. (2009), the SI analysis shows good results, but it needs very-expensive and complex tools. Besides that, there is a lack of standardization regarding these methodologies, once different probes and contact velocities can be used, altering the results. In addition, the lack of uniformity in bone size and format difficult for the test uniformity. Determination of bone chemical composition (MM, Ca, and P contents) presents a high level of assertiveness and a significant correlation with bone quality (Donkó et al., 2018). Nevertheless, this analysis increases costs and labor because of the time needed to prepare samples added to costs related to chemical reagents and, of course, the installation and maintenance of the laboratory.

Thus, considering the fragility of the bone structure from laying hens at the end of the production cycle, the complexity of mobilization and reabsorption of calcium in the bone tissues, and the number of methodologies to analyze bone quality, it is important to investigate the reliability of correlation analysis. In this sense, path analysis is a methodology often used in breeding programs that allows understanding of the causes involved in associations between characters and decompound these correlations into direct and indirect effects (Cruz, 2013). Thereby, it is possible to determine, with good accuracy, which methodologies can characterize the bone quality directly or indirectly, determining which ones reflect, or no, that quality (Cruz, Regazzi, & Carneiro, 2006). Therefore, these results may contribute to obtaining accurate and reliable data regarding the bone density of old hens, aiming for the improvement of their performances and welfare.

Based on this context, this study aimed to investigate direct or indirect correlations among assessment methodologies of bone quality from brown egg layers, at final of the production cycle.

Material and methods

The experiment was carried out at the Laboratory of Animal Products Analysis, and at the Laboratory of Bromatology from Animal Science Institute of Rural Federal University of Rio de Janeiro (UFRRJ), besides the Soil Analyzes Center from UFRRJ, at Campos dos Goytacazes municipality, Rio de Janeiro. All procedures were done under authorization of the Ethics Committee about the use of animals from the Animal Science Institute (CEUA-IZ) of UFRRJ, under number protocol 23083.005984/2017-02.

Femurs used for the analyses were collected from the right member of Dekalb Brown laying hens, which were allocated into cages and remained under the same conditions of management and feeding, since 14-week-old. The hens were fed with isoproteic and isoenergetic diets based on soybean meal and ground corn, according to the nutritional requirements proposed by Rostagno et al. (2011). During all experiment periods, the laying hens had an *ad libitum* water consumption and daily received 17 hours of light (sunlight and artificial). Twelve laying hens were euthanized at 85-week-old through cervical dislocation, according to recommendations of Conselho Nacional de Controle de Experimentação Animal (CONCEA, 2013).

Thereafter the euthanasia procedures, bones were collected yet with their cover tissues (muscles and cartilage) and frozen at -25°C aiming for posterior analyzes. Bone structures were previously defrosted and drought into boiling water for 10 minutes, aiming to facilitate the removal of adjacent tissues adhered to the bones, according to the methodology described by Orban, Roland, Bryant, and Williams (1983). Dissection of the material was done with the aid of bistouries, scissors, and tweezers aiming to improve the extraction of residues adhered to the bone structures (Faitarone et al., 2012). According to Paz et al. (2009), this methodology allows extracting almost 80% of the fat content from bone structures.

The following analyzes were made to determine bone quality: Seedor index (SI), breaking strength (BS); measurements of cortical (CorD), medullar (MedD), and epiphysis diameters (EpiD); besides determination of bone mineral matter (MM), phosphorous (P) and calcium (Ca) contents.

The SI was evaluated using a manual pachymeter. For that, the bones were measured considering their highest epiphysis length, and their weights were obtained on a semi-analytic digital scale. Thereafter, the femur weight (mg) was divided by its length (mm), following the methodology proposed by Seedor, Quartuccio, and Thompson (1991).

Breaking strength was made according to the methodology described by Faitarone et al. (2012), using the texturometer *Stable Micro Systems Texture Analysers®*, model TA.XT Plus. Bones were allocated to the center of the texturometer with adequate support and free space of a 6-cm distance from the probe. The probe's down-speed was 2.0 mm second^{-1}, with 1.0 mm second^{-1} during the contact with the bone. Besides that, the post-test speed was 4.0 mm second^{-1}. A specific software registered the necessary strength to break the femurs, which was expressed as kilogram-force.

Morphometric analyzes of CorD, MedD and EpiD were done after the BS analysis, with the structures under natural status using a simple-saw bistoury (STARRET model) and a manual pachymeter. Bone structures were sectioned in their distal diaphysis, specifically in the transversal direction, and measurements were made close to the epiphyses.

Defatted dry matter content was determined according to Kim et al. (2004), and the bones were weighted on an analytic digital scale. Thereafter, they were allocated into a forced-air oven and dried at 105°C for 72 hours. After cooling down, the bones were weighed again to calculate the dry matter content. The MM was obtained by the bones-burn into a muffle furnace, at 550°C for 6 hours (942.05 methods, Association of Official Analytical Chemists [AOAC], 2005). The remaining ashes were macerated in pistil and porcelain grail until they passed through a 1-mm mesh sieve. Approximately 1 g of ground ashes were subjected to acid digestion to prepare the mineral solution (method 968.08D; AOAC, 2005). Furthermore, the phosphorus contents were determined through colorimetric technique (Detmann et al., 2012) and calcium contents analyzes were performed using the atomic absorption method (Zenebon, Pascuet, & Tiglea, 2008).

Pearson's linear correlations among all bone quality methodologies were analyzed through the PROC CORR of SAS® University version. The path analyzes were made with the aid of PROC CALIS, also from SAS® University (Statistical Analysis System Institute [SAS], 2015). For that, direct and indirect effects of all variables were verified on the breaking strength. Thereby, femur breaking strength was pointed out as the goal method, because of its direct and quantitative measure of bone resistance, and quality. All results were considered significant at 5% of error probability.

Results and discussion

The results observed from methodologies of femur bone quality, assessed on 12 laying hens, are described in Table 1.

Table 1. Results obtained from methodologies of femur bone quality from laying hens at 85- week-old.

N° of the hen	SI[a]	BS[b] (kgf)	MM[c] ($g\ kg^{-1}$)	P[d] ($g\ kg^{-1}$)	Ca[e] ($g\ kg^{-1}$)	CorD[f] (mm)	MedD[g] (mm)	EpiD[h] (mm)
1	102.51	12.73	54.55	19.73	25.00	1.9	2.3	9.3
2	116.10	10.80	52.04	19.53	26.13	1.2	5.8	10.0
3	113.43	11.78	47.50	18.78	25.05	2.1	3.3	9.9
4	113.05	18.96	60.49	18.38	29.60	2.5	2.3	9.8
5	108.48	14.72	54.14	18.54	25.50	2.1	3.3	9.9
6	94.63	10.78	48.41	19.00	24.90	3.9	3.5	7.2
7	107.33	11.39	53.52	19.06	24.89	1.7	5.3	11.7
8	93.34	13.49	50.99	18.36	24.78	2.1	3.3	9.9
9	108.48	14.72	54.14	18.54	25.50	2.1	3.3	9.9
10	108.48	14.72	54.14	18.54	25.50	2.1	3.3	9.9
11	107.08	13.84	52.10	17.62	24.56	2.2	3.4	11.8
12	116.75	15.26	56.05	17.61	25.50	3.8	3.3	12.1
Mean	108.48	14.72	54.14	18.54	25.50	2.1	3.3	9.9
SD[i]	7.56	3.03	3.87	0.57	0.83	0.75	0.89	1.36

[a]Seedor index. [b]Breaking strength (kilogram-force). [c]Mineral matter bone content (basis on dry matter). [d]Phosphorous bone content (basis on mineral matter). [e]Calcium bone content (basis on mineral matter). [f]Cortical diameter (cm). [g]Medullar diameter (cm). [h]Epiphysis diameter (cm). [i]Standard deviation of the mean.

The SI average found in this study is according to those of literature and similar to that one obtained by Paz et al. (2009), which studied bone calcium and phosphorous mobilizations to form the eggshell, during the production phase of heavy laying hens until 40-week-old. Nunes et al. (2013) pointed out that the provision of well-formulated diets attends to the mineral requirements, avoiding bone illnesses during the high production phase. Besides that, the hens are able to deposit the necessary amount of minerals into their bones, making them denser.

About the BS, the average obtained also is according to those found in hens of similar age. Donkó et al. (2018) found an average of 13.25 kgf (from 8.97 to 20.80 kgf) in tibias from laying hens aged 90-week-old when the authors determined the tibia breaking strength.

Compared to the present study, Oliveira, Freitas, Filgueira, Cruz, and Nascimento (2013) observed lower values of SI and BS in tibias from slight laying hens, which were fed with different levels and granulometry of limestone, and were submitted to artificial light.

Mineral matter content found in bones (54.14 g kg^{-1}) diverged from those verified in the literature. These differences may occur due to the method of fatty extraction regarding the prepared bones for the analysis but also can depend on both the age and lineage of hens, besides the own assessed bone. Donkó et al. (2018) evaluated the tibia of laying hens from Tetra SL lineage with 90-week-old and found MM content of 31.9 g kg^{-1}. However, the authors did not report if they extracted the fatty from bones during the preparation. Reis et al. (2020) used the same fatty extraction methodology performed in this study and found MM content of about 46.0 g kg^{-1}, in laying hens 85-week-old. In an assay with laying hens from Hy-line lineage aged 32-week-old, Nie, Wang, Gao, Guo, and Wang (2018) found MM contents from 52.79 to 55.01 g kg^{-1}, near to those observed in this study.

The P and Ca contents were 18.54 and 25.50 g kg^{-1}, respectively, based on mineral matter content. The calcium contents reported in this study were lower than those found in literature, considering laying hens of 32-week-old (Nie et al., 2018), 85-week-old (Robison & Karcher, 2019), and those varying from 64-to 96-week-old (Gebhardt-Henrich et al., 2017). The P content was greater than that one reported by Nie et al. (2018), for laying hens of 32-week-old. The capability of calcium storage in the skeleton decreases as the laying hen ages, while egg production often remains at great levels (Wistedt et al., 2019). Regarding it, a physiological disorder may occur when the total requirement of calcium cannot be attended to.

The average values of CorD, MedD and EpiD from femurs were 2.09, 3.28 and 9.95 mm, respectively (Table 1). Sorza (2019) reported the importance of these measurements to assess the structural development of these animals during the first weeks of life (until 13-week-old), besides their functional development that occurs from 14- to 24-week-old, and on the maintenance evaluation of the bone quality until the final production cycle. Thus, these measurements are important to allow the observation of bone status from laying hens.

Pearson's correlations were high and positive between the methodologies of BS and MM content p = 0.0011) and between MM and Ca contents (p = 0.0101). Moreover, correlations were low and negative between the methodologies of BS and DMed (p = 0.0299). These results are demonstrated in Table 2.

Table 2. Pearson's correlation among methodologies of femur bone quality from brown egg layers with 85-week-old.

	BS[b]	MM[c]	P[d]	Ca[e]	CorD[f]	MedD[g]	EpiD[h]
SI[a]	0.2908[i]	0.3980	-0.1600	0.4210	-0.1812	0.2127	0.5284
	0.3595	0.2001	0.6195	0.1729	0.5731	0.5068	0.0774
BS[b]		0.8213	-0.5612	0.7074	0.1740	-0.6247	0.2193
		0.0011*	0.0576	0.0101*	0.5886	0.0299*	0.4935
MM[c]			-0.2212	0.7221	-0.0044	-0.3029	0.3194
			0.4896	0.0080*	0.9891	0.3385	0.3115
P[d]				-0.0464	-0.4481	0.3106	-0.5352
				0.8861	0.1440	0.3258	0.0729
Ca[e]					0.0067	-0.2428	-0.0817
					0.9834	0.4470	0.8007
CorD[f]						-0.3913	-0.1963
						0.2084	0.5409
MedD[g]							0.2403
							0.4519

[a]Seedor index. [b]Breaking strength. [c]Femur mineral matter content. [d]Femur phosphorous content. [e]Femur calcium content. [f]Cortical diameter. [g]Medullar diameter. [h]Epiphysis diameter. [i]Pearson's correlation coefficients. *Significant p-values ($p \leqslant 0.05$).

Considering the Pearson's correlation, the MM content stood out as a strong predictor of BS. This corroborates with other scientific research results, in which correlation coefficients were 0.92 (Zhang & Coon, 1997), 0.77 (Hester et al., 2004), and 0.71 (Donkó et al., 2018). Nevertheless, both results of the present study and those verified in the literature did not corroborate results found by Neijat, Casey-Trott, Robinson, Widowski, and Kiarie (2019), which did not observe a significant correlation between the BS and MM content on the tibia, however, they found an association between these variables on the humerus (r = 0.60).

It is worth pointing out that the bones are mainly formed by mineral matter (54%), and the calcium content stands out with 25% of this total. That fact explains the high linear correlation between MM and Ca contents (r = 0.72), considering that long bones are the greatest calcium storage for laying hens, and they are essential for the metabolism of this mineral for eggshell formation. Bone hardness results from organic and inorganic components of the tissue, which demonstrates the importance to apply technics that measure mineral matter content and breaking strength of bone structures (Olgun & Aygun, 2016).

Negative Pearson's correlation between BS and MedD (r = -0.62) can be explained by the function of medullar bone, which acts like a labile reserve of calcium composed of apatite crystals randomly distributed all over the organic matrix. The medullar bone tissue differs from that of cortical bone from laying hens, displaying lower mineral content and lower levels of mineral organization. Besides that, the medullar tissue has shorter particles and it is not a structural bone tissue (Nys & Le Roy, 2018). This negative correlation indicates a reduction of bone mass, and according to Sorza (2019), this points out a reduction of bone hardness and can directly reflect in a worsening of the eggshell quality, due to the lower amount of mobilized calcium to form this eggshell.

It is worth pointing out that there was no significant Pearson's correlation among the methodologies and SI, P content, CorD, and EpiD. These results do not corroborate those verified by Schreiweis et al. (2004), which found an association between hens' bone density, body weight, and egg quality. Donkó et al. (2018) also suggest a significant correlation between tibia density and breaking strength. Nevertheless, Zhang and Coon (1997) observed that the bone's density and length showed low or no correlation, related to the mineral matter content and the breaking strength. This scenario can be observed when laying hens have calcium dietary deficiency, and consequently, display a reduction of bone calcium content, but without changes in the bone size (Cheng & Coon, 1990).

All these results may be considered contradictory to each other but can be explained by the different experimental conditions of the respective studies, mainly related to the diets and the laying hens' age. According to Rath, Huff, Huff, and Balog (2000), due to the intensity of metabolism in the laying hen bone and because of its modification in the different physiological phases, many variables may influence the bone status with great or low relevance, considering the hen age.

Direct effects (obtained from the path analysis) were observed on almost all methodologies of bone quality related to the BS. The only exception was the SI (Table 3). As previously mentioned, Zhang and Coon (1997) and Hester et al. (2004) found reduced, or no association between these two variables.

Greater direct effects on the femur BS were observed from MM (r = 0.53) and P contents (r = -0.48), which were middling correlations, however, positive and negative ones, respectively. The Ca content and EpiD presented lower direct effects on the BS, with positive and negative correlations, respectively. Nevertheless, these variables had their correlations indirectly improved by the physical characteristics of laying hens' femurs (Table 3). These results corroborate those reported by Zhang and Coon (1997), which observed a lower direct association between morphometric measurements and calcium levels, regarding the bone breaking strength.

Thereby, the indirect effect of EpiD reduced the correlation between MM content and BS (from r = 0.53 to r = 0.39). The indirect effects of CorD and EpiD reduced the negative correlation between P content and the BS (from r = -0.48 to r = -0.12). Moreover, the other indirect and direct effects were quite low, according to the classification of Kaps and Lamberson (2017), and considering the path analysis.

Conversely, the correlation between MM content and BS (Table 4) indirectly increased through the Ca content, due to this mineral composes a significant part of the total MM content in the skeleton. Therefore, the correlation coefficient virtually returned to its original value, regarding the direct effect on femur BS (r = 0.53). These results suggest that the correlation between MM content and BS is low affected by other methodologies of bone quality. In addition, these variables had the greatest coefficient of Pearson's correlation (Table 2), observed in this study (r = 0.82). Therefore, it is possible to point out the methodology of MM analysis as the principal one associated with femur BS, and because of that, it could be used to indirectly estimate this breaking strength.

Table 3. Path analysis of bone quality correlations, divided into direct and indirect effects on the femur breaking strength (BS), via methodologies of bone quality assessments from brown egg layers with 85-week-old.

Methodology		r[a]	P-value
SI[1]			
	Direct effect on the BS	0.0645	0.2263
	Indirect effect via CorD	-0.0480	0.5404
	Indirect effect via MedD	-0.0002	0.9808
	Indirect effect via EpiD	-0.0928	0.0937
	Subtotal	0.0645	0.2263
MM[2]			
	Direct effect on the BS	0.5345	<0.0001*
	Indirect effect via SI	-0.0264	0.4183
	Indirect effect via CorD	-0.0843	0.4207
	Indirect effect via MedD	0.1910	0.1003
	Indirect effect via EpiD	-0.1439	0.0418*
	Subtotal	0.3906	<0.0001*
P[3]			
	Direct effect on the BS	-0.4822	0.0002*
	Indirect effect via SI	0.0241	0.4303
	Indirect effect via CorD	0.2078	0.0402*
	Indirect effect via MedD	-0.1867	0.0972
	Indirect effect via EpiD	0.1552	0.0346*
	Subtotal	-0.1192	0.0002*
Ca[4]			
	Direct effect on the BS	0.1791	0.0023*
	Indirect effect via IS	0.0524	0.9009
	Indirect effect via CorD	0.1034	0.3327
	Indirect effect via MedD	-0.0967	0.4545
	Indirect effect via EpiD	0.1546	0.0379*
	Subtotal	0.3337	0.0026*
DCor[5]			
	Direct effect on the BS	-0.2355	0.0044*
	Indirect effect via IS	0.0109	0.5786
	Indirect effect via MedD	-0.0193	0.8209
	Indirect effect via EpiD	0.0759	0.1272
	Subtotal	-0.2355	0.0044*
DMed[6]			
	Direct effect on the BS	-0.2816	0.0004*
	Indirect effect via IS	-0.0312	0.9808
	Indirect effect via CorD	-0.0160	0.8208
	Indirect effect via EpiD	-0.0676	0.1428
	Subtotal	-0.2816	0.0004*
DEpi[7]			
	Direct effect on the BS	-0.2611	0.0303*
	Indirect effect via IS	0.0617	0.2819
	Indirect effect via CorD	0.2220	0.0973
	Indirect effect via MedD	-0.2391	0.1222
	Subtotal	-0.5002	0.0195*
R^2		0.9889	

[a] Correlation coefficient after the deployment of direct and indirect effects of the quality methodologies of femur from brown egg layers with 85-week-old. [1] Seedor index. [2] Bone mineral matter content. [3] Bone phosphorous content. [4] Bone calcium content. [5] Cortical diameter. [6] Medullar diameter. [7] Epiphysis diameter. R^2 = determination coefficient. r = Pearson's correlation coefficient. *Significant at 5% of error probability ($p \leq 0.05$).

The P content maintained its low correlation with the BS, under a strong negative influence of CorD and EpiD. Regarding the Ca content, an increase in the correlation coefficient occurred again, but this time, due to MM content (Table 4). Thereby, the methodology of Ca determination was the most positively influenced by the others, considering its total correlation with the BS (r = 0.78), and its low direct effect on this variable (r = 0.18).

It is worth pointing out that the retention efficiency of Ca absorbed by a hen at final of the production cycle is relatively low, only about 40%, compared to the 60% often observed in younger hens. Moreover, calcium homeostasis is one of the most relevant mechanisms for maintaining hens' bone integrity, because almost 99% of this mineral is found in the bone tissue. Calcium and phosphorous are linked in the bone tissue, and because of that, under a deficiency of some of them on a diet, the bone quality is worsened (Saldanha et al., 2009). Nonetheless, in the present study, both the Ca and P contents had their correlations, with the BS, affected by other variables (Tables 3 and 4). These results do not allow suggest nutritional discrepancy of Ca and P, low mineral contents on the bone, or else a low femur BS of the laying hens. It is important emphasizing again, the complexity of calcium mobilization and reabsorption on the bone tissues, mainly at final of the production cycle.

Table 4. Path analysis of bone quality correlations, divided into direct and indirect effects on the femur breaking strength (BS), via methodologies of bone quality assessments from brown egg layers with 85-week-old.

Methodology		r[a]	P-value
SI[1]			
	Indirect effect via MM	-0.1211	0.3131
	Indirect effect via P	-0.1378	0.3228
	Indirect effect via Ca	0.0759	0.1148
	Subtotal[a]	0.0645	0.2263
	Total	0.0645	0.2263
MM[2]			
	Indirect effect via P	-0.2602	0.1513
	Indirect effect via Ca	0.1393	0.0337*
	Subtotal	0.3906	<0.0001*
	Total	0.5300	<0.0001*
P[3]			
	Indirect effect via MM	0.2088	0.1683
	Indirect effect via Ca	-0.0685	0.2037
	Subtotal	-0.1192	0.0002*
	Total	-0.1192	0.0002*
Ca[4]			
	Indirect effect via MM	0.4420	0.0010*
	Indirect effect via P	0.2706	0.1358
	Subtotal	0.3337	0.0026*
	Total	0.7757	<0.0001*
CorD[5]			
	Indirect effect via MM	-0.1649	0.4252
	Indirect effect via P	0.2704	0.0357*
	Indirect effect via Ca	-0.0340	0.3661
	Subtotal	-0.2355	0.0044*
	Total	0.0349	0.0200*
MedD[6]			
	Indirect effect via MM	-0.1649	0.1054
	Indirect effect via P	-0.2008	0.0835
	Indirect effect via Ca	0.0263	0.4713
	Subtotal	-0.2816	0.0004*
	Total	-0.2816	0.0004*
EpiD[7]			
	Indirect effect via MM	0.4395	0.0273*
	Indirect effect via P	0.5910	0.0091*
	Indirect effect via Ca	-0.1489	0.0512*
	Subtotal	-0.2611	0.0195*
	Total	0.6205	0.0472*
R^2		0.9889	

[a] Correlation coefficient after the deployment of the indirect effects of quality methodologies of the femur from brown egg layers with 85-week-old (Table 3). [1] Seedor index. [2] Bone mineral matter content. [3] Bone phosphorous content. [4] Bone calcium content. [5] Cortical diameter. [6] Medullar diameter. [7] Epiphysis diameter. R^2 = determination coefficient. R^2 = determination coefficient. r = Pearson's correlation coefficient. *Significant at 5% of error probability ($p \leqslant 0.05$).

These results reinforce the importance of the association between MM content and BS, instead of the Ca and P contents under singular perspectives (Figure 1). Besides that, the Ca content displayed a high-positive linear correlation with MM content and BS (Table 2). Therefore, it can be affirmed that Ca content also strongly associates itself with the BS, directly or indirectly.

The total correlation between EpiD and BS was positive (Table 4), despite the direct effect on BS had been negative (Table 3). In this sense, the EpiD cannot be precisely correlated with the BS (Cruz et al., 2006). Finally, the total correlations of CorD and MedD with this variable were quite low. Some authors (Zhang & Coon, 1997; Hester et al., 2004) already reported low associations among these variables. However, Harash et al. (2020) found a positive correlation (r = 0.89) between CorD and BS, when they assessed double-purpose hens. The authors reported that these characteristics might vary according to the lineage and age of the hens.

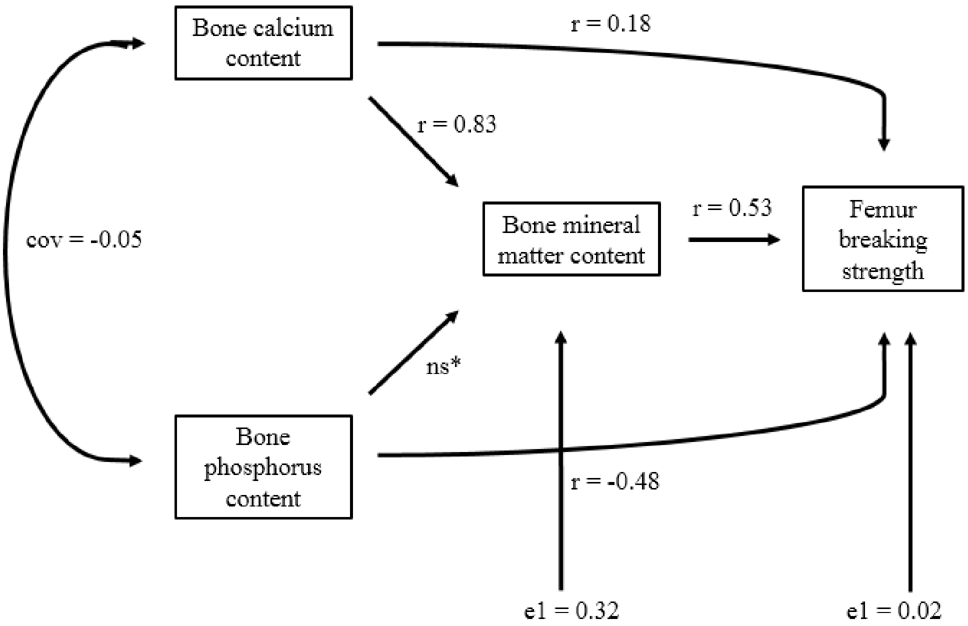

Figure 1. Path diagram of correlations among bone calcium, phosphorus, and mineral matter contents, besides the femur breaking strength, considering direct and indirect effects via mineral matter content. Cov = covariance between calcium and phosphorus contents; r = correlation coefficient; e1 = residual error associated with bone mineral matter content; e2 = residual error associated with the femur breaking strength; ns = non-significant correlation path at 5% of error probability.

Conclusion

Mineral matter content stood out as a feasible parameter to estimate indirectly the femur breaking strength. The calcium content, despite the high correlation with this breaking strength, was indirectly influenced by other parameters. Therefore, the determination of mineral matter content is the best methodology to characterize the bone quality of brown egg layers, at final of the production cycle, considering that the calcium determination requires more labor and presents a great cost.

Epiphysis diameter is not a precise parameter associated with the femur breaking strength from brown egg layers with 85-week-old.

References

Association of Official Analytical Chemists [AOAC]. (2005). *Official methods of analysis* (16th ed.). Washington, DC: AOAC.

Cheng, T. K., & Coon, C. N. (1990). Sensitivity of various bone parameters of laying hens to different daily calcium intakes. *Poultry Science, 69*(12), 2209–2213. DOI: http://doi.org/10.3382/ps.0692209

Conselho Nacional de Controle de Experimentação Animal [CONCEA]. (2013). *Diretrizes da Prática de Eutanásia do Concea*. Brasília, DF: Ministério da Ciência, Tecnologia e Inovações.

Cruz, C. D. (2013). GENES: A software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy, 35*(3), 271-276. DOI: http://doi.org/10.4025/actasciagron.v35i3.21251

Cruz, C. D., Regazzi, A. J., & Carneiro, P. C. S. (2006). *Modelos biométricos aplicados ao melhoramento genético*. Viçosa, MG: UFV.

Detmann, E., Souza, M. A. d., Valadares Filho, S. d. C., Queiroz, A. C. d., Berchielli, T. T., Saliba, E. d. O. S., ... Azevedo, J. A. G. (2012). *Métodos para análise de alimentos*. Visconde do Rio Branco, MG: Suprema.

Donkó, T., Tischler, A., Csóka, Á., Kovács, G., Emri, M., Petneházy, Ö., ... Bajzik, G. (2018). Estimation of bone mineral density and breaking strength of laying hens based on scans of computed tomography for body composition analysis. *British Poultry Science, 59*(4), 365–370. DOI: http://doi.org/10.1080/00071668.2018.1471662

Faitarone, A. B. G., Garcia, E. A., Artoni, S. M. B., Sgavioli, S., Silva, M. D. P., Gonçalves, H. C., & Pelícia, K. (2012). Qualidade óssea de poedeiras comerciais leves alimentadas com rações suplementadas com diferentes óleos vegetais. *Veterinária e Zootecnia, 19*(3), 356-365.

Gebhardt-Henrich, S. G., Pfulg, A., Fröhlich, E., Käppeli, S., Guggisberg, D., Liesegang, A., & Stoffel, M. H. (2017). Limited associations between keel bone damage and bone properties measured with computer tomography, three-point bending test, and analysis of minerals in swiss laying hens. *Frontiers in Veterinary Science, 4(*128), 1-9. DOI: http://doi.org/10.3389/fvets.2017.00128

Harash, G., Richardson, K. C., Alshamy, Z., Hünigen, H., Hafez, H. M., Plendl, J., & Al Masri, S. (2020). Basic morphometry, microcomputed tomography and mechanical evaluation of the tibiotarsal bone of a dual-purpose and a broiler chicken line. *PLoS ONE, 15*(3), e0230070. DOI: http://doi.org/10.1371/journal.pone.0230070

Hester, P. Y., Schreiweis, M. A., Orban, J. I., Mazzuco, H., Kopka, M. N., Ledur, M. C., & Moody, D. E. (2004). Assessing bone mineral density in vivo: dual energy X-ray absorptiometry. *Poultry Science, 83*(2), 215–221. DOI: http://doi.org/10.1093/ps/83.2.215

Kaps, M., & Lamberson, W. R. (2017). *Biostatistics for animal science* (3rd ed.). Oxford, UK: CABI. DOI: http://doi.org/10.1079/9781786390356.0000

Kim, W. K., Bloomfield, S. A., Sugiyama, T., & Ricke, S. C. (2012). Concepts and methods for understanding bone metabolism in laying hens. *World's Poultry Science Journal, 68*(1), 71-82. DOI: http://doi.org/10.1017/S0043933912000086

Kim, W. K., Donalson, L. M., Herrera, P., Woodward, C. L., Kubena, L. F., Nisbet, D. J., & Ricke, S. C. (2004). Research note: Effects of different bone preparation methods (fresh, dry, and fat-free dry) on bone parameters and the correlations between bone breaking strength and the other bone parameters. *Poultry Science, 83*(10), 1663–1666. DOI: http://doi.org/10.1093/ps/83.10.1663

Molino, A. d. B., Garcia, E. A., Gonçalves, H. C., Pelícia, K., Berto, D. A., & Silva, A. d. P. (2009). Avaliação de medidas de qualidade da casca dos ovos de poedeiras comerciais. São Pedro, SP: APA-Congresso de Produção, Comercialização e Consumo de Ovos.

Moraes, J. E. d., Borges, M. R., Amoroso, L., Reis, T. L., Calixto, L. F. L., Lagassi, T. C., ... Pizzolante, C. C. (2020). Bien estar de gallinas ponedoras y la osteoporosis. *Research, Society and Development, 9*(3), e150932588. DOI: http://doi.org/10.33448/rsd-v9i3.2588

Neijat, M., Casey-Trott, T. M., Robinson, S., Widowski, T. M., & Kiarie, E. (2019). Effects of rearing and adult laying housing systems on medullary, pneumatic and radius bone attributes in 73-wk old Lohmann LSL lite hens. *Poultry Science, 98*(7), 2840–2845. DOI: http://doi.org/10.3382/ps/pez086

Nie, W., Wang, B., Gao, J., Guo, Y., & Wang, Z. (2018). Effects of dietary phosphorous supplementation on laying performance, egg quality, bone health and immune responses of laying hens challenged with *Escherichia coli* lipopolysaccharide. *Journal of Animal Science and Biotechnology, 9*, 53. DOI: http://doi.org/10.1186/s40104-018-0271-z

Nunes, J. K., Santos, V. L., Rossi, P., Anciuti, M. A., Rutz, F., Maier, J. C., & Silva, J. G. C. (2013). Qualidade de ovos e resistência óssea de poedeiras alimentadas com minerais orgânicos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 65*(2), 610-618. DOI: http://doi.org/10.1590/S0102-09352013000200043

Nys, Y., & Le Roy, N. (2018). Calcium homeostasis and eggshell biomineralization in female chicken. In D. Feldman (Ed.), *Vitamin D* (4th ed., p. 361-382). Stanford, CA: Elsevier.

Olgun, O., & Aygun, A. (2016). Nutritional factors affecting the breaking strength of bone in laying hens. *World's Poultry Science Journal, 72*(4), 821-832. DOI: http://doi.org/10.1017/S0043933916000696

Oliveira, A. N. d., Freitas, E. R. d., Filgueira, T. M. B., Cruz, C. E. B., & Nascimento, G. A. J. (2013). Limestone particle size and artificial light for laying hens in the second laying cycle. *Revista Brasileira de Zootecnia, 42*(7), 481-488. DOI: http://doi.org/10.1590/S1516-35982013000700004

Orban, J. I., Roland, D. A. SR., Bryant, M. M., & Williams, J. C. (1993). Factors influencing bone mineral content, density, breaking strength, and ash as response criteria for assessing bone quality in chickens. *Poultry Science, 72*(3), 437-446. DOI: http://doi.org/10.3382/ps.0720437

Paz, I. C. L. A., Mendes, A. A., Balog, A., Komiyama, C. M., Takahashi, S. E., Almeida, I. C. L., ... Cardoso, K. F. G. (2009). Efeito do cálcio na qualidade óssea e de ovos de poedeiras. *Archivos de Zootecnia, 58*(222), 173-183.

Paz, I. C. L. A., Mendes, A. A., Balog, A., Komiyama, C. M., Takahashi, S. E., Almeida I. C. L., ... Bueno, L. G. F. (2008). Qualidade óssea, produção e qualidade de ovos de poedeiras semi-pesadas em primeiro ciclo de produção. *Pubvet, 2*(23), 1-14.

Rath, N. C., Huff, G. R., Huff, W. E., & Balog, J. M. (2000). Factors regulating bone maturity and strength in poultry. *Poultry Science, 79*(7), 1024-1032. DOI: http://doi.org/10.1093/ps/79.7.1024

Reis, T. L, Palomino, J. C., Dielis, F., Moraes, J. E. d., Pizzolante, C. C., & Calixto, L. F. L. (2020). Qualidade óssea, excreção mineral e digestibilidade da matéria seca de poedeiras semipesadas alimentadas com caulim no final do ciclo produtivo. *Archives of Veterinary Science, 25*(2), 56-70. DOI: http://doi.org/10.5380/avs.v25i2.72441

Robison, C. I., & Karcher, D. M. (2019). Analytical bone calcium and bone ash from mature laying hens correlates to bone mineral content calculated from quantitative computed tomography scans. *Poultry Science, 98*(9), 3611–3616. DOI: http://doi.org/10.3382/ps/pez165

Rodriguez-Navarro, A. B., McCormack, H. M., Fleming, R. H., Alvarez-Lloret, P., Romero-Pastor, J., Dominguez-Gasca, N., ... Dunn, I. C. (2018). Influence of physical activity on tibial bone material properties in laying hens. *Journal of Structural Biology, 201*(1), 36–45. DOI: http://doi.org/10.1016/j.jsb.2017.10.011

Rostagno, H. S., Albino, L. F. T., Donzele, J. L., Gomes, P. C., Oliveira, R. F. d., Lopes, D. C., ... Euclides, R. F. (2011). *Tabelas brasileiras para aves e suínos: composição dos alimentos e exigências nutricionais* (3a ed.). Viçosa, MG: UFV.

Saldanha, E. S. P. B., Garcia, E. A., Pizzolante, C. C., Faittarone, A. B. G., Sechinato, A. d., Molino, A. B., & Laganá, C. (2009). Effect of organic mineral supplementation on the egg quality of semi-heavy layers in their second cycle of lay. *Brazilian Journal of Poultry Science, 11*(4), 241-247. DOI: http://doi.org/10.1590/S1516-635X2009000400005

Statistical Analysis System Institute [SAS Institute]. (2015). *Base SAS 9.4 procedures guide*. SAS Institute.

Schreiweis, M. A., Orban, J. I., Ledur, M. C., Moody, D. E., & Hester, P. Y. (2004). Effects of ovulatory and egg laying cycle on bone mineral density and content of live White Leghorns as assessed by dual-energy X-ray absorptiometry. *Poultry Science, 83*(6), 1011–1019. DOI: http://doi.org/10.1093/ps/83.6.1011

Seedor, J. G., Quartuccio, H. A., & Thompson, D. D. (1991). The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. *Journal of Bone and Mineral Research, 6*(4), 339–346. DOI: http://doi.org/10.1002/jbmr.5650060405

Sorza, J. D. (2019, 12 de julho). A importância dos ossos da galinha nos ciclos produtivos longos. *AviNews Brasil*, 1-9. Recovered on June 19,2020 from https://avicultura.info/pt-br/a-importancia-dos-ossos-da-galinha-nos-ciclos-produtivos-longos

Wang, J., Qiu, L., Gong, H., Celi, P., Yan, L., Ding, X., ... Zhang, K. (2020). Effect of dietary 25-hydroxycholecalciferol supplementation and high stocking density on performance, egg quality, and tibia quality in laying hens. *Poultry Science, 99*(5), 2608–2615. DOI: http://doi.org/10.1016/j.psj.2019.12.054

Wistedt, A., Ridderstråle, Y., Wall, H., & Holm, L. (2019). Age-related changes in the shell gland and duodenum in relation to shell quality and bone strength in commercial laying hen hybrids. *Acta Veterinaria Scandinavica, 61*(14). DOI: http://doi.org/10.1186/s13028-019-0449-1

Zenebon, O., Pascuet, N. S., & Tiglea, P. (2008). *Métodos físico-químicos para análise de alimentos*. Núcleo de Informação e Tecnologia. São Paulo, SP: Instituto Adolfo Lutz.

Zhang, B., & Coon, C. N. (1997). The relationship of various tibia bone measurements in hens. *Poultry Science, 76*(12), 1698-1701. DOI: http://doi.org/10.1093/ps/76.12.1698