Effect of limestone particle size on performance, eggshell quality, bone strength, and in vitro/in vivo solubility in laying hens: a meta-analysis approach

F. Hervo,[*,†,1] A. Narcy,[†] Y. Nys,[†] and M.-P. Létourneau-Montminy[*]

[*]Département des sciences animales, Université Laval, Québec city, Québec, G1V 0A6, Canada; and [†]INRAE, Université de Tours, BOA, 37380, Nouzilly, France

ABSTRACT Numerous publications over the past 5 decades have investigated the effect of limestone particle size (**LmPS**) on production performance, bone mineralization, and limestone solubilization in laying hens. Coarse limestone particles have been shown to improve eggshell quality and bone mineralization. However, there is a large variability of responses in birds to this factor, indicating the need to better quantify the effect of modulating factors related to coarse particles that could explain this variability. The objective of this meta-analysis was to study the impact of LmPS on the digestive and metabolic fate of Ca to optimize its utilization by laying hens. Fifty-eight papers published between 1971 and 2019, including 71 experiments were included in this study. Four categories of dependent variables were identified: Ca solubility, production performance, eggshell quality, and bone strength. Independent variables tested were LmPS and age. Results showed that the in vitro solubilization of limestone linearly decreased ($P < 0.001$; $R^2 = 0.91$) while in vivo solubilization linearly increased with LmPS ($P < 0.001$; $R^2 = 0.91$). Coarse limestone particles were retained longer in the gizzard ($P < 0.001$; $R^2 = 0.60$), inducing higher solubilization by gastric juices than fine limestone. LmPS showed no effect on production performance while all eggshell quality parameters increased with LmPS ($P < 0.001$; $R^2 > 0.91$): increasing specific gravity by 0.8%, eggshell thickness by 1.1%, and eggshell breaking strength by 3% when increasing from 0.15 mm to 1.5 mm. LmPS had an effect on tibia breaking strength dependently of age (Age × LmPS, $P < 0.001$; $R^2 = 0.89$): coarse limestone particles increased tibia breaking strength with aging compared to fine limestone particles. The current study renders it possible to quantify the effects of age and LmPS on eggshell quality and tibia breaking strength. This work showed an interaction between eggshell quality and bone strength and showed that LmPS increases bone strength in older laying hens.

Key words: laying hen, limestone particle size, eggshell quality, bone strength, limestone solubilization

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INTRODUCTION

Eggshell formation takes place in the shell gland during a daily cycle of mineralization (Nys et al., 2010). The hen has to export two grams of calcium (**Ca**) daily into the eggshell that is mainly composed of calcium carbonate (95%). Part of the Ca is provided by the diet (60−70%), but due to an offset of Ca intake during the light period and the Ca requirement to produce the eggshell during the dark period, another part of Ca is mobilized from bone, especially from a specific tissue: medullary bone (Nys, 2017). This type of bone, produced at the onset of sexual maturity (Hadley et al., 2016), represents a transitory and labile Ca reserve (Rodriguez-Navarro et al., 2018). As osteoclasts resorb medullary bone, cortical bone is also degraded over time, leading to bone weakness with aging (Kim et al., 2005). This weakness increases the incidence of osteoporosis in layer flocks and causes economic losses for producers and welfare issues (Whitehead and Fleming, 2000). Moreover, with aging, laying rate decreases by 13.5% during the production period (Herrera et al., 2018), and eggshell quality is reduced from 49 wk onward (Wistedt et al., 2019). Thus, the rate of cracked eggs increases, causing economic losses for producers. With aging, the capacity of intestinal absorption of Ca decreases (Beck and Hansen, 2004), associated with a decrease in plasma 1,25-OH_2-D_3 (Abe et al., 1982). Indeed, 1,25-OH_2-D_3 has been clearly shown to stimulate Ca absorption by stimulating calbindin expression in the intestine (Bar, 2008).

One of the solutions to reduce the decrease in eggshell quality and bone strength with aging is to increase the use of coarse limestone particles (i.e., >0.8 mm). There is no consensus according the optimal ratio of coarse to

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[1]Corresponding author: fabien.hervo.1@ulaval.ca

fine limestone with age. However, a ratio of 75% coarse: 25% fine is often used in old hens. Several authors have shown a positive effect of coarse limestone on eggshell quality (Jardim-Filho et al., 2005a; Guo and Kim, 2012) and bone health in laying hens (Jardim-Filho et al., 2005b; de Araujo et al., 2011). According to Rao and Roland (1990), coarse limestone particles are retained longer in the gizzard and provide a greater amount of soluble Ca and for an extended time during the dark period compared to fine limestone particles. Additionally, hens fed with coarse limestone particles showed a continuous soluble Ca diffusion during the night and reduced their bone Ca mobilization (Fleming et al., 2006). Thus, coarse limestone particles improve the synchronization between the high Ca requirements to produce the eggshell and the dietary Ca supply.

The beneficial effects of coarse limestone particles on egg production, eggshell quality, and bone strength have been demonstrated in numerous publications, but results remain variable. In order to better quantify these effects and highlight the modulating factors, a meta-analysis tool has been used to describe to what extent coarse limestone particles can modulate eggshell quality and bone strength.

MATERIALS AND METHODS

Data Sourcing

Studies used in this meta-analysis were found on several databases: CAB Abstracts, Google Scholar, MEDLINE, and Food Science and Technology Abstracts by using the following keywords: *calcium particle size, ground limestone, laying hen, hen, poultry, production performance, egg quality, eggshell quality, bone quality, bone strength, digestibility*, and *solubility*. Further publications were identified in the references of previous articles. This resulted in 82 publications which described the effect of calcium particle size on distinct parameters in poultry.

Inclusion Criteria

Only publications on laying hens and with limestone as a Ca source were retained (Figure 1). As there is no consensus, fine limestone particles (**FP**) were defined lower than 0.8 mm, and large particles (**LP**) were greater or equal to 0.8 mm, based on Rao and Roland (1990). Variations in dietary levels of Ca and phosphorus (**P**) within and between publications were low and were mostly due to the variation of age of animals between publications. Therefore, based on the NRC (1994), only studies in which animals received enough dietary Ca and P to cover their requirements, according to their age, were kept (i.e., Ca between 3.2 and 4.5% and total P between 0.36 and 0.72%). All publications on the effect of Ca particle size in hens, from 18 wk to 90 wk were retained. The final dataset included 58 publications published between 1971 and 2019.

Three different databases were built. The first included data on in vitro/in vivo solubilization and

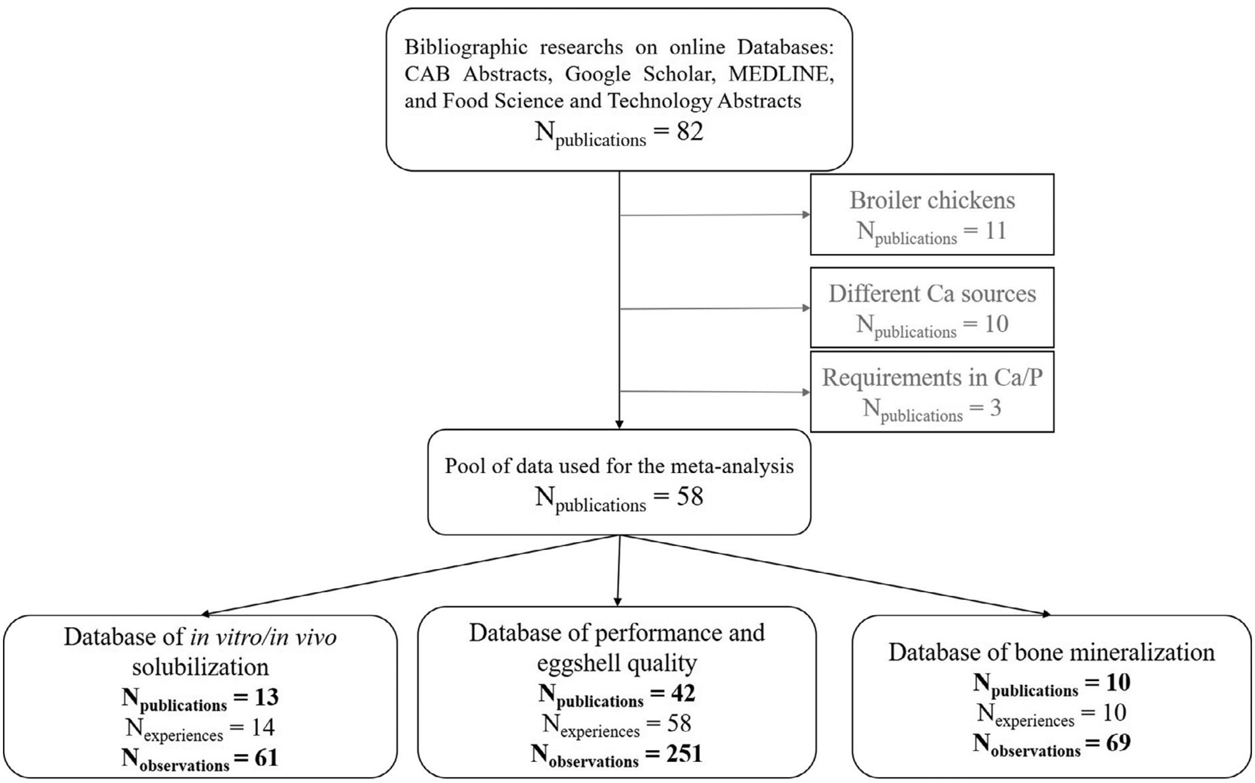

Figure 1. Prisma diagram describing the data sourcing and the main inclusion criteria used to build the different databases used in the meta-analysis.

gizzard retention. This database had 13 publications published between 1971 and 2011; 8 publications dealt with in vitro solubilization, 5 with in vivo solubilization, and only 2 with both. In all publications, the in vitro solubilization was determined using the method described by Zhang and Coon (1997a). The in vivo solubilization was calculated according to the method of Rao and Roland (1989). The second database included data on performance and eggshell quality and had 42 publications published between 1971 and 2019. The third database included data on tibia breaking strength, comprising ten publications published between 1990 and 2015.

Calculations

A few studies (about 24%) tested the effect of limestone particle size crossed with different levels of inclusion of large particles in dietary treatments. Additionally, several authors gave just a range of particle sizes. In order to standardize the limestone particle size within and between publications, the mean calcium particle size and the proportion of fine and coarse particles in the limestone supply were considered. Limestone particle size (**LmPS**) was calculated as follow:

$$\begin{aligned}\text{LmPS} = &(\text{mean coarse limestone particle size (mm)}\\ &\times \text{proportion of coarse limestone (\%)})\\ &+(\text{mean fine limestone particle size (mm)}\\ &\times \text{proportion of fine limestone (\%)})\end{aligned}$$

Tibia breaking strength is an indicator of bone mineralization frequently measured due to its link with bone fracture risk (Kim et al., 2012). However, several studies measured the force at yield stress (kg), while others measured the stress (kg/cm^2). To standardize these variables, all breaking strength data were converted into force at yield stress (kg) by using the method of Crenshaw et al. (1981):

$$\text{stress}\ (\text{kg/cm}^2) = \frac{(\text{F} \times \text{L} \times \text{C})}{(4 \times \text{MI})}$$

with F the force at yield stress (kg), L the length between supporting fulcra points (cm), C the $^1/_2$ diameter of bone parallel to force applied (cm), and MI the area moment of inertia (cm^4).

Statistical Analysis

Dependent variables measured in publications were classed into 4 categories (Table 1): 1) Fate of coarse limestone in the digestive tract: in vitro and in vivo solubility (%) and quantity of Ca recovered after 24 h in the gizzard (g); 2) Production performance: laying rate (%) and egg weight (g); 3) Eggshell quality: specific gravity, eggshell thickness (mm), and eggshell breaking strength (kg); 4) Bone strength: tibia breaking strength (kg).

Each dependent variable was analyzed with a linear mixed model as follows:

$$Y_{ij} = B_0 + S_i + B_i\, X_{ij} + b_i X_{ij} + e_{ij}$$

with Y_{ij} the dependent variable of the j^{th} treatment of the i^{th} study, B_0 the intercept of the study, X_{ij} the independent variable of the j^{th} treatment of the i^{th} study, S_i the random effect of the study on the intercept, b_i the random effect of the study on the regression coefficient, and e_{ij} the residual error unexplained by the model. For each of the dependent variables, linear and quadratic effects of particle size and age were tested in order to evaluate the behavior of the model and its tendency to plateau ($LmPS^2$, Age^2). The interaction LmPs × Age was also tested. Statistical analysis was conducted with R open-source software, version 3.02 (R core team, 2013). Linear mixed model studies were performed with the *lme* function of the package *nlme*, version 3.1 − 117 (Pinheiro et al., 2015).

RESULTS

Fate of Coarse Limestone in the Digestive Tract

In vitro solubility significantly decreased with LmPS ($P < 0.001$; Table 2). As an example, in vitro solubilization of a limestone particle considered coarse (1.5 mm) was 17% less than with a finer limestone particle (0.15 mm). In vivo solubility significantly increased ($P < 0.01$; Table 2) with increasing LmPS; in vivo solubilization of coarse limestone (1.5 mm) was 8% greater than fine limestone (0.15 mm). Statistical analysis of the database showed that, after 24 h, gizzard Ca retention was increased with LmPS ($P < 0.001$; data not shown). The quantity of Ca recovered in the gizzard after 24 h

Table 1. Descriptive statistics.

	Number of observations	Number of publications	Mean	SD	Min.	Max.
Limestone particle size (mm)	309	58	1.251	0.824	0.039	6.500
In vitro solubility of calcium (%)[a]	61	8	50.76	15.66	24.20	100.00
In vivo solubility of calcium (%)[b]	43	5	79.23	9.47	54.00	96.00
Gizzard retention (g)	32	2	4.50	3.48	0.10	15.40
Egg production (%)	251	42	81.7	10.4	52.0	98.5
Egg weight (g)	251	37	62.44	3.56	54.70	70.20
Specific gravity	85	15	1.081	0.005	1.070	1.091
Eggshell thickness (mm)	137	19	0.370	0.035	0.260	0.446
Eggshell breaking strength (kg)	127	16	3.71	0.42	2.58	5.13
Tibia breaking strength (kg)	69	10	20.80	3.29	15.40	27.30

[a]In vitro solubility of calcium was determined according to the method of Zhang and Coon (1997a).
[b]In vivo solubility of calcium was determined according to the method of Rao and Roland (1989).

Table 2. Response of in vitro and in vivo solubility (%) of particulate limestone to limestone particle size (mm).

	In vitro solubility[a]			In vivo solubility[b]		
	Slope	SD	*P*-value	Slope	SD	*P*-value[d]
Intercept	73.454	2.916	**<0.001**	70.167	3.671	**<0.001**
LmPS[c]	−9.168	0.713	**<0.001**	4.166	0.901	**<0.001**
RMSE			5.534			2.389
R^2			0.843			0.905

[a]In vitro solubility of calcium was determined according to the method of Zhang and Coon (1997a).
[b]In vivo solubility of calcium was determined according to the method of Rao and Roland (1989).
[c]Limestone particle size.
[d]Boldface values respresent significant effects.

linearly increased between 0.10 g for fine particles (0.15 mm) and 5.2 g for coarse particles (1.5 mm), and reached a plateau at around 2 mm.

Production Performance

The interaction between LmPS and age was tested and showed no significant effect on egg production or on egg weight. LmPS showed no significant effect on egg production or egg weight (Table 3). As expected, age showed a significant effect on egg production (Age and Age^2; $P < 0.001$) with maximum laying at around 30 to 35 wk and a decrease of 10% of laying rate from 35 to 80 wk, while before the 25 wk the laying rate increased linearly until the laying peak (supplementary data). Age also influenced egg weight ($P < 0.001$). As an example, egg weight increased by 4.77 g from 35 to 80 wk and reached a plateau at around 80 wk (supplementary data).

Table 5. Response of tibia breaking strength (kg) to calcium particle size (mm) and age (weeks) in laying hens.

	Tibia breaking strength (kg)		
	Slope	SD	*P*-value[b]
Intercept	16.921	3.229	**< 0.001**
LmPS[a]	−0.045	0.376	0.907
Age	0.291	0.174	0.111
Age^2	−0.0038	0.018	**< 0.05**
LmPS × Age	0.0164	0.0074	**< 0.05**
RMSE			1.306
R^2			0.894

[a]Limestone particle size.
[b]Boldface values respresent significant effects.

Eggshell Quality

The interaction between LmPS and age showed no significant effect on specific gravity, eggshell breaking strength, or eggshell thickness (Table 4). Current models showed that specific gravity, eggshell thickness, and eggshell breaking strength linearly increased with LmPS ($P < 0.001$). For example, increasing LmPS from 0.15 to 1.5 mm resulted in an increase of 0.08, 1.1, and 3.0% of specific gravity, eggshell thickness, and breaking strength, respectively. Age showed a significant linear effect on specific gravity ($P < 0.001$; Table 4); it decreased by 0.6% from 35 to 70 wk. Age showed no significant effect on eggshell thickness or eggshell breaking strength.

Tibia Breaking Strength

The interaction LmPS × Age showed a significant linear effect on tibia breaking strength ($P < 0.05$; Table 5).

Table 3. Response of egg production (%) and egg weight (g) to limestone particle size (mm) and age (week) in laying hens.

	Egg production (%)			Egg weight (g)		
	Slope	SD	*P*-value[a]	Slope	SD	*P*-value[a]
Intercept	75.072	2.904	**<0.001**	52.419	0.902	**<0.001**
LmPS[b]	0.958	0.635	0.135	354×10^{-5}	0.191	0.985
Age	0.495	0.104	**<0.001**	0.270	0.031	**<0.001**
Age^2	-605×10^{-5}	931×10^{-6}	**<0.001**	-142×10^{-5}	251×10^{-6}	**<0.001**
LmPS × Age	-480×10^{-5}	102×10^{-4}	0.638	-198×10^{-5}	269×10^{-5}	0.463
RMSE			2.183			0.878
R^2			0.905			0.882

[a]Boldface values respresent significant effects.
[b]Limestone particle size.

Table 4. Response of specific gravity, eggshell thickness (mm), and eggshell breaking strength (kg) to calcium particle size (mm) and age (weeks) in laying hens.

	Specific gravity[a]			Eggshell thickness (mm)			Eggshell breaking strength (kg)		
	Slope	SD	*P*-value[c]	Slope	SD	*P*-value[c]	Slope	SD	*P*-value[c]
Intercept	1,088	2.271	**<0.001**	0.367	0.018	**<0.001**	3.359	0.276	**<0.001**
LmPS[b]	1.493	0.512	**<0.001**	0.005	0.001	**<0.001**	0.121	0.045	**<0.001**
Age	−0.127	0.038	**<0.001**	720×10^{-4}	323×10^{-3}	0.825	0.003	0.005	0.468
LmPS × Age	-150×10^{-6}	900×10^{-8}	0.089	-430×10^{-5}	270×10^{-5}	0.114	-762×10^{-5}	835×10^{-5}	0.364
RMSE			704×10^{-6}			546×10^{-5}			0.168
R^2			0.989			0.983			0.912

[a]Value of parameters for specific gravity has been multiplied by 1,000.
[b]Limestone particle size.
[c]Boldface values respresent significant effects.

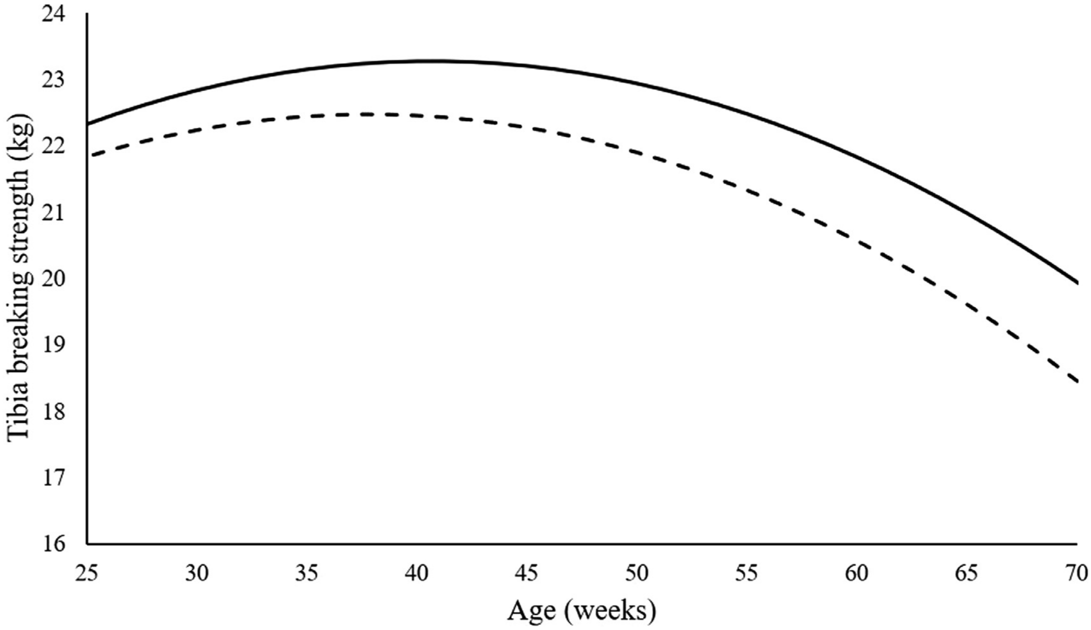

Figure 2. Impact of age on tibia breaking strength (kg) in laying hens (Y= 16.921+0.291 × Age −0.044 × LmPS −0.004 × Age^2 + 0.016 × LmPS × Age; R^2 = 0.894; RMSE = 1.306; $P < 0.001$); Full line represents hens fed with coarse limestone particles (1.5 mm) and dotted line represents hens fed with fine limestone particles (0.15 mm).

Tibia breaking strength significantly decreased with age ($P < 0.05$; Figure 2), but this decrease was more pronounced with a smaller particle size. For example, tibia breaking strength was decreased by 30% and 24% from 35 to 80 wk of age when hens received 0.15 mm and 1.5 mm limestone, respectively.

DISCUSSION

From in Vitro to in Vivo Solubilization of Limestone

The present results show that in vivo solubility increased by 8% when LmPS increased from 0.15 mm to 1.5 mm while the opposite was observed with in vitro solubility, with a decrease of 17% from 0.15 mm to 1.5 mm. These results are in accordance with Zhang and Coon (1997b) who compared the in vivo and in vitro solubilization of limestone particles. They showed that 4 mm limestone particles increased in vivo solubilization by 10% and decreased in vitro solubilization by 50% compared to 0.65 mm limestone particles. Note that the term "in vivo solubilization" is commonly used in publications to describe the chemical reaction of solubilization of limestone in the gizzard. However, this term confounded the chemical reaction of solubilization and the physical disappearance of limestone from the gizzard by passing the pylorus. Here, we distinguish in vivo solubilization which describes the chemical process, and mean retention time which describes the physical one. The current observations can be explained by 2 main effects. First, coarse limestone particles are specifically retained in the gizzard (Scott et al., 1971) and also show a slower disappearance compared to fine limestone. Rao et al. (1992) distributed 10 g of limestone with 25% of fine particles (≤0.84 mm) and 75% of coarse particles (comprised between 0.84 mm and 4 mm, with an average of 1.56 mm) and showed that 4 h after ingestion, 43% of coarse limestone particles had passed through the gizzard vs. 68% of fine limestone particles. Based on these data, we calculated the mean retention time of coarse and fine limestone particles in the gizzard, according to the calculation method of van der Klis et al. (1990) in broilers. Coarse limestone particles showed a slower disappearance in the gizzard, compared to fine limestone particles; the mean retention time was 345 min and 64 min for coarse and fine limestone particles, respectively. Secondly, coarse particles stimulate acidic secretion by the proventriculus. Jimenez-Moreno et al. (2009) showed that the gizzard pH decreases with the size of cereals. Svihus (2011) noted that whole or coarsely ground cereals added to the diet decreased the gizzard pH by 0.2 to 1.2 units. The decrease in gizzard pH is due to a stimulation of the H^+/K^+-ATPase in the proventriculus (Guinotte et al., 1995), which secretes hydrochloric acid. Guinotte et al. (1993) showed that coarse limestone inclusion (>1.2 mm) in the diet enhanced the H^+/K^+-ATPase activity by 11%. The stimulation of the pump is mediated by mechanoreceptors (Duke, 1986) located in the crop (Ruoff and Sewing, 1971). Thus, stimulation of acidic secretion by the coarse particles creates a favorable environment for limestone solubilization. Due to a transitory accumulation of coarse particles in the gizzard, coarse limestone particles stay longer in an acidic environment, and therefore, show a more complete solubilization compared to fine limestone particles which pass rapidly through the gizzard (Bo-Linn et al., 1984). This effect is not described in the crop where limestone particles pass through independently of their size (Rao et al., 1992).

Performance and Eggshell Quality

As expected, LmPS did not affect production performance. In all experiments of this meta-analysis, laying hens received enough energy and nutrients to cover their requirements. The current study showed that egg production decreased with age while egg weight increased. These results agree with the observations of Tumova and Gous (2012) that showed a 13% decrease of laying rate from the onset of laying to the end of the laying cycle, associated with an increase in egg weight of 9.9 g during the same period. After the peak production period, the sequence length (i.e., the period during which the hen lays 1 egg a day between 2 rest days) decreases and time between 2 sequences increases (Johnston and Gous, 2003). These subsequent effects partly explain the decrease of laying rate with age (Sauveur, 1988). The increase in egg weight is due to the increase of the relative proportion of the yolk during the laying cycle (Travel et al., 2010).

The present study showed that LmPS linearly improved eggshell quality. Lukic et al. (2009) showed that the response of the eggshell to coarse limestone particles is greater if the hen is fed a suboptimal Ca level. The enhancement of eggshell quality described in this meta-analysis could be due to the longer retention of coarse particles in the gizzard inducing an extended diffusion of soluble Ca during the night. As eggshell formation lasts approximatively 20 h and mainly takes place during the night, coarse limestone particles reduce desynchronization between availability and requirement of Ca for the eggshell (Saki et al., 2019). Additionally, laying hens receiving coarse limestone mobilize less bone Ca, and also P, than hens receiving fine limestone, in accordance with Gloux et al. (2020).

The present study did not show a decrease in eggshell quality with aging. It is noteworthy that the genetic selection has contributed to a relative flattening of the egg weight curve as the hen ages (Bain et al., 2016). Egg weight being correlated negatively with the eggshell strength (Johnston and Gous, 2007), thus the genetic selection may have an impact on the eggshell quality. As such, it cannot be excluded that the genetic selection has masked the age effect. However, the decrease in eggshell quality with aging is a well-known phenomenon. These results are explained by the meta-design in which the variability within-experimentation is based on LmPS. Zita et al. (2012) observed a decrease of 7% of eggshell thickness and 8% of eggshell breaking strength from 30 to 60 weeks. The rate of cracked eggs increases throughout the laying period by up to 16% (Travel et al., 2010). The decrease in eggshell quality with age seems to be partly due to a decrease in the calcium absorption capacity of the intestine (Al-Batshan et al., 1994; Franco-Jimenez and Beck, 2005) and to a decrease in the Ca transfer capacity of the shell gland to eggshell (Navikis et al., 1979). These decreases could be attributed to a decrease in plasma 1,25-OH_2-D3 and kidney 1-α-hydroxylase with age (Abe et al., 1982; Bar and Hurwitz, 1987).

Tibia Breaking Strength

This meta-analysis showed that bone breaking strength quadratically decreased with age, and that the incorporation of coarse limestone particles lessened this decrease. The impact of age on bone strength is well known (Whitehead and Fleming, 2000; Yamada et al., 2021). Rath et al. (2000) showed a decrease of tibia breaking strength by 11% from 35 to 55 wk. High laying rate associated with an offset of calcium intakes and needs during the dark period induce heavy bone calcium mobilization (Kim et al., 2012). This bone mobilization also affects cortical bones and thus, increases the incidence of osteoporosis with age (Whitehead, 2004). Additionally, with aging, the Ca absorption capacity of the intestine decreases (Franco-Jimenez and Beck, 2005), contributing to the deterioration of bone integrity. Coarse limestone particles provide soluble calcium later during the dark period to supply the eggshell formation (Rao et al., 1992) and reduce the desynchronization between the Ca supply and demand to produce the eggshell. Finally, coarse limestone particles reduce bone mobilization (Fleming, 2008). Thereby, osteoclast bone resorption is reduced when the hen is fed with coarse limestone particles (Guinotte and Nys, 1991) and inversely, fine limestone particles stimulate bone resorption (Gloux et al., 2020). This leads to increased bone breaking strength with coarse limestone particles in accordance with our results (Figure 2). De Witt et al. (2009) did not observe a coarse limestone effect on tibia breaking strength before 66 wk. Xavier et al. (2015a) indicated a positive effect of coarse limestone on tibia breaking strength from 36 wk to 90 wk. Although authors do not agree on the pivotal age after which coarse limestone had a significant positive effect, they all reported a significant positive effect at the end of the laying period.

At 70 wk of age, coarse limestone particles (1.5 mm) increased tibia breaking strength by 7.6% compared to fine limestone particles. With aging, daily bone resorption to produce the eggshell induces bone weakness, because of a gradual loss of cortical bone without replacement (Whitehead, 2004). This could suggest a prioritization for eggshell production at the expense of bone integrity. However, the relationship between bone integrity and eggshell quality remains unclear. Sauveur et al. (1983) showed that eggshell weight decreased with bone Pi mobilization ($Y = 5.74 - 0.0066 \times$ bone Pi mobilization). Based on works of Miles and Harms (1982), the increase in plasma Pi following bone mobilization could disturb the Ca deposition on the eggshell *in utero*. Furthermore, increased plasma Pi due to bone resorption induces the secretion of FGF23, a phosphatemia regulator, by osteocytes and osteoblasts (Ren et al., 2017). FGF23 achieves negative feedback on bone resorption (Erben and Andrukhova, 2017) and reduces Ca absorption in the intestine by inhibiting 1,25-OH_2-D3 production in the kidney (Ren et al., 2017). Therefore, FGF23 limits the Ca uptake from intestine and bone to the eggshell. To support this hypothesis, Keshavarz and Austic (1990) showed that high dietary phosphorus levels (i.e., 1%) led to decreased eggshell quality. According to Kim et al. (2005), high eggshell quality is associated with poorer bone quality, because hens that show high eggshell Ca deposition also show high bone Ca mobilization, increasing the incidence of fractures. Moreover, Eusemann et al. (2018) showed that the bone fracture incidence was reduced in a low-producing line compared to a high-producing line Conversely, Alfonso-Carrillo et al. (2021) did not show any correlation between eggshell quality (eggshell percentage, eggshell thickness, and eggshell breaking strength) and bone quality during an extended laying cycle of 100 wk. According to these authors, bone characteristics and eggshell characteristics are independent and could be improved separately. These results are in agreement with Jansen et al. (2020) who showed no correlation between bone breaking strength and total eggshell production in genetically divergent layer lines based on performance.

Limits of the Meta-analysis Approach

It is clear from the literature that in vivo solubility of calcium particles depends on their size. Additionally, their geological origin, their composition in other minerals, and the internal capacity of the hen to dissolve the calcium has been shown to have an effect in a few studies (Guinotte et al., 1991; Saunders-Blades et al., 2009). The multifactorial dependence of limestone solubility limits the quantification of LmPS' effects in laying hens. Other factors could modulate LmPS' effects in laying hens, but the lack of data prevents such analysis. First, the incorporation level of coarse limestone particles. According to Xavier et al. (2015a), tibia breaking strength linearly increases with the proportion of coarse limestone in the diet. However, de Oliveira et al. (2013) showed a quadratic effect of incorporation level of coarse limestone with an optimal level at 60%. Molnar et al. (2018) also described a quadratic effect with an optimal level of 70% of coarse limestone. Second, the timing of coarse limestone distribution could also modulate its effects. During the early light period, the hen deposits calcium and phosphorus into medullary bone (Kebreab et al., 2009). The hen also needs a rapidly available calcium source such as fine limestone. During the dark period, the hen produces its eggshell (Nys et al., 2010). The hen needs a long-term calcium source, like coarse limestone, to reduce the desynchronization between the availability and the requirement of soluble Ca, and therefore, reduce bone mobilization. Split feeding could be an interesting way to ensure calcium requirements. To support this hypothesis, Molnar et al. (2018) showed a positive effect of an evening distribution of coarse limestone on eggshell quality and a negative effect of a morning distribution of coarse limestone.

The aim of this meta-analysis was to quantify the effect of LmPS on the digestive availability and metabolic utilization of Ca by the laying hen. Current results show that coarse limestone particles improve eggshell quality, most likely by increasing mean retention time of Ca in the gizzard and increasing Ca absorption during the night. It also showed that coarse limestone particles improve eggshell quality regardless of age while its effect on bone strength is age dependent. Thus, coarse limestone particles help prevent bone weakness in layer flocks.

DISCLOSURES

The authors (Hervo et al.) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101686.

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