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Título:

Effects of the source and level of digestible phosphorus in the diet on performance and egg quality of brown laying hens from 64 to 76 weeks of age

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ABSTRACT

We studied the influence of the source of phosphorus (P) and level of digestible P (dP) in the diet on performance and egg quality traits of brown hens from 64 to 76 wk of age. The diets were based on corn and soybean meal and all contained 4.1% Ca. The design was completely randomized with 8 treatments arranged as a 2x4 factorial with 2 sources of P [monocalcium phosphate (MCP) and calcined bone phosphate (CBP)] and 4 levels of dP (0.27, 0.31, 0.35, and 0.39%) as main effects. Each treatment was replicated 14 times and the experimental unit was an enriched cage with 6 hens. The experiment lasted for 12 wk (3 periods of 4 wk each). Egg production and hen mortality were recorded daily. Feed intake and body weight (BW) of the hens were determined by period and cumulatively. Egg weight was estimated by period by weighing all the eggs produced the last day of each week on trial. From these data, average daily feed intake, egg mass, feed conversion ratio, and BW gain (BGW) were calculated by period and cumulatively. Egg quality traits, including Haugh Units and shell resistance to breakage, were determined per cage in 8 eggs collected at random the last two days of each experimental period. In addition, the percentage of dirty, broken, and shell-less eggs was recorded in all eggs produced. Data were analized as a completely randomized design with source of P and level of dP of the feed as main effects, and the interaction between them was also analized. In addition, the effect of the level of dP on the different variables studied was partitioned into linear (L) and quadratic (Q) components. No interactions between main effects were detected for any of the traits studied and therefore, only main effects are presented. Neither source of P nor level of dP affected any of the productive traits studied, except BWG that increased (L; $P < 0.05$) as the level of dP of the diet increased. An increase in dP of the diet from 0.27 to

0.39% tended to reduce (L, P= 0.077; Q, P =0.058) shell resistance to breakage. Haugh units decreased linearly (P < 0.01) as the level of dP increased. The percentage of non-sealable eggs (dirty, broken, and shell-less eggs) was higher in hens fed CBP than in hens fed MCP (P < 0.05). In conclusion, from 64 to 76 wk of age, hens might not require no more than 0.27% dP in the diet for optimal egg production and egg quality. An excess of dP (≥ 0.39%) might reduce shell quality. Hens responded similarly to both sources of P but the percentage of non-saleable eggs increased with the use of calcined bone phosphate.

Key words: calcined bone phosphate, digestible phosphorus, egg quality, laying hen, monocalcium phosphate.

RESUMEN

En el presente experimento se estudió el efecto de la fuente y el nivel de fósforo (P) digestible (Pd) de la dieta sobre la productividad y la calidad del huevo en ponedoras rubias desde las 64 a las 76 semanas de edad. Las dietas estaban basadas en maíz y harina de soja, y todas contenían un 4.1% de calcio. El diseño experimental consistió en 8 tratamientos, organizados de forma factorial 2x4 cuyos efectos principales fueron la fuente de P [fosfato monocálcico (FMC) y fosfato de huesos calcinados (FHC)] y el nivel de Pd (0,27, 0,31, 0,35 y 0,39%). Hubo 14 réplicas por tratamiento, y la unidad experimental era una jaula con 6 gallinas. El experimento duró 12 semanas (3 períodos de 4 semanas). La producción de huevos y la mortalidad de las gallinas se registraron diariamente. El consumo de pienso y el peso de las gallinas se registraron por período y de forma global. El peso del huevo se determinó por período, pesando todos los huevos producidos el último día de cada semana. Con estos datos, el consumo medio diario, masa de huevo, índice de conversión y la ganancia media diaria (GMD) se calcularon por período y de forma acumulativa. Los parámetros de calidad del huevo, incluyendo las Unidades Haugh (UH) y la resistencia de cáscara a la rotura, se registraron en 8 huevos cogidos al azar de cada jaula, durante los últimos dos días de cada período. Además, el porcentaje de huevos sucios, rotos y en fárfara se determinó a partir de un registro diario. Los datos se analizaron como un diseño aleatorio con la fuente de P y el nivel de Pd como efectos principales, y la interacción entre ambos también fue analizada. Además, el efecto del nivel de Pd sobre las distintas variables estudiadas fue particionado en sus componentes lineal (L) y cuadrático (Q). No se detectó ninguna interacción significativa entre los efectos principales sobe ninguna de las variables, de modo que sólo se presentan los efectos principales. Ni la fuente de P ni el nivel de Pd

tuvieron efecto sobre ninguno de los parámetros productivos estudiados, excepto sobre la GMD, que incrementó (L; P< 0,05) conjuntamente con el nivel de Pd de la dieta. Un incremento en el nivel de Pd de la dieta de 0,27 a 0,39% tendió a reducir la resistencia de la cáscara a la rotura (L, P = 0,077; Q= 0,058). Las UH disminuyeron linealmente (P < 0,01) a medida que el nivel de Pd incrementó. El porcentaje de huevos no comercializables (suma de sucios, rotos y en fárfara) fue mayor en gallinas alimentadas con FHC que en gallinas alimentadas con FMC (P < 0,05). En conclusión, de 64 a 76 semanas de edad, las gallinas ponedoras podrían no requerir más de 0,27% Pd en la dieta para alcanzar una producción y una calidad de huevo óptima. Un exceso de Pd (≥ 0,39%) puede reducir la calidad de la cáscara. Las gallinas respondieron de forma similar a ambos tipos de fosfatos, pero el porcentaje de huevos no comercializables fue mayor cuando la dieta contenía FHC.

Palabras clave: calidad del huevo, fosfato de huesos calcinados, fosfato monocálcico, fósforo digestible, gallina ponedora.

RÉSUMÉ

Le présent travail a été menée afin d'étudier l'effet de la source et du niveau du régime en phosphore (P) digestible (Pd) sur la productivité et la qualité des œufs chez les poules pondeuses de 64 à 76 semaines. Les régimes étaient à base de maïs et tourteau de soja et contenaient tous 4.1% de calcium. Le plan expérimental comprenait 8 traitements, établis par une factorielle 2x4 dont les effets principaux étaient la source de P [phosphate monocalcique (PMC) et phosphate osseux calciné (POC)] et le niveau de Pd (0,27, 0,31, 0,35 et 0,39%). Il y avait 14 répétitions par traitement, et l'unité expérimentale était une cage avec 6 poules. L'expérience a duré 12 semaines (3 périodes de 4 semaines). La production d'oeufs et la mortalité des poules ont été enregistrées quotidiennement. La consommation d'aliments (C) et le poids des poules ont été enregistrés par période et globalement. Le poids de l'œuf a été déterminé par période, en pesant tous les œufs produits le dernier jour de chaque semaine. Avec ces données, la C quotidienne moyenne, la masse en œufs, le taux de conversion et le gain quotidien moyen (GQM) ont été calculés par période et de manière cumulative. Les paramètres de qualité des œufs, y compris les unités de Haugh (UH) et la résistance à la rupture de la coquille, ont été enregistrés dans 8 œufs prélevés au hasard dans chaque cage, au cours des deux derniers jours de chaque période. De plus, le pourcentage d'œufs sales, cassés et sans coquille a été déterminé à partir d'un relevé quotidien. Les données ont été analysées sous forme de plan randomisé avec la source de P et le niveau de Pd comme effets principaux, et l'interaction entre les deux a également été analysée. En outre, l'effet du niveau de Pd sur les différentes variables étudiées a été divisé en ses composantes linéaires (L) et quadratiques (Q). Aucune interaction significative n'a été détectée entre les effets principaux sur l'une des variables,

de sorte que seuls les effets principaux sont présentés. Ni la source de P ni le niveau de Pd n'ont eu d'effet sur aucun des paramètres de production étudiés, à l'exception du GQM, qui a augmenté (L; P <0,05) en même temps que le niveau de Pd du régime alimentaire. Une augmentation du niveau de Pd de l'alimentation de 0,27 à 0,39% tend à réduire la résistance de la coquille à la rupture (L, P = 0,077, Q = 0,058). Les UH a diminué linéairement (P <0,01) à mesure que le niveau de Pd augmentait. Le pourcentage d'œufs non commercialisables (somme des œufs sales, cassés et sans coquille) était plus élevé chez les poules nourries avec du POC que chez les poules nourries avec du PMC (p <0,05). En conclusion, à partir de l'âge de 64 à 76 semaines, une valeur de plus de 0,27% de Pd dans l'alimentation des poules pondeuses peut-être pas nécessaire pour obtenir une production et une qualité d'œufs optimales. Un excès de Pd (≥ 0,39%) peut réduire la qualité de la coquille. Les poules ont réagi de manière similaire aux deux types de phosphates, mais le pourcentage d'œufs non commercialisables était plus élevé lorsque le régime alimentaire contenait du POC.

Mots clés: qualité des œufs, phosphate osseux calciné, phosphate monocalcique, phosphore digestible, poule pondeuse.

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LIST OF ABBREVIATIONS

ADFI: Average daily feed intake

AP: Available phosphorus

BW: Body weight

BWG: Body weight gain

Ca: Calcium

CBP: Calcined bone phosphate

DCP: Dicalcium phosphate

DFP: Defluorinated phosphate

dP: Digestible phosphorus

EM: Egg mass

EP: Egg production

EU: European Union

EW: Egg weight

FCR: Feed conversion ratio

GIT: Gastrointestinal tract

GMD: Geometric mean diameter

GSD: Geometric standard deviation

MCP: Monocalcium phosphate

NPP: Non-phytate phosphorus

P: Phosphorus

1. INTRODUCTION

1.1. Egg production in the world

Commercial livestock production has increased all over the world and it's enough, combined with traditional production systems, to meet the enormous global demand of food from animal origin (FAO, 2018a). Within the main livestock markets, poultry has been the most developed systems in the past few decades due to the growing demand of meat and eggs, the technological advances of the industry and the implementation of economies of scale (FAO, 2008). Nowadays poultry, large-scale intensive production systems are characterized by the breeding of selected commercial genotypes, which are housed in environmentally controlled farms, under strong biosecurity measures that prevent the large flocks from diseases. These commercial strains also demand an accurate nutrition management, to meet the high nutritional requirements of the birds in order to reach their productive potential. A good nutritional management is crucial for large farms profitability, as feed represent the highest cost input for intensive poultry farms (FAO, 2018b).

The egg is one of the most important sources of animal products in the world. Egg production reached 81 million t in 2016, after increasing by more than 150% in the last three decades (FAO, 2018b). The main egg producing countries are China, United States, and India, with approximately 39, 8, and 5 % of the total egg production, respectively (FAO, 2015). The European Union (EU) reached a production of 7.5 million t in 2018 (European Commission, 2019).

In 2018, 50% of the laying hens in EU were kept in enriched cages systems. Egg production in barn, free-range and organic systems, was 28, 16 and 5%, respectively

(European Commission, 2019). In EU, the production regime under which the eggs are produced must be reflected by law (European Commission, 2008) in the eggs labelling, by means of a numeric code. The first number of the code marked in the shell, corresponds to the production system, being 3 for eggs from housed hens, 2 for barn eggs, 1 for free range eggs, and 0 for organic eggs. The identification code of the country and farm of origin where the eggs were produced must appear in this code.

Approximately, 75% of the 7.5 million t of eggs produced in 2018 in EU, were produced by 7 countries: France (13%), Germany (12%), Italy (11%), Spain (10%), the United Kingdom (10%), the Netherlands (9%), and Poland (8%) (European Commission, 2019).

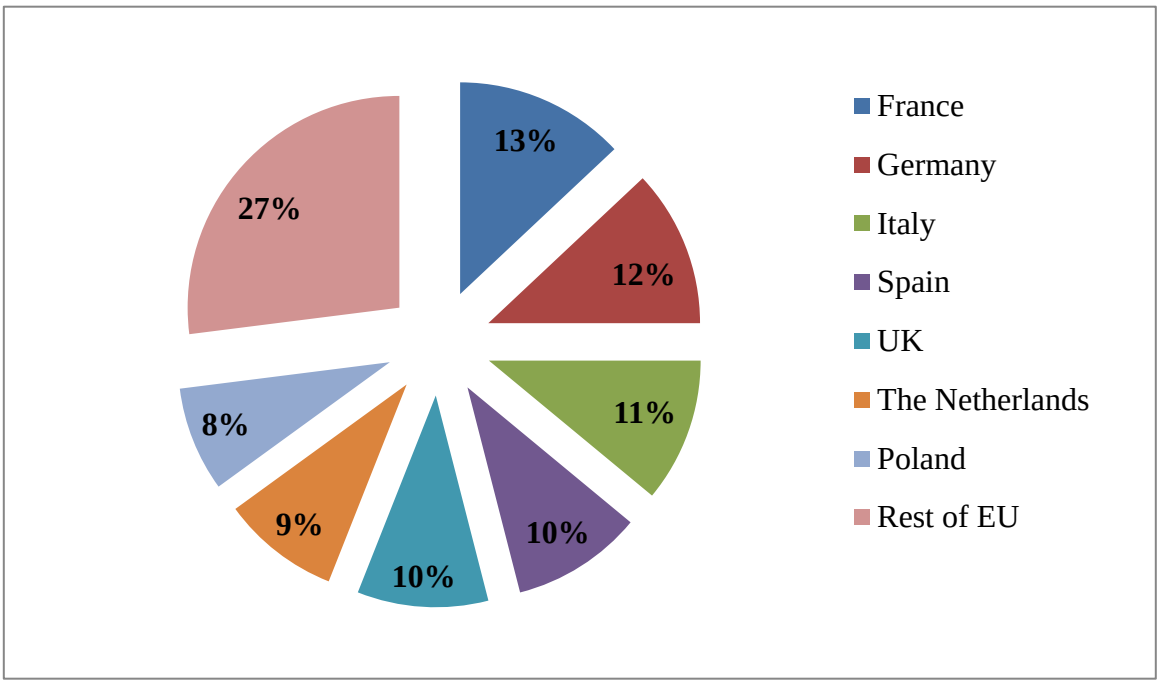

Figure 1. Main egg producers in EU during 2018 (European Commission, 2019)

Spain produced 10.3% of the total production (0.76 million t approximately) in 2017, and is placed fourth in terms of egg production in EU 28. Most of these eggs were

produced under enriched cages systems (European Commission, 2019). Only 12.2% of them were produced under alternative production systems (MAPAMA, 2018). However the continuously growing customer's concern on sustainability and animal welfare are modifying these values quickly (MAPAMA, 2017). In fact, it is expected that, more than 25% of the eggs produced in Spain in 2022 will come from hens with access to floor (Rubén Martínez, personal communication, April 2019). The most productive region in Spain is Castilla La Mancha, with 25.2% of the total production, followed by Castilla y León, Aragón, and Cataluña, with 17.3, 11.7 and 11.2%, respectively (MAPAMA, 2018).

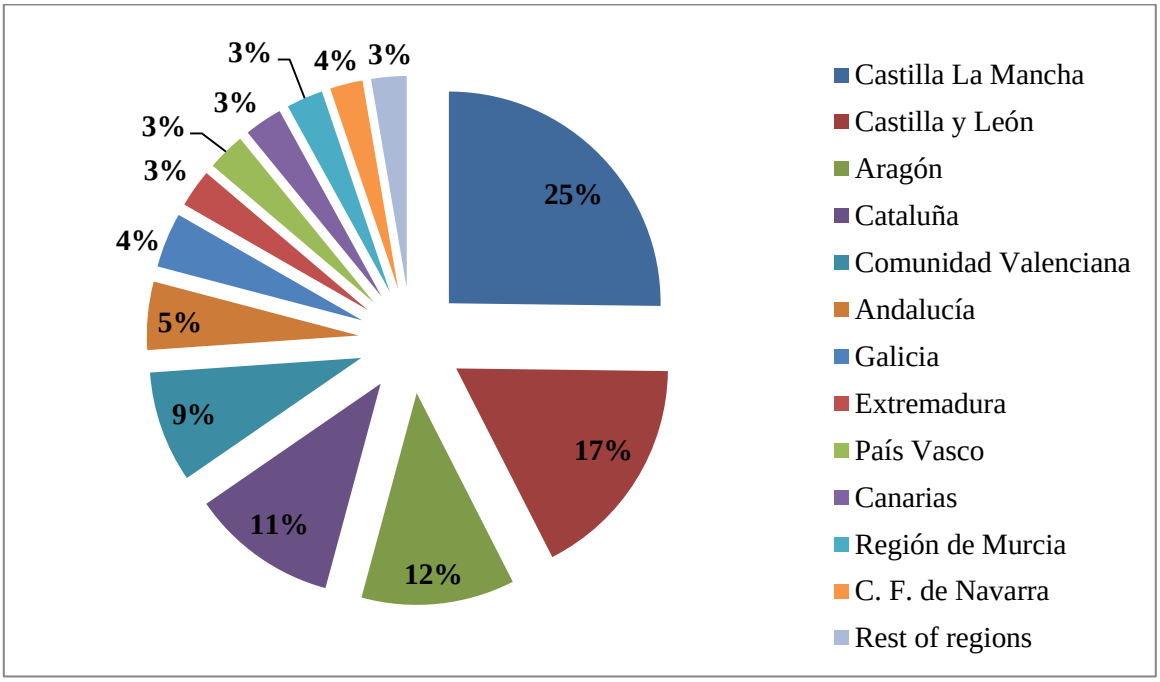

Figure 2. Distribution of egg production in Spain during 2016 (MAPAMA, 2018)

In the past years, the average consumption in Spain increased from approximately 13 Kg eggs/year in 2012 to 17 Kg eggs/year per capita in 2017. In EU, the average consumption per capita in 2016 was around 13 Kg eggs/year (MAPAMA, 2018).

Poultry meat and eggs play an important role in human nutrition all over the world, especially in developing and underdeveloped countries. Poultry products are considered as high quality sources of protein, vitamins, and minerals, and, compared with other protein sources, poultry meat and eggs are relatively cheap and easily available (FAO, 2013).

The egg is formed by 64% of albumen, 27% of yolk, and 9% of shell (Lambert et al., 2014). A 60g egg provides from 3 to 4% of the daily energy requirements of an adult, as well as around 6.5g of high quality protein. Eggs contain approximately 11% of fat (mainly stored in the yolk), which is compound by 44% monounsaturated, 29% saturated and 11% polyunsaturated fatty acids, approximately (Sparks, 2006). Eggs also contain important levels of vitamins (B2, B6, B12, folic acid, choline, A, D, and E) as well as minerals (phosphorus, selenium, iron and zinc, among others) (FAO, 2015).

The profitability of the egg industry depends on many factors, including length of the laying period, number of eggs produced per hen housed, egg size, and percentage of eggs that reach the table of the final consumer. Nowadays, genetics, management of the flock, and feeding practices, including mineral nutrition, are key points for successful egg production (Herrera, 2017).

2. LITERATURE REVIEW

2.1. Phosphorus in poultry nutrition: importance and problematic

Phosphorus (P) is an essential nutrient for poultry and it is necessary for growth, production, and normal development of vital functions (Waldroup, 1999; Ahmadi and Rodehutscord, 2012; Rodehutscord and Rosenfelder, 2016). A failure to provide P to poultry, according to their needs, may cause important economic losses due to physiological problems (Waldroup, 1999).

One of the main problems faced by modern agriculture is the environmental impact generated by its activity (Gerber et al., 2013). In particular, animal production contributes to the environmental damage by the contamination of ecosystems as a consequence of nitrogen and P wasted in the manure (FAO, 2006). Phosphorus present in the excreta or in the water wasted from the farms is an important cause of eutrophication of the surface water, which affects drastically water quality and the maintenance of aquatic ecosystems. This situation forces poultry and livestock industries to improve the management of P nutrition by reducing inputs and outputs at farm level (FAO, 2006).

Phosphorus in the feed can be provided by either inorganic sources, such as rock phosphates, or organic sources, such as animal by-products or plant feedstuffs (FEDNA, 2010). Most of the organic P present in plants is stored as phytic acid. Phytates (phytic acid and its salts) are mostly present in seeds and their by-products, which are main ingredients in poultry feeds (Rodehutscord et al., 2016). However, phytate-bound P is hardly digested by monogastrics (Witzig et al., 2018). Consequently, the use of inorganic sources of P, such as rock phosphates, is needed in order to meet birds P requirements (Vieira et al., 2018).

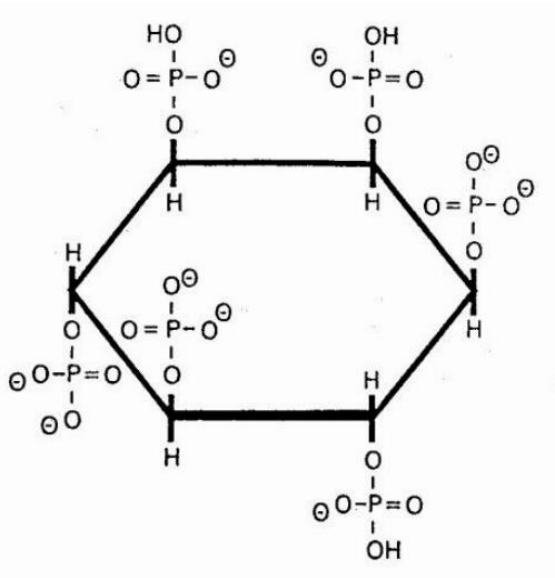

Figure 3. Phytic acid molecule

Nevertheless, recent studies (Rodehutscord and Rosenfelder, 2016; Sommerfeld et al., 2018) reported that broiler chickens are able to use P from phytates when fed a diet with low P and calcium (Ca) supplementation. At higher and commonly used levels, however, these minerals may interfere with the endogenous phytase produced by the birds, and reduce their activity.

There are different ways for assessing the P content of the feedstuffs as well as reporting P requirements of poultry:

a) The bioavailability of P might be defined by the expression of non-phytate P (NPP) as the difference between total P and phytate-bound P. This implies that mineral and animal sources of P have similar bioavailabilities, due to the lack of phytate-bound P in these ingredients (FEDNA, 2018).

b) Available P (AP) values had been widely used to assess P bioavailability for poultry (Gorrachategui, 2018). However, the concept of P availability has been defined in

several ways. For example, in the 1990's a value of 30 or 100% AP was given to feedstuffs from vegetable and not-vegetable origin, respectively. Recently, the P availability of an ingredient has been expressed compared to a 100% value given to a pattern source, established for monocalcium phosphate (MCP) (FEDNA, 2018) or monosodium phosphate (Gorrachategui, 2018). Sometimes, AP could be referred as NPP by the literature, but they don't mean the same (Rodehutscord, 2009), as a part of the phytic P can be utilized also by poultry (Rodehutscord and Rosenfelder, 2016). Finally, Rodehutscord (2009) defined the AP as the proportion of dietary total P that, at marginal levels of P supply, can be utilised to cover the P requirement of an animal.

c) Probably, the most suitable way to assess P biological value for poultry is using digestible P (dP) values, which are obtained in experimental *in vivo* procedures. One inconvenient of this system, is the lack of a standardized method to determine the digestibility of P (Shashtak and Rodehutscord, 2013; Rodehutscord et al., 2017). Nevertheless methods like calculating the P retention or the pre-caecal digestibility of P have been considered as suitable methods for assessing the utilization of P provided by phosphates (Shastak et al., 2012).

However, there is still a tendency to formulate poultry diets using AP requirements, since genetics corporations use them, rather than digestible P requirements, in their commercial guidelines (Lohmann, 2018; ISA-Brown, 2019).

Because of its nutritional importance in poultry, it is a common practice to include higher levels of P in the feed than those needed. The use of large safety margins ensure that P supplementation will not limit bird productivity (Waldroup, 1999), although this practice

presents two major inconvenient: the high cost of rock phosphates due to the limited resources left in the world (Rodehutscord, 2009), and the increase in the environmental damage resulting from the high concentration of non-digested P in the manure (Maenz and Classen, 1998). This manure, rich in P, is frequently used for crop fertilization (Penn et al., 2004) and, when used in excess, may result in eutrophication of the reservoirs and surface water (Rodehutscord, 2009).

2.2. Strategies to reduce P supplementation in poultry

In order to reduce the impact of P wastes and to improve sustainability of poultry production, professional nutritionists may perform different strategies such as phytases supplementation of poultry diets, reducing safety margins in diet formulation with more accurate P levels, and the use of P sources with higher biological value for poultry (Waldroup, 1999).

2.2.1. Inclusion of exogenous phytases in poultry diets

Phytases, usually obtained from transgenic fungi, yeasts, and bacteria, have become a constant additive included in poultry diets (Vieira et al., 2018). Phytase inclusion improves significantly animal performance, especially when extra doses of inclusion are used (Walk et al., 2014). Phytases catalyze the hydrolysis of phytates, releasing the P bounded to the inositol molecule, which becomes available to be absorbed and used by the organism (Vieira et al., 2018). The endogenous phytases produced in the gastro intestinal tract (GIT) of the birds are able to hydrolyze the phytates (Rodehutscord and Rosenfelder, 2016), although this effect is limited under practical conditions. Poultry diets usually include high levels of P and Ca which interfere with endogenous phytase activity and

reduce phytate hydrolysis (Rodehutscord and Rosenfelder, 2016; Angel et al., 2015). Furthermore, intrinsic phytase activity of plant feedstuffs contributes to the release of the phytate-bound P. Nevertheless, this intrinsic phytase activity is very variable within feedstuffs and its relevance in poultry diets is not well defined (Rodehutscord and Rosenfelder, 2016).

The low availability of the phytate-bound P leads to the use of exogenous phytases, which has become an extended commercial practice worldwide to meet the P requirements of poultry. The inclusion of exogenous phytases in the feed increases significantly P digestibility and consequently, the P excretion to the environment is evidently reduced (Waldroup et al., 2000; Penn et al., 2004; Angel et al., 2006).

Li et al. (2016; 2018) reported that exogenous phytases increases the utilization of phytate-bound P throughout the entire GIT. Nevertheless, these studies confirmed that Ca has a negative effect on phytase activity increasing the pH of the GIT of the birds, reducing the solubilisation of phytates, and increasing their chelation with Ca. Also, when low doses of phytases are used, an increase in the level of Ca and NPP in the diet may reduce the phytase activity which in turn, can be solved with the inclusion of higher doses of phytases (Li et al., 2016; 2018).

2.2.2. Adjusting P level of supplementation

Phosphorus requirements in poultry have been widely studied in the past, resulting in nutritional recommendations for poultry feeds by different research institutions (NRC, 1994; FEDNA 2018, CVB 2018b, Rostagno et al., 2017). Phosphorus recommendations for broilers and brown egg laying hens established by FEDNA (2018) are sown in Table 1.

Table 1. Phosphorus recommendations for commercial broilers and housed brown laying hens (FEDNA, 2018).

	Total P (%)	Available P (%)	Digestible P (%)
Broilers (0-14 d)	0.66	≥0.48	0.45
Broilers (15-23 d)	0.58	0.43	0.40
Broilers (24-36 d)	0.56	0.38	0.34
Broilers (>37 d)	0.52	0.35	0.32
Layers (16-25 wk)	0.60	0.38-0.40	0.33-0.37
Layers (26-50 wk)	0.56	0.36-0.39	0.31-0.33
Layers (>50 wk)	0.51	0.32-0.37	0.29-0.32
Layers (shell problems)	0.48	0.27	0.23

Several authors (Angel et al., 2005; 2006; Ahmadi and Rodehutscord, 2012; Rama Rao et al., 2019; Jing et al., 2018) have reported that, the NPP requirements for poultry might be lower than those currently recommended (NRC, 1994). Consequently, P excretion could be minimized by reducing the P content of the diets without any negative effect on productive performance of the birds (Angel et al., 2006).

Former recommendations of NPP for broiler chickens (NRC, 1994) ranged between 0.45 to 0.30% (Table 2). Nevertheless, Angel et al. (2006) reported that feeding 0.36% NPP in the starter diet (0-18 d of age), decreasing this level up to 0.12% in the withdrawal phase (42-49 d of age), resulted in good productive performance and bone mineralization and reduced the amount of P excreted to the environment, in broilers supplemented with phytases and vitamin D.

Table 2. Phosphorus recommendations for broiler chickens (NRC, 1994)

Age of the birds	0-3 wk	3-6 wk	6-8 wk
Non-phytate phosphorus, %	0.45	0.35	0.30

For growing pullets, Keshavarz (2000b) reported that diets with no supplemental phytases containing 0.20, 0.15, and 0.10% NPP from 0 to 6, 6 to 12 and 12 to 18 wk, respectively, were enough to achieve normal growing and development of the pullets during the rearing period, as well as a normal egg production at the start of the laying phase. These levels of NPP are lower than those recommended by NRC (1994) which are 0.40, 0.35 and 0.30% NPP for each phase of the rearing period (Table 3).

Table 3. Phosphorus recommendations for growing pullets (NRC, 1994)

Age of the pullets	0-6 wk	6-12 wk	12-18 wk	18 wk to first egg
Non-phytate phosphorus, %	0.40	0.35	0.30	0.32-0.35

From 22 to 34 wk of age, Lohmann laying hens fed a diet with 0.15% AP resulted in similar productive performance, and minimized P excretion, even in the absence of phytases, compared to hens fed higher levels of AP up to 0.45% (Jing et al., 2018). This level of AP is much lower than that currently recommended by the Lohmann guide (2018) for white laying hens, which ranges from 0.32 to 0.42% AP depending on the age and feed intake of the birds.

2.2.3. The importance of the P source used in the diet

Inorganic P sources, such as rock phosphates, are usually included in poultry diets to reach birds requirements, with or without phytases supplementation (Vieira et al., 2018). Consequently, the prediction of the P availability or digestibility of the different phosphates might result in a reduction of the P concentration of the feed (Bikker et al., 2016; Rodehutscord et al., 2012).

In general, P from rock origin is considered of high availability (FEDNA, 2010). Generally, monocalcium phosphates are more available than bicalcium phosphates, and these are more available than tricalcium phosphates. Also, sodium phosphates are more soluble than calcium or magnesium phosphates, and consequently, more available. Finally, the hydrated phosphates are more available than those non hydrated (Gorrachategui, 2018).

The P digestibility of the different phosphates for poultry varies from 60 to 90% (FEDNA, 2010; CVB, 2018a), although these values could be affected by the methodology used and even vary within the same phosphates. Therefore, special attention should be taken when comparing the biological value of the different phosphates (Shastak et al., 2012). Phosphorus digestibility values for different phosphates are shown in Table 4.

Phosphorus sources from animal origin are considered a source of high P availability, especially when obtained from grounded bone components (FEDNA, 2010). The main P sources from animal origin used in the EU come from the extraction of collagen or gelatine from non-ruminant bones. Bones can be thermally treated and, tricalcium phosphate or hydroxyapatite is obtained. When the bones are chemically treated, dehydrated dicalcium phosphate is obtained (DCP) (Gorrachategui, 2018). According to

Van Harn et al. (2017), when organic P sources from animal bones origin are treated either by thermal or chemical processes, they can reach pre-caecal digestibility values equivalent to those obtained for rock phosphates. Processed animal P sources might be considered as interesting alternatives to reduce the use of rock phosphates sources and the P excretion, as their P digestibility could be comparable to that of mineral sources (Van Harn et al., 2017).

Table 4. Poultry digestibility values for phosphorus from different phosphates

P source*	Total P	FEDNA, 2010	CVB, 2018a	Rostagno et al., 2017	Van Harn et al., 2017
MSP, $1H_2O$	22.5	85	91	74	-
MCP, $1H_2O$	22.7	81.7	85	71	88.5
DCP, $2H_2O$	17.7	80.2	78	70	94.5
CBP	14.9	-	-	60	86.9

*MSP: Monosodium phosphate; MCP: Monocalcium phosphate; DCP: Dicalcium phosphate; CBP: Calcined bone phosphate

2.3. Phosphorus importance and management in diets for laying hens

Phosphorus is considered an essential nutrient for poultry, which is needed for skeletal growth, energy metabolism, maintenance of cellular structure, and also for egg formation (Ahmadi and Rodehutscord, 2012). The eggshell contains little amounts of P (de Vries et al., 2010), whereas it is the most abundant mineral in the shell-less egg (AEB, 2017). Calcium however is barely present within the egg, but each eggshell is compounded of 98% calcium carbonate (Rose, 1997).

In commercial laying hens, the time between consecutive ovipositions, is slightly higher than 24h (Keshavarz, 1998). The formation of the shell takes place during the dark hours, in which the Ca available from digestion is low (de Vries et al., 2010). This situation forces the hen to mobilize Ca from bone reservoirs for shell formation (Lambert et al.,

2014). In the morning, when lights are on, the egg is laid and the hen starts to eat and the Ca concentration in the plasma increases. Consequently, the bones can be recalcified (Lambert et al., 2014). The Ca dynamics is similar to that for P. Calcium is stored in the bone as hydroxyapatite, which contains both Ca and P, and the two minerals are mobilized at the same time (Lambert et al., 2014). However, P is not needed for shell formation, and therefore, it is excreted by the urine (de Vries et al., 2010). The mobilization of Ca and P from bones results in an increase of the intake of these minerals during the first hours of the day (Keshavarz, 1998).

There are many factors affecting the P requirements for laying hens, such as the P level of the feed, the level and particle size of the Ca source, the age of the hens, the housing system, or ambient temperature and health status (Lambert et al., 2014).

Nowadays, there is a trend to reduce P levels in diets for laying hens in order to reduce the environmental impact as well as feed costs associated with the use of inorganic phosphates (Keshavarz and Austic, 2004; Ahmadi and Rodehutscord, 2012; Jing et al., 2018). Furthermore, Ahmadi and Rodehutscord (2012) recommended a minimum 0.22% NPP in the feed, without supplementing with phytases, to maintain hen performance from 36 to 76 wk. Furthermore it could be diminished to 0.14% when phytases are supplemented. Low levels of P in diets for layers increase P retention (Keshavarz and Austic, 2004) and may improve shell thickness (Jing et al., 2018).

High P levels in the diets of laying hens might interfere with the absorption of Ca, resulting in reduced shell quality (Boorman and Gunaratne, 2001) and reduced P absorption (Angel et al., 2015). This effect is due to the formation of insoluble complexes (de Vries et

al., 2010). Nevertheless, the detrimental effect of high Ca level on P absorption can be decreased by supplementing with high doses of phytases (Li et al., 2018).

The age of the hens may affect P requirements, and the information available on the effect of hen age on P requirements is scarce (Lambert et al., 2014). Old laying hens may be more sensible to a P deficiency than younger hens (Moshtagian et al., 1991; Boling et al., 2000). Boling et al. (2000) reported that old laying hens (older than 70 wk of age) consuming a diet with 0.10% AP supplemented with phytases showed similar productive performance compared to hens consuming a 0.45% AP. However, Bar et al. (2002) reported that when the Ca level of the diet of aged laying hens is increased, the level of AP should be increased or phytases should be supplemented. Lambert et al. (2014) concluded that old layers may require diets containing between 0.20 and 0.25% AP. However, genetics companies (ISA-Brown, 2019; Lohmann, 2018) and research institutions (FEDNA, 2018; CBV, 2018b; Rostagno et al., 2017; NRC, 1994) usually recommend higher levels of P for old hens than the ones appointed by Lambert et al. (2014) (Table 5).

Table 5. Phosphorus recommendations for old hens (>50 wk of age)

	FEDNA, 2018	CVB, 2018b	Rostagno et al., 2017	ISA-Brown, 2019	NRC, 1994
Available phosphorus, %	0.32-0.37	-	0.32	0.27-0.36	-
Digestible phosphorus, %	0.29-0.32	0.28	0.29	-	-
Non phytate phosphorus, %	-	-	-	-	0.25

Recommended levels of P for poultry diets are excessive in many cases compared with P requirements according to published data (Angel et al., 2005; 2006; Ahmadi and Rodehutscord, 2012; Jing et al., 2018). Therefore, there is a chance to minimise P

supplementation and excretion by using lower security margins, supplementing with exogenous phytases, and using mineral sources of high biological value (Waldroup, 1999).

3. OBJECTIVE

The objective of the present study was to compare the effects of monocalcium phosphate and calcined bone phosphate at four levels of digestible phosphorus on productive performance and egg quality in diets for brown laying hens from 64 to 76 weeks of age.

4. MATERIALS AND METHODS

4.1. Husbandry and experimental diets

All the experimental procedures used in this experiment were in compliance with the Spanish legislation for the care and use of animals in research (Boletín Oficial del Estado, 2013).

The experiment was conducted at the facilities of Camar Agroalimentaria S.L., placed at Cedillo del Condado (Toledo, Spain). A total of 672 ISA Brown laying hens were taken from a 65,000 hen commercial flock at 64 wk of age, and were randomly allotted into 112 enriched cages. The experimental unit consisted of an enriched cage with 6 hens. The cages were 63.5cm width, 120 cm length and 45 cm height, and were equipped with a 120 cm lineal feeder, 2 pressure nipple drinkers and a 120 cm long perch. Water and feed were provided for *ad libitum* consumption throughout the entire experiment. Eight experimental diets were randomly distributed within the 112 replicates. All the birds were kept under adequate environmental conditions (light, temperature, humidity, and air ventilation) according to the management guidelines of the commercial strain for cage housing (ISA Brown, 2019).

The experimental diets were commercial diets based on corn, soybean meal, and sunflower meal, in mash form. Diets were formulated to meet or exceed the nutrient recommendations of FEDNA (FEDNA, 2018) for aged brown laying hens housed in cages. Diets were formulated to have the same AMEn (2,710 Kcal/Kg), CP (16%), and Ca (4.1%) content, but to differ in the source of P [MCP or calcined bone phosphate (CBP)] and in the level of dP used (0.27, 0.31, 0.35, and 0.39%).

4.2. Analytical evaluation of feeds and phosphates

All the diets were analysed in the laboratory of Animal Science of the Universidad Politécnica de Madrid. A representative sample of each diet was collected and then grounded in a laboratory mill (Retsch model punishments, Stuttgart, Germany) equipped with a 1 mm diameter screen. Dry matter was determined by the oven-drying method (method 930.15), and total ash content (method 942.05) by a muffle furnace (model 12PR/300, Forns Hobersal, Barcelona, Spain). Crude protein was analysed (method 990.03) using a Leco FP-528 Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Gross energy was determined using an adiabatic bomb calorimeter (model 6400, Parr Instrument Company, Moline, USA). Neutral detergent fiber was analized as indicated by Pérez-Bonilla et al. (2011). Ingredient composition and chemical analyses (%, as fed basis) of the experimental diets are presented in Table 6.

Particle size distribution and mean particle size of the feeds (Table 7), expressed as the geometric mean diameter (GMD) and geometric standard deviation (log normal SD; GSD), were determined in 100 g samples using a shaker equipment (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 μm as indicated by ASAE (1995). All the analyses were conducted in triplicate.

Both phosphates were analysed by Jernbro Industrial Services AB (Helsinborg, Sweden) (Table 8). For both phosphates, we assumed a P availability of 100%, and a P digestibility of 88.5 and 86.9% for MCP and CBP, respectively, as reported by Van Harn et al. (2017).

Table 6. Ingredient composition and chemical analysis of the diets (%)

	Calcined bone phosphate				Monocalcium phosphate			
	0.39	0.35	0.31	0.27	0.39	0.35	0.31	0.27
Ingredient								
Soybean meal, 47% CP	14.4	14.4	14.3	14.3	15.3	15.1	14.8	14.6
Sunflower meal, 35% CP	13.4	13.5	13.7	13.8	12.6	12.9	13.2	13.5
Corn	56.8	56.8	56.7	56.7	56.6	56.6	56.6	56.6
Fine calcium carbonate	3.68	3.77	3.87	3.96	3.95	3.98	4.01	4.04
Coarse calcium carbonate	5.58	5.72	5.87	6.01	5.98	6.03	6.08	6.13
Soybean soapstocks	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80
Calcined bone phosphate	1.24	0.95	0.66	0.37	-	-	-	-
Monocalcium phosphate	-	-	-	-	0.75	0.58	0.39	0.22
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
DL-Methionine, 98%	0.13	0.13	0.12	0.12	0.13	0.12	0.11	0.10
L-Lys HCL, 78%	0.10	0.10	0.09	0.09	0.08	0.08	0.09	0.09
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Determined analysis								
Moisture	11.2	11.5	11.4	11.6	11.0	11.2	11.2	11.3
Gross Energy, kcal/kg	3,648	3,604	3,779	3,722	3,575	3,594	3,695	3,697
Crude protein	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Total ash	14.7	12.8	11.8	10.4	15.1	14.0	12.7	12.5
Neutral detergent fiber	10.7	10.7	10.8	10.8	10.5	10.6	10.7	10.7
Calculated analysis								
AMEn, kcal/kg	2,746	2,746	2,745	2,745	2,745	2,745	2,745	2,745
Ether extract	6.10	6.10	6.09	6.08	6.07	6.07	6.07	6.07
Crude fibre	4.30	4.32	4.34	4.36	4.18	4.23	4.27	4.32
Calcium	4.12	4.12	4.12	4.12	4.12	4.12	4.12	4.12
Phosphorus	0.58	0.54	0.49	0.45	0.56	0.52	0.48	0.44
Available phosphorus	0.41	0.37	0.33	0.29	0.40	0.36	0.32	0.28
Digestible phosphorus	0.39	0.35	0.31	0.27	0.39	0.35	0.31	0.27
Sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chloride	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Digestible amino acids								
Lys	0.68	0.68	0.67	0.67	0.69	0.68	0.68	0.68
Met	0.39	0.39	0.38	0.38	0.39	0.38	0.37	0.36
Met+Cys	0.61	0.61	0.60	0.60	0.61	0.60	0.59	0.58
Thr	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.51
Added fat	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80

[1]Provided the following (per kilogram of diet): vitamin A (*trans*-retinyl acetate), 8,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (dl-α-tocopheryl acetate), 8,000 IU; vitamin K, 1 mg; vitamin B1, 1.0 mg; vitamin B2, 4 mg; vitamin B6, 1.5 mg; vitamin B12 (cyanocobalamin), 10 mg; niacin, 20 mg; pantothenic acid (d-calcium pantothenate), 8.2 mg; folic acid, 1 mg; biotin, 10 mcg; choline (choline chloride), 200 mg; manganese (MnO), 70 mg; zinc (ZnO), 50 mg; iron ($FeSO_4.H_2O$), 30 mg; copper ($CuSO_4$ $5H_2O$), 6 mg; iodine [$Ca(IO_3)2$], 0.5 mg; selenium (Na_2SeO_3), 0.3 mg; 300,000 U of 4a24 6-fitasa (EC 3.13.26)152,00 U of endo-1,3 (4)-β-glucanase (EC 3.2.1.6), and 1220,00 U of endo-1,4-β-xylanase (EC 3.2.1.8) supplied by Trouw. All diets included 0.18 g/kg and 0.32 g/kg, respectively of the ester of canthaxantin/kg and β-apo-8-carotenoic/kg (supplied by Trouw Nutrition)

Table 7. Particle size of the diets

	Calcined bone phosphate				Monocalcium phosphate			
	0.39	0.35	0.31	0.27	0.39	0.35	0.31	0.27
GMD[1], µm	954	977	985	975	975	1,006	1,015	999
GSD[2], µm	2.33	2.31	2.22	2.28	2.17	2.23	2.18	2.22

[1]GMD: Geometric mean diameter.
[2]GSD: Geometric standard deviation.

Table 8. Chemical composition of the phosphates and calcium carbonate

Analytical results	Monocalcium phosphate	Calcined bone phosphate	Calcium carbonate (limestone)
Ca, %	16.8	31.2	38.6
Mg, %	1.4	0.60	0.30
Na, %	< 0.10	0.20	0.07
P, %	22.5	14.9	0
Al, mg/kg	2,600	< 2	-
Fe, mg/kg	4,400	32	620
K, mg/kg	1,600	400	70
S, mg/kg	2,000	< 30	70
Si, mg/kg	840	16	-
As, µg/kg	2,400	< 200	-
Cd, µg/kg	290	< 30	-
Pb, µg/kg	1,300	160	-
Hg, µg/kg	< 5	< 5	-
F, %	0.12	0.02	-
P neutr.ammon.-citr. 20°C, %	22	7.8	-
P soluble in citric acid, %	22.3	11.3	-
P soluble in water 20°C, %	17.1	0.20	-

4.3. Productive performance and egg quality

Egg production (EP) and mortality were recorded daily by replicate. Feed disappearance and body weight (BW) of the hens were recorded by replicate at 4 weeks intervals. All the eggs produced on the last day of each week were weighed. Egg production, egg weight (EW), egg mass (EM), average daily feed intake (ADFI), feed

conversion ratio (FCR) by kilogram, and BW gain (BWG) were calculated from these data by period and cumulatively.

The total number of broken, dirty and shell-less eggs was recorded daily. Eggs were considered dirty when a spot of any kind was detected on the shell, as indicated by Safaa et al. (2008). Eight eggs were collected randomly per replicate on the last two days of each 4 week period to measure the shell resistance to breakage (Kg/cm^2), as indicated by Herrera et al. (2018). The method consisted in using a press meter (Egg Force Reader, Sanovo Technology A/S, Odense, Denmark) that applies pressure gradually to the broad pole of the egg, until it breaks. Haugh units (HU) were determined by using a multitester equipment (QCM System, Technical Services and Supplies, Dunnington, UK) as indicated by Safaa et al (2008).

From these data, percentage of dirty, broken and non-saleable eggs (the sum of dirty, broken and shell-less eggs), shell resistance to breakage (Kg/cm^2) and HU were determined by replicate cage, by period and globally.

4.4. Statistical analysis

The experiment was conducted as a completely randomized design, with 8 treatments arranged as a 2x4 factorial design with 2 sources of P (MCP vs CBP) and 4 levels of dP (0.27, 0.31, 0.35, or 0.39%) as main effects. The experiment lasted for a total of 12 weeks, and was divided in three 4 weeks periods.

Main effects and their interaction were analized using the MIXED procedure of SAS (SAS Inst. Inc., Cary. NC). Also, the effect of dP level of the diets was partitioned

into linear and quadratic components, using the REG procedure of SAS (SAS Inst. Inc., Cary. NC).

Results in tables are shown as means. All differences were considered significant at $P < 0.05$ and P values between 0.05 and 0.1 were considered a trend.

5. RESULTS

5.1. Productive performance

The data on the productive performance traits of the hens are shown in Table 9. There were no significant interactions between P source and dP level of the diet, and consequently, only main effects are presented.

An increase in the dP content of the diet from 0.27 to 0.39% tended to reduce (Q; $P = 0.051$) EW for the whole experimental period (Figure 4). Egg production, EM and FCR, however, were not affected by the level of dP of the diet. BWG increased (L; $P < 0.05$) as the level of dP increased (Figure 5). ADFI tended to be greater in hens fed 0.35% dP (L, $P = 0.098$; Q, $P= 0.078$). There were no significant effects of P source observed for any of the productive traits studied. The effect of the level of dP in the diet on ADFI was more noticeable from 72 to 75 wk (Q; $P = 0.054$), with a maximum intake observed with 0.35% dP (Figure 6). Phosphorus source did not affect any productive performance trait, although a trend for reduced ADFI of hens fed CBP compared with hens fed MCP was observed ($P = 0.066$) from 64 to 67wk.

Egg production, ADFI, EM, and BWG decreased ($P < 0.01$) as the age of the hens increased. EW increased ($P < 0.001$) with hen age but FCR was not affected. A trend ($P = 0.063$) between P source x age of the hens on ADFI was observed; ADFI decreased with age but the reduction was more pronounced in hens fed CBP than in hens fed MCP (Figure 7).

Table 9. Effect of phosphorus source [monocalcium phosphate (MCP) vs calcined bone phosphate (CBP)] and level of inclusion of digestible phosphorus (dP, %) on performance of brown laying hens from 64 to 76 weeks of age

	MCP, %				CBP, %				Phosphate		dP (%)				SEM*	P**					
	0.27	0.31	0.35	0.39	0.27	0.31	0.35	0.39	MCP	CBP	0.27	0.31	0.35	0.39	n=14	1	2	3	4	5	6
Egg production %																					
64-67 wk	87.1	89.4	90.6	86.4	87.8	86.8	87.7	89.6	88.4	88.0	87.5	88.1	89.2	88.0	2.00	0.795	0.627	0.728	0.774		
68-71 wk	85.4	88.6	89.0	84.8	87.1	86.6	85.0	88.4	86.9	86.8	86.2	87.6	87.0	86.6	2.05	0.905	0.867	0.811	0.704		
72-75 wk	82.7	85.9	86.4	83.7	84.5	84.1	82.9	85.4	84.7	84.2	83.6	85.0	84.7	84.5	2.33	0.794	0.708	0.813	0.931		
Global	85.0	87.9	88.7	85.0	86.5	85.8	85.2	87.8	86.7	86.3	85.8	86.9	87.0	86.4	2.01	0.819	0.558	0.505	0.362	<0.001	0.919
ADFI, g																					
64-67 wk	121	122	121	121	119	118	121	121	121	119	120	120	121	121	1.38	0.066	0.313	0.602	0.364		
68-71 wk	119	119	119	118	118	119	119	119	119	119	118	119	119	119	1.42	0.954	0.680	0.713	0.987		
72-75 wk	113	115	118	115	112	115	115	114	115	114	113	115	117	115	1.54	0.273	0.132	0.054	0.184		
Global	117	119	119	118	116	117	118	118	118	117	117	118	119	118	1.23	0.242	0.098	0.078	0.931	<0.001	0.063
Egg weight, g																					
64-67 wk	65.4	65.4	65.5	64.9	65.5	66.1	66.4	64.6	65.3	65.7	65.4	65.7	65.9	64.8	0.555	0.321	0.301	0.113	0.403		
68-71 wk	66.1	65.4	66.0	65.6	65.8	66.7	66.4	65.5	65.8	66.1	65.9	66.1	66.2	65.5	0.566	0.376	0.560	0.517	0.689		
72-75 wk	65.7	65.6	65.6	65.7	65.8	66.7	66.4	65.5	65.7	66.1	65.7	66.1	66.0	65.6	0.555	0.277	0.782	0.607	0.779		
Global	65.7	65.4	65.7	65.4	65.7	66.5	66.4	65.2	65.6	66.0	65.7	66.0	66.0	65.3	0.522	0.291	0.272	0.051	0.585	<0.001	0.957
Egg mass, g																					
64-67 wk	56.8	58.4	58.7	56.1	57.6	57.3	58.2	57.9	57.5	57.8	57.2	57.9	58.4	57.0	1.41	0.812	0.991	0.596	0.924		
68-71 wk	56.3	57.9	58.1	55.6	57.4	57.7	56.5	57.9	57.0	57.4	56.8	57.8	57.3	56.8	1.49	0.725	0.920	0.761	0.913		
72-75 wk	54.2	56.3	56.6	55.0	55.6	56.1	55.0	56.0	55.5	55.7	54.9	56.2	55.8	55.5	1.56	0.886	0.778	0.712	0.964		
Global	55.8	57.6	57.8	55.6	56.9	57.0	56.5	57.3	56.7	56.9	56.3	57.3	57.2	56.4	1.39	0.796	0.908	0.351	0.700	<0.001	0.920
FCR																					
64-67 wk	2.15	2.10	2.07	2.17	2.07	2.07	2.09	2.09	2.12	2.08	2.11	2.09	2.08	2.13	0.047	0.206	0.733	0.527	0.720		
68-71 wk	2.12	2.08	2.07	2.14	2.07	2.07	2.13	2.07	2.10	2.09	2.10	2.08	2.10	2.11	0.051	0.701	0.755	0.890	0.952		
72-75 wk	2.09	2.05	2.11	2.11	2.03	2.07	2.11	2.05	2.09	2.07	2.06	2.06	2.11	2.08	0.051	0.484	0.494	0.714	0.901		
Global	2.12	2.08	2.09	2.14	2.06	2.07	2.11	2.07	2.11	2.08	2.09	2.07	2.10	2.11	0.046	0.404	0.435	0.636	0.699	0.224	0.455
BWG, g																					
64-67 wk	0.94	1.42	0.91	0.37	0.55	0.66	0.86	1.40	0.91	0.87	0.74	1.04	0.89	0.89	0.333	0.859	0.742	0.794	0.262		
68-71 wk	0.35	-0.22	0.61	1.18	0.32	0.60	0.47	0.57	0.48	0.49	0.34	0.19	0.54	0.88	0.338	0.974	0.071	0.122	0.241		
72-75 wk	-0.18	0.58	0.54	0.68	-0.02	0.32	0.48	0.05	0.41	0.21	-0.10	0.45	0.51	0.36	0.347	0.422	0.195	0.150	0.552		
Global	0.37	0.59	0.69	0.74	0.28	0.53	0.60	0.67	0.60	0.52	0.33^{b}	0.56^{ab}	0.65^{ab}	0.71^{a}	0.160	0.495	<0.05	0.120	1.00	<0.01	0.839

*SEM: n = 56 for main effect of P source; n = 28 for main effect of dP level; n = 14 for the interaction between P source and dP level.

**1: P source; 2: Lineal effect of dP level (%); 3: Quadratic effect of dP level (%); 4: P source*dP level; 5: Age of the hens; 6: P source*Age. There were no significant interactions between, dP Level*Age and P source*dP Level*Age.

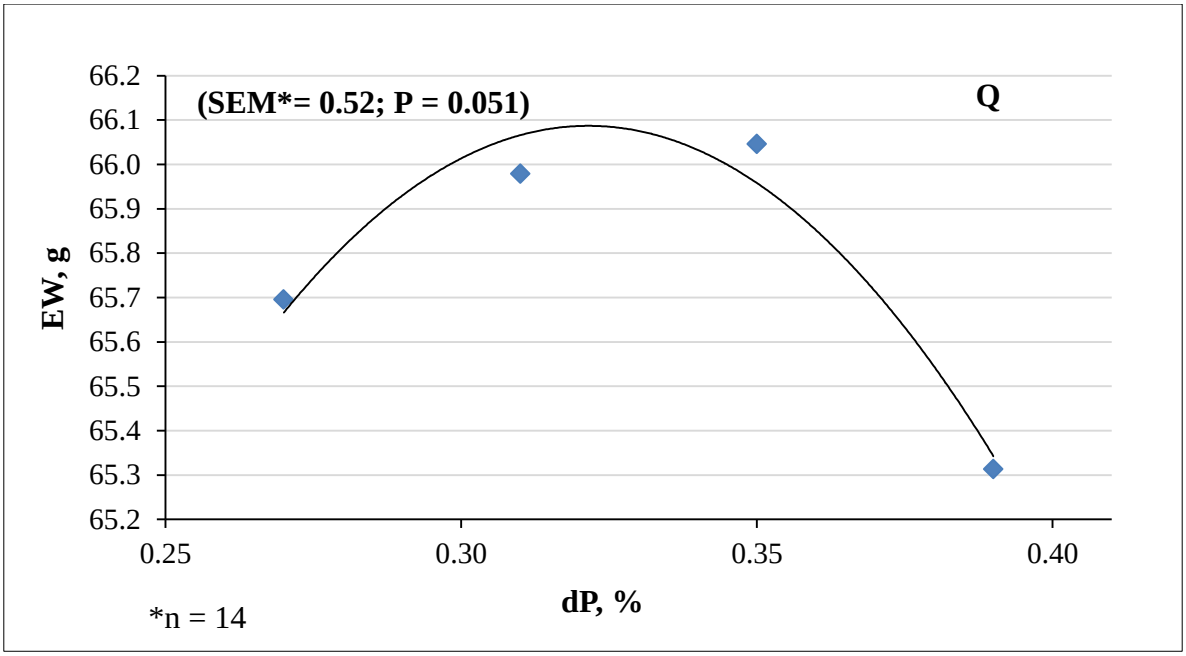

Figure 4. Effect of digestible phosphorus level (dP, %) on egg weight (EW, g)

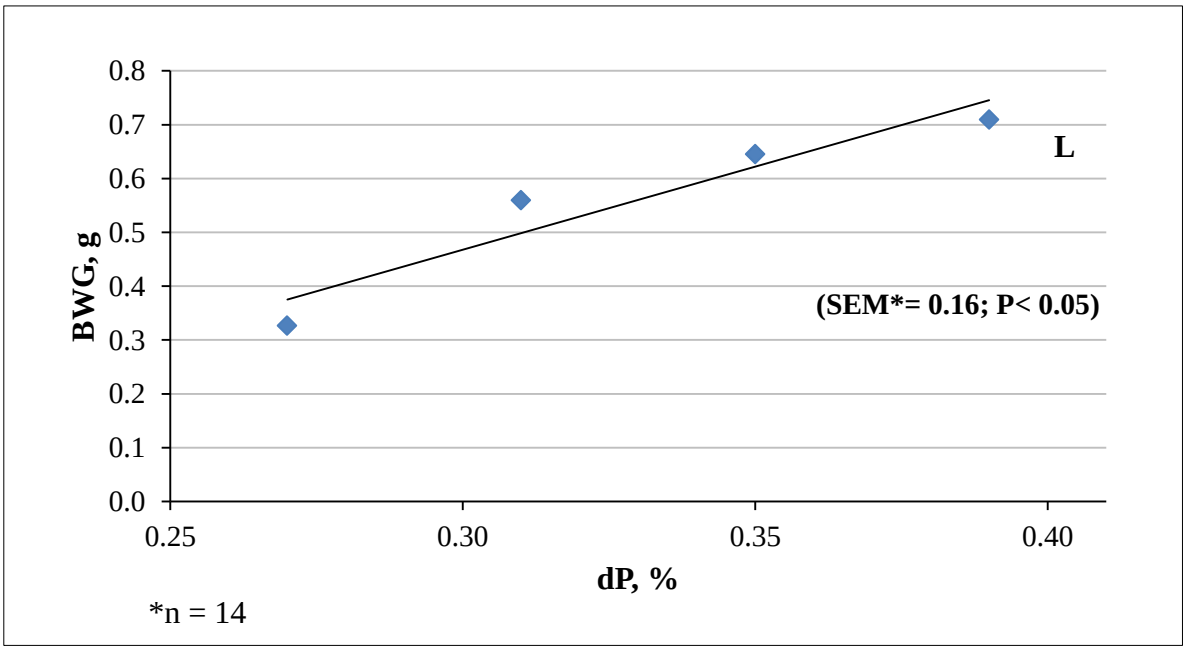

Figure 5. Effect of digestible phosphorus level (dP, %) on body weight gain (BWG, g)

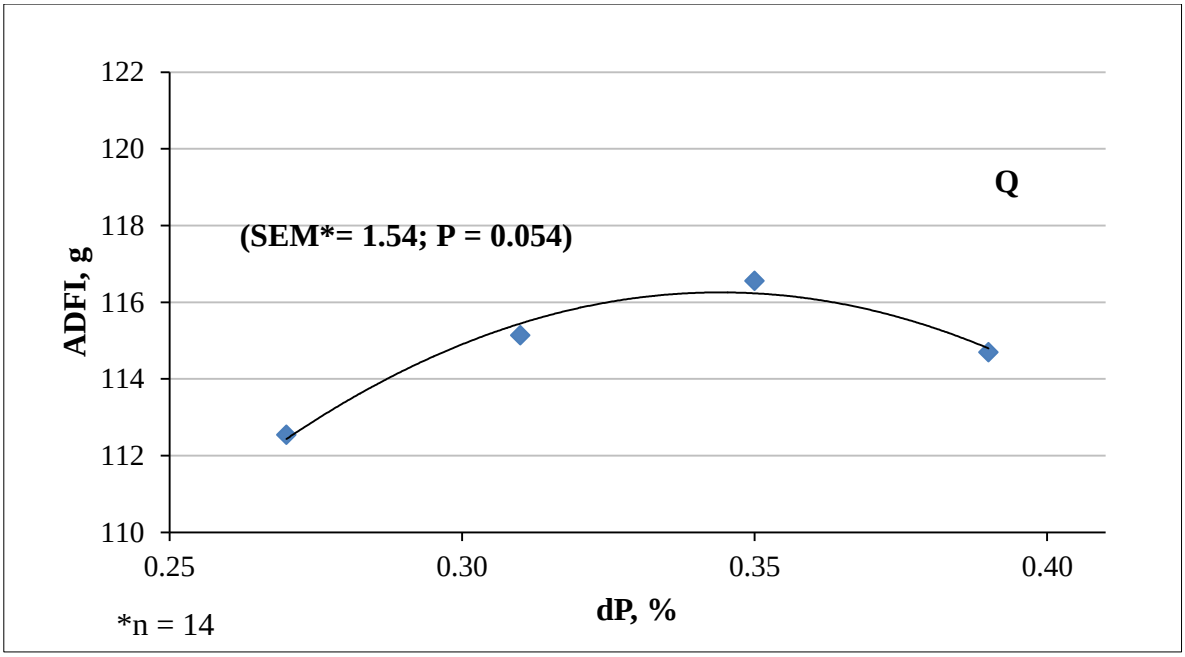

Figure 6. Effect of digestible phosphorus level (dP %) on average daily feed intake (ADFI, g) during the third period (72-75 wk)

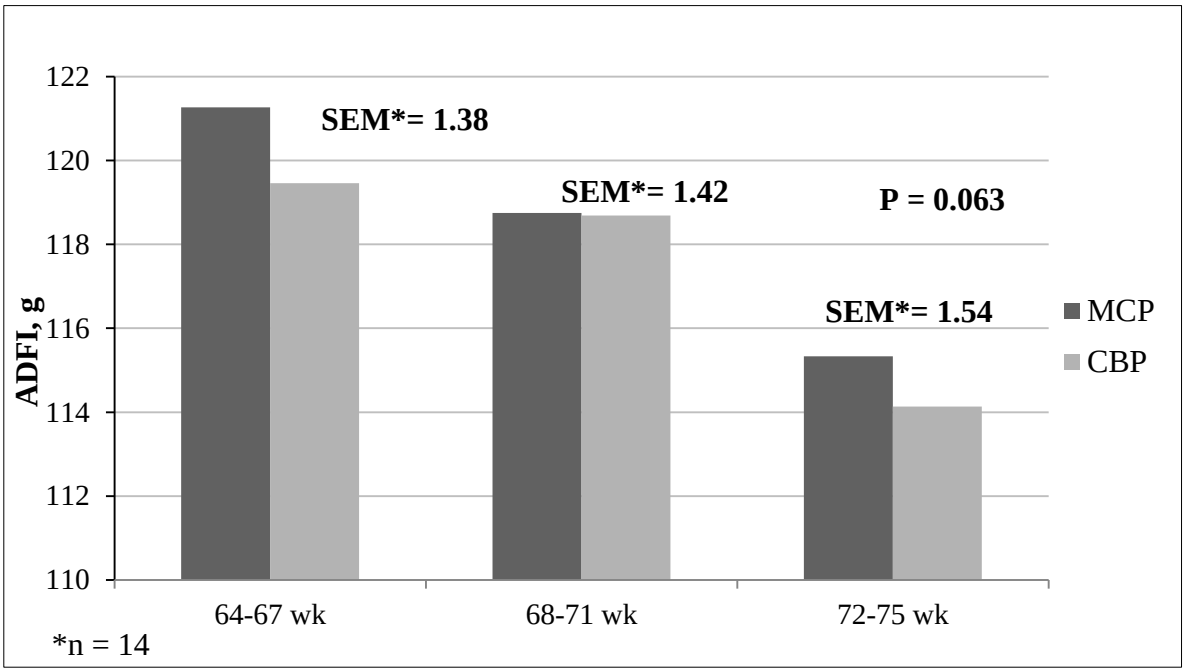

Figure 7. Interaction between phosphorus source [monocalcium phosphate (MCP) and calcined bone phosphate (CBP)] and age on average daily feed intake (ADFI, g)

5.2. Egg quality

The data on egg quality traits are shown in Table 10. An interaction trend between P source and dP level on shell resistance to breakage was observed from 68 to 71 wk (Figure 8). The use of MCP improved shell resistance to breakage in hens fed 0.31% dP but an opposite effect was observed at higher levels of dP inclusion ($P = 0.064$).

The level of dP in the feed did not affect the percentage of broken, dirty, and non-saleable eggs. However, shell resistance to breakage tended to decrease as the level of dP of the diet increased (L, $P = 0.077$; Q, $P = 0.058$) (Figure 9).

Phosphorus source did not affect shell resistance to breakage. Nevertheless, hens fed CBP tended to have a higher incidence of broken eggs for the whole experimental period than hens fed MCP ($P = 0.099$). Similarly, the percentage of dirty eggs tended to be higher for hens fed CBP than for hens fed MCP ($P= 0.089$). The percentage of non-saleable eggs was higher for hens fed CBP than for hens fed MCP ($P< 0.05$).

From 68 to 71 wk of age shell resistance to breakage significantly decreased (L; $P < 0.05$) with increasing of dP level from 0.27 to 0.39% (Figure 10).

The effects of the P source on the percentage of broken eggs were higher for hens fed CBP than for hens fed MCP ($P < 0.05$), and were more noticeable from 64 to 67 wk of age. Also, the negative effect on the percentage of non-saleable eggs observed in hens fed CBP was more noticeable from 64 to 71 wk of age ($P< 0.05$).

Shell resistance to breakage decreased with age throughout the trial ($P<0.001$) and an opposite effect was observed for percentage of broken ($P < 0.001$), dirty ($P < 0.001$), and non-saleable eggs ($P < 0.001$).

For the whole experimental period, an increase in dP level from 0.27 to 0.39% decreased HU (L, $P < 0.01$) (Figure 11). On the contrary, P source did not affect albumen quality. The adverse effect of dP level on HU was more evident from 72 to 75 wk of age (L; $P < 0.05$) than in the previous periods (Figure 12). Haugh units decreased significantly as the age of the hens increased ($P<0.001$).

Table 10. Effect of phosphorus source [monocalcium phosphate (MCP) vs calcined bone phosphate (CBP)] and level of inclusion of digestible phosphorus (dP, %) on Haugh Units and shell quality of eggs produced by brown laying hens from 64 to 76 weeks of age

	MCP, %				CBP, %				Phosphate		dP, %				SEM*	P**				
	0.27	0.31	0.35	0.39	0.27	0.31	0.35	0.39	MCP	CBP	0.27	0.31	0.35	0.39	n=14	1	2	3	4	5
Haugh Units																				
64-67 wk	91.7	88.4	89.2	85.2	89.6	86.4	88.9	85.9	88.6	87.7	90.7	87.4	89.1	85.5	3.11	0.676	0.156	0.938	0.836	
68-71 wk	83.1	85.3	83.4	78.8	85.2	84.2	80.2	84.1	82.6	83.4	84.2	84.7	81.8	81.4	1.86	0.545	0.076	0.753	0.154	
72-75 wk	82.7	80.9	81.3	78.9	84.8	82.3	77.1	79.1	80.9	80.8	83.7^{a}	81.6^{ab}	79.2^{ab}	79.0^{b}	2.32	0.932	<0.05	0.607	0.369	
Global	85.8	84.9	84.6	80.9	86.5	84.3	82.1	83.0	84.1	84.0	86.2^{a}	84.6^{ab}	83.4^{ab}	82.0^{b}	1.50	0.935	<0.01	0.988	0.462	<0.001
Shell resistance, Kg/cm²																				
64-67 wk	3.92	3.80	3.86	3.71	3.66	3.85	3.82	3.74	3.82	3.77	3.79	3.83	3.84	3.72	0.101	0.452	0.579	0.472	0.624	
68-71 wk	3.95	4.05	3.82	3.58	3.89	3.89	3.82	3.88	3.85	3.87	3.92^{a}	3.97^{a}	3.82^{ab}	3.73^{b}	0.095	0.771	<0.05	0.051	0.064	
72-75 wk	3.70	3.67	3.72	3.68	3.64	3.68	3.72	3.53	3.69	3.64	3.67	3.68	3.72	3.60	0.117	0.568	0.661	0.670	0.963	
Global	3.86	3.84	3.80	3.66	3.73	3.81	3.79	3.72	3.79	3.76	3.79	3.82	3.79	3.69	0.078	0.622	0.077	0.058	0.669	<0.001
Broken eggs, %																				
64-67 wk	0.89	0.90	1.06	1.42	2.23	2.35	1.42	1.97	1.07	1.99	1.56	1.62	1.24	1.70	0.632	<0.05	0.980	0.901	0.515	
68-71 wk	1.44	1.65	1.48	2.10	3.96	2.31	1.53	2.96	1.67	2.69	2.70	1.98	1.51	2.53	0.772	0.063	0.704	0.276	0.227	
72-75 wk	1.39	1.85	1.40	2.09	2.84	2.09	1.07	2.50	1.69	2.13	2.12	1.97	1.24	2.30	0.787	0.429	0.952	0.571	0.779	
Global	1.24	1.47	1.31	1.87	3.01	2.25	1.34	2.48	1.47	2.27	2.13	1.86	1.33	2.17	0.680	0.099	0.793	0.176	0.637	<0.001
Dirty eggs, %																				
64-67 wk	0.39	1.27	1.27	0.85	1.13	1.54	1.19	1.35	0.94	1.30	0.76	1.40	1.23	1.10	0.358	0.157	0.420	0.210	0.423	
68-71 wk	2.57	2.85	3.27	2.78	3.08	3.07	3.86	3.54	2.87	3.39	2.83	2.96	3.56	3.16	0.616	0.231	0.396	0.597	0.869	
72-75 wk	0.88	0.78	0.89	1.24	0.86	1.51	1.06	1.42	0.95	1.21	0.87	1.15	0.97	1.33	0.292	0.200	0.196	0.428	0.480	
Global	1.28	1.63	1.81	1.62	1.69	2.04	2.04	2.10	1.59	1.97	1.49	1.84	1.92	1.86	0.317	0.089	0.178	0.257	0.982	<0.001
Non-sealable eggs*, %**																				
64-67 wk	1.40	2.33	2.40	2.40	3.61	4.01	2.66	3.45	2.13	3.43	2.50	3.17	2.53	2.92	0.926	<0.05	0.802	0.945	0.537	
68-71 wk	4.17	4.61	4.80	5.05	7.29	5.75	5.62	6.64	4.66	6.33	5.73	5.18	5.21	5.85	1.00	<0.05	0.895	0.687	0.335	
72-75 wk	2.39	2.73	3.19	3.44	4.00	4.06	2.29	4.14	2.94	3.62	3.20	3.40	2.74	3.79	0.896	0.281	0.682	0.764	0.668	
Global	2.65	3.22	3.46	3.63	4.97	4.61	3.52	4.75	3.24	4.46	3.81	3.92	3.49	4.19	0.846	<0.05	0.668	0.699	0.620	<0.001

*SEM: n = 56 for main effect of P source; n = 28 for main effect of dP level; n = 14 for the interaction between P source and dP level.

**1: P source; 2: Lineal effect of dP level (%); 3: Quadratic effect of dP level (%); 4: P source*dP level; 5: Age of the hens. There were no significant interactions between, P source*Age, dP Level*Age and P source*dP Level*Age.

**∑ dirty, broken, and shell-less eggs.

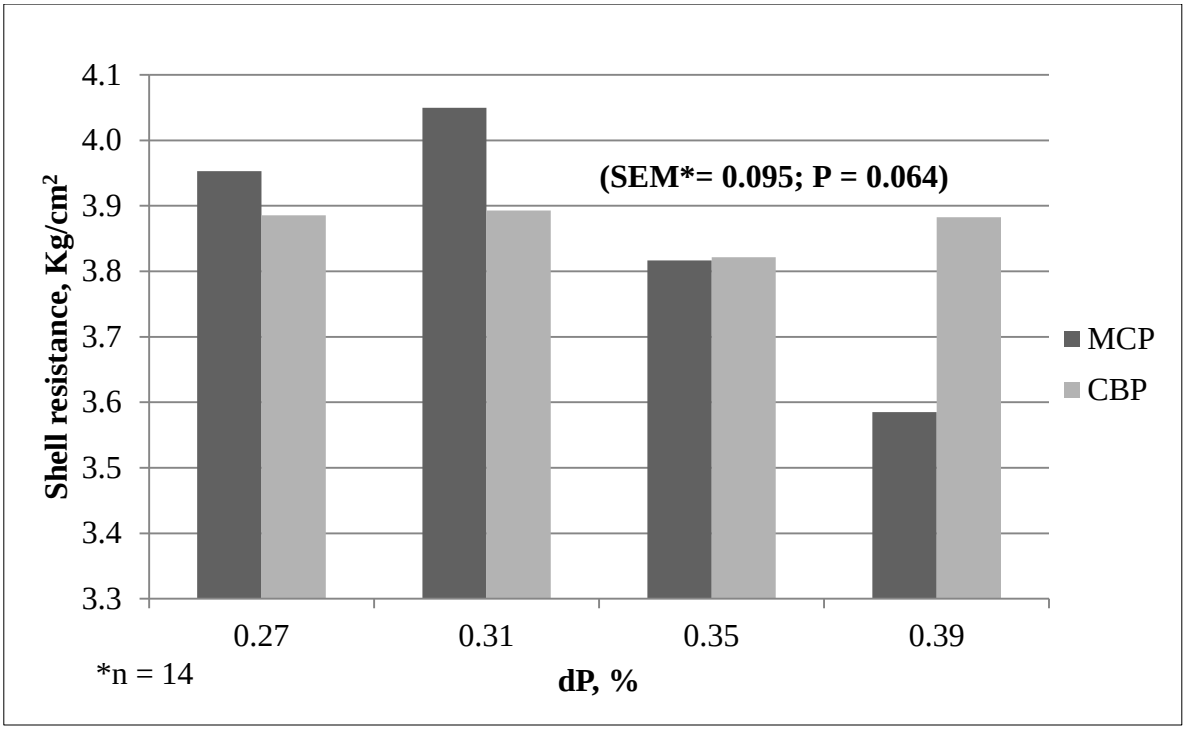

Figure 8. Interaction between source [monocalcium phosphate (MCP) and calcined bone phosphate (CBP)] and level of digestible phosphorus (dP, %) on shell resistance (Kg/cm^2) during the second period (68-71 wk)

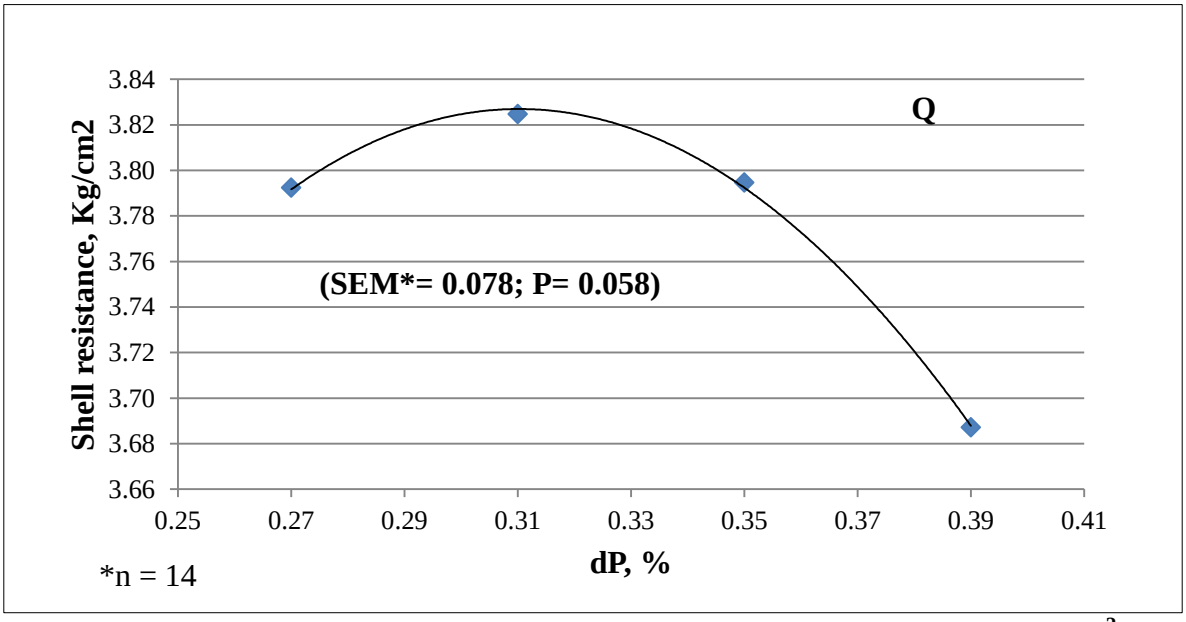

Figure 9. Effect of digestible phosphorus level (dP, %) on shell resistance (Kg/cm^2)

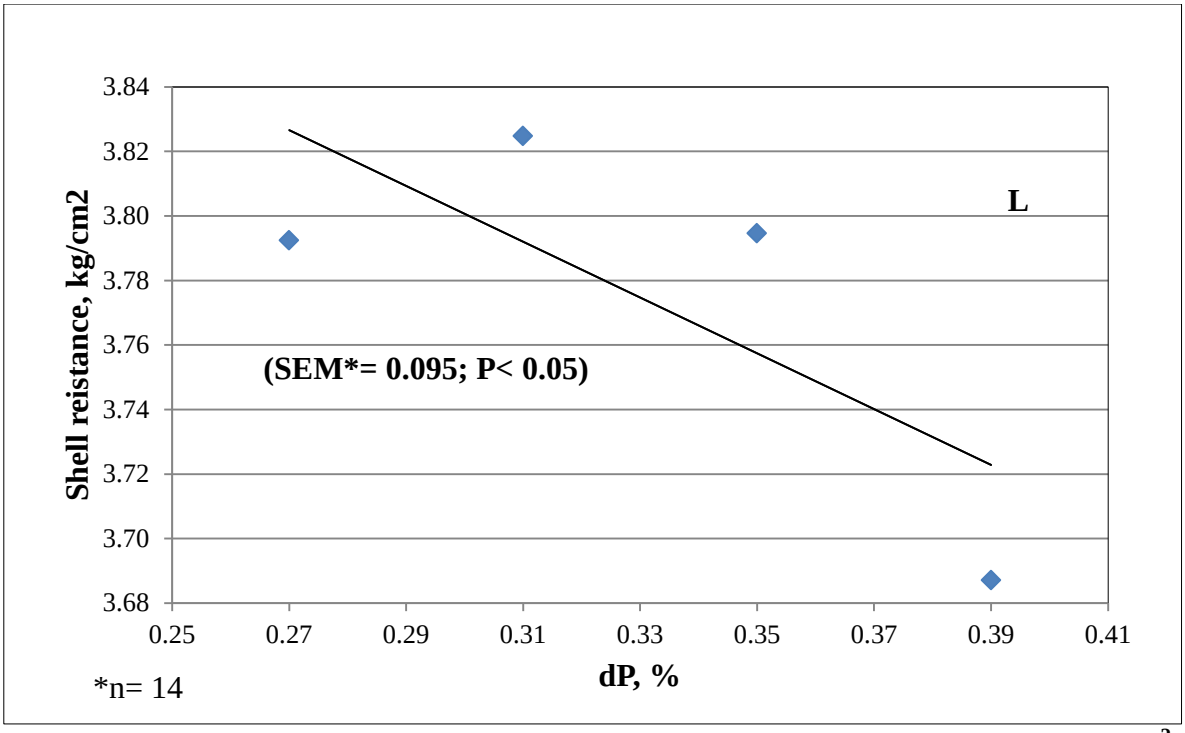

Figure 10. Effect of digestible phosphorus level (dP, %) on shell resistance (Kg/cm^2) during the second period (68-71 wk)

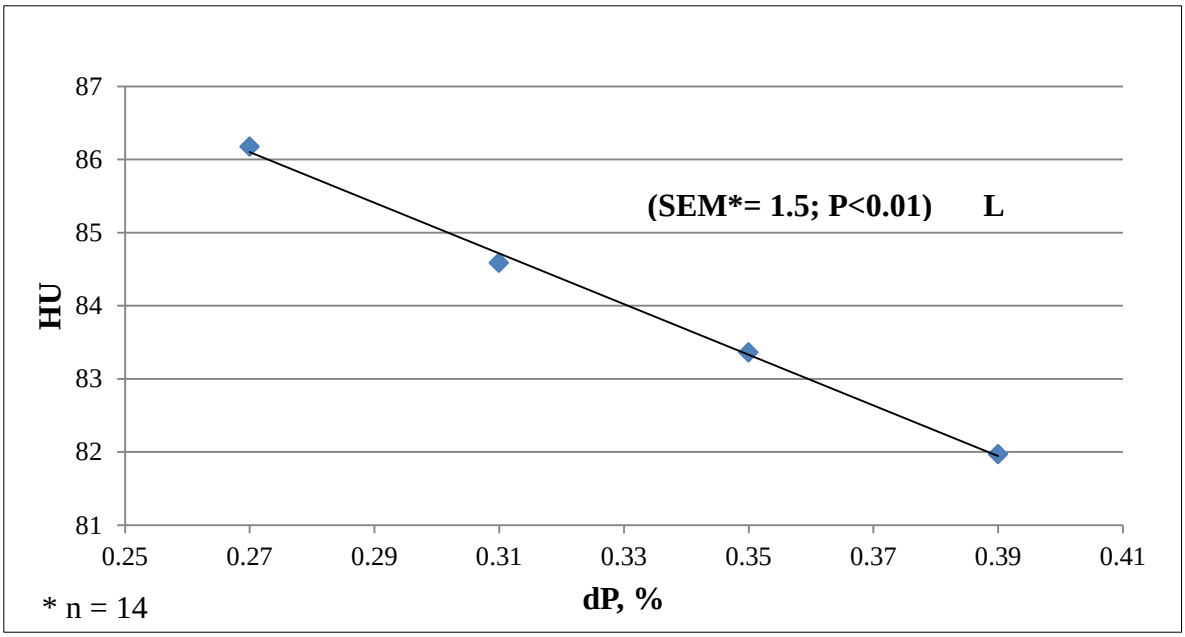

Figure 11. Effect of digestible phosphorus level (dP, %) on Haugh units (HU)

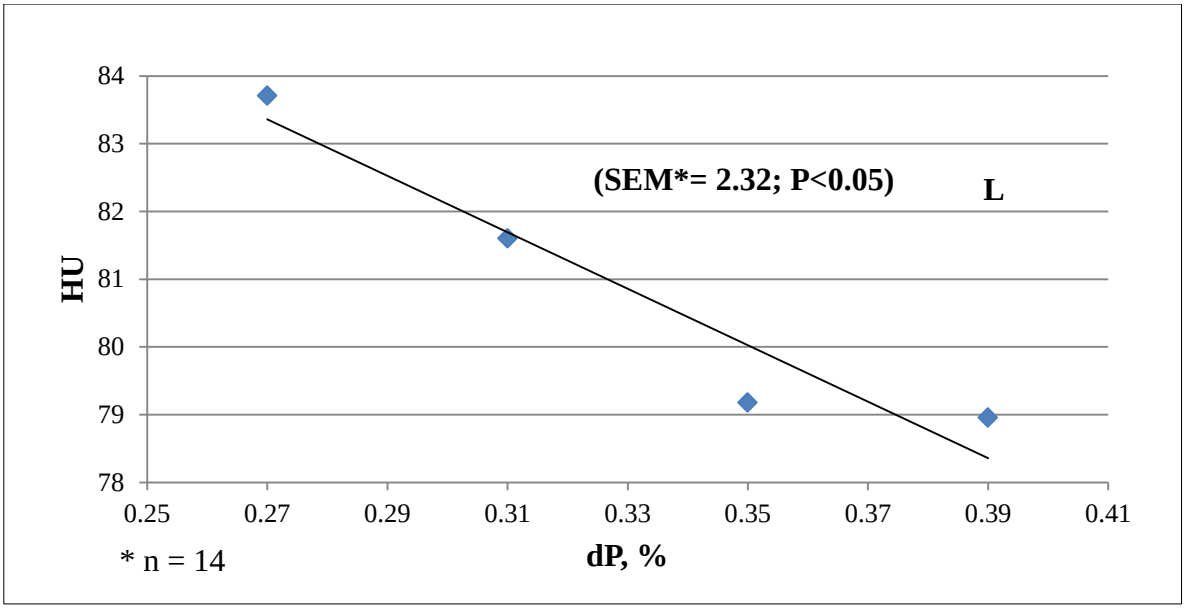

Figure 12. Effect of digestible phosphorus level (dP, %) on Haugh units (HU) during third period (72-75 wk)

6. DISCUSSION

6.1. Productive performance

In the current experiment, none of the productive traits studied (EP, EM, and FCR) was affected by the level of dP of the diet. Moshtaghian et al. (1991) reported a decrease in EP and EM when hens where fed diets with 0.10 or 1.80% NPP, compared with hens fed diets with 0.45% NPP. Boling et al. (2000) reported a rapid decrease in EP in old hens fed a diet containing 0.10% AP without added phytase. However, no differences were observed when a phytase was included in the diets. Keshavarz (2000a) also did not report any effect of NPP level on EP, EM and FCR of hens fed diets with supplemental phytases and 0.15, 0.10, and 0.10% NPP from 30 to 42 wk, 42 to 54 wk, and 54 to 66 wk, respectively, compared to a control group fed 0.40, 0.35, and 0.30% NPP for each phase, with no phytases used. Even in young hens, recent investigation suggest that, a level of 0.15% AP seems to be sufficient to maintain a good EP at least from 22 to 34 wk of age (Jing et al., 2018).

Egg weight was not significantly affected by the level of dP of the diet. However, EW tended to be greater in hens fed 0.31 or 0.35% dP diets (corresponding with 0.32 and 0.36% AP) than in hens fed diets with 0.27 or 0.37% dP. These results are in agreement with data of Skřivan et al. (2010) that reported that a level of 0.30% AP, but not of 0.20%, ensures normal EW and performance of laying hens from 47 to 61 wk of age. Nevertheless, Bar et al. (2002) reported no effect of P level on EW in Lohmann hens from 66 to 78 wk of age, fed diets containing 0.43, 0.59, and 0.75% total dietary P.

The BWG increased as the level of dP of the diet increased. Similarly, Keshavarz (2000a) reported higher BWG in hens fed a NPP regime of 0.40, 0.35, and 0.30% from 30 to 42, 42 to 54 and 54 to 66 wk of age, respectively, than in hens fed 0.15, 0.10, and 0.10% NPP for each phase. However Jing et al. (2018) in younger hens, and Bar et al. (2002) in hens from 66 to 78 wk, reported no significant effect of P level on BWG, when hens were fed diets containing 0.15 to 0.45% AP, or 0.43 to 0.75% total dietary P, respectively.

In the current experiment, hens fed diets with 0.35% dP tended to increase ADFI. In contrast, Keshavarz (2000a) did not observe any significant effect of NPP level on feed intake in hens fed diets with 0.10 to 0.30% NPP from 54 to 66 wk of age. Similarly, Jing et al. (2018) also reported no effect of AP level on ADFI in hens fed diets with 0.15 to 0.45% AP from 22 to 34 wk of age.

In the current study, dP at the lower level used (0.27%) had no effect on hen performance. In contrast, the trends for EW and ADFI reported that a dP level below 0.31% may be too low, and a value proximate or over 0.41% may be excessive, and both situations penalized laying hens performance. These results are in agreement with those of Ahmadi and Rodehutscord (2012) that reported a quadratic response of laying hens performance to increasing levels of NPP in the diets, resulting in a penalization on performance at a low or at an excessive P supplementation.

No significant effects of P source on any of the productive traits studied were observed in the current experiment. Ahmadi and Rhodehutscord (2012) conducted a meta-analysis revision about P requirements in laying hens. The authors reported that the most used P sources in laying hens studies were mono-dicalcium phosphate, dicalcium

phosphate (DCP) and MCP, but their data set contained not enough observation to estimate a variance component for the source of P. Most P studies in laying hens focus on P level, P-Ca interactions or supplemental phytases effects, rather than on the P source used in the diets.

The results of the current experiment agree with data obtained comparing deflourinated phosphate (DFP) and DCP (Vandepopuliere and Lyons, 1992). Nevertheless, this study found significant differences on ADFI of hens fed different phosphates. A recent study (Skřivan et al., 2010) used either DCP or MCP as sources of P in laying hen diets, without finding any significant effect of P source on productive performance of the hens.

Rama Rao et al. (2019) tested a P source of animal origin (DCP obtained from the residue of gelatin extraction from bones), in the diets of laying hens from 54 to 72 wk. The authors did not report any effect on productive performance of the hens related to the use of this source of P, in agreement with our results.

Similar type of CBP used in the current experiment was tested recently in broilers (Van Harn et al., 2017) to determine the pre-cecal digestibility, compared to other P sources as MCP, DCP and other bones phosphates. The authors reported no significant effect of the phosphate used on productive performance of broilers from 14 to 24 d of age.

As expected, productive performance of the hens declined with age, as indicated by Lambert et al. (2014), resulting in a decrease of EP, EM and ADFI. On the other hand, and as expected, EW increased with the age (Roberts, 2004).

6.2. Egg quality

The treatments that contained 0.27 and 0.31% dP, showed a higher shell resistance to breakage when the hens were fed MCP. However, when the dP level of the diet increased to 0.35 and 0.39%, shell resistance to breakage decreased in hens fed MCP, being similar or higher for the eggs laid by hens fed CBP. This effect was more accentuated during the second period (68 to 71 wk) and may imply that the P digestibility rate assumed for CBP (86.9%, against 88.5% digestibility for MCP) in this experiment were overestimated.

In the present experiment, the number of eggs with shell defects, classified as broken, dirty and non-saleable (the sum of broken, dirty and shell-less eggs), were not affected by the level of dP of the diet. Against these findings, Vandepopuliere and Lyons (1992), in laying hens from 48 to 63 wk of age, found a higher number of eggs classified as broken and thin-shelled in hens fed diets containing 0.50% total P than in hens fed diets containing 0.40% total P. In addition, these authors found that an increase in the total P level from 0.40 to 0.70% decreased the shell quality at least from 38 to 54 wk of age.

The maximum shell resistance to breakage was observed with a level of 0.31% dP, whereas the minimum value was observed with a level of 0.39% dP (corresponding to 0.32% and 0.40% AP, respectively). Skřivan et al. (2010) reported a significant decrease in shell resistance to breakage when the level of AP of the diet increased from 0.25 to 0.41%, in laying hens from 47 to 59 wk of age.

Haugh units are often used as a measure of the internal quality of the egg, as they are obtained as a relation between albumen height and egg weight, and represent the freshness of the albumen (Roberts, 2004). Skřivan et al. (2010), in hens from 47 to 59 wk

of age, reported that HU reached the optimum value with 0.20 to 0.30% AP and decreased when the AP of the diet was higher. In the current study, HU decreased linearly when the level of AP of the diet increased from 0.28 to 0.40% (0.27 to 0.39% dP). These results are in agreement with the results reported by Englmaierová et al. (2014), who showed that HU decreased when the AP level increased from 0.20 to 0.30% in the diet of hens from 37 to 49 wk of age. Conversely, Um and Paik (1999) did not detect significant differences in HU of eggs laid by hens fed diets containing from 0.17 to 0.30% NPP, from 21 to 40 wk of age.

Phosphorus source had no effect on shell resistance to breakage or HU. In the same line Skřivan et al. (2010) did not find any effect of the P source (either DCP or MCP) on external and internal quality parameters of eggs laid by hens from 47 to 59 wk of age. Also, in a study previously mentioned (Rama Rao et al., 2019), where hens from 54 to 72 wk of age were fed with DCP from bones, authors did not report any effect on egg quality traits due to the P source used.

Nevertheless, the percentage of broken and dirty eggs tended to be higher and, the percentage of non-saleable eggs was significantly higher for hens fed diets with CBP. Vandepopuliere and Lyons (1992) reported a significant interaction between the P source and the P level of the diet on the number of thin-shell and broken eggs layed by hens fed either coarse DFP or DCP, based on on-farm observation. Specifically, there were more eggs with quality defects laid by hens fed DFP, when hens were fed diets containing 0.5% total P in one experiment, and 0.4% total P in another experiment; meaning that maybe DFP was less available for the hens than DCP. The results obtained in the present experiment indicate that the availability or the digestibility of P of the CBP may be slightly

lower than that of MCP. Probably we overestimated these values when assuming the results reported by Van Harn et al. (2017).

The external quality of the eggs was impaired with age with a decrease in shell resistance to breakage and an increase in the percentage of broken, dirty and non-saleable eggs. The reduction in shell quality with age was expected due to the decrease in Ca absorbability (Bar et al., 2002) and the increase in egg size but not in the amount of shell deposited per day, resulting in eggs with thinner (and often weaker) shells (Roberts, 2004).

In accordance with Roberts (2004), HU were affected by the age of the hens decreasing with age throughout the trial.

7. CONCLUSIONS

We conclude that a level of dP of 0.27% in the diet may satisfy the requirements of P for old hens, allowing to maintain good productive performance and adequate egg quality.

Moreover, despite the similar response of the hens to both sources of P for egg production, the negative effects of CBP on the percentage of non-saleable eggs suggest that the P of this source was probably less available or digestible for the hens than that of MCP. New studies are necessary to document the bioavailability of P sources derived from bones, in comparison with the phosphates of common use in hen diets.

8. REFERENCES

AEB (American Egg Board). (2017) Nutrient composition tables. aeb.org. Consulted in May 2019.

Ahmadi, H; Rodehutscord, M. (2012) A meta-analysis of responses to dietary nonphytate phosphorus and phytase in laying hens. *Poult. Sci.* 91, 2072–2078.

Angel, R; Saylor, WW; Dhandu, AS; Powers, W; Applegate, TJ. (2005) Effects of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on performance of broiler chickens grown in floor pens. *Poult. Sci.* 84, 1031-1044.

Angel, R; Saylor, WW; Jimenez-Moreno, E; Li, W; Kim, SW; Proskowiek-Weglarz, M; Escribano, F. (2015) Dietary calcium, phosphorus and phytase interactions. *FEDNA* 31, 111-121.

Angel, R; Saylor, WW; Mitchell, AD; Powers W; Applegate, TJ. (2006) Effect of dietary phosphorus, phytase, and 25- hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. *Poult. Sci.* 85, 1200-1211.

AOAC International. (2005) Official Methods of Analysis, 18th ed. AOAC Int., Gaithersburg, MD, USA.

ASAE (American Society of Agriculture Engineers). (1995) Method of determining and expressing fineness of feed material by sieving. Standard S319.2. In: Agriculture Engineers Yearbook of Standard. ASAE, St Joseph, USA.

Bar, A; Razaphkovsky, V; Vax, E. (2002) Re-evaluation of calcium and phosphorus requirements in aged laying hens. *Br. Poult. Sci.* 43, 261–269.

Bikker, P; Spek, JW; Van Emous RA; Van Krimpen, M. (2016) Precaecal phosphorus digestibility of inorganic phosphate sources in male broilers. *Br. Poult. Sci.* 57, 810-817.

Boletín Oficial del Estado. (2013) Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. BOE 34, 11370-11421.

Boling, SD; Douglas, MW; Johnson, ML; Wang, X; Parsons, CM; Koelkebeck, KW; Zimmerman, RA. (2000) The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. *Poult. Sci.* 79, 224–230.

Boorman, KN; Gunaratne, SP. (2001) Dietary phosphorus supply, egg-shell deposition and plasma inorganic phosphorus in laying hens. *Br. Poult. Sci.* 42, 81-91.

CVB (Central Veevoeder Bureau). (2018a) Chemichal Composition and Nutritional Values of Feedstuffs. Wageningen, The Netherlands.

CVB (Central Veevoeder Bureau). (2018b) Table Booklet Feeding of Poultry. Wageningen, The Netherlands.

De Vries, S; Kwakkel, RP; Dijkstra, J. (2010) Dynamics of calcium and phosphorus metabolism in laying hens. In: Vitti, DMSS; Kebreab, E. (Eds) Phosphorus and Calcium Utilization and Requirements in Farm Animals. CABI Publishing, Wallingford, UK.

Englmaierová, M; Skřivanová, V; Skřivan, M. (2014) The effect of non-phytate phosphorus and phytase levels on performance, egg and tibia quality, and pH of the digestive tract in hens fed higher-calcium-content diets. *Cze. J. Anim. Sci.* 59, 107-115.

European Commission. (2008) Commission Regulation No 589/2008 of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs. OJ L 163, 6–23.

European Commission. (2019) Agricultural and Rural Development – Eggs. ec.europa.eu. Consulted in April 2019.

FAO (Food and Agriculture Organization of the United Nations). (2006) Livestock's long shadow: Environmental issues and options. Rome: Food and Agriculture Organization of the United Nations. Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations). (2008) Poultry in the 21st Century: Avian Influenza and Beyond. Thieme, O; and Pilling, D (Eds); FAO Animal Production and Health Proceedings, No. 9. Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations). (2013) Poultry Development Review. fao.org. Consulted in January 2019.

FAO (Food and Agriculture Organization of the United Nations). (2015) El huevo en cifras. fao.org. Consulted in January 2019.

FAO (Food and Agriculture Organization of the United Nations). (2018a) Animal Production. fao. org. Consulted in March 2019.

FAO (Food and Agriculture Organization of the United Nations). (2018b) Gateway to poultry production and products. fao. org. Consulted in March 2019.

FEDNA (Fundación Española para el Desarrollo de la Nutrición Animal). (2010) Tablas FEDNA de Composición y Valor Nutritivo de Alimentos para la Fabricación de Piensos Compuestos. 3ª ed. De Blas, C; Mateos, GG; Rebollar, PG. (Eds) Madrid, Spain.

FEDNA (Fundación Española Desarrollo Nutrición Animal). (2018) Normas FEDNA-Necesidades Nutricionales para Avicultura. 2ª ed: Santomá, G; Mateos, GG. (Eds) Madrid, Spain.

Gerber, PJ; Steinfeld, H; Henderson, B; Mottet, A; Opio, C; Dijkman, J; Falucci, A; Tempio, G. (2013) Enfrentando el cambio climático a través de la ganadería: una evaluación global de las emisiones y oportunidades de mitigación. Roma: Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO). Rome, Italy.

Gorrachategui, M. (2018) Actualización de la tercera edición de las tablas FEDNA: minerales y aditivos. *FEDNA* 34, 43-78.

Herrera, J. (2017) Influence of particle size of the cereal of the diet on egg production, egg quality, and the development of the gastrointestinal tract of brown-egg laying hens. PhD Thesis, Universidad Politécnica de Madrid.

Herrera, J; Saldaña, B; Cámara, L; Berrocoso, JD; Mateos, GG. (2018) Influence of grinding size of the main cereal of the diet on egg production and eggs quality of brown egg laying hens from 33 to 65 weeks of age. *Poult. Sci.* 97, 2506-2515.

ISA Brown. (2019) Nutrition management guide. Hendrix Genetics. Boxmeer, the Netherlands.

Jing, M; Zhao, S; Rogiewicz, A; Slominski, BA; House, JD. (2018) Assessment of the minimal available phosphorus needs of laying hens: Implications for phosphorus management strategies. *Poult. Sci.* 97, 2400-2410.

Keshavarz, K. (1998) Investigation on the possibility of reducing protein, phosphorus, and calcium requirements of laying hens by manipulation of time of access to these nutrients. *Poult. Sci.* 77, 1320-1332.

Keshavarz, K. (2000a) Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poult. Sci.* 79, 748-763.

Keshavarz, K. (2000b) Reevaluation of nonphytate phosphorus requirement of growing pullets with and without phytase. *Poult. Sci.* 79, 1143-1153.

Keshavarz, K; Austic, RE. (2004) The use of low-protein, low phosphorus, amino acid- and phytase-supplemented diets on laying hen performance and nitrogen and phosphorus excretion. *Poult. Sci.* 83, 75-83.

Lambert, W; de Lange, L; Van Krimpen, MM; Van der Klis, J.D; Star, L. (2014) P-Requirement in modern laying hens - a literature review. Report nº 1326. Schothorst Feed Research B.V. Livestock Research Wageningen, The Netherlands.

Li, W; Angel, R; Kim, SW; Brady, K; Yu, S; Plumstead, PW. (2016) Impacts of dietary calcium, phytate, and nonphytate phosphorus concentrations in the presence or absence of phytase on inositol hexakisphosphate (IP6) degradation in different segments of broilers digestive tract. *Poult. Sci.* 95, 581-589.

Li, W; Angel, R; Kim, SW; Jiménez-Moreno, E; Proszkowiec-Weglarz, M; Plumstead, PW. (2018) Impacts of age and calcium on Phytase efficacy in broiler chickens. *Anim. Feed Sci. Technol.* 238, 9-17.

Lohmann. (2018) Lohmann LSL - Classic - Guía de Manejo Sistemas de Jaulas. Cuxhaven, Germany.

Maenz, DD; Classen, HL. (1998) Phytase activity in the small intestinal brush border membrane of the chicken. *Poult. Sci.* 77, 557-563.

MAPAMA (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente). (2017) Caracterización del Sector Avícola de Puesta en España. Año 2016. Madrid, Spain.

MAPAMA (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente). (2018) El Sector de la Avicultura de Puesta en Cifras. Principales Indicadores Económicos en 2017. Madrid, Spain.

Moshtaghian, J; Parsons, CM; Leeper, RW; Harrison, PC; Koelkebeck, KW. (1991) Effect of sodium aluminosilicate on phosphorus utilization by chicks and laying hens. *Poult. Sci.* 70, 955–962.

National Research Council (NRC). (1994) Nutrient Requirements of Poultry: Ninth Revised Edition. The National Academies Press. Washington DC, USA.

Penn, CJ; Mullins, GL; Zelazny, LW; Warren, JG; McGrath, JM. (2004) Surface runoff losses of phosphorus from Virginia soils amended with turkey manure using phytase and high available phosphorus corn diets. *J. Environ. Qual.* 33, 1431-1439.

Pérez-Bonilla, A; Frikha, M; Mirzaie, S; García,J; Mateos, GG. (2011) Effects of the main cereal and type of fat of the diet on productive performance and egg quality of brown egg-laying hens from 22 to 54 weeks of age. *Poult. Sci.* 90, 2801-2810.

Rama Rao, SV; Raju, MVLN; Paul, SS; Prakash, B. (2019) Effect of supplementing graded concentrations of non-phytate phosphorus on performance, egg quality and bone mineral variables in White Leghorn layers. *Br. Poult. Sci.* 60, 56-63.

Roberts, JR. (2004) Factors affecting egg internal quality and eggshell quality in laying hens. *J. Poult. Sci.* 41, 161-177.

Rodehutscord, M. (2009) Approaches and challenges for evaluating phosphorus sources for poultry. Pages 2–6 in Proc. 17th Eur. Sym. Poult. Nutr. WPSA UK branch, Edinburgh, UK.

Rodehutscord, M; Adeola, O; Angel, R; Bikker, P; Delezie, E; Dozier, WA; Umar Faruk, M; Francesch, M; Kwakernaak, C; Narcy, A; Nyachoti, CM; Olukosi, OA. (2017) Results of an international phosphorus digestibility ring test with broiler chickens. *Poult. Sci.* 96, 1679-1687.

Rodehutscord, M; Dieckmann, A; Witzig, M; Shastak, Y. (2012) A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. *Poult. Sci.* 91, 965-971.

Rodehutscord, M; Rosenfelder, P. (2016) Update on phytate degradation pattern in the gastrointestinal tract of pigs and broiler chickens. In: Walk, CL; Kühn, I; Stein, HH; Kidd, MT; Rodehutscord, M. (Eds) Phytate destruction-consequences for precision animal nutrition. Wageningen Academic Publishers, Wageningen, the Netherlands.

Rodehutscord, M; Rückert, C; Maurer, HP; Schenkel, H; Schipprack, W; Bach Knudsen, BE; Schollenberger, M; Laux, M; Eklund, M; Siegert, W; Mosenthin, R. (2016) Variation in chemical composition and physical characteristics of cereal grains from different genotypes. *Arch. Anim. Nutr.* 70, 87-107.

Rose, SP. (1997) Principles of Poultry Science. CAB International, Wallingford, UK.

Rostagno, HS; Albino, LFT; Hannas, MI; Donzele, JL; Sakomura, NK; Perazzo, FG; Saraiva, A; de Abreu, MLT; Rodrigues, PB; de Oliveira, RF; Barreto, SLT; Brito, CO. (2017) Brazilian Tables for Poultry and Swine. Feedstuff Composition and Nutritional Requirements. 4th Edition. Rostagno, HS (Ed). Department of Animal Science, Federal University of Viçosa, Brazil.

Safaa, H; Serrano, MP; Valencia, DG; Frikha, M; Jiménez-Moreno, E; Mateos, GG. (2008) Productive performance and egg quality of brown egg-laying hens in the late phase of production as influenced by level and source of calcium in the diet. *Poult. Sci.* 87, 2043-2051.

SAS Institute. 2004. SAS/STAT User's Guide, Version 9,1, SAS Inst. Inc., Cary, NC.

Shastak, Y; Rodehutscord, M. (2013) Determination and estimation of phosphorus availability in growing poultry and their historical development. *World's Poult. Sci. J.* 69, 569-586.

Shastak, Y; Witzig, M; Hartung, K; Rodehutscord, M. (2012). Comparison of retention and prececal digestibility measurements in evaluating mineral phosphorus sources in broilers. *Poult. Sci.* 91, 2201-2209.

Skřivan, M; Englmaierova, M; Skřivanova, V. (2010) Effect of different phosphorus levels on the performance and egg quality of laying hens fed wheat- and maize-based diets. *Czech J. Anim. Sci*. 55, 420–427.

Sommerfeld, V; Schollenberger, M; Kühn, I; Rodehutscord, M. (2018) Interactive effects of phosphorus, calcium, and phytase supplements on products of phytate degradation in the digestive tract of broiler chickens. *Poult. Sci.* 97, 1117-1118.

Sparks, NHC. (2006) The hen's egg - is its role in human nutrition changing? *World's Poult. Sci. J.* 62, 308-315.

Um JS; Paik IK. (1999) Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. *Poult. Sci.* 78, 75-79.

Van Harn, J; Spek, JW; van Vuure, CA; van Krimpen, MM. (2017) Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers. *Poult. Sci.* 96, 1334-1340.

Vandepopuliere, JM; Lyons, JJ. (1992) Effect of inorganic phosphate source and dietary phosphorus level on laying hen performance and eggshell quality. *Poult. Sci.* 71, 1022-1031.

Vieira, B; Caramori, J; Oliveira, C; Correa, G. (2018) Combination of phytase and organic acid for broilers: role in mineral digestibility and phytic acid degradation. *World's Poult. Sci. J.* 74, 711-726.

Waldroup, PW. (1999) Nutritional approaches to reducing phosphorus excretion by poultry. *Poult. Sci.* 78, 683-691.

Waldroup, PW; Kersey, JH; Saleh, EA; Fritts, CA; Yan, F; Stilborn, HL; Crum Jr, RC; Raboy, V. (2000). Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. *Poult. Sci.* 79, 1451-1459.

Walk, CL; Santos, TT; Bedford, MR. (2014) Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. *Poult. Sci.* 93, 1172-1177.

Witzig, M; Inglemann, CJ; Möhring, J; Rodehutscord, M. (2018) Variability of prececal phosphorus digestibility of triticale and wheat in broiler chickens. *Poult. Sci.* 97, 910-919.