UNIVERSIDAD POLITÉCNICA DE MADRID

**ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRÓNOMICA,
ALIMENTARIA Y DE BIOSISTEMAS**

DEPARTAMENTO DE PRODUCCIÓN AGRARIA

**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**

TESIS DOCTORAL

JORGE HERRERA LLORENTE

VETERINARIO

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ABBREVIATIONS

ABBREVIATIONS

AA	Amino acid
ADFI	Average daily feed intake
AMEn	Apparent metabolisable energy, corrected for nitrogen
BMI	Body mass index
BW	Body weight
cm	Centimeter
CF	Crude fiber
CP	Crude protein
d	Day
DM	Dry matter
EE	Ether extract
EMAn	Energía metabolizable aparente, corregida en nitrógeno
ES	Enzyme supplementation
EU	European union
FCR	Feed conversion ratio
FI	Feed intake
g	Gram
GIT	Gastrointestinal tract
GMD	Geometric mean diameter
GSD	Geometric standard deviation
h	Hour
kg	kilogram
LNL	Linoleic acid
mm	Millimeters
MT	Million tons

NDF	Neutral detergent fiber
NSP	Non-starch polysaccharides
SCWL	Single comb white leghorn
SEM	Standard error of the mean
TSAA	Total sulfur amino acids
μm	Micrometer
wk	Week

RESUMEN

RESUMEN

El objetivo general de esta tesis doctoral fue estudiar los efectos del tamaño de la criba utilizado para moler el cereal principal del pienso sobre el consumo, los rendimientos productivos y las características del tracto gastrointestinal (TGI) en gallinas ponedoras rubias. Con este proposito, se estudió la inclusión de maíz, trigo y cebada en piensos de gallinas ponedoras en tres experimentos.

En el experimento 1 (capítulo 2), se estudió la influencia del tamaño de partícula del cereal principal del pienso en la producción y la calidad del huevo en gallinas ponedoras rubias de 33 a 65 sem de edad. El experimento se diseñó completamente al azar, con 6 tratamientos organizados en un factorial 3 x 2 en base a 3 cereales (cebada, maíz y trigo) y 2 tamaños de molienda del cereal (criba de 6 y 10 mm). Cada tratamiento se replicó 11 veces (10 gallinas por repetición). Las dietas se formularon para ser isonutritivas y contenían 2.740 Kcal/kg EMAn y 16.8% de proteína bruta. La producción de huevos, el consumo de pienso, el peso del huevo y el índice de conversión se calcularon por período (4 sem) y para el global del experimento. Así mismo se midieron los parámetros de calidad del huevo: porcentaje de huevos sucios, rotos y sin cáscara; proporción de albumen, yema y cáscara y espesor, fuerza y color de la cáscara al final de cada período. Al final de la prueba, no se observaron interacciones entre el cereal principal y el diámetro geométrico medio del pienso para ninguna de las variables estudiadas. Ni el pienso ni el tamaño de partícula afectaron el consumo de pienso, la producción de huevos o la ganancia de peso corporal. Los huevos fueron más pesados ($P < 0,01$) en las gallinas alimentadas con cebada que en las gallinas alimentadas con maíz o trigo, probablemente debido al mayor contenido de grasa de los piensos de cebada. Además, el Índice de Conversión tendió a ser mejor ($P < 0,07$) en las

gallinas alimentadas con cebada que en las gallinas alimentadas con maíz o trigo. El tipo de pienso no afectó a ninguno de los parámetros de calidad del huevo. En resumen, cebada y trigo convenientemente suplementados con enzimas, pueden utilizarse en sustitución del maíz a niveles de hasta el 55%, en piensos para gallinas ponedoras, sin efecto adverso alguno sobre la producción o los parámetros de calidad del huevo. Además, la sustitución del maíz por una combinación de cebada y grasa aumentó el tamaño del huevo. En consecuencia, el uso de uno u otro cereal del pienso dependerá principalmente de su coste relativo y de la preferencia del mercado por el huevo grande. Dentro del rango estudiado, el tamaño de partícula de los cereales no afectó a la producción de las aves.

En el experimento 2 (capítulo 3), se estudió la influencia del tamaño de la criba utilizado para moler el cereal sobre la producción de huevos, el desarrollo del TGI y las medidas corporales en gallinas de 17 a 49 sem de edad. Se utilizaron 10 piensos, utilizando un factorial 2×5 con 2 cereales principales (maíz vs. cebada) y 5 tamaños de criba del cereal (4, 6, 8, 10 y 12 mm). Cada tratamiento se replicó 5 veces (10 gallinas por repetición). No se observaron interacciones entre el cereal principal y el tamaño de la criba, para ninguno de los factores estudiados. El tipo de cereal y el tamaño de la criba no afectaron al consumo de pienso, la producción de huevos, o ganancia de peso vivo o parámetros de calidad de los huevos. Los huevos tendieron a ser más grandes ($P = 0.09$) en las gallinas alimentadas con cebada que en las gallinas alimentadas con maíz. Además, el índice de conversión tendió a aumentar ($P = 0,08$) al moler el cereal del pienso con una criba de 4 mm comparado con la media de los otros piensos. A las 49 sem de edad, el peso relativo (% BW) del TGI y de la molleja fue superior ($P < 0,05$) en las gallinas alimentadas con cebada que en las alimentadas con maíz. Un aumento del tamaño de la criba aumentó linealmente el peso relativo del TGI ($P = 0,09$), de la

molleja ($P < 0,01$) y del hígado ($P = 0,06$). Ninguna de las otras medidas del TGI o corporal, se vieron afectadas por el cereal principal del pienso o por el tamaño de la criba. En resumen, la cebada puede sustituir hasta un 45% del maíz en piensos para gallinas ponedoras sin ningún efecto adverso sobre la producción de huevos. Por lo tanto, el uso de uno u otro cereal dependerá de su coste relativo. Al aumentar el tamaño de la criba mejoró el desarrollo de la molleja, sin llegar a afectar la productividad de las aves. Dentro del rango estudiado, el tamaño de la criba utilizada para moler el cereal tuvo poco efecto sobre la productividad de las gallinas, aunque la criba de 4 mm tendió a incrementar los índices de conversión pero al mismo tiempo mejoró el desarrollo de la molleja.

En el experimento 3 (capítulo 4), se estudió la influencia del tamaño de partícula del cereal principal del pienso sobre la preferencia en el consumo de las gallinas ponedoras. Los piensos se diseñaron formando un factorial 2×5 con 2 cereales principales (maíz vs. cebada) y 5 tamaños de molienda del cereal (4, 6, 8, 10 y 12 mm de criba). Cada tratamiento se replicó 5 veces (10 gallinas por repetición). Tras un período de ayuno de 8 h, las gallinas recibieron sus respectivos piensos experimentales de 6,00 a.m. a 14,00 p.m. El tamaño geométrico medio y la desviación estándar geométrica de los piensos se determinaron cada 2 h. Además se determinó el contenido en proteína bruta, cenizas y Ca de los piensos al inicio y al final del experimento. El diseño experimental fue completamente al azar y los datos se analizaron con medidas repetidas con el tamaño de partícula y el cereal como factores principales. El tamaño geométrico medio de los piensos originales aumentó al aumentar el tamaño de la criba y fue mayor para los piensos basados en cebada que para los piensos basados en maíz. La diferencia en el tamaño geométrico medio entre las piensos originales y los residuos de este pienso medidos a intervalos de 2 h, disminuyó a medida que transcurría el

experimento ($P < 0,001$ para la interacción). La proteína bruta, la ceniza y el Ca de piensos y residuos en comederos se concentraron en la fracción grosera tanto en los piensos originales como en el pienso sobrante con los efectos más pronunciados para los minerales. Independientemente del tamaño del tamiz, el contenido en cenizas y Ca fue mayor en el pienso sobrante no consumido a las 14.00 h p.m que en los piensos iniciales. Las gallinas mostraron una clara preferencia por las partículas groseras, independientemente de la concentración de proteína bruta, ceniza o Ca en las diferentes fracciones de los piensos. Los datos mostraron que las gallinas consumen menos Ca durante la mañana, un período en el que los requisitos para la deposición mineral son bajos. En resumen, las gallinas mostraron una preferencia significativa por las partículas más gruesas, un efecto que fue más evidente cuando los cereales fueron molidos gruesos. Sin embargo, las gallinas no mostraron ninguna preferencia por consumir aquellas fracciones de pienso con mayores contenidos en proteína bruta, cenizas o Ca.

SUMMARY

SUMMARY

The general objective of this thesis was to study the effects of the screen size used to grind the main cereal of the diet on FI, performance, and GIT traits of brown-egg laying hens. With this purpose, the inclusion of corn, wheat, and barley in the diet of laying hens was studied in three experiments.

In experiment 1 (chapter 2), the influence of particle size of the main cereal of the diet on production and egg quality traits was studied in brown hens from 33 to 65 wk of age. The experiment was completely randomized with 6 treatments arranged as a 3 x 2 factorial with 3 main cereals (barley, corn, and wheat) and 2 grinding size of the cereal (6 and 10 mm screen). Each treatment was replicated 11 times (10 hens/replicate). Diets were isonutritive and contained 2,740 kcal/kg AMEn and 16.8% CP. Egg production, ADFI, egg weight, and feed conversion ratio (FCR) were determined by period (4 wk) and for the entire experiment. Egg quality traits (percentage of undergrads, proportion of albumen, yolk, and shell, Haugh units, and thickness, strength, and color of the shell) were measured also by period. No interactions between main cereal and geometric mean diameter of the diet were observed for any of the traits studied. Feed intake, egg production, and BW gain were not affected by diet or particle size. Eggs were heavier ($P < 0.01$) in hens fed barley than in hens fed corn or wheat, probably because of the higher fat content of the barley diets. Also, FCR tended to be better in hens fed barley than in hens fed corn or wheat ($P = 0.07$). Diet did not affect any of the egg quality traits studied. In summary, barley and wheat conveniently supplemented with enzymes, can be used in substitution of corn at levels of up to 55% in diets for laying hens, without any adverse effect on egg production or egg quality traits. Moreover, the substitution of corn by a combination of

barley and supplemental fat increased egg size. Consequently, the inclusion of one or other cereal in the diet will depend primarily on their relative cost. Within the range studied, particle size of the cereal did not have any effect on hen production.

In experiment 2 (chapter 3), the influence of the screen size used to grind the main cereal of the diet on egg production, GIT development, and body measurements was studied in hens from 17 to 49 wk of age. Diets were arranged as a 2×5 factorial with 2 main cereals (corn vs. barley) and 5 screen sizes of the cereal (4, 6, 8, 10, and 12 mm). Each treatment was replicated 5 times. No interactions between main cereal and screen size were observed for any of the traits studied. Cereal type and screen size did not affect feed intake, egg production, BW gain, or quality traits of the eggs. Eggs tended to be larger ($P = 0.092$) in hens fed the barley than in hens fed corn. Also, feed conversion ratio tended to increase ($P = 0.081$) when the cereal of the diet was ground with a 4-mm screen as compared with the average of the other diets. At 49 wk of age, the relative weight (% BW) of the GIT and gizzard was greater ($P < 0.05$) in hens fed barley than in hens fed corn. An increase in the screen size increased linearly the relative weight of the GIT ($P = 0.089$), gizzard ($P < 0.01$), and liver ($P = 0.056$). None of the other GIT traits or body measurements was affected by the main cereal or the screen size. In summary, barley can substitute up to 45% of the corn in diets for laying hens without any adverse effect on egg production. Therefore, the use of one or other cereal will depend on their relative cost. An increase in screen size improved gizzard development but had little effect on hen productivity. Within the range studied, the size of the screen used for grinding the cereal had little effect on hen productivity, although the use of a 4-mm screen might increase feed conversion ratio and gizzard development.

In experiment 3 (chapter 4), we studied the influence of particle size of the main cereal of the diet on preference behavior of laying hens. Diets were arranged as a 2×5

factorial with 2 main cereals (corn vs. barley) and 5 grinding sizes of the cereal (4, 6, 8, 10, and 12 mm screen). Each treatment was replicated 5 times (10 hens per replicate). After a fasting period of 8 h, hens received their respective experimental diets from 6.00 a.m. to 14.00 p.m. The geometric mean diameter (GMD) and the geometric standard deviation of the feeds were determined every 2 h. In addition, CP, ash, and Ca contents of the feeds were determined at the start and at the end of the experiment. The experimental design was completely randomized with data analyzed as repeated measures with particle size and cereal as main effects. The GMD of the original feeds increased with increases in screen size and was greater for the barley than for the corn diets. The difference in GMD between the original diets and the residuals measured at 2 h intervals decreased as the experiment progressed ($P < 0.001$ for the interaction). Crude protein, ash, and Ca concentrated in the coarse fraction of the original diets and of the uneaten feed with effects being more pronounced for the minerals. Independent of the coarseness of the feed sieve, ash and Ca contents were higher in the uneaten feed at 14.00 p.m than in the original diets. Hens showed a clear preference for coarse particles irrespective of the concentration of CP, ash, or Ca in the different fractions of the diets. The data showed that hens under-consumed Ca during the morning, a period in which the requirements for mineral deposition are low. In summary, hens showed a significant preference for coarser particles, an effect that was more evident when the cereals were ground coarse. Hens, however, did not show any preference for consuming those feed fractions with greater CP, ash, or Ca contents.

CHAPTER 1:

LITERATURE REVIEW AND OBJECTIVES

1. Literature review

1.1. Introduction

From 2000-2014, world egg production increased by 36.5% an average of 2.8% per year. In 2014, 7,200 M hens produced 1,320 billion eggs, worldwide. In 2016, there were 374 M hens in the European Union (EU-28), with a total egg production of 7.5 MT of eggs (European Commission, 2017). Spain, with 44 M hens (13.4 % of the total EU-28 census) and 828,000 tons of kg of eggs production was the fourth largest egg producer in the EU-28, after France, Germany, and Italy (USDA-FAS, 2015; European Commission, 2017) (Figure 1). Moreover, in 2014, Spain exported approximately 170,041 tons of eggs, mostly to other European countries with some exports to Africa and Asia.

In 2016, world cereal production was 2,569 MT an increase of 15.7% compared with the production of 2,221 Mt in 2006 (FAO, 2016) (Figure 2). In this year, cereal production reached 316.4 Mt in the EU-28 of which, 19.2 MT were produced in Spain (FAO, 2016). The production of corn, wheat, and barley in Spain was 4.6, 6.4, and 6.7 MT, respectively blow the requirements of the industry, (MAPAMA, 2017) (Figure 3). The demand for grains in Spain is increasing and the feed production sector needs to import from other countries. In fact, Spain is one of the main customer for cereals of other EU-28 countries.

Genetics, management of the flock, and feeding practices are key points for successful egg production. The profitability of the egg industry depends of many factors, including length of the laying period, number of eggs per hen housed, egg size, and percentage of eggs that reach the table of the final consumer. Cereals are the main constituents of laying hen diets with levels of use of up to 60%. Consequently, cereal

selection is important in the control of cost because they provide up to 70% of the AMEn and 40% of the protein content of diets for laying hens.

One of the most critical points to improve egg rate and egg quality is proper management and feeding practices. Feed technology is of paramount importance in this respect (Amerah et al., 2007; Amerah et al., 2008; Frikha et al. 2011). Type of cereal and particle size of the cereal used in diets have gained increasing attention from the poultry industrial in recent years.

Figure 1. Egg production in the 10 largest country of the EU-28 in 2016 (European Commission, 2017).

Figure 2. Cereal production in the world (FAO, 2016).

Figure 3. Production of cereals (corn, wheat, and barley) in Spain (MAPAMA, 2017).

1.2. Main cereal of the diet

Corn, wheat, and barley are the most common cereals used in poultry diets. Corn has less crude protein (7.5% vs. 11.2% vs. 11.3%) and fiber (2.3% vs. 2.8% and 4.5%) and more starch (63.3% vs. 59% vs. 51.1 %) and energy (3.280 vs. 3.150 vs. 2.800 kcal AMEn/kg) than wheat and both more than barley (FEDNA, 2010). One of the main objectives of this thesis was to compare the effects of the main cereal of the diet (corn, wheat, and barley) on egg production, egg quality, gastrointestinal tract (GIT) traits, and body measurements of brown-egg laying hens. The hypothesis was that wheat and barley in conveniently formulated diets, and with the inclusion of adequate enzyme complexes, could be excellent alternatives to corn in feeding programs for laying hens.

1.2.1. Effect on poultry performance

Cereals are the most widely used energy sources in poultry feeds. The starch fraction of the grains provides a major proportion of the total energy of the diets. In addition, cereals provide part of the CP and amino acids (AA) required by the birds. Starch digestion depends on factors such as the nature, protection, and chemical characteristics of the starch, as well as the presence of anti-nutritional factors in the grain (soluble cell-wall polysaccharide content) and the age and the GIT capacity of the birds (Classen, 1996; Svihus and Hetland, 2001; Zimonja et al., 2007; Abdollahi et al., 2011; Svihus, 2014). Consequently, starch utilization varies with the cereal used which in turn depends on the properties of the starch ingested which will affect its digestibility (Svihus et al., 2005; Svihus, 2014). However, there is still a lack of knowledge on the exact causes for these variations in starch digestibility. Factors such as the ratio between amylose and amylopectin, granule size, and content and properties of proteins, lipids, and phosphates on the surface of starch granules, have been identified as potential

causes for low starch digestibility (Svihus et al., 2005; Tester et al., 2006; Singh et al., 2010). In this respect, more pronounced benefits of fine grinding are expected for ingredients with highly protected starch, such as peas and hard wheat, than for ingredients with less protected starch such as rice (Parera et al., 2010; Abdollahi et al., 2011; Yegani and Korver, 2012). In fact, in the case of wheat the correlation between starch digestibility and grain hardness tends to be negative (Carré et al., 2005). Finally, pelleting might release the lipids contained in the oil bodies of ingredients such as corn, increasing energy utilization.

The level of inclusion of corn, wheat, and barley in the diet and its nutritional value for poultry depend on many factors such as the species considered, age, enzyme supplementation, and chemical characteristics of the batch, including AMEn, CP, and non-starch polysaccharide (NSP) contents (Classen, 1996; Gutiérrez-Alamo et al., 2008; Abdollahi et al., 2011; Jacob and Pescatore, 2012). Many reports compared the inclusion of these cereals in the poultry diets. In broilers (Cowieson, 2005) reported that corn has higher nutritional value for poultry than wheat or barley. The AMEn content (as fed basis) for wheat, reported in tables by the different institutions ranges from 2.870 kcal/kg for Feedipedia (2017) to 3.120 kcal/kg for NRC (1994), whereas for barley the values range from 2.350 kcal/kg (Feedipedia, 2017) to 2.820 kcal/kg (WPSA, 1989). These values are more variable than those reported for corn from 3.110 kcal/kg (Feedipedia, 2017) to 3.350 kcal/kg (NRC, 1994). The energy content of the cereals depends on the moisture content of the grain and the proportion and physico-chemical characteristics of the starch and fiber fractions (closely and negatively related) as well as on the concentration of viscous carbohydrates. A higher variability in energy is expected for wheat and barley than for corn. Fortunately, the inclusion in the diet of adequate enzyme complexes reduce the problems created by the NSP and digest

viscosity resulting in more uniform energy and protein value setting, closer for energy to that of corn.

The composition of corn, especially for ether extract and moisture content, varies substantially among countries and even from batch to batch, resulting in considerable variation in its energy value for poultry. Diverse studies have been conducted on the use of exogenous enzymes to ameliorate the inconsistencies in the nutritional value of corn and thus, to improve BW gain, feed conversion ratio (FCR), and flock uniformity. However, the inclusion of enzymes, such as carbohydrases and proteases, in corn based diets has shown inconsistent results with some research showing improvements (Zanella et al. 1999; Ouhida et al. 2000; Gracia et al. 2003; Cowieson and Adeola, 2005) and others showing no effects (Marquardt et al. 1994; Çiftci et al. 2003). Nir et al (1994a) reported better FCR for broilers fed corn than in broilers fed wheat from 1 to 21 d of age. Ruiz et al. (1987) reported similar BWG and FCR in broilers fed mash when corn was substituted by wheat. Moreover, Moran et al. (1993) reported better growth performance in birds fed wheat than in birds fed corn from 1 to 42 d of age. Marquardt et al. (1994) compared corn, wheat, and barley as the main ingredient in broiler diets from 7 to 21 d of age, and reported greater feed intake (FI) and FCR in birds fed corn. In this last report the benefits of enzyme supplementation was greatest for the barley than for the wheat diet, without observing any improvement in the case of the corn based diet. On the other hand, Edney et al. (1989) reported that when the diets were supplemented with an adequate enzyme complex, growth performance was better in broilers fed barley than in broilers fed wheat from 1 to 21 d of age. In contrast, Yasar and Forbes (1999) observed similar performance when wheat or barley were used as the main ingredient in the diet in broilers from 7 to 21 d or from 28 to 42 d of age. To mention than in this report no

enzymes were added to the diets. In brown egg laying pullets, Frikha et al. (2011) reported that the substitution of 500 g of corn by 500 g of wheat/kg as the main cereal in the diet did not affect productive performance from 1 to 120 d of age. However, in a previous report, Frikha et al. (2009a) reported significantly greater BW gain from 1 to 120 d of age in pullets fed corn than in pullets fed wheat, although FCR was not affected. Moreover, Saldaña et al. (2015a) reported that pullets fed corn had similar BW gain but poorer FCR than pullets fed wheat from hatching to 17 wk of age. In laying hens, Lillie and Denton (1968) reported similar production and FCR in Single Comb White Leghorn (SCWL) fed diets based on corn, wheat, or barley, although in this report no enzymes were added to the diet. Lázaro et al. (2003a) reported that the substitution of corn by wheat or barley did not affect FI, egg production, or egg quality of SCWL hens from 20 to 44 wk of age. Similarly, Safaa et al. (2009) and Pérez-Bonilla et al. (2011) observed similar performance when corn was substituted by wheat as the main ingredient of the diet in brown-egg laying hens from 20 to 48 wk and from 22 to 54 wk of age, respectively. In contrast, Kim et al. (1976) found that SCWL hens fed a diet based on corn ate more feed and produced bigger eggs than hens fed a diet based on wheat from 21 to 43 wk of age. In addition, Pérez-Bonilla et al. (2014) reported that feed intake was greater in brown egg-laying hens fed wheat or corn than in hens fed barley from 24 to 59 wk of age. However, none of the other productive or egg quality traits was affected by the main cereal of the diet. Overall, the information available indicates that poultry responds well to the inclusion of barley or wheat in the diets as a substitute of corn. The data indicate also that the results of the different cereals on poultry production are less variable when adequate enzyme complexes are included in the diet.

1.2.2. Effects on gastrointestinal tract development

There is a growing interest on the influence of the main cereal of the diet on the development of the GIT of chickens (Ravindran et al., 2006; Gabriel et al., 2008; Jiménez-Moreno et al., 2009c; Saldaña et al., 2015a). The GIT is a major consumer of the nutrients supplied by the diet in all animals, especially in those with rapid cell turnover which utilizes significant quantities of both AA and energy (Thomas and Ravindran, 2008). An increase in the size of the GIT will increase nutrient demand by the gut and will reduce feed efficiency in birds. It has been shown that cereals with a high NSP content increase the size and length of the GIT (Jorgensen et al., 1996; Steinfeldt, 2001; Thomas and Ravindran, 2008). In broilers, the growth of the different organs of the GIT and the production of HCL and of endogenous digestive enzymes depends on age, feed form, and diet composition (González-Alvarado et al., 2007; Svihus, 2011). Gizzard function reduces the particle size of feed ingredients by applying increasing amount of mechanical grinding pressure. The gizzard, the most muscular organ of the GIT, requires a large amount of energy in birds. Nir et al. (1994a) reported heavier relative weights (% BW) of the gizzard of broilers at 21 d of age when fed corn than when fed wheat. Similar differences between corn and wheat diets have been reported by Abdollahi et al. (2013b) comparing mash and pelleted diets. In contrast, Amerah et al. (2008) did not observe any difference in the relative weight of the gizzard at 21 d of age of broilers fed a corn or a wheat based diet, although the relative length (cm/kg BW) of the different segments of the GIT was shorter in birds fed wheat. Thomas and Ravindran (2008) reported similar relative weight of the GIT and the gizzard at 14 d of age in birds fed diets based on corn or wheat. In brown egg laying pullets, Frikha et al. (2009a) observed heavier relative weight of the gizzards in pullets fed corn than in pullets fed wheat at 45 d of age. In this report, gizzard pH and the

relative length of the different segments of the GIT were not affected by the main cereal of the diet at this age. Similar data in young pullets has been produced by Saldaña et al. (2015a) which indicated that because of its chemical composition and structure, the corn grain is more difficult to grind than the wheat grain. Consequently, the gizzard needs to work harder for corn than for wheat based diets. In contrast, Frikha et al. (2011) did not observe any difference in relative weight of the GIT or the gizzard, or gizzard pH at 120 d of age in pullets fed corn or wheat. The author has not found any report on the influence of the main cereal of the diet on the development of the GIT in laying hens.

1.3. Particle size of the diet

The term ‘particle size’ refers to the average diameter of the individual particles of a given feed, or simply to the “fineness of grind” of the feed (Amerah et al., 2007). The reduction in size of feed particles is the largest cost of energy in feed manufacturing for the layer industry (Deaton et al., 1989) and the second largest energy cost, after pelleting, in feed manufacturing for broilers industry (Reece et al., 1985). Consequently, any reduction in energy consumption during the grinding process, prior to ingredient mixing, can reduce significantly nutrition cost. In nutritional studies the particle size of a diet is defined by two variables: the geometric mean diameter (GMD) and the geometric standard deviation (GSD), variables that are calculated as indicated by the (ASAE, 1999). Feed particle size is typically determined by dry sieving a representative sample of 100 g (Baker and Herrman, 2002). The feed sample is passed through a sieve stack on a shaker for 10 minutes. The amount of particles retained on each screen size is then determined, and the GMD and GSD of the sample calculated using standard formula or computer software (ASAE, 1999). A reduction in particle size allows for a better mixing of the ingredients but influences also GIT development, nutrient utilization, and bird performance (Amerah et al., 2007; Koch, 2008; Jiménez-Moreno et

al., 2010). In the 50's and 60's, scientific reports on the effects of to the particle size of the feeds on animal performance defined particle size by using very general qualitative terms, such as fine, medium, and coarse (Davis et al., 1951). However, this approach does not allow to define accurately the size, uniformity, and the physical characteristics of the feed.

The interest on the effects of particle size of the diet on poultry growth, health, and production has increased in recent years, as the industry continues to search for new practices to optimize feed efficiency (Frikha et al., 2009b; Safaa et al., 2009; Svihus, 2010; Ruhnke et al., 2015). In addition, the renewed attention on this area is driven partly by the increasing interest in feeding whole grains to birds specifically broilers, to reduce the incidence of digestive problems in the absence of in feed antibiotics. Physical form of the feed, complexity of the diet, grinding technology, grain type, endosperm hardness, and particle size distribution, might modify the effects of screen size on poultry production. Consequently, research efforts on this area of laying hen feeding, is of paramount interest for both feed cost and egg production.

1.3.1. Methods for particle size reduction

Two major processing equipments available to reduce the particle size of the grains are the hammer mill and the roller mill (Koch, 1996; Waldroup, 1997; Pérez-Bonilla et al., 2014). The hammer mill consists of a set of hammers moving at high speed in the grinding chamber, which reduce the size of the grains until the particles are able to pass through a screen of designated size. Thus, the size and spectrum of particles produced depends not only on the screen size but also on the equipment and the speed of the hammer pieces (Koch, 1996; Amerah et al., 2007). The roller mill consists of one or more pairs of horizontal rollers in a supporting frame, the distance between which may be varied according to the particle size required. The size of the grain is reduced by

a constant compression force as they pass between the rotating rollers (Amerah et al., 2007). Again, numerous factors affect the final size and uniformity of the ultimate feed.

Irrespective of the cereal used, the GSD of the diets is reduced when roller mills rather than hammer mills are used, results that agree with data of Koch (1996) who indicated that the uniformity of the particle size of the diet was higher when using a roller mills than when using a hammer mills. Pérez-Bonilla et al., (2014) reported that the GMD of a barley diet was not affected by the type of mill used but those of the corn and wheat diet were lower when using a roller mill. These recorded differences reflect probably, differences in NDF content among the 3 cereals. Also, roller mills are more efficient and require less energy for grinding than the hammer mill. Other differences among mills, are that the undersized grains may escape the grinding process in the roller mill (Douglas et al., 1990) and that the shape of the particles produced is more irregular, being cubic or rectangular compared to those from a hammer mill, which tend to be spherical with more uniform shape (Reece et al., 1985; Koch, 1996). Hammer mill equipment however, are cheaper, easier to handle, and have lower maintenance cost than roller mill equipment, which favours its use by the feed compound industry (Koch, 1996; Amerah et al., 2007; Pérez-Bonilla et al., 2014).

1.3.2. Effects on poultry performance

Most studies on the effects of particle size reduction of corn, barley, wheat, and sorghum of poultry diets have been conducted in broilers. Nir et al. (1994a) reported that birds fed mash had the best performance when the GMD of the grain portion of the diet was within the range of 1,130 to 1,230 μm , independent of the type of cereal used (corn, wheat, or sorghum). These authors reported lower ADFI when the feed particles were smaller than 570 μm or larger than 2,100 μm . Also, Nir et al. (1995) reported that broilers fed diets based on wheat or sorghum, in mash form, had heavier BW and better

FCR when ground coarse than when ground fine. Hamilton and Proudfoot (1995a) observed that BW gain was improved when birds were fed mash diets containing coarse or very coarse corn particles compared to those containing fine corn particles. Similar results were found by Reece et al. (1985). In contrast, Lott et al. (1992) reported that corn ground through a hammer mill with a screen opening of 9.59 mm, which resulted in coarse particles (GMD $\approx$ 1,200 μ m), significantly decreased 21 d BW and increased FCR compared with corn ground through a screen with an opening of 3.18 mm (GMD $\approx$ 680 μ m). In brown-egg laying pullets, Frikha et al. (2011) reported that pullets fed the finer diet (6-mm screen) had higher BW gain and better FCR than pullets fed the medium and the coarser diets (8- and 10-mm screen) from 1 to 45 d of age, but not thereafter. These data emphasize the importance of age on FI and FCR of the bird. In brown-egg laying hens, Safaa et al. (2009) reported similar production but higher ADFI in hens fed diets with the cereal (corn or wheat) ground through a 10-mm screen (GMD of 1,165 μ m and 1,250 μ m, respectively) than in hens fed the same cereals ground through a 6-mm screen (GMD of 774 μ m and 922 μ m for corn and wheat diets, respectively). However, Deaton et al. (1989) did not observe any difference in ADFI or productive performance when SCWL hens were fed corn based diets with a GMD varying between 873 and 1,501 μ m. Similar results have been reported by Hamilton and Proudfoot (1995b) in hens fed mash diets based on wheat either coarsely or finely ground.

The existing interactions between cereals, particle size, and feed form of the diet on growth performance and productivity of poultry is well documented (Hamilton and Proudfoot, 1995b; Amerah et al. 2007; Abdollahi et al., 2013a,b). Many studies suggest that the particle size of the grain is more critical in mash feeds than in pelleted or crumbled feeds (Reece et al., 1985; Hamilton and Proudfoot, 1995a,b; Nir et al., 1995;

Svihus et al., 2004). The improvements observed for BW gain and FCR were consistent with the greater FI and the reduction in feed wastage detected. Amerah et al. (2008) reported higher ADFI and poorer efficiency in broilers from 1 to 21 d of age fed pelleted diets, based on fine corn or wheat (1-mm screen), than in those fed diets based on coarse corn or wheat (7-mm screen). Fine particles are retained for less time in the gizzard than coarse particles (Abdollahi et al., 2013a). Consequently, fine particles pass rapidly to the small intestine with minor effects on muscle development or gizzard function (Amerah et al. 2007; Svihus, 2011). However, a decrease in particle size might increase the voluntary FI of the birds, because of the faster emptying of the upper part of the GIT but also the dysfunction of the proventriculus-gizzard complex (Svihus et al., 2010). Hamilton and Proudfoot (1995b), however, reported that layers fed a mash diet performed better than those fed a crumbled diet, although this is not a current finding in laying hens (Wahlström et al., 1999). Similarly, Berg and Bearse (1948) reported that the rate of egg production and BW gain of laying hens were not influenced by the particle size of the cereal in mash diets.

1.3.3. Effects on gastrointestinal tract development

The development of digestive tract of poultry, especially the gizzard, is known to be influenced by feed particle size, which is evident already in chickens at 7 d of age (Amerah et al. 2007). Nir et al. (1994b) reported greater gizzard development and lower gizzard pH in 7 d of age chicks fed medium or coarse particle size diets compared with those fed fine particle size diets. Also, Nir et al. (1994a) reported that the weight of the gizzard and its contents were positively related to the coarseness of the feed (1.13 vs. 1.23 mm) in broilers at 21 d of age. In this report, the pH of the gizzard contents was greater in broilers fed the fine ground diet than in broilers fed the medium or coarse mash diets. The rate of passage of the digesta through the gizzard is faster with fine

particles than with coarse particles, because coarse particles need to be grinded before exiting the gizzard and therefore, coarse particle remain for longer in this organ. Hetland et al. (2004) indicated that the gizzard has a remarkable ability to grind all the constituents of the feed to a consistent fine size, regardless of the original particle size of the feed. When the digesta is retained in the gizzard for long, organ function is improved because of greater muscular development which in turn improves gizzard function. A more functional gizzard increases HCl and enzyme secretion and improves GIT motility and anti-peristaltic movements and gastro-duodenal refluxes of the GIT, facilitating the exposition of nutrient to digestive enzymes. An improvement in digesta mixing may increase nutrient digestibility (Duke, 1992; Carré, 2000; Svihus, 2011). In contrast, fine grinding reduces gizzard size and increase the incidence of proventriculitis (Taylor and Jones, 2004a,b). When the feed is finely ground, the small particles will pass quickly through the gizzard to the distal part of the GIT without any further reduction in size. Parsons et al. (2006) reported that at 42 d of age, broilers had heavier gizzards when fed mash diets based on corn with coarse particle size than when fed the same diet with fine particle size. Amerah et al. (2008) reported that at 21 d of age, broilers had heavier gizzards when fed coarse corn (7-mm screen) than when fed fine corn (1-mm screen), but no difference between coarse and fine grinding was observed in birds fed wheat based diets. Also, fine grinding of the corn but not of the wheat, reduced the relative length of the different segments of the GIT. In 120 d of age brown egg laying pullets, Frikha et al. (2011) reported no differences in relative weight of the GIT and of the gizzard and on gizzard pH when fed mash diets based on corn or wheat, with fine, medium, or coarse particle size. Moreover, Saldaña et al. (2015a) reported at 17 wk of age, that pullets had heavier gizzard and GIT when fed mash diets based on corn or wheat than when fed crumble diets based on the same cereals. This information

indicates the importance of feed particle size which affects the residence time of the feed particles in the gizzard, an effect that will depend on the type and age of the bird as well as on the ingredient composition of the diet. Probably, response to these stimuli is greater in young birds than in older birds.

When diets are fed mash diets, feed particle size and relative gizzard weight are positively related (Nir and Ptichi, 2001). In pelleted diets, however, the relation is not clear and probably will depend on the particle size and uniformity of the digesta. Svihus et al. (2004) found no effect of pre-pelleting particle size on gizzard weight in birds fed a hard wheat with a GMD varying between 600 and 1,700 μm . The reason for this finding probably lies in the reduction of the difference in particle size distribution after pelleting. In contrast, Péron et al. (2005) reported that particle size differences remained even after pelleting in very hard wheat-based diets, with GMD values varying from 380 to 955 μm . The authors indicated that pelleted diets made with ingredients coarsely ground significantly increased gizzard weights compared to those made from fine wheat. Probably, the high resistance of the hard particulate material to reduction by the pelleting process, might explain the outcome of this experiment. Chewning et al. (2012) reported that 44 d of age, broilers had heavier gizzards when fed mash diets than when fed pellet diets based on coarsely or finely ground corn. The author has not found any information on the influence of particle size of the main cereal of the diet on GIT of laying hens fed mash or pelleted diets.

2. Objectives of the thesis

The general objective of this thesis was to study the effects of the size of the screen used to grind the main cereal of the diet on FI, production performance, and GIT traits of brown-egg laying hens. With this purpose, the inclusion of corn, wheat, and barley in the diet of laying hens was studied in three experiments.

The individual objectives of the three assays of this research were as follow:

In experiment 1 (chapter 2), we evaluated the effects of the screen size (6 mm vs. 10 mm) used to grind the cereal on egg production and egg quality of brown-egg laying hens fed diets based on barley, corn, and wheat from 33 to 65 wk of age.

In experiment 2 (chapter 3), we evaluated the effects of the screen size used to grind the cereal on egg production, egg quality, GIT traits, and body measurements of brown egg laying hens fed diets based on corn or barley from 17 to 49 wk of age.

In experiment 3 (chapter 4), we evaluated the effect of the size of the screen used to grind the cereal on preference behavior of brown-egg laying hens fed diets based on corn or barley from 06.00 a.m. to 14.00 p.m., after a night fasting period of 8 h.

CHAPTER 2:

***INFLUENCE OF PARTICLE 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***

1. Introduction

Corn and wheat are the main sources of energy in poultry diets worldwide, although barley is also commonly used in laying hens (Frikha et al. 2009; Jacob and Pescatore, 2012). Barley and wheat contain variable amounts of non-starch polysaccharides (NSP) which increase digesta viscosity and reduce nutrient absorption and performance in broilers (Edney et al., 1989; Wang et al., 2005; García et al., 2008) and laying hens (Lázaro et al., 2003; Mirzaie et al., 2012). In layers, a high NSP content of the diet is associated with an increase in dirty eggs (Francesch and Brufau, 2004). However, the incidence of dirty eggs decreases and even disappear with the use of convenient enzyme complexes (Lázaro et al., 2003; Garcia et al., 2008; Saki et al., 2010).

Egg weight has been associated traditionally to the level of methionine, the most limiting amino acid in hen diets, as well as to the linoleic acid (LNL) content (Shutze et al. 1962; Balnave 1970, 1971). Corn contains more LNL than wheat or barley, which favors its use in laying hen diets (Grobas et al., 1999a, b). However, it is unclear if the increase in egg size observed is due to the LNL concentration per se or to an increase in the level of supplemental fat, because under most practical conditions both factors are confounded (Grobas et al., 1999a).

Numerous factors affect voluntary feed intake (FI) in poultry fed mash diets. In this respect, the optimal geometric mean diameter (GMD) of the diet that maximizes FI and hen production is a major challenge for the egg industry. Most studies indicate that when allowed to choose, hens show a clear performance for coarse particles (Isa Brown, 2009). Herrera et al. (2017) reported that feed efficiency tended to improve when diets based on corn or barley were ground with a 6 to 12 mm screen compared with a 4-mm screen but no other production or egg quality traits were affected. The hypothesis of this

research was that the size of the screen used to grind the main cereal of the diet could affect hen production, and effect that could depend on the type of cereal used. The objective of this research was to evaluate the effects of the GMD of the main cereal of the diet on production and egg quality traits of brown-egg laying hens fed diets based on barley, corn, and wheat from 33 to 65 wk of age.

2. Materials and methods

2.1. Husbandry, diets, and experiment design

The procedures described in this research were approved by the Animal Ethics Committee of Universidad Politécnica de Madrid and followed the Spanish guidelines for the care and use of animals in research (Boletín Oficial del Estado, 2007). In total, 660 Lohmann Brown Classic hens were selected at random from an 80,000-bird flock at 30 wk of age and housed in an environmentally controlled barn. Hens were randomly allotted in groups of 10 into 66 enriched cages (120 cm × 63 cm × 45 cm; Facco S.p.A., Padova, Italy) with similar average BW per cage. Then, the hens were fed a common diet based on equal proportions of corn, wheat, and barley for 3 wk and weighed again ($1,811 \pm 31.9$ g BW). The cages were provided with an open trough feeder and 2 low pressure nipple drinkers. The lighting program consisted in 16 h light/day throughout the experiment. Room temperature was recorded daily during the experiment, with a maximum average value of $26 \pm 3^{\circ}\text{C}$ (June, last period of the experiment) and a minimum of $21 \pm 3^{\circ}\text{C}$ (February). Feed in mash form and water were provided for ad libitum consumption.

Three diets with similar CP (16.8%) and AMEn (2,740 kcal/kg) contents but differing in the main cereal used (corn, wheat, or barley) were formulated. Two batches of the experimental diets, with same ingredient composition were manufactured and used from 33 to 44 wk and from 45 to 64 wk of age, respectively. The diets met or

exceeded the nutrient requirements of laying hens as recommended by FEDNA (2008). All diets were supplemented with a commercial enzyme complex with xylanase and β -glucanase activity (Roxazyme, DSM SA., Madrid, Spain). In the formulation of the diets it was assumed that the inclusion of the enzyme complex increased the AMEn content of the barley and wheat grains by 2% (from 2,800 to 2,856 kcal/kg of barley and from 3,150 to 3,213 kcal/kg of wheat) but had no effects on the energy content of the corn or of the other ingredients of the diets (FEDNA, 2010). Before feed manufacturing, a batch of each of the 3 cereals were divided into 2 portions and each portion was ground using a horizontal hammer mill (Mecafa S.A., Ciudad Real, Spain) provided with a 6- or a 10-mm screen and included as such in their respective experimental diets.

The experiment was conducted as a completely randomized design with 6 diets arranged factorially with 3 main cereals and 2 GMD of the cereal. Each treatment was replicated 11 times and the experimental unit was the cage with 10 hens for all measurements.

2.2. Laboratory analysis

Representative samples of the feeds were ground using a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) equipped with a 1-mm screen and analyzed for moisture by oven-drying (method 930.15), total ash in a muffle furnace (method 942.05), and nitrogen by Dumas (method 968.06) using a Leco analyzer (Model FP-528, Leco Corp., St. Joseph, MI) as described by AOAC International (2005). Ether extract was determined by Soxhlet analysis after 3 N HCl acid hydrolysis and crude fiber (CF) by sequential extraction with diluted acid and alkali (Boletín Oficial Estado, 1995). Gross energy was determined using an adiabatic bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL, USA) with benzoic acid as the calibration standard.

The LNL content of the diets was determined by gas-liquid chromatography (GC-14B, Shimadzu, Kyoto, Japan) as shown by Grobas et al. (1999a) and the amino acid content by ion-exchange chromatography (Hewlett-Packard 1100, Waldbronn, Germany) after acid hydrolysis, as indicated by de Coca-Sinova et al. (2008). Particle size distribution and mean particle size of the diets, expressed as the GMD and geometric standard deviation (GSD; log normal SD), 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 duplicate except for the GMD of the diets that were determined in triplicate. The ingredient composition and chemical analyses of the diets are shown in Table 1. The GMD and GSD of the two batches of the diets used during the experiment are shown in Table 2.

2.3. Measurements

2.3.1. *Hen production*

Egg production, feed disappearance, and BW of the hens were determined by cage at 4 wk intervals. Eggs produced the last 2 d of each week were weighed, and the average value of the 4 wk was used to estimate egg weight by period. From these data, ADFI, egg production, egg weight, egg mass, feed conversion ratio (FCR) per kilogram and per dozen of eggs, and BW gain of the hens were calculated for each of the 8 periods and for the entire experiment (33 to 65 wk of age). Any mortality was recorded and weighed as produced.

2.3.2. *Egg quality*

Undergrades (dirty, broken, and shell-less eggs) were recorded daily by replicate in all eggs produced. An egg was considered as dirty when a spot of any kind or size

was detected on the shell (Lázaro et al., 2003). Other egg quality traits, including haugh units, shell strength, shell color, and shell thickness, were measured in 12 fresh eggs collected randomly from each cage replicate the last 2 d of each of the 8 experimental periods. Haugh units was measured using a multitester equipment (QCMSystem, Technical Services and Supplies, Dunnington, York, UK) as indicated by Pérez-Bonilla et al. (2012). Shell thickness, was measured at the two pole ends and at the middle section of the egg shell with a digital micrometer (model IT-014UT, Mitotuyo, Kawasaki, Japan). The average of the 3 measurements of each of the 12 eggs was used for further analyses (Safaa et al., 2008). Shell strength and shell color were measured in 6 eggs collected randomly from each replicate on the same days. Shell strength, expressed in g/cm², was evaluated applying increased pressure to the broad pole of the egg using a press meter (Egg Force Reader, SANOVO Technology A/S, Odense, Denmark) as indicated by Safaa et al. (2008). Shell color was measured using a Minolta colorimeter (Chroma Meter Model CR-200, Minolta Corp., Ramsey, NJ) and the Hunter color values, L* (lightness), a* (green to red), and b* (blue to yellow), were recorded (Table 3).

2.4. Statistical analysis

Data were analyzed as a completely randomized design with 6 treatments arranged as a 3x2 factorial with cereal and GMD of the diet as main effects using the MIXED procedure of SAS (SAS Institute, 2004). Results in tables are presented as means and differences were considered significant at $P < 0.05$.

3. Results

The GMD was higher for the barley than for the corn diets with the wheat diets being intermediate in both periods (Table 2). Also, the GMD of the diets increased as the screen size used to grind the cereals increased. The GSD was lower for the barley diets than for the wheat or the corn diets and in all cases decreased as the screen size increased.

3.1. Hen production and egg quality

No interactions between the main cereal of the diet and the screen size used to grind the cereal were observed for any of the traits studied and consequently, only main effects are discussed.

3.1.1. Main cereal of the diet

Egg production, egg mass, ADFI, and BW gain were not affected by the main cereal of the diet in any of the periods considered (Figure 1). Mortality tended to be higher in hens fed corn than in hens fed wheat or barley (4.9% vs. 2.6 and 1.9%; $P=0.06$). Cumulatively (33 to 65 wk of age), egg weight was greater in hens fed barley than in hens fed corn or wheat (65.7 g vs. 64.7 and 64.4 g; $P < 0.05$; Table 3). Also, FCR tended to be better for the barley- than for the corn- or the wheat diets (1.94 vs. 1.98 and 1.98; $P = 0.07$). None of the egg quality traits studied (percentage of broken and dirty eggs, egg shell-less eggs, Haugh units, strength, thickness, and color of the shell, and percentage of albumen, yolk, and shell of the eggs) was affected by the main cereal of the diet (Table 3).

3.1.2. Screen size

The screen size used to grind the cereal did not affect any of the egg production or egg quality traits studied.

4. Discussion

The GMD of the diets was higher for the barley than for the corn diet with that of the wheat diet being intermediate. The differences reported were expected because the barley grain had very flexible glumes and contain more neutral detergent fiber than the corn grain. Consequently, more barley particles pass intact through the screen, increasing the GMD of the diet. The GSD was higher for the corn- and wheat diets than for barley diets. On the other hand, the GMD and the GSD of the diets increased as the screen size used to grind the cereals increased. Similar results comparing these three cereals were reported by Perez-Bonilla et al. (2014) and Herrera et al. (2017), indicating that the structure of the feed depends not only on the size of the screen used but also on the characteristics of the grain.

4.1. Main cereal of the diet

Egg production, egg mass, ADFI, and BW gain were not affected by the main cereal of the diet, consistent with most published research in which the wheat and barley diets were supplemented with an adequate combination of exogenous enzymes (Pérez-Bonilla et al., 2011; Herrera et al., 2017). However, hens fed barley produced heavier egg than hens fed corn or wheat. The information available on the effects of main cereal of the diet on egg size is scarce. Herrera et al. (2017) observed that egg size was greater in hens fed barley than in hens fed corn, in agreement with the data reported herein. In contrast, Coon et al. (1988) and Pérez-Bonilla et al. (2011) reported similar egg size in hens fed barley based diets than in hens fed corn based diets. The reason for the inconsistencies reported among researches is not known but might be related to differences in the physico-chemical characteristics of the cultivars and batch of cereal used in the different experiments or to changes in the ingredient composition of the diets which result of the different nutrient value of the cereals. In this respect, when

corn is substituted by barley, the level of supplemental fat and the LNL content of the diet increases to compensate for the lower AMEn content of barley. As has been shown by Jensen et al. (1958), Grobas et al. (1999 a,c), and Ravindran et al. (2014) an increase in these 2 dietary components might increase egg size. Published data on the minimum requirement of LNL to optimize egg size in laying hens are not consistent. Scragg et al. (1987) reported that brown egg-laying hens required more than 2.0% LNL to maximize egg size. Moreover, Lohmann (2016) recommend a minimum of 1.8% in diets for commercial laying hens. In contrast, Pérez-Bonilla et al. (2014) observed that egg size was maximized with as little as 1.1% of LNL in the diet. In fact, Grobas et al. (1999c) and Safaa et al. (2008) demonstrated that an increase in the LNL content of the diet from 1.15% to 1.65% and from 1.12% to 1.60%, respectively, did not affect egg weight in brown egg-laying hens from 22 to 65 wk and 59 to 70 wk of age, respectively. In these two experiments, however, egg size increased significantly when extra amounts of supplemental fat were included in the diet, irrespective of their LNL content. In the current experiment, feeding barley resulted in heavier eggs compared with feeding corn or wheat. Probably, the higher level of supplemental fat of the barley diets, compared with that of the corn and wheat diets (4.9% vs. 1.8 and 1.6%, respectively) rather than the LNL content that was high and above 1.8% in all cases, was responsible for the increase in egg weight observed in hens fed barley. The results reported herein are consistent with data of Herrera et al. (2017) who reported heavier eggs in hens fed diets based on barley containing 5.3% supplemental fat and 3.4% LNL than in hens fed diets based in corn containing 4.7% supplemental fat and 3.7% LNL. These results confirm that once the LNL requirement of the hens are reached (around 1.0 to 1.2% of the diet), an increase in the level of supplemental fat, rather than a further increase in LNL, is responsible for the increase in egg weight often observed.

For the entire experimental period, FCR tended to be better with the barley- than with the wheat- and corn diets. In the current research, we accepted that the exogenous enzymes increase the energy content of barley by 2%. Probably, the enzyme complex used improved the AMEn content of barley more than expected (Mateos et al., 2017a). In this respect, Lázaro et al. (2003) reported that the supplementation of the diet with a complex based on β -glucanase and xylanase, similar to that used in the current research, improved energy utilization of the barley diet by up to 5.5% whereas no improvement was observed for the wheat and corn diets.

Mortality tended to be higher in hens fed corn than in hens fed wheat or barley, probably because of differences in CF among the diets (Mateos et al., 2012; 2017). Similarly, Hartini et al. (2002) reported higher mortality in laying hens fed corn diets than in laying hens fed barley diets (2.9% vs. 5.3% CF, respectively) from 17 to 24 wk of age. Diets with a low fiber content increase aggressiveness and feather pecking in poultry resulting in higher mortality. In this respect, Francesch et al. (2005) reported greater mortality in laying hens fed corn based diets than in laying hens fed barley based diets (4.3% vs. 5.1%, respectively) from 22 to 46 wk of age.

4.2. Screen size

It is widely accepted that, when allowed to choose, hens show a preference for consuming the larger feed particles. Consequently, finely ground diets might reduce voluntary FI (Safaa et al., 2009; Lohmann, 2016; Herrera et al., 2017, 2018) and therefore, egg size and egg production (Isa Brown, 2009). In the current research, however, the size of the screen used to grind the cereals had little effect on hen production or egg quality traits. Similarly, Herrera et al. (2017) did not detect any difference on these traits using screens varying in size from 6 to 12 mm. In contrast, Safaa et al. (2009) reported a significant 2.5% increase in FI in hens fed corn or wheat

ground with a 10-mm screen than in hens fed these same diets ground with a 6-mm screen, although, no differences in egg production or egg weight were detected. In general, the data reported herein show a trend for increases in FI, egg production, and egg weight in hens fed the 10-mm ground diets compared with hens fed the 6-mm ground diets, although the differences were significant only for some of the traits during the first part of the experiment. For example, egg mass was greater and FCR was better when the cereal was ground with a 10-mm screen from 33 to 49 wk of age with no effects from 49 to 65 wk of age. The data might indicate that laying hens respond better to increases in particle size of the diet at younger ages, with effects disappearing with age. In the current research, the differences in GMD between the coarse and the fine diets were 199 and 141 μm for barley, 260 and 155 μm for wheat, and 265 and 151 μm for corn for the first (33 to 44 wk) and second (45 to 64 wk) period, respectively, differences that were less than expected for the second period (Safaa et al., 2009; Herrera et al., 2017). The reason for the small difference in GMD between the 2 diets could be related to differences in the use time and worn-out of the screens or of other parts of the hammer used to grind the cereals in the two periods considered. For example, in the experiment of Safaa et al. (2009), in which the authors reported greater FI in hens fed diets based on corn or wheat ground with a 10-mm screen than in hens fed the same cereals ground with a 6-mm screen, the difference in GMD reported was of 491 and 252 μm , respectively, greater than the differences reported in the current research. The possibility that the greater effect of increased particle size in the first period of the experiment compared to the second period could be due to differences in GMD of the diets cannot be ruled out.

In conclusion, the main cereal of the diet did not affect FI, egg production, BW gain of the hens, or eggs quality traits of the hens. However, hens fed barley had less

mortality and produced heavier eggs than hens fed wheat or corn, probably because of the higher level of fiber that reduces feather pecking and of supplemental fat, that increases egg size, of the barley diets. Feed conversion ratio tended to be better in hens fed barley than in hens fed wheat or corn, suggesting that the enzyme complex used improved energy and nutrient utilization more than expected. The size of the screen size used to grind the cereals had little effect on egg production or egg quality. Barley and wheat, supplemented with adequate enzymes, can substitute up to 55% of the corn in diets for laying hens, without any adverse effect on production or egg quality traits.

Table 1. Ingredient composition and chemical analyses (% , as fed basis) of the experimental diets.

Ingredient	Corn	Wheat	Barley
Barley	-	-	55.12
Corn	60.89	9.92	4.81
Wheat	-	55.08	-
Soybean meal (47% CP)	23.80	16.40	24.30
Calcium carbonate	8.20	8.50	8.50
Sunflower meal (35% CP)	2.70	6.20	-
Soy oil soapstock	1.80	1.60	4.90
Dicalcium phosphate	1.54	1.14	1.29
Sodium chloride	0.34	0.34	0.34
L-Lisine-HCl (78%)	0.02	0.10	0.02
DL-Methionine (99%)	0.21	0.22	0.22
Premix ¹	0.50	0.50	0.50
Calculated analysis ²			
AMEn (kcal/kg)	2,740	2,740	2,740
Digestible amino acid			
Lys	0.76	0.76	0.77
Met	0.39	0.38	0.39
Met + Cys	0.62	0.62	0.62
Thr	0.55	0.50	0.53
Total ash	12.00	12.00	12.00
Neutral detergent fiber	8.56	10.33	12.62
Available P	0.38	0.38	0.38
Determined analysis ³			
Dry matter	90.10	90.20	90.0
Gross energy (kcal/kg)	3,661	3,601	3,752
Ether extract	3.70	2.90	5.80
Linoleic acid (C18:2)	2.20	1.80	2.60
Crude fiber	2.70	3.60	3.40
Crude protein	17.30	17.00	17.40
Total amino acid			
Lys	0.94	0.93	0.95
Met	0.39	0.40	0.42
Met + Cys	0.63	0.62	0.64
Thr	0.56	0.54	0.55
Total ash	11.80	12.10	11.80
Calcium	3.80	3.86	3.70
Phosphorus	0.60	0.63	0.58

¹Provided the following (per kilogram of diet): vitamin A (*trans*-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,750 IU; vitamin E (dl- α -tocopheryl acetate), 10 mg; vitamin B1, 1.3 mg; vitamin B2, 5 mg; vitamin B6, 2 mg; vitamin B12 (cyanocobalamin), 15 mg; niacin, 25 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 1 mg; biotin, 15 mcg; choline (choline chloride), 250 mg; manganese (MnO), 90 mg; zinc (ZnO), 60 mg; iron (FeSO₄·H₂O), 40 mg; copper (CuSO₄ 5H₂O), 8 mg; iodine [Ca(IO₃)₂], 0.7 mg; selenium (Na₂SeO₃), 0.3 mg; Roxazyme, 200 mg [1,600 U of endo-1,4- β -glucanase (EC 3.2.1.4), 3,600 U of endo-1,3 (4)- β -

glucanase (EC 3.2.1.6), and 5,200 U of endo-1,4- β -xylanase (EC 3.2.1.8)] supplied by DSM S.A., Madrid, Spain; Natuphos 5,000 (300 FTU/kg supplied by Basf Española, S.A., Tarragona, Spain), 60 mg. All diets included 0.18 g canthaxantin/kg. The barley and wheat diets included also 0.18 g/kg and 0.16 g/kg, respectively of the ester of β -apo-8-carotenoic/kg (supplied by Basf Española, S.A., Tarragona, Spain).

²According to FEDNA (2010).

³Data correspond to the average of duplicate analyses of the 2 diets differing in the GMD of the cereal used in the 2 batches manufactured (33 to 44 wk and 45 to 64 wk of age). Within each cereal type, the determined chemical analyses were similar for the 2 diets (CV below 4%).

Table 2. Geometric mean diameter (GMD, μm) and geometric standard deviation (GSD) of the experimental diets

Period	Screen size	Corn	Wheat	Barley	Mean
33 to 44 wk of age	6 mm	940 ± 2.13	$1,010 \pm 2.14$	$1,181 \pm 2.09$	$1,044 \pm 2.13$
	10 mm	$1,205 \pm 2.16$	$1,270 \pm 2.12$	$1,380 \pm 2.05$	$1,285 \pm 2.12$
	Mean	$1,072 \pm 2.17$	$1,140 \pm 2.14$	$1,280 \pm 2.08$	$1,164 \pm 2.14$
45 to 54 wk of age	6 mm	972 ± 2.21	$1,116 \pm 2.26$	$1,245 \pm 2.11$	$1,111 \pm 2.19$
	10 mm	$1,123 \pm 2.19$	$1,271 \pm 2.19$	$1,386 \pm 2.02$	$1,260 \pm 2.13$
	Mean	$1,047 \pm 2.20$	$1,193 \pm 2.23$	$1,315 \pm 2.06$	$1,185 \pm 2.14$

Table 3. Influence of the main cereal of the diet and the screen size used to grind the cereal on hen production and egg quality from 33 to 65 wk of age

	Cereal			Screen size (mm)			Probability ²	
	Corn	Wheat	Barley	6	10	SD ¹	Cereal	Screen size
Hen production								
Egg production (%)	89.1	89.4	89.2	89.0	89.4	1.45	0.96	0.68
Feed intake (g/d)	114.2	114.5	113.8	113.5	114.1	2.63	0.54	0.13
Egg weight (g)	64.7 ^b	64.4 ^b	65.7 ^a	64.8	65.1	1.36	0.01	0.48
Egg mass (g/d)	57.6	57.6	58.6	57.7	58.2	2.86	0.40	0.47
FCR ³ (kg/kg)	1.98	1.98	1.94	1.96	1.96	0.09	0.07	0.94
FCR ³ (kg/dozen)	1.55	1.55	1.54	1.55	1.55	0.09	0.69	0.81
BW (g)	1,875	1,851	1,844	1,865	1,849	62.0	0.22	0.31
Mortality (%)	4.9	2.6	1.9	3.4	2.8	0.92	0.06	0.58
Egg quality								
Haugh units ⁴	88.6	88.7	88.5	88.6	88.6	1.35	0.78	0.95
Dirty eggs ⁵ (%)	1.8	1.5	1.5	1.4	1.7	0.75	0.23	0.16
Broken eggs ⁵ (%)	0.5	0.6	0.7	0.7	0.6	0.65	0.35	0.72
Shell-less eggs ⁵ (%)	0.2	0.2	0.2	0.2	0.2	0.37	0.95	0.61
Shell thickness ⁵ (mm)	0.372	0.375	0.371	0.372	0.375	0.01	0.31	0.35
Shell strength ⁵ (g/cm ²)	4,299	4,419	4,237	4,328	4,309	0.34	0.25	0.82
Shell color ^{5,6}								
L	18.4	18.1	18.4	18.2	18.3	0.85	0.12	0.39
A	30.0	29.6	29.6	29.7	29.8	0.86	0.33	0.23
B	62.6	63.3	62.8	62.9	62.8	1.48	0.21	0.48
Egg constituents (% of the eggs)								
Albumen	59.7	59.8	60.0	59.7	60.0	1.13	0.49	0.28
Yolk	27.0	27.0	26.8	27.0	26.9	0.84	0.39	0.40
Shell	13.2	13.2	13.2	13.3	13.2	0.70	0.54	0.50

^{a-b}Within a row, means without a common superscript differ significantly.

¹22 and 33 replicates for the main cereal and for the screen size effects.

²The interactions between main effects was not significant for all variables ($P > 0.10$).

³Feed conversion ratio.

⁴Haugh units

⁵Data for dirty, broken, and shell-less eggs correspond to the average of all eggs produced during the 5 periods of the experiment.

⁶Hunter color values, L* (lightness), a* (green to red), and b* (blue to yellow).

⁷Data for these variables correspond to the means of 12 fresh eggs of the 5 periods (28 days each one)

Egg production (A)

Average daily feed intake (B)

Egg weight (C)

Figure 1. Effect of the main cereal of the diet on egg production (A), ADFI (B), egg weight (C), egg mass (D), and feed conversion ratio (FCR) per kg of eggs (E) from 33 to 65 wk of age

^{NS} $P > 0.1$; ⁺ $0.1 > P > 0.05$; * $0.05 > P > 0.01$.; ** $0.01 > P > 0.005$; *** $0.005 > P > 0.001$.

Figure 2. Effect of screen size used to grind the cereal of the diet on egg production (A), ADFI (B), egg weight (C), egg mass (D), and feed conversion ratio per kg of eggs (E), of the hens from 33 to 65 wk of age

^{NS} $P > 0.1$; ⁺ $0.1 > P > 0.05$; * $0.05 > P > 0.01$.; ** $0.01 > P > 0.005$; *** $0.005 > P > 0.001$.

CHAPTER 3:

***INFLUENCE OF PARTICLE SIZE OF THE
MAIN CEREAL OF THE DIET ON EGG
PRODUCTION, GASTROINTESTINAL TRACT
TRAITS, AND BODY MEASUREMENTS OF
BROWN LAYING HENS***

1. Introduction

Corn (*Zea mays* L.) and barley (*Hordeum vulgare* L.) are extensively used in diets for laying hens. As an average, corn has less protein (7.5 vs. 11.3%) but more starch (63.3 vs. 51.1%), EE (3.6 vs. 2.0%), linoleic acid (**LNL**; 1.81 vs. 0.78%), and energy (3,280 vs. 2,800 kcal AMEn/kg) than barley (FEDNA, 2010). In addition, the nutritive value of corn is less variable than that of barley, probably because of its lower content in non-starch polysaccharides (**NSP**) (García et al., 2008; Jacob and Pescatore, 2012). The β -glucans and arabino-xylans present in barley increase digesta viscosity and might affect the development of the gastrointestinal tract (**GIT**) (Mateos et al., 2002), nutrient digestibility and absorption (Lázaro et al., 2003a), and bird performance (García et al., 2008). A high content in NSP is associated also with an increase in the incidence of dirty eggs (Francesch et al., 1995; Lázaro et al., 2003a). Enzyme supplementation (ES), however, reduces or even eliminates the negative effects of NSP on nutrient digestibility and egg production, improving the feeding value of barley (Gracia et al., 2003; Saki et al., 2010).

Ingredient composition and size of the screen used to grind the cereals affect the structure and particle size of the diet. Mechanoreceptors located in the beak detect differences in texture which might affect feed intake (FI) and performance in broilers (Amerah et al., 2007; Jiménez-Moreno et al., 2016) and laying hens (Safaa et al., 2009; Pérez-Bonilla et al., 2014). A reduction in particle size facilitates the contact between nutrients and endogenous enzymes, improving nutrient digestibility (Parsons et al., 2006). However, fine particles result often in a less developed gizzard and GIT (Hetland et al., 2002) which might affect poultry performance (Nir et al., 1994a; González-Alvarado et al., 2007). On the other hand, when the diets are coarsely ground, bird selection increases which may affect feed efficiency (Nir et al., 1994b). These opposite

effects might counteract each other and the final effect on hen productivity might depend on factors such as the characteristic and the ingredient composition of the experimental diets. However, no research is available comparing the influence of corn or barley of diets ground with different screen size on productive performance of hens.

Body measurements are useful criteria to predict body composition and the size and future performance of birds, including broilers (Van Rooy-Reijnders, 2013), pullets (Guzmán et al., 2015), and laying hens (Guzmán et al., 2016). However, the information available on the effects of the particle size and the characteristics of the diet on these variables in laying hens is scarce.

The hypothesis of this research was that screen size used to grind the cereal could affect FI, GIT development, and egg production in laying hens, effects that could vary depending on the cereal. The objective of this research was to compare the effects of the screen size used to grind the cereal on egg production, egg quality, GIT traits, and body measurements of brown egg laying hens fed diets based on corn or barley.

2. Materials and methods

2.1. Husbandry, diets, and experiment design

The procedures described in this research were approved by the Animal Ethics Committee of Universidad Politécnica de Madrid, in compliance with the Spanish guidelines for the care and use of animals in research (Boletín Oficial del Estado, 2007). In total, 500 Lohmann Brown Classic hens were housed at 16wk of age in an environmentally controlled barn. Hens were weighed individually at 17 wk of age ($1,407 \pm 31.9$ g BW) and randomly allotted in groups of 10 into 50 enriched cages (40 cm $\times$ 80 cm $\times$ 68 cm; Faccò S.p.A., Padova, Italy) with similar average BW per cage. The cages were provided with an open trough feeder and 2 low pressure nipple drinkers. Room temperature was recorded daily throughout the experiment, with a maximum

average value of $26 \pm 3^{\circ}\text{C}$ (July, first period of the experiment) and a minimum of $21 \pm 3^{\circ}\text{C}$ (February, last period of the experiment). Feed in mash form and water were provided for ad libitum consumption. The lighting program consisted in 16 h of light per day throughout the experiment.

Two diets with similar AMEn (2,750 kcal/kg) and CP (17.5%) content but differing in the main cereal used (corn vs. barley) were formulated. The diets met or exceeded the nutrient requirements of laying hens as recommended by FEDNA (2008). All diets were supplemented with the same dose of a commercial enzyme complex with xylanase and β -glucanase activity (Roxazyme, DSM S.A., Madrid, Spain). In the formulation of the diets it was accepted that the inclusion of the enzyme complex increased the AMEn content of the barley by 2% (from 2,800 to 2,856 kcal/kg) but had no effects on the energy content of any of the other ingredients of the diet (FEDNA, 2010). Before feed manufacturing, the batch of each of the 2 cereals was divided into 5 portions, and each portion was ground using a horizontal hammer mill (Mecafa S.A., Ciudad Real, Spain) provided with a 4, 6, 8, 10, or 12 mm screen and then included in their respective experimental diets.

The experiment was conducted as a completely randomized design with 10 diets in a factorial arrangement with 2 main cereals and 5 screen size used to grind the cereal. Each treatment was replicated 5 times and the experimental unit was the cage with 10 hens for all measurements.

2.2. Laboratory analysis

Representative samples of the feeds were ground using a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) equipped with a 1-mm screen and analyzed for moisture by the oven-drying (method 930.15), total ash in a muffle furnace (method 942.05), and nitrogen by Dumas (method 968.06) using a Leco analyzer (Model FP-528, Leco Corp., St. Joseph, MI) as indicated by AOAC International (2005). Ether extract, gross energy, and the neutral detergent fiber were determined as indicated by Pérez-Bonilla et al. (2011) and expressed on an ash-free basis. The LNL content of the diets was determined by gas-liquid chromatography (GC-14B, Shimadzu, Kyoto, Japan) as shown by Grobas et al. (1999a) and the amino acid (AA) content by ion-exchange chromatography (Hewlett-Packard 1100, Waldbronn, Germany) after acid hydrolysis, as indicated by de Coca-Sinova et al. (2008). Particle size distribution and mean particle size of the diets, 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 duplicate except for the GMD of the diets that was determined in triplicate. The ingredient composition and chemical analyses of the diets and their GMD and particle size distribution are shown in Tables 1 and 2 and Figure 1, respectively.

2.3. Measurements

2.3.1. *Hen productivity*

Feed disappearance, egg production, and BW of the hens were determined by age at 4 wk intervals. Any mortality was recorded and weighed as produced. All eggs produced the last 2 d of each week were weighed, and the average value of the 4 weeks was used to estimate egg weight by period. From these data, ADFI, egg production, egg

weight, egg mass, feed conversion ratio (FCR) per kilogram and per dozen of eggs, and BW gain were calculated by period as well as for the entire experiment (17 to 49wk of age).

2.3.2. Egg quality

The number of undergrades, dirty, broken, and shell-less eggs was recorded daily by replicate in all eggs produced. An egg was considered as dirty when a spot of any kind or size was detected on the shell. Other egg quality traits, including yolk color, Haugh units, and shell thickness were measured in 12 fresh eggs collected randomly from each cage replicate the last 2 d of each of the 8 experimental periods. Haugh units and yolk color (Roche Color Fan) were measured using a multitester equipment (QCMSsystem, Technical Services and Supplies, Dunnington, York, UK). Shell thickness was measured at the two pole ends and at the middle section of the egg shell with a digital micrometer (model IT-014UT, Mitotuyo, Kawasaki, Japan) and the average of the 3 measurements of each of the 12 eggs was used for further analyses. Shell strength and shell color were measured in 6 eggs collected randomly from each replicate the same days. Egg shell strength, expressed in g/cm², was evaluated applying increased pressure to the broad pole of the egg using a press meter (Egg Force Reader, SANOVO Technology A/S, Odense, Denmark). Shell color was measured using a Minolta colorimeter (Chroma Meter Model CR-200, Minolta Corp., Ramsey, NJ) and the Hunter color values, L* (lightness), a* (green to red), and b* (blue to yellow), were recorded.

2.3.3. *Gastrointestinal tract traits and body measurements*

At 49 wk of age, after the corresponding performance control, 2 hens per replicate were randomly selected, weighed individually, and euthanized by CO₂ inhalation. The digestive tract, from the post-crop esophagus to the cloaca, including the digesta content and the annex organs (liver, pancreas, and spleen) was removed and weighed. Then, the proventriculus, gizzard, and the liver were excised and weighed and the weight expressed relative to BW. In addition, the gizzard was emptied from any digesta content, cleaned, dried with desiccant paper, and weighed again. The weight of the digesta content of the gizzard was expressed relative to the full organ weight (%). Gizzard pH was measured in situ in all these hens in duplicate using a digital pH meter fitted with a fine tip glass electrode (model 507, Crison Instruments S.A., Barcelona, Spain) as indicated by Jiménez-Moreno et al. (2009a). The length of the duodenum (from the gizzard to the pancreo-biliary ducts), jejunum (from the pancreo-biliary ducts to the Meckel's diverticulum), ileum (from the Meckel's diverticulum to the ileo-cecal valve), and the two ceca (from the ostium to the tip of the right and left ceca) were measured on a glass surface using a flexible tape with a precision of 1 mm. The length of the small intestine was determined by adding that of the duodenum, jejunum, and ileum. In addition, hen length, from the tip of the beak to the end of the longest phalanx, was measured in extended birds as indicated for the small intestine and body mass index (BMI) was estimated. Also, the length and diameter of the tarsus of these hens were measured with the aid of a digital caliper and expressed relative to BW. The average value of the 2 hens was used for further statistical analysis.

2.4. Statistical analysis

Data were analyzed as a completely randomized design with 10 treatments arranged as a 2×5 factorial with main cereal of the diet and screen size used as main effects using the MIXED procedure of SAS (SAS Institute, 2004). In addition, treatment sum of squares of the effects of screen size on all variables studied was partitioned into the linear and quadratic components. Also, a non-orthogonal contrast comparing the effects of the average of the 2 diets based on corn or barley ground through a 4-mm screen and the average of all the other diets (ground through a 6- to 12-mm screen size) was included in the statistical analysis. The Pearson correlation analyses (SAS Institute, 2004) was used to study the effect of diet on the relation between BW and BMI, body length, tarsus length, and tarsus diameter of the hens at 49 wk of age. Results in tables are presented as means and differences were considered significant at $P < 0.05$.

3. Results

The GMD was higher for the barley than for the corn diets and increased as the screen size used to grind the cereals increased (Table 2). The GSD, however, was higher for the corn than for the barley diets and increased with the size of the screen used.

3.1. Hen productivity and egg quality

Mortality was 0.6% and was not related to any treatment (data not shown). No interactions between main cereal of the diets and the screen size used were observed for any of the traits studied and therefore, only main effects are discussed.

3.1.1. Main cereal of the diet

For the entire experimental period, none of the production traits studied, except egg weight that tended ($P = 0.092$) to increase with barley, were affected by the main cereal of the diet (Table 3). In fact, egg production, ADFI, and BW gain were not

affected by the main cereal in any of the periods studied. Egg mass, however, increased with barley feeding in some of the periods considered (Figure 2). The main cereal of the diet did not affect any of the egg quality traits studied (dirty, broken, and shell-less eggs, Haugh units, and strength, thickness, and color of the shell) (Table 3).

3.1.2. Screen size

From 17 to 49 wk of age, the screen size used to grind the cereal did not affect any of the production or egg quality traits studied, except for FCR that tended ($P = 0.081$) to be higher in hens fed the 4- mm screen ground diet as compared with the average of hens fed the 6- to 12-mm screen ground diets (Table 3). Egg production, egg mass, and FCR were affected by the screen size used to grind the cereal in some of the periods considered (Figure 3). The screen size used to grind the cereal did not affect any of the egg quality traits studied.

3.2. Gastrointestinal tract traits and body measurements

3.2.1. Main cereal of the diet

At the end of the experiment, the GIT (126 vs. 122 g/kg BW; $P < 0.05$) and the gizzard (29.1 vs. 27.7 g/kg BW; $P < 0.05$) were heavier in hens fed barley than in hens fed corn. Gizzard pH, gizzard content, and body measurements traits, however, were not affected by the main cereal of the diet (Tables 4 and 5).

3.2.2. Screen size

The relative weight of the full gizzard ($P < 0.01$), GIT ($P = 0.089$), and liver ($P = 0.056$) increased linearly as the screen size used increased (Table 4). Body measurements, however, were not affected by the screen size (Table 5).

A significant positive relation between BW of the hens and BMI ($P < 0.001$), body length ($P < 0.05$), and tarsus length and diameter ($P < 0.01$) were observed at 49 wk of age (Table 6).

4. Discussion

The chemical composition of the experimental diets was close to expected values, confirming that the ingredients were mixed correctly. The GMD was greater for the barley than for the corn diets, in agreement with data of Pérez-Bonilla et al. (2011). The glumes of the barley grains are coarse and flexible and barley has a higher NDF content than corn. Consequently, the chances of the fiber fraction of barley passing intact through the screen increase, resulting in an increase in the GMD of the diet. In contrast, the GSD was higher for the corn than for the barley diets, in agreement with data of Pérez-Bonilla et al. (2014) comparing different types of grinding in diets for laying hens. The data suggest that the structure of the feed (mean particle size and uniformity) depends not only on the size of the screen but also on the characteristics of the grain used. As expected, the GMD of the diets increased as the screen size increased. In fact, the GMD of the diet (average of the corn and barley diet) increased by 42.3% (1,003 to 1,427 μm) as the size of the screen increased from 4 to 12 mm. The GSD tended to increase with increases in screen size.

4.1. Hen productivity and egg quality

4.1.1. Main cereal of the diet

For the entire experimental period, the main cereal of the diet did not affect egg production, ADFI, FCR, or egg quality traits, results that agree with previous studies (Safaa et al., 2009; Pérez-Bonilla et al., 2011). In contrast, Coon et al. (1988) reported higher ADFI but reduced feed efficiency in hens fed barley than in hens fed corn but in this research the diets were not supplemented with enzymes. Yu et al. (1998) reported

also better growth performance of broilers when fed a corn diet than when fed a barley diet supplemented with enzymes. The chemical composition and nutritive value of barley is less uniform than that of corn because of its higher content in NSP (Jacob and Pescatore, 2012). Consequently, the use of corn favors poultry performance as compared with the use of barley. However, the potential differences in nutritive value among the two cereals might be reduced and even disappear when the barley diets are supplemented with adequate exogenous enzymes (Lázaro et al., 2003a; Gracia et al., 2003; Pérez-Bonilla et al., 2011), as occurred in the current experiment. On the other hand, moisture content is more variable in corn than in barley (FEDNA, 2010). Consequently, FCR could increase in those flocks fed diets based on corn with a higher moisture content.

Eggs tended to be heavier in hens fed barley than in hens fed corn. The information available on the effects of the main cereal of the diet on egg weight is limited and conflicting, with reports showing higher (Nahashon et al., 1994; Lázaro et al., 2003a), similar (Brufau et al., 1994; Pérez-Bonilla et al., 2011), or reduced (Coon et al., 1988) egg weight with barley. Total sulfur AA, LNL, and supplemental fat are the main dietary factors affecting egg weight in commercial layer operations (Grobass et al., 1999b; Safaa et al., 2008). In the current research, the contents in TSAA and all others indispensable AA of the 2 diets was above recommendations (FEDNA, 2008; Lohmann, 2014) and the LNL content was also in excess of hen requirements for optimal hen productivity (Grobass et al., 1999a). Consequently, no effect of an excess of TSAA or LNL on egg production was expected. On the other hand, the level of supplemental fat was higher for the barley than for the corn diet (5.3 vs. 4.7%) which might have resulted in the small increase in egg weight observed (Pérez-Bonilla et al., 2011).

4.1.2. Screen size

In the current research, FI was not affected by the screen size used to grind the cereal. Most published research, however, showed that hens had a preference for larger feed particles and that fine grinding reduced voluntary FI in laying hens (Safaa et al., 2009). In this respect, Safaa et al. (2009) observed a 2.5% greater FI in hens fed corn or wheat ground with a 10-mm screen than in hens fed the same cereals ground with a 6-mm screen. Moreover, many laying hens management guides (i.e., Lohmann, 2014) recommend the use of coarse texture diets when the objective is to maximize FI. In this respect, Nir et al. (1994b) indicated that coarse particles are better adapted to the size of the beak than fine particles. The lack of effect of the screen size used to grind the cereal on FI of the hens observed in the current experiment is not consistent with most published research and we do not have a clear explanation for the difference in behavior observed. In our research, the proportion of very fine particles ($<315\ \mu\text{m}$) was low in all the diets (between 7.9% for the 4-mm screen diet and 4.0% for the 12-mm screen diet) which might have reduced the negative impact of fine grinding on FI. In addition, all diets included extra amounts of supplemental fat (4.7 to 5.3%) which could have reduced dust formation and hen selection. The data suggest that laying hens adapt well their voluntary FI to diets varying widely in particle size, provided that the proportion of very fine particles is limited and that some extra supplemental fat is included in the diet.

In the current research, FCR from 17 to 49 wk of age tended to be reduced in hens fed the 4-mm screen ground diets as compared with the average of the hens fed the other diets. Also, a reduction in egg production and egg mass was observed in hens fed the 4-mm screen ground diet in some of the feeding periods. The available data on the effects of coarse particle size on nutrient digestibility and feed efficiency in poultry are contradictory with some research showing an improvement (Nir et al., 1994a,b; Röhe et

al., 2014; Ruhnke et al., 2015) and some research showing no effects (MacIsaac and Anderson, 2007; Safaa et al., 2009). Coarsely ground diets have a stimulating effects of the development of the GIT, improving gizzard and pancreas weight (Röhe et al., 2014) and starch digestibility (Ruhnke et al., 2015). Fine particles, however, are not retained for long in the gizzard, which reduce gizzard activity and nutrient digestibility in poultry (Nir et al., 1994a; Mateos et al., 2012). In this respect, Amerah et al. (2007) suggested that coarse particles increase the antiperistaltic movements in the GIT of the birds, leading to better mixing and utilization of the nutrients. Also, a reduction in particle size increases the surface of contact between nutrients and endogenous enzymes which might favor nutrient digestibility (Amerah et al., 2007). On the other hand, the vitamins, minerals, and crystalline AA might concentrate in the fine fraction of the diet. Consequently, a preference for coarse particles affects the balance of nutrients ingested, which eventually might reduce egg weight and hen production. These opposite effects of particle size on feed efficiency might counteract each other, and the final outcome might depend on factors such as ingredient composition of the diet and age, management, and health status of the birds.

The screen size used to grind the cereal did not affect any of the egg quality traits studied. Most published data (Safaa et al., 2009) do not show any influence of particle size on egg traits, including different measurements of shell and albumen quality. Moreover, Hafeez et al. (2015) indicated that differences in albumen weight and shell quality due to particle size were of little practical interest. Also, these authors recorded that the proportion of shell, yolk, and albumen of the eggs were not affected by particle size. Consequently, the screen size use to grind the cereal can be modified according to specific requirements without any effect on egg quality.

4.2. Gastrointestinal tract traits and body measurements

4.2.1. *Main cereal of the diet*

None of the GIT traits studied was affected by the main cereal of the diet, except the gizzard that was heavier in hens fed barley. The GMD of the diets was greater for the barley than for the corn diets and coarser particles are associated with greater development of the gizzard in broilers (Nir et al., 1994a) and pullets (Saldaña et al., 2015b). Also, the NDF content was higher (11.3 vs. 10.4%) in the barley than in the corn diets and consequently, barley feeding might stimulate more gizzard and GIT development than corn feeding (González-Alvarado et al., 2007; Mateos et al., 2012; Sacranie et al., 2012).

4.2.2. *Screen size*

None of the GIT traits studied were affected by the screen size, except gizzard weight that increased as the screen size used to grind the cereal increased, in agreement with the data of Röhe et al. (2014). Fine particles pass faster through the upper part of the GIT than coarse particles (Hetland et al., 2002) and consequently, gizzard size and function will increase with coarse grinding (Jiménez-Moreno et al., 2009b; Mateos et al., 2012).

Body mass index, body length, and tarsus length and diameter are used to estimate the BW and size of broilers (Van Roover-Reijrink, 2013), pullets (Saldaña et al., 2015b), and laying hens (Guzmán et al., 2016). In the current research, none of these traits was affected by the main cereal of the diet or the screen size used to grind the cereal, results that are consistent with the lack of effect of the characteristics of the diets on BW gain. On the other hand, the significant positive relation detected between BW and BMI, body length, and tarsus length and diameter, confirm that these traits can be used as good estimators of BW in hens.

In summary, barley can substitute up to 45% of the corn in diets for laying hens provided that the feed is supplemented with adequate enzymes. The size of the screen used to grind the cereal had no impact on hen productivity or egg quality provided that the proportion of very fine particles ($<160\ \mu\text{m}$) remains low and that the diets are supplemented with fat. Gizzard development, however, improves as the screen size increases. The data suggest that corn and barley ground through a 6 to 10-mm screen can be used indistinctly in diets for laying hens, without any effect on hen performance.

Table 1. Ingredient composition and chemical analyses (% , as fed basis, unless otherwise indicated) of the experimental diets

Ingredient	Corn	Barley
Corn	45.00	12.50
Barley	8.80	45.00
Soybean meal, 47% CP	20.80	25.20
Sunflower meal, 34% CP	10.00	1.20
Soy oil soapstock	4.70	5.30
Dicalcium phosphate	1.49	1.42
Calcium carbonate	8.22	8.36
Sodium chloride	0.35	0.35
DL-methionine, 99%	0.14	0.17
Vitamin and mineral premix ^{1,2}	0.50	0.50
Calculated analyses ³		
DM	89.20	90.50
AME _n (kcal/kg)	2,750	2,750
Ether extract	7.10	6.90
Linoleic acid (C18:2)	3.60	3.30
Crude fiber	4.40	3.60
Neutral detergent fiber	10.40	11.30
CP	17.50	17.50
Digestible amino acid ^c		
Lys	0.79	0.80
Met	0.36	0.36
Met+cys	0.60	0.60
Thr	0.57	0.56
Ash	12.40	12.60
Calcium	3.80	3.80
Digestible phosphorus	0.35	0.35
Determined analyses ⁴		
DM	89.60	90.30
Gross energy (kcal/kg)	4,305	4,290
Ether extract	7.10	7.50
Linoleic acid (C18:2)	3.75	3.40
Neutral detergent fiber	10.00	11.30
CP	17.80	18.00
Total amino acid		
Lys	0.85	0.82
Met	0.40	0.43
Met+cys	0.64	0.66
Thr	0.61	0.63
Ash	12.30	12.80

¹Provided the following (per kilogram of diet): vitamin A (*trans*-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,750 IU; vitamin E (dl- α -tocopheryl acetate), 10 mg; vitamin B1, 1.3 mg; vitamin B2, 5 mg; vitamin B6, 2 mg; vitamin B12 (cyanocobalamin), 13 mg; niacin, 25 mg; pantothenic acid (d-calcium pantothenate), 10 mg;

folic acid, 1 mg; biotin, 13 mcg; choline (choline chloride), 250 mg; manganese (MnO), 88 mg; zinc (ZnO), 63 mg; iron (FeSO₄.H₂O), 38 mg; copper (CuSO₄ 5H₂O), 8 mg; iodine [Ca(IO₃)₂], 0.7 mg; selenium (Na₂SeO₃), 0.3 mg; Roxazyme, 200 mg [1,600 U of endo-1,4-β-glucanase (EC 3.2.1.4), 3,600 U of endo-1,3 (4)-β-glucanase (EC 3.2.1.6), and 5,200 U of endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM S.A., Madrid, Spain; Natuphos 5,000 (300 FTU/kg supplied by Basf Española, S.A., Tarragona, Spain), 60 mg.

²All diets included 2.4 g canthaxantin/kg. The barley diet included also 1.7 g of ester of β-apo-8-carotenoic/kg (supplied by Basf Española, S.A., Tarragona, Spain).

³According to FEDNA (2010).

⁴Data correspond to the average of analyses conducted by duplicate in the 5 diets that differed in the size of the screen used per each cereal. Within each cereal, the determined chemical analyses were similar for the 5 diets, with a CV below 5% in all cases

Table 2. Geometric mean diameter (GMD¹ ± GSD², μm) of the experimental diets

Screen size (mm)	Cereal		Mean
	Corn	Barley	
4	960 ± 2.07	1,045 ± 2.01	1,003 ± 2.04
6	1,041 ± 2.12	1,165 ± 2.07	1,103 ± 2.10
8	1,180 ± 2.20	1,335 ± 2.06	1,258 ± 2.13
10	1,232 ± 2.21	1,458 ± 2.08	1,345 ± 2.15
12	1,302 ± 2.20	1,551 ± 2.07	1,427 ± 2.14
Mean	1,143 ± 2.16	1,311 ± 2.06	

¹GMD = Geometric mean diameter.

²GSD = Geometric standard deviation (Log normal SD).

Table 3. Influence of the main cereal of the diet and the screen size used to grind the cereal on hen productivity and egg quality from 17 to 49 wk of age

	Main cereal		Screen size (mm)					SD ¹	Probability ^{2,3}		
	Corn	Barley	4	6	8	10	12		1	2	3
Productivity											
Egg production (%)	93.3	93.3	92.7	93.4	93.4	93.8	93.2	1.44	0.946	0.342	0.140
ADFI (g/d)	112.1	112.9	112.4	112.4	112.3	112.6	112.8	2.07	0.220	0.571	0.824
FCR ⁴ (kg/kg)	1.96	1.95	1.98	1.95	1.95	1.95	1.95	0.041	0.310	0.304	0.081
FCR (kg/dozen)	1.44	1.45	1.46	1.44	1.44	1.44	1.45	0.027	0.308	0.653	0.236
Egg weight (g)	61.5	62.2	61.5	62.0	61.8	61.7	62.3	1.44	0.092	0.398	0.413
Egg mass (g/d)	57.4	58.1	57.1	58.0	57.8	58.0	58.1	1.48	0.119	0.195	0.109
BW gain (g)	565	564	599	550	538	574	561	36.5	0.998	0.856	0.672
Egg quality traits ⁵											
Dirty eggs (%)	1.47	1.30	1.30	1.34	1.50	1.43	1.46	0.402	0.295	0.309	0.628
Broken eggs (%)	0.46	0.65	0.49	0.73	0.44	0.31	0.80	0.460	0.140	0.684	0.163
Shell-less eggs (%)	0.13	0.15	0.18	0.16	0.10	0.13	0.14	0.142	0.686	0.526	0.723
Haugh units	88.5	88.8	88.8	88.5	88.4	88.4	89.1	1.27	0.360	0.672	0.595
Yolk color	10.7	10.7	10.6	10.7	10.6	10.7	10.7	0.25	0.955	0.355	0.200
Shell strength (g/cm ²)	5,165	5,147	5,194	5,232	5,221	5,155	4,977	412	0.880	0.221	0.993
Shell thickness (mm)	0.382	0.382	0.383	0.381	0.380	0.382	0.383	0.0071	0.710	0.793	0.786
Shell color ⁶											
L	61.3	61.6	61.3	62.4	60.9	61.2	61.4	1.07	0.371	0.321	0.431
a	15.6	15.4	15.6	14.9	16.0	15.6	15.6	0.75	0.391	0.341	0.667
b	32.6	32.6	32.7	32.4	32.7	32.6	32.7	0.52	0.690	0.617	0.943

¹25 replicates for the main cereal and 10 replicates for the screen size effects.²1) Effect of the main cereal; 2) Linear effect of the screen size; 3) Comparison between the average of the 2 diets ground at 4 mm vs. the average of all the other diets (ground at 6 to 12 mm).³The interactions between main effects, and the quadratic effect of the screen size were not significant for all variables ($P > 0.10$).⁴Feed conversion ratio.⁵The data correspond to the means of 8 periods (28-d each) measured in 12 fresh eggs per period.⁶Hunter color values, L* (lightness), a* (green to red), and b* (blue to yellow).

Table 4. Influence of the main cereal of the diet and the screen size used to grind the cereal on the relative weight (g/kg full BW) and length (cm/kg full BW) of the gastrointestinal tract (GIT) traits and gizzard content (% organ weight) and pH of the hens¹ at 49 wk of age

	Cereal		Screen size (mm)					SD ²	Probability ^{3,4}		
	Corn	Barley	4	6	8	10	12		1	2	3
Hen BW (g)	1,782	1,771	1,770	1,783	1,804	1,747	1,780				
GIT relative weight											
GIT ⁵	122 ^b	126 ^a	122	123	124	124	126	5.7	0.031	0.089	0.163
Full proventriculus	5.51	5.65	5.43	5.67	5.59	5.58	5.44	0.548	0.362	0.649	0.676
Full gizzard	27.8 ^b	29.1 ^a	27.1	28.3	28.5	28.8	29.5	1.85	0.018	0.006	0.017
Liver	26.4	26.8	25.3	26.7	27.2	26.7	27.3	2.16	0.495	0.056	0.232
GIT relative length											
Duodenum	14.3	14.6	14.4	14.0	13.9	15.0	14.9	1.45	0.600	0.189	0.269
Jejunum	40.6	41.2	40.9	40.5	41.4	41	40.7	3.01	0.527	0.966	0.617
Ileum	46.0	46.0	45.6	45.1	46.7	46.7	45.9	3.63	0.957	0.542	0.935
Small intestine	101	102	101	100	102	103	102	7.16	0.860	0.625	0.766
Ceca	20.2	20.6	19.5	20.0	20.9	21.2	20.5	2.33	0.567	0.180	0.403
Gizzard content	29.3	29.0	30.4	29.3	27.7	28.8	29.7	3.50	0.767	0.577	0.730
Gizzard pH	3.93	3.99	4.02	4.01	3.90	3.86	4.01	0.269	0.456	0.536	0.495

^{a-b}Within a row, means without a common superscript differ significantly.

¹Average of 2 hens per replicate chosen at random.

²25 replicates for the main cereal and 10 replicates for the screen size effects.

³1) Effect of the main cereal; 2) Linear effect of the screen size; 3) Comparison between the average of the 2 diets ground at 4 mm vs. the average of all the other diets (ground at 6 to 12 mm).

⁴The interactions between main effects, and the quadratic effect of the screen size were not significant for all variables ($P > 0.1$).

⁵From the post-crop esophagus to the cloaca, including the digesta content and the annex organs (liver, pancreas, and spleen).

Table 5. Influence of the main cereal of the diet and the screen size used to grind the cereal on the body mass index (BMI, g/body length²) and the relative length¹ (cm/kg full BW) of the hens and tarsus at 49 wk of age

	Cereal		Screen size (mm)					SD ²	Probability ^{3,4}		
	Corn	Barley	4	6	8	10	12		1	2	3
BMI	0.400	0.407	0.399	0.417	400	0.399	0.401	0.0262	0.363	0.615	0.545
Hen length	37.6	37.4	37.8	36.7	37.4	38.0	37.6	2.18	0.752	0.682	0.586
Tarsus length	4.70	4.73	4.71	4.69	4.65	4.77	4.77	0.267	0.740	0.508	0.248
Tarsus diameter	0.726	0.739	0.721	0.730	0.734	0.735	0.744	0.0413	0.267	0.226	0.089

¹Average of 2 hens per replicate chosen at random (BW are show in Table 4).

²25 replicates for the main cereal and 10 replicates for the screen size effects.

³1) Effect of the main cereal; 2) Linear effect of the screen size; 3) Comparison between the average of the 2 diets ground at 4 mm vs. the average of all the other diets (ground at 6 to 12 mm).

⁴The interactions between main effects, and the quadratic effect of the screen size were not significant for all variables ($P > 0.10$).

Table 6. Correlations between BW of the hens¹ at 49 wk of age and BMI², body length, and tarsus length and diameter

	BMI (g/cm ²)	Body length (cm)	Tarsus length (cm)	Tarsus diameter (cm)
r	0.6829	0.2449	0.3624	0.3602
Probability	<.001	0.048	0.010	0.010

¹Average of 2 hens per replicate chosen at random (BW are show in Table 4).

²Body mass index.

A.

B.

Figure 1. Particle size distribution¹ of the corn (A) and barley (B) diets

¹ The percentage of particles smaller than 160 µm and bigger than 2,500 µm were negligible for all diets.

Screen size (mm)   ···◆··· 4   -■- 6   —▲— 8   —×— 10   —*— 12

Figure 2. Effect of the main cereal of the diet on egg production (A), feed intake (B), egg weight (C), and feed conversion ratio per kg of eggs (D) of the hens from 17 to 49 wk of age

+ $P < 0.10$; * $P < 0.05$

--◇-- Barley   --■-- Corn

Figure 3. Effect of screen size used to grind the cereal of the diet¹ on egg production (A), feed intake (B), egg weight (C), and feed conversion ratio per kg of eggs (D) of the hens from 17 to 49 wk of age

¹L = Linear effect of screen size used to grind the cereal; Q = Quadratic effect of screen size used to grind the cereal; C = Comparison between diet ground at 4 mm vs. all other diets (6 to 12 mm).

⁺ $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

—◆— 4 mm   —■— 6 mm   —▲— 8 mm   —✕— 10 mm   —✕— 12 mm

CHAPTER 4:

***PARTICLE SIZE AFFECTS SHORT TERM
PREFERENCE BEHAVIOR OF BROWN-EGG
LAYING HENS FED DIETS BASED ON CORN
OR BARLEY***

1. Introduction

Several researchers (Benabdeljelil and Arbaoui, 1994; Lázaro et al., 2003a; Herrera et al. 2017) have compared the effects of diets based on corn or barley on feed intake (FI), egg production, and egg quality in laying hens. In most cases, the main cereal of the diet did not affect egg production provided that the feed was supplemented with an adequate enzyme complex. Berg (1959) reported also similar egg production but greater BW gain and lower FI in hens fed corn than in hens fed barley. Pérez-Bonilla et al. (2011) compared diets based on corn or barley and reported similar egg production but greater BW gain in hens fed corn. In contrast, Coon et al. (1988) reported higher FI, lower egg size, and reduced feed efficiency and BW gain in hens fed a barley diet than in hens fed a corn diet, probably because in this research the diets were not supplemented with exogenous enzymes.

The information available on particle size preference by brown-egg laying hens is limited. Schiffman (1968) and Allen and Perry (1977) observed that poultry had a pecking preference for coarse textured feed over non-textured feed, a preference that increased with age. ISA Brown (1999) recorded a 3.4% increase in FI and egg production in hens fed coarsely ground feeds compared with hens fed finely ground feeds (9% vs. 31% of particles smaller than 0.5 mm, respectively). More recently, Safaa et al. (2009) recorded higher FI in hens fed diets with a geometric mean diameter (GMD) of 1,375 μm than in hens fed diets with a GMD of 1,007 μm . In fact, most guidelines for laying hens (ISA Brown, 2010; Lohmann, 2016) recommend feeding coarse diets with less than 15% to 20% particles smaller than 0.5 mm when the objective is to increase FI. However, Herrera et al. (2017) did not detect any difference in FI or egg production in brown egg-laying hens fed diets in which the cereal was

ground using sieves varying in screen size from 4 to 12 mm (GMD of 1,003 μm and 1,427 μm , respectively).

The physico-chemical characteristics of the cereal and the diameter of the screen used to grind the cereal affect diet structure (Amerah et al., 2007; Jiménez-Moreno et al., 2016) and FI (Schiffman, 1968; Xu et al., 2015). The mechanoreceptors located in the beak of the bird detect differences in feed texture and discriminate against the consumption of fine particles (Gentle, 1979; González-Alvarado et al., 2007; Pérez-Bonilla et al., 2014) which may reduce voluntary FI. On the other hand, coarse particles are retained for longer in the upper part of the gastrointestinal tract (GIT) than fine particles, which might result in better development and function of the gizzard and in an improvement in nutrient utilization (Jiménez-Moreno et al., 2010; Svihus, 2011). The relative smaller surface area of the coarse particles, however, penalizes the contact between digestive enzymes and nutrients, reducing feed utilization (Amerah et al., 2007). Moreover, coarse grinding reduces feed uniformity and might induce a preference of the hens for coarse particles, resulting in the consumption of unbalanced diets and a reduction in feed efficiency (Davis et al., 1951; Nir et al., 1994a). In this respect, mash diets based on cereals with different physico-chemical characteristics might result in feeds with different particle size distribution and GMD, even when ground using the same screen size (Douglas et al., 1990; Nir et al., 1995; Jiménez-Moreno et al., 2009c; Perez-Bonilla et al., 2014). All these contrasting effects might counteract each other and the final outcome might depend on other uncontrolled variables, such as age of the bird, management practices, and nutritional characteristics of the diet (Mateos and Sell, 1980; Grobas et al., 1999a; González-Alvarado et al., 2010).

Laying hens show a specific appetite for some components of the diet, such as protein, fiber, and Ca. When given a choice, birds might be able to balance the final diet by selecting nutrients and increasing the consumption of those feed particles rich in the deficient nutrient (Hughes and Wood-Gush, 1971; Mongin and Sauveur, 1974; Holcombe et al., 1975; Abdollahi et al., 2016). For example, Forbes and Shariatmadari (1994) showed that poultry offered diets differing in CP content, choose the correct amount of the different feeds to meet protein requirements. Poultry show also an appetite for structural dietary components, and when fed low fiber diets, they consume extra amounts of fibrous components if have free access to the litter (Hetland et al., 2003; Mateos et al., 2012).

We hypothesized that grinding size could affect FI and preference behavior in laying hens, with effects that could depend on the physico-chemical characteristics of the main cereal used. Also, it was hypothesized that differences in the nutritional composition of the coarse and the fine fractions of the diets, could affect the feeding behavior of the hens. The objective of this research was to compare the effect of the size of the screen used to grind the cereal on the preference behavior of brown-egg laying hens fasted for 8 h and then fed diets based on corn or barley from 06.00 a.m. to 14.00 p.m.

2. Materials and methods

2.1. Husbandry, diets, and experiment design

All procedures were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish guidelines for the care and use of animals in research (Boletín Oficial del Estado, 2007). Details on husbandry, ingredient composition, feeding program, and nutrient content of the diets,

were reported by Herrera et al. (2017). Briefly, two diets with similar nutrient content but differing in the main cereal used (corn vs. barley) were formulated. Before feed manufacturing, the batches of the 2 cereals were divided into 5 portions, and each portion was ground using a horizontal hammer mill (Mecafa S.A., Ciudad Real, Spain) provided with a 4, 6, 8, 10, or 12 mm screen and included in their respective experimental diets. Each of the 10 diets was fed to 5 replicates that consisted in an enriched cage with 10 brown egg-laying hens.

The soybean meal used in the manufacturing of the diets was used as received whereas the the sunflower meal (received in pellets) was ground using a 10-mm screen. The GMD $\pm$ geometric standard deviation (**GSD**; log normal SD) of the soybean meal and sunflower meal were $1,069 \pm 1.79$ and 699 ± 2.23 μm , respectively (Table 1). The limestone included in the diets was presented 50% as powder (GMD = 597 ± 2.31 μm) and 50% in granular (GMD = $2,166 \pm 1.39$ μm) form. This ratio was representative of diets used in laying hens under Spanish commercial conditions (Pérez-Bonilla et al., 2014). The ingredient composition and the calculated (FEDNA, 2010) and determined nutrient content of the experimental diets are shown in Table 2. As reported by Herrera et al. (2017), feed intake, egg production, and BW gain of the hens from 17 to 48 wk of age (previous to the start of the experiment) were not affected by the main cereal or the screen size used to grind the cereal.

The night before the start of the experiment, feeders were emptied at 22.00 p.m. and the hens fasted for 8 h. At 06.00 a.m. of the following day, all replicates were offered 2.2 kg of their respective experimental feeds for ad libitum consumption. The amount of feed supplied at the start of the experiment was chosen to allow a complete filling of the feeders while avoiding feed wastage. Consequently, hens consumed the different fractions of the original feeds according to their preference throughout the

experiment. Representative samples (300 g) of the feed remaining in the feeders were collected every 2 h during the experiment (from 06.00 a.m. to 14.00 p.m.) and the particle size distribution and the $GMD \pm GSD$ were determined per cage at the end of each interval. Feed intake was measured by replicate as the difference between the amount of feed present in the feeder at the start and end of the experiment. Feed wastage was not determined.

The experiment was conducted as a completely randomized design with 10 diets in a factorial arrangement with 2 cereals and 5 screen sizes. Each treatment was replicated 5 times and the experimental unit was the cage with 10 hens for all measurements.

2.2. Laboratory analysis

Representative samples of the feeds were ground with a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) equipped with a 1-mm screen and analyzed in duplicate for major nutrients as indicated by AOAC International (2005) (Table 2). The particle size distribution of the main ingredients, the original diets, and the uneaten feed remaining in the feeders was determined in triplicate in representative samples (100 g) every 2 h (Tables 1 and 3, and Figure 1). The data was expressed as $GMD \pm GSD$ (ASAE, 1995).

At the end of the experiment (14.00 p.m.), the feed remaining in the feeders of all the replicates was collected and pooled by treatment. The reason for pooling the uneaten feed of the 5 replicates of each of the 10 treatments, was the limited amount of residual feeds present in some replicates, which did not allow to estimate precisely the nutrient composition of each of the sieve fractions in the replicates. Representative samples of the 5 sieve fractions of each of the 10 original diets and of the 50 composite uneaten feed samples collected at 14.00 p.m. (10 treatments and 5 sieve fractions per

treatment) were ground (1-mm screen) and analyzed for moisture, CP, total ash, and Ca as indicated by Herrera et al. (2017).

2.3. Statistical analysis

The experiment was conducted as a completely randomized design with 10 treatments arranged as a 2x5 factorial with 2 main cereals (corn and barley) and 5 mean particle size of the cereal (4, 6, 8, 10, and 12 mm screen). The effects of type of cereal and grinding size of the cereal on FI and their interactions were analyzed by the GLM procedure of SAS (SAS Institute, 2004). For particle size selection, the same effects and the interaction between sampling time and the main effects were analyzed by the MIXED procedure of SAS (SAS Institute, 2004), using time as repeated measure. The individual cage represented the experimental unit for all measurements. When the model was significant, treatment means were separated using the Tukey test. Results in tables are presented as means and differences were considered significant at $P < 0.05$. For data on CP, ash, and Ca content of the different sieve fractions of the original diets and of the residual feeds at 14.00 p.m., no replicates were available and therefore, only average values per treatment are presented.

3. Results

The effects of the cereal and screen size on the GMD and the GSD of the diets are shown in Table 3. Independent of the screen used, the GMD of the feeds was greater (average of 1,311 μm vs. 1,143 μm) for the barley- than for the corn diets. However, an opposite effect was observed for the GSD (2.06 vs. 2.16). Feed intake from 06.00 a.m. to 14.00 p.m. (56.6 ± 5.4 g) was not affected by the main cereal or the screen size used to grind the cereal (data not shown).

The effects of the main cereal, screen size, and time on particle size distribution of diets and feed refusals are shown in Table 4. The GMD and the GSD of the diets

decreased ($P < 0.001$) as the experiment progressed, irrespective of the size of the screen used to grind the cereal. The differences in GMD between the corn and barley based diets decreased with time ($P < 0.001$ for the interaction; Figure 2A). For the GSD, the decrease with sampling time observed was more pronounced for the corn than for the barley diets ($P < 0.001$ for the interaction; Figure 2B).

As an average of the corn and barley diets, both GMD (from 948 μm to 1,246 μm) and the GSD (from 2.00 to 2.10) increased ($P < 0.001$) as the screen size used to grind the cereal increased from 4 to 12 mm (Table 4). The differences in GMD among screen sizes of the corn and barley diets decreased with time ($P < 0.001$ for the interaction; Figure 3) but no interactions were detected for the GSD.

The CP content of the sieved fractions of the original diets and of the residual feeds, measured after 8 h of ad libitum feeding, is shown in Table 5. When the cereal, either corn or barley, was finely ground (4 and 6 mm screen), the protein accumulated primarily in the coarser fraction (average of the 1,250 and 2,500 μm sieves) of the original diets. However, no such an effect was observed when the cereal was coarsely ground (10 and 12 mm screen), with more protein accumulating in the finer fraction (160 and 315 μm sieves). In fact, the CP content of the coarser fraction was 18.6% and 19.2% for the corn and barley diets, when the cereal was ground with a 4 mm screen but 14.3% and 14.4% when ground with a 12 mm screen. Similar but less pronounced effects were observed in the residual feeds after 8 h of ad libitum feeding; the CP content of the coarser fractions was 17.1% and 16.8% for the corn and barley diets when the cereal was finely ground (4 mm screen) but 15.0% and 16.6% when the cereal was coarsely ground (12 mm screen).

The ash and Ca contents of the sieving of the original diets and of the residual feeds, measured after 8 h of ad libitum feeding are shown in Tables 6 and 7,

respectively. Ash and Ca concentrated in the coarser fractions of the original and the residual feeds in both set of diets, with differences being more pronounced when the cereals were ground fine than when ground coarse. In fact, the Ca content of the coarser fraction (1,250 and 2,500 μm sieves) of the original corn and barley diets was 8.2% and 7.8% when the cereal was ground at 4 mm but 4.4% and 3.7% when ground at 12 mm (Table 7). Similarly, the Ca content of the coarser fraction (1,250 and 2,500 μm sieves) of the residual feed after 8 h of ad libitum feeding was 13.2% and 11.7% for the corn and barley diets when the cereal was ground at 4 mm but 9.7% and 6.7% when ground at 12 mm. Similar response was observed for the ash, which concentrated in the coarser fraction of the original and of the residual feeds, with more pronounced effects when the cereal was ground fine (4- and 6 mm screen) than when ground coarse (10- and 12 mm screen).

4. Discussion

The GMD of the original diets was greater for the barley- than for the corn diets (1,311 vs. 1,143 μm), consistent with data of Pérez-Bonilla et al. (2011) comparing the two cereals. The glumes of the barley grains are quite flexible, and when ground, a high proportion passes intact through the screen, resulting in an increase in the GMD of diets based on barley compared with diets based on corn. These results confirm that the structure of the feed depends not only on the size of the sieve but also on the main cereal of the diet, consistent with data of Douglas et al. (1990) and Nir et al. (1995).

The beak of the chicken is not well adapted to consume fine particles and consequently, coarse particles tended to be preferred (Picard et al., 1997; Mateos et al., 2002). In fact, most management guides (ISA Brown, 2010; Lohmann, 2016) recommended coarse grinding when the objective is to maximize FI. In this respect, Safaa et al. (2009) observed a significant 2.5% increase in FI in hens fed corn or wheat

ground with a 10-mm screen compared with hens fed the same cereals ground with a 6-mm screen. However, in the current research in which diets based on corn or barley were compared, no differences in FI because of particle size were observed. The reasons for the discrepancy among experiments is not apparent but could be related to the proportion of very fine particles present in the diets rather than to their GMD. In this respect, Portella et al. (1988a) suggested that hen preference for coarse particles might be altered not only by the average size of the particles of the diet but also by the proportion of very fine particles. Herrera et al. (2017) suggested that supplemental fat might agglomerate the fines of the diet, improving palatability and reducing the rejection rate for fine particles. In the current research supplemental, fat was high in both corn and barley diets (4.7% and 5.3%, respectively) which might have reduced the preference of the hens for coarse vs. fine particles.

The GMD of the diets decreased with time, reflecting the preference behavior of the hens for coarse particles, in agreement with previous studies with broilers (Schiffman, 1968; Portella et al., 1988b; Xu et al., 2015) and laying hens (Portella et al., 1988a; Safaa et al., 2009). The data confirm that when allowed to choose, laying hens prefer coarse particles. In contrast, Herrera et al. (2017) reported no differences in FI of brown laying hens fed diets from 17 to 49 wk of age when the cereal was ground using screens varying in size from 4 to 12 mm. The reasons for the discrepancy among researches are not known but might depend on the chemical composition of the experimental diets as well as on the feeding strategy used (i.e., frequency of feeding) and feeder design which might affect feed particle size distribution. When allowed to choose, hens might prefer coarse particles as observed in the current experiment. However, when new feed is constantly added to the uneaten feed or when the hens are

obliged to clean the feeders at specific times, as occurred often under practical conditions, the effects might be less pronounced or even disappear.

The differences in GMD of the residual feeds between the two cereals based diets and among screen sizes, decreased with time, reflecting the increased uniformity of the particles in the uneaten feed. Portella et al. (1988a) reported also that the disappearance of coarse particles was overwhelming when the feed had an excess of large particles (> 2.36 mm). However, the preference was less obvious as the experiment progressed and the percentage of coarse particle was reduced, consistent with the results reported herein. The GSD of the diets was greater for the corn- than for the barley diets, differences that increased with increases in the size of the screen. In contrast, the differences in GSD for both main effects (type of cereal and screen size) decreased with time, reflecting the higher rate of disappearance of the coarser particles of the uneaten feed for the first hours after the start of the experiment.

Birds show a specific appetite for some components of the diet, such as CP, fiber, or Ca (Hughes and Wood-Gush, 1971; Mongin and Sauveur, 1974; Holcombe et al., 1975). When the diet is deficient in a given nutrient, birds might choose to eat those sieving fractions richer in the deficient nutrient (Portella et al., 1988a). As a result, hen preference might depend not only on particle size but also on the physical distribution of the limiting nutrient within the sieving fractions. In the current research, however, the differences in CP, ash, and Ca content among the sieving fractions of the original diets were similar to those of the residuals at the end of the experiment, suggesting that feed preference was not modulated by differences in nutrient content of the sieve fractions of the diet.

An observation that deserves further attention is the difference in Ca concentration between the original diets and the residual feeds after 8 h of ad libitum

consumption. For the corn diet, the percentage of Ca across fractions of the diet, independent of the sieving fraction considered and of the screen size used to grind the corn, was 8.6% to 63.4% higher in the residual feeds than in the original diets. Similar data were observed for the barley diets, in which the percentage of Ca was 11.1% to 58.3% higher in the residual feed than in the original diets. Similar distribution pattern was observed for ash content but not for CP. The reasons of these contrasting observations are unknown but might be related to the timing at which the feed (and the Ca included in the feed) was available for consumption and the Ca needs for shell formation according to the period of the day. Hughes et al. (1972) reported that laying hens under-consume Ca during the morning but tend to over-consume Ca late in the day, when most of the shell formation process occurs. In the evening, hens try to balance Ca intake and Ca requirements, giving preference to those particles rich in the deficient nutrient, regardless of feed coarseness, whereas an opposite pattern might occur during the morning.

The current research was conducted from 06.00 a.m. to 14.00 p.m., a period of the day in which Ca deposition is relatively low. Consequently, hens chose to under-consume Ca because Ca requirements are lower. The higher Ca concentration of the residual feeds after 8 h of ad libitum consumption, compared to that of the original diets observed, is consistent with this suggestion. Also, it was noticed that Ca concentrated in the coarse fractions of the original feeds, probably because 50% of the CaCO_3 of the diets was supplied in granular form. Similar Ca distribution pattern was observed in the residual feeds of all diets after 8 h of ad libitum consumption, confirming that hens preference for coarse particles was independent of its Ca content. In contrast, Portella et al. (1988a) observed that the proportion of Ca in the different sieving fractions of a mash diet was not homogenous and decreased as the GMD of the diet increased,

opposite to the results reported herein. The reasons for the discrepancy are not known. We do not have information on the time of the day and the size of the Ca carbonate used by Portella et al. (1988a) and thus, no comparison of the results of the two experiments can be made.

The consumption pattern observed was different for CP than for Ca. Hens showed a clear preference for consuming coarse particles and were unable to choose those fractions of the feed with a higher or lower protein content. Traineau et al. (2015), however, reported that when hens were allowed to choose between two feeds varying in CP content, they over-consumed of the diet rich in protein after oviposition, early in the morning, a period in which the albumen of the egg is formed. In the research of Traineau et al. (2015), hens were offered two different feeds to choose, whereas a single feed was supplied in the current research, limiting the capacity of the hens for selecting feed particles based on its protein content. In this respect, Forbes and Shariatmadari (1994) showed that the ability of the hens to choose ingredients according to its CP needs, when offered a single diet, was limited. Moreover, Pousga et al. (2005) reported that hens offered a single conventional feed were not able to choose those feed fractions with higher CP content, consistent with the data reported herein.

In conclusion, type of cereal and size of the screen used to grind the cereal affected feed preference behavior of the hens. The CP, ash, and Ca contents of the diet were not uniform and varied with the screen used to grind the cereal and with the sieving fraction (fine vs. coarse) of the diets. The data demonstrate that under the condition of the current research, hen preference for coarse particles was not modulated by the desire to meet any potential requirement for the missing nutrient. In fact, in relative terms, the concentration of CP, ash, and Ca of the sieve fractions of the original diets and the residuals after 8 h of ad libitum consumption, followed a similar pattern, demonstrating

that the composition of the sieving fractions of the diet did not affect the preference behavior of the hens for coarse particles. When allowed to choose, and no extra quantities of feed are added to the feeder during the experimental period, hens showed a clear preference for consuming coarse particles. Crude protein content of the different sieve fractions, did not affect hen preference behavior. In fact, hens did not show any preference for consuming extra amounts of protein during the early part of the day. In contrast, Ca intake of the hens was reduced during the morning, resulting in residual feeds with greater concentration of ash and Ca than in the original diets, irrespective of the size of the feed fractions. It should be noticed that in the current research hens were offered at all times the leftovers of the previous feeding periods, reducing its potential capacity to choose coarse particles. Hen preference behavior might be different under commercial conditions, when the residuals are remixed constantly with new feed coming from the auger and the hens are obliged often to consume the fine particles previously refused.

Table 1. Particle size distribution and geometric mean diameter (GMD) and Log normal SD (GSD) of the main ingredients¹

Sieve diameter, μm	Soybean meal (47% CP)	Sunflower meal (34% CP)	Calcium carbonate	
			Powder	Granular
5,000	0.0	0.5	0.0	0.2
2,500	3.6	3.6	0.1	29.2
1,250	36.0	18.5	18.5	70.3
630	47.7	34.6	35.2	0.2
315	10.2	27.3	23.4	0.0
160	1.4	11.8	14.8	0.0
80	0.7	3.2	6.0	0.1
40	0.4	0.5	1.9	0.0
0	0.0	0.0	0.1	0.0
GMD	1,069	699	597	2,166
GSD	1.79	2.23	2.31	1.39

¹Measured in the original ingredients before the manufacturing of the diets.

Table 2. Ingredient composition and chemical analyses (% as fed basis) of the experimental diets

Ingredient	Corn	Barley
Corn	45.00	12.50
Barley	8.80	45.00
Soybean meal, 47% CP	20.80	25.20
Sunflower meal, 34% CP	10.00	1.20
Soy oil soapstock	4.70	5.30
Dicalcium phosphate	1.49	1.42
Calcium carbonate (powder)	4.11	4.18
Calcium carbonate (granular size)	4.11	4.18
Sodium chloride	0.35	0.35
DL-methionine, 99%	0.14	0.17
Vitamin and mineral premix ¹	0.50	0.50
Calculated analyses ²		
AME _n (Kcal/kg)	2,750	2,750
Crude fiber	4.40	3.60
Digestible amino acids ²		
Lys	0.80	0.80
Met	0.35	0.35
Met + cys	0.59	0.59
Thr	0.56	0.56
Determined analyses ³		
Dry matter	89.60	90.30
Gross energy (Kcal/kg)	4,305	4,290
Crude protein	17.70	17.90
Neutral detergent fiber	10.00	11.30
Ether extract	7.10	7.50
Linoleic acid (C18:2)	3.75	3.40
Ash	12.30	12.80
Calcium	3.85	3.94
Total phosphorus	0.65	0.62

¹Provided the following (per kilogram of diet): vitamin A (*trans*-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,750 IU; vitamin E (dl- α -tocopheryl acetate), 10 mg; vitamin B1, 1.3 mg; vitamin B2, 5 mg; vitamin B6, 2 mg; vitamin B12 (cyanocobalamin), 13 mg; niacin, 25 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 1 mg; biotin, 0.13 mg; choline (choline chloride), 250 mg; manganese (MnO), 90 mg; zinc (ZnO), 65 mg; iron (FeSO₄·H₂O), 40 mg; copper (CuSO₄·5H₂O), 8 mg; iodine [Ca(IO₃)₂], 0.7 mg; selenium (Na₂SeO₃), 0.3 mg; Roxazyme, 200 mg [1,600 U of endo-1,4- β -glucanase (EC 3.2.1.4), 3,600 U of endo-1,3 (4)- β -glucanase (EC 3.2.1.6), and 5,200 U of endo-1,4- β -xylanase (EC 3.2.1.8)] supplied by DSM S.A., Madrid, Spain; Natuphos 5,000 (300 FTU/kg supplied by Basf Española, S.A., Tarragona, Spain), 60 mg.

²According to FEDNA (2010).

³Data correspond to the average of duplicate analyses of the 5 diets ground with different screen size of each cereal. Within each cereal type, the determined chemical analyses were similar for all the diets (CV below 5%), independent of the screen size.

Table 3. Geometric mean diameter (GMD, μm) and geometric standard deviation (GSD) of the experimental diets¹

Screen size (mm)	Cereal		Average
	Corn	Barley	
4	960 $\pm$ 2.07	1,045 $\pm$ 2.01	1,003 $\pm$ 2.04
6	1,041 $\pm$ 2.12	1,165 $\pm$ 2.07	1,103 $\pm$ 2.10
8	1,180 $\pm$ 2.20	1,335 $\pm$ 2.06	1,258 $\pm$ 2.13
10	1,232 $\pm$ 2.21	1,458 $\pm$ 2.08	1,345 $\pm$ 2.15
12	1,302 $\pm$ 2.20	1,551 $\pm$ 2.07	1,427 $\pm$ 2.14
Average	1,143 $\pm$ 2.16	1,311 $\pm$ 2.06	

¹ Determined before the start of the experiment.

Table 4. Influence of the main cereal and the particle size distribution of the diet on preference behavior of the hens

	Cereal		Screen size (mm)					Time	SD ¹	Probability ^{2,3}				
	Corn	Barley	4	6	8	10	12			1	2	3	4	5
GMD ⁴									88.5	<.001	<.001	<.001	<.001	<.001
06.00 h	1,143	1,311	1,003	1,103	1,258	1,345	1,427	1,227x						
08.00 h	1,060	1,202	950	1,109	1,103	1,239	1,255	1,131y						
10.00 h	1,048	1,149	936	1,076	1,063	1,206	1,212	1,098y						
12.00 h	1,023	1,158	933	1,070	1,079	1,165	1,206	1,091y						
14.00 h	928	1,053	884	991	981	1,036	1,061	991z						
Average	1,048b	1,175a	948c	1,069b	1,108b	1,198a	1,246a							
GSD ⁵									0.051	<.001	<.001	<.001	<.001	0.601
06.00 h	2.16	2.06	2.04	2.10	2.13	2.15	2.14	2.11y						
08.00 h	2.13	2.06	2.03	2.07	2.12	2.12	2.14	2.10x						
10.00 h	2.08	2.04	2.01	2.04	2.09	2.09	2.09	2.06y						
12.00 h	2.03	1.98	1.96	1.98	2.01	2.02	2.06	2.01z						
14.00 h	2.03	1.99	1.97	1.99	2.01	2.02	2.05	2.01z						
Average	2.09a	2.03b	2.00c	2.04b	2.07a	2.08a	2.10a							

^{a-b-c} Within a line, means without a common superscript differ significantly.

^{x-y-z} Within a column, means without a common superscript differ significantly.

¹25 replicates for the main cereal effect and 10 replicates for the screen size effect.

²1 = Effect of type of cereal; 2 = effect of screen size; 3 = effect of time; 4 = interaction between type of cereal and time; 5 = interaction between screen size and time.

³The interaction between type of cereal and screen size was not significant (P > 0.10).

⁴Geometric mean diameter.

⁵Geometric standard deviation.

Table 5. Crude protein content (%) of the sieved fractions of the original diets at the start of the experiment (06.00 h) and of the residual feeds at the end of the experiment (14.00 h)

Original diet ¹										
Main cereal	Corn					Barley				
Screen size (mm)	4	6	8	10	12	4	6	8	10	12
Sieve diameter, μm										
160	15.0	14.7	15.1	15.5	16.0	13.0	13.4	13.7	14.3	16.9
315	15.9	16.6	16.2	17.1	19.3	14.6	14.6	16.0	16.2	18.3
630	17.4	18.4	19.3	20.9	20.9	17.9	18.4	19.0	21.2	21.6
1,250	17.4	17.6	19.8	18.8	18.5	18.9	18.2	19.1	21.0	18.9
2,500	19.9	19.5	12.3	11.4	10.1	19.4	15.2	13.4	11.0	9.8
Pooled fractions										
Fine fraction ²	15.5	15.7	15.7	16.3	17.7	13.8	14.0	14.9	15.3	17.6
Coarse fraction ³	18.6	18.5	16.1	15.1	14.3	19.2	16.7	16.3	16.0	14.4
Residual feed ⁴										
Main cereal	Corn					Barley				
Screen size (mm)	4	6	8	10	12	4	6	8	10	12
Sieve diameter, μm										
160	14.1	15.0	15.7	15.5	15.7	12.4	12.8	13.2	13.4	13.5
315	16.3	17.7	18.1	17.4	19.2	14.9	16.6	17.1	17.6	19.5
630	19.4	20.1	20.6	21.0	22.2	19.2	21.4	21.2	23.5	23.5
1,250	19.0	18.3	18.7	19.9	17.9	18.8	18.9	18.9	18.7	19.4
2,500	15.1	13.7	15.9	13.2	12.1	14.7	12.4	15.0	13.9	13.8
Pooled fractions										
Fine fraction	15.2	16.4	16.9	16.5	17.5	13.7	14.7	15.2	15.5	16.5
Coarse fraction	17.1	16.0	17.3	16.6	15.0	16.8	15.7	17.0	16.3	16.6

¹The analyzed CP content was 17.7% and 17.9% for the corn and barley diets, respectively.

²The fine fraction was defined as the proportion of particles with a geometric mean diameter no more than 315 μm .

³The coarse fraction was defined as the proportion of particles with a geometric mean diameter of at least 1,250 μm .

⁴The feed remaining in the feeders of all the replicates of each of the 10 experimental diets was collected and pooled by treatment. Consequently, only average data are presented.

Table 6. Ash content (%) of the sieved fractions of the original diets at the start of the experiment (06.00 h) and of the residual feeds at the end of the experiment (14.00 h)

Original diet ¹										
Main cereal	Corn					Barley				
Screen size (mm)	4	6	8	10	12	4	6	8	10	12
Sieve diameter, μm										
160	7.3	7.5	8.7	8.3	8.7	10.5	10.8	10.8	12.3	12.6
315	6.6	7.3	7.4	7.7	7.6	8.3	9.1	8.9	9.7	9.7
630	11.3	12.1	12.2	12.7	12.9	11.2	13.6	12.9	14.6	14.7
1,250	12.7	15.0	12.1	16.4	15.5	10.4	17.2	12.3	14.8	14.2
2,500	34.1	21.5	22.1	16.5	14.7	33.2	20.2	15.1	15.3	10.3
Pooled fractions										
Fine fraction ²	7.0	7.4	8.1	8.0	8.2	9.4	10.0	9.9	11.0	11.2
Coarse fraction ³	23.4	18.3	17.1	16.5	15.1	21.8	18.7	13.7	15.1	12.3
Residual feed ⁴										
Main cereal	Corn					Barley				
Screen size (mm)	4	6	8	10	12	4	6	8	10	12
Sieve diameter, μm										
160	9.7	10.6	10.9	10.5	9.3	11.3	14.7	16.0	17.1	15.7
315	9.3	9.4	9.8	9.1	10.4	8.9	11.5	11.9	12.7	12.2
630	11.8	12.0	13.3	12.5	13.7	12.1	15.6	15.2	15.8	15.5
1,250	21.7	19.6	24.0	18.3	19.8	18.1	19.2	17.6	16.8	18.2
2,500	47.9	36.0	42.7	33.0	33.0	45.4	29.1	27.4	27.6	21.4
Pooled fractions										
Fine fraction	9.5	10.0	10.4	9.8	9.9	10.1	13.1	14.0	14.9	14.0
Coarse fraction	34.8	27.8	33.4	25.7	26.4	31.8	24.2	22.5	22.2	19.8

¹The analyzed ash content was 12.3% and 12.8% for the corn and barley diets, respectively.

²The fine fraction was defined as the proportion of particles with a geometric mean diameter no more than 315 μm .

³The coarse fraction was defined as the proportion of particles with a geometric mean diameter of at least 1,250 μm .

⁴The feed remaining in the feeders of all the replicates of each of the 10 experimental diets was collected and pooled by treatment. Consequently, only average data are presented.

Table 7. Calcium content (%) of the sieved fractions of the original diets at the start of the experiment (06.00 h) and of the residual feeds at the end of the experiment (14.00 h)

Original diet ¹										
Main cereal	Corn					Barley				
Screen size (mm)	4	6	8	10	12	4	6	8	10	12
Sieve diameter, μm										
160	1.3	1.3	1.6	1.5	1.7	2.2	2.3	2.3	2.8	2.9
315	0.9	1.2	1.1	1.7	1.5	1.8	1.9	1.7	1.9	2.0
630	3.2	3.2	3.2	3.3	3.3	3.0	3.8	3.5	4.2	4.0
1,250	4.0	4.2	3.3	4.6	4.3	3.5	5.3	3.7	4.5	4.3
2,500	12.4	7.1	7.4	5.5	4.5	12.1	8.1	4.4	4.9	3.0
Pooled fractions										
Fine fraction ²	1.1	1.3	1.4	1.6	1.6	2.0	2.1	2.0	2.4	2.5
Coarse fraction ³	8.2	5.7	5.4	5.1	4.4	7.8	6.7	4.1	4.7	3.7
Residual feed ⁴										
Main cereal	Corn					Barley				
Screen size (mm)	4	6	8	10	12	4	6	8	10	12
Sieve diameter, μm										
160	2.5	3.0	3.1	2.9	3.5	3.1	4.3	4.6	5.0	4.2
315	2.3	2.4	2.4	2.4	2.5	2.1	3.2	3.1	3.5	3.1
630	3.6	3.5	4.0	3.7	4.0	3.7	4.8	4.4	4.9	4.5
1,250	7.8	6.9	9.0	6.4	7.0	6.0	7.1	5.6	5.5	6.2
2,500	18.6	13.8	16.4	12.6	12.3	17.4	11.4	10.5	10.7	7.2
Fine fraction	2.4	2.7	2.8	2.7	3.0	2.6	3.8	3.9	4.3	3.7
Coarse fraction	13.2	10.4	12.7	9.5	9.7	11.7	9.3	8.1	8.1	6.7

¹The analyzed Ca content was 3.85% and 3.94% for the corn and barley diets, respectively.

²The fine fraction was defined as the proportion of particles with a geometric mean diameter no more than 315 μm .

³The coarse fraction was defined as the proportion of particles with a geometric mean diameter of at least 1,250 μm .

⁴The feed remaining in the feeders of all the replicates of each of the 10 experimental diets was collected and pooled by treatment. Consequently, only average data are presented.

A) Corn diet

B) Barley diet

Figure 1. Particle size distribution¹ of the original diets at the start of the experiment²

¹ The percentage of particles smaller than 160 µm and bigger than 2,500 µm were negligible for all diets.

²The average geometric mean diameter and geometric standard deviation (GMD ± GSD), across sieve diameters was $1,143 \pm 2.16$ µm for the corn diets and $1,311 \pm 2.06$ µm for the barley diets.

A) Geometric mean diameter (GMD, μm)

B) Geometric standard deviation (GSD, μm)

Figure 2. Interaction between the main cereal of the diet and sampling time on geometric mean diameter (GMD) and Log normal SD (GSD) of the diets

Figure 3. Interaction between the screen size (mm) used to grind the cereal and sampling time on the geometric mean diameter (GMD) of the diet

CHAPTER 5:
GENERAL DISCUSSION AND CONCLUSIONS

1. General discussion

The aim of this thesis was to gain scientific insights on the use of the most available cereals (corn, wheat, and barley), ground at different particle size, in diets for laying hens. In all the experiments exogenous enzymes complexes were used as a supplement of the diets. In this chapter, the main findings of the three experiments conducted are combined to generate a general discussion.

1.1. Main cereal

1.1.1. Influence on hen production

In experiment 1, egg production, egg mass, ADFI, BW gain, and FCR were not affected by the main cereal of diet (corn, wheat, and barley) from 33 to 65 wk of age. Similarly, in experiment 2, the main cereal of the diet (corn vs. barley) did not affect egg production, ADFI, FCR, or BW gain of brown-egg laying hens from 17 to 49 wk of age. Similarly, FI was not affected by the main cereal of diet (corn vs. barley) as indicated in experiment 3, confirming previous results. All these data are in agreement with previous studies (Craig and Goodman, 1993; Safaa et al., 2009; Pérez-Bonilla et al., 2011). Safaa et al. (2009) did not observe any effect on productive performance of layers from 20 to 48 wk of age when 50% corn was substituted by wheat. Similarly, Pérez-Bonilla et al. (2011) reported that the substitution of 45% of corn by the same amount of wheat or barley did not affect productive performance of brown-egg laying hens from 22 to 54 wk of age. In contrast, Coon et al. (1988) reported higher FI and poorer FCR per kilogram of eggs in hens fed barley based diets than in hens fed corn based diets, although in this research diets were not supplemented with an adequate exogenous enzyme complex. Also, Pérez-Bonilla et al. (2014) reported greater FI in brown-egg laying hens fed corn and wheat than in hens fed barley from 24 to 59 wk of

age, although in this research none of the other production traits was affected by the main cereal of the diet.

In the current experiment, all diets, including those based on corn, were supplemented with exogenous carbohydrases and therefore, no effect of NSP content of the diet on productive performance was expected. Lázaro et al. (2003a) reported in SCWL hens that corn could be substituted by high soluble fiber content cereals (wheat, barley, or rye) without any negative effect on performance. These authors observed also that the supplementation of the wheat, barley, and rye diets with enzymes (a combination of β -glucanases and xylanases) improved nutrient digestibility, probably because of the decrease in digesta viscosity caused by enzyme supplementation. Lillie and Denton (1968) reported also similar production and FCR in SCWL fed diets based on corn, wheat, or barley, even though in this report no enzyme were added to the diet. Because of its higher content in NSP, barley and wheat are less uniform in chemical composition and nutritive value than corn (Jacob and Pescatore, 2012). Consequently, under commercial practices the use of corn tends to favor poultry performance compared with the use of wheat or barley. However, the potential differences in nutritive value among cereals are reduced and even disappear when the small, white grain based diets are supplemented with an adequate exogenous enzyme complex (Lázaro et al., 2003a; Gracia et al., 2003; Pérez-Bonilla et al., 2011).

In experiments 1 and 2, the only variable affected by the main cereal of the diet was egg weight that was heavier for hens fed barley than for hens fed corn. The scientific information available on the effects of the main cereal of the diet on egg weight is limited and conflicting (Coon et al., 1988; Lázaro et al., 2003a, Pérez-Bonilla et al., 2011). For example, Nahashon et al. (1994) and Lázaro et al. (2003a) reported heavier eggs in hens fed barley than in hens fed corn, results that are consistent with the

data of the current research. However, Pérez-Bonilla et al. (2011) reported similar egg weight when corn was substituted by wheat or barley as the main ingredient of the diet in brown-egg laying hens from 22 to 54 wk of age. Mathlouthi et al. (2002) reported also similar egg weight when wheat was substituted by barley as the main ingredient of the diet of white laying hens from 28 to 40 wk of age. In contrast, Coon et al. (1988) reported heavier eggs in hens fed corn than in hens fed barley as the main ingredient of the diet in white hens from 36 to 64 wk of age. Total sulfur AA, linoleic acid (LNL), and supplemental fat are the main dietary factors affecting egg weight in commercial layer operations (Coon et al., 1988; Grobas et al., 1999a; Safaa et al., 2008). In this respect, Coon et al. (1988) reported that eggs from hens fed barley diets with lower lysine and methionine that was 0.68% and 0.29% respectively, were about 1 g lighter than weights of eggs from layers fed the corn and a second barley diets in hens from 36 to 64 wk of age. In the current experiments, the contents in TSAA and all others indispensable AA of the diets was above recommendations (FEDNA, 2008; Lohmann, 2016) and the LNL content was also in excess of hen requirements for optimal hen productivity (Grobas et al., 1999a). Consequently, no effect of an excess of TSAA or LNL on egg production was expected. In experiments 1 and 2 of the current thesis, the levels of supplemental fat was higher for the barley- than for the corn diet (4.9% vs. 1.8% in experiment 1 and 5.3% vs. 4.7% in experiment 2) which might have resulted in heavier in eggs (Pérez-Bonilla et al., 2011). The results of the current experiments confirm that wheat and barley, supplemented with adequate enzymes, are a good alternative to corn in diets for brown-egg laying hens.

1.1.2. Influence on egg quality traits

The effects of the main cereal of the diet on egg quality traits were studied in experiments 1 and 2. The hypothesis was that the inclusion of barley and wheat in the

diet in substitution of corn could not affect any of the variables studied, except the incidence of dirty eggs that could increase and yolk color that could be reduced. However, in none of the two experiments the cereal used affected any of the egg quality traits studied, including HU, yolk color, shell strength, shell thickness, and shell color. To notice that in the current researches, an enzyme complex and adequate amounts of canthaxanthin and β -apo-8-carotenoid, yellow and red pigments, were added to the diets as needed, and therefore no effects of diet on the percentage of dirty eggs or yolk color were expected. In experiment 1, the only quality traits affected were the percentage of undergrades and of dirty eggs, that tended to be higher in hens fed corn and wheat than in hens fed barley, and the number of broken eggs that tended to be greater in hens fed the barley diet. Francesch et al. (1995) reported that the inclusion of barley and wheat in diets without any enzyme supplementation, increased the incidence of dirty eggs compared with the inclusion of corn. The available information on the effect of the main cereal of the diet on egg quality traits, other than incidence of dirty eggs, is scarce. To our knowledge, the only reports available comparing egg quality in hens fed these 3 cereals are those of Lázaro et al. (2003a) and Pérez-Bonilla et al. (2011). Lázaro et al. (2003a) reported that hens fed barley or wheat based diets showed an increase in the percentage of dirty eggs compared with hens fed a control corn diet. However, none of the other quality traits studied (shell weight, percentage of shell-less, and HU) was affected. The authors reported that when an enzyme complex was added to the wheat and barley diets, the incidence of dirty eggs decreased to levels similar to those found for the corn diet. In this respect, Mathlouthi et al. (2002), Lázaro et al. (2003b, 2004), and García et al. (2008) reported that enzymes reduced intestinal viscosity in poultry, improving the consistency of the excreta, and thus, reducing or even eliminating the incidence of dirty eggs. Pérez-Bonilla et al. (2011) observed that the substitution of corn

by wheat and barley in enzyme supplemented diets fed to brown-egg laying hens from 22 to 54 wk of age did not affect any of the egg quality traits studied (HU, shell thickness, shell density, and number of undergrads eggs) except yolk color, that was higher for hens fed corn than for hens fed wheat or barley. Moreover, Çiftci et al. (2003) and Safaa et al. (2009) reported that the substitution of corn by wheat in enzyme supplemented diets did not affect any egg quality trait in SCWL or brown-egg laying hens, respectively. Also, Jamroz et al. (2001) reported similar egg quality in hens fed wheat or barley diets supplemented with enzymes

1.1.3. Influence on gastrointestinal tract development and body measurements

The effect of the main cereal of the diet on GIT traits was studied in experiment 2, exclusively. The authors have not found any published report on the effects of the main cereal of the diet on GIT measurements in brown-egg layer hens to compare with the data of the current research. In this experiment the relative weight of the GIT and the gizzard at 49 wk of age was greater in hens fed the barley diets than in hens fed the corn diets. However, none of the others GIT traits studied (proventriculus and liver weights) was affected by the main cereal of the diet. The GMD of the diets was greater for the barley than for the corn feeds (1,311 vs. 1,143 μm) and coarser particles are associated with greater development of the gizzard in broilers (Nir et al., 1994a) and pullets (Saldaña et al., 2015b). Also, the NDF content was higher (11.3 vs. 10.4%) in the barley than in the corn diets and consequently, barley feeding might stimulate more the muscular layers of the gizzard and the development of the GIT than corn feeding (González-Alvarado et al., 2007; Mateos et al., 2012; Sacranie et al., 2012).

Body mass index (BMI), body length, and tarsus length are useful criteria to evaluate body size and predict future performance of broilers (Mendes et al., 2007; Van Roover-Reijrink, 2013), pullets (Saldaña et al., 2015b), and layers (Guzmán et al.,

2016). In experiment 2, none of these traits was affected by the main cereal of the diet. The only report available comparing the relative length of the pullets and the tarsus in pullets fed the corn or wheat diets is that of Saldaña et al. (2015b). These authors reported that the relative length of the pullets tended to be higher in birds fed corn than in birds fed wheat at 5 and 17 wk of age. However, tarsus length was not affected. The authors have not found any published report on the effects of the main cereal of the diet on hens or tarsus length to compare with the results reported herein.

1.2. Particle size

1.2.1. Influence on hen production

The effects of the particle size of the diet on productive performance of the hens were studied in experiments 1, 2, and 3. In all these experiments, the GMD of three cereals increased as the screen size used to grind the ingredient increased but the magnitude of the increase was greater for barley and wheat than for corn, in agreement with data of Pérez-Bonilla et al. (2011). The glumes of barley are coarser and flexible and also, barley grains have higher NDF content than corn. Consequently, the chances of the different fractions of barley passing intact through the screen increased compared with corn, resulting in an increase in the GMD of the diet. Safaa et al. (2009) reported that the GMD of wheat was greater than that of corn when both cereals were hammer milled through an 8 or a 10-mm screen. In contrast, the GSD was higher for the corn and wheat diets than for the barley diets in these experiments, in agreement with data of Pérez-Bonilla et al. (2011, 2014) comparing different cereals in diets for laying hens. The data suggest that the structure of the feed (mean particle size and uniformity) depends not only of the size of the screen used for grinding but also of the characteristics of the ingredient (i.e., cereal) used.

The effect of particle size of the diet on poultry production is a subject of debate. The authors have not found any scientific report in the literature comparing the influence of particle size of corn, wheat, and barley on production of brown-egg laying hens. In experiment 2, from 17 to 49 wk of age, the screen size used to grind the cereals (corn or barley) did not affect any of the production traits studied, except for FCR that tended to increase in hens fed the cereals ground with a 4- mm screen compared with the average of hens fed the 6- to 12-mm screen ground cereals. In experiment 1, from 33 to 65 wk of age, the screen size (6 mm vs. 10 mm) used to grind the cereal (corn, wheat or barley) did not affect any of the production traits studied, in agreement with data of Deaton et al. (1989) who did not observe any effect on ADFI, FCR, or egg production in SCWL hens when fed diets based on coarse (873 μm) or fine (1,501 μm) corn from 23 to 71 wk of age. Similarly, MacIsaac and Anderson (2007) reported that coarsely ground corn and wheat (milled to pass through a 7-mm vs. a 5-mm screen) did not affect egg production, egg weight, FI, or FCR of SCWL from 20 to 64 wk of age. Moreover, Hamilton and Proudfoot (1995b) observed that BW, egg production, egg weight, and FCR were not affected when diets based on coarse or fine soft wheat were fed to SCWL from 20 to 70 wk of age. In any of the 3 experiments of this thesis, FI was affected by the screen size used to grind the cereal. Most published research, however, showed that hens had a preference for larger feed particles and that coarse grinding increased voluntary FI in laying hens. In this respect, Safaa et al. (2009) observed a 2.5% increase in FI in hens fed corn or wheat ground with a 10-mm screen compared with hens fed the same cereals ground with a 6-mm screen. In fact, many laying hen management guides (i.e., Lohmann, 2016) recommend the use of coarse texture diets when the objective is to maximize FI. It is worthy to notice that in experiment 3 (50 wk of age) laying hens showed a clear preference for ingesting coarse particles from diets

based on corn or barley, with differences being more notorious for the first hours after feed provision. The reasons for the discrepancy among experiments is not apparent but could be related to the proportion of very fine particles of the diet rather than to the GMD. In experiments 2 and 3 less than 20% of the feed particles of the original diets had a GMD of less than 0.5 mm, a percentage recommended as maximum by most guidelines for maximizing FI in laying hens. Moreover, supplemental fat of the diets might agglomerate the very fine particles, improving palatability and reducing the rejection rate for fine particles and thus, limiting the preference of the hens for coarse vs. fine particles. In fact, in these two experiments, the supplemental fat was high for both corn and barley diets (4.7% and 5.3%, respectively) which might have reduced the rejection of fine particles by the hen.

The data available on the effects of coarse particle size on nutrient digestibility and FCR in poultry are contradictory with some research showing an improvement (Nir et al., 1994a,b; Röhe et al., 2014; Ruhnke et al., 2015) and some research showing no effects (MacIsaac and Anderson, 2007; Safaa et al., 2009). In experiment 2, FCR tended to increase in hens fed the 4-mm screen ground diets compared with the average FCR of the hens fed the other diets from 17 to 49 wk of age. Ouart et al. (1986) reported no differences in FCR between SCWL hens fed diets based on coarse or fine wheat from 20 to 68 wk of age. In this respect, Amerah et al. (2007) suggested that coarse particles increase the antiperistaltic movements in the GIT of the birds, leading to better mixing and utilization of the nutrients. Also, a reduction in particle size increases the surface of contact between nutrients and endogenous enzymes which might favor nutrient digestibility. On the other hand, vitamins, minerals, and crystalline AA might concentrate in the fine fraction of the diet. Consequently, a preference for coarse particles affects the balance of nutrients ingested, which eventually might reduce egg

weight and hen production. These opposite effects of particle size on feed efficiency might counteract each other, and the final outcome might depend on factors such as ingredient composition of the diet, age, management, and health status of the birds.

1.2.2. Influence on egg quality traits

The effects of particle size of the diet on egg quality were studied in experiments 1 and 2. In both experiments, an increase in the size of the screen used to grind the cereal did not affect any of the egg quality traits studied, consistent with data of Safaa et al. (2009) who observed that egg quality traits were similar in brown egg-laying hens fed fine-, medium-, or coarse- ground corn and wheat diets from 20 to 48 wk of age. Hamilton and Proudfoot (1995b) observed that specific gravity, HU, and percentage of blood spots were similar in SCWL hens fed fine- or coarse-ground wheat diets from 20 to 70 wk of age. Moreover, Deaton et al. (1989) reported that an increase in the GMD of a corn diet from 814 to 873 μm did not affect shell-breaking strength of the eggs from 23 to 71 wk of age. In contrast, Ouart et al. (1986) noticed that SCWL hens fed diets based on coarsely ground wheat from 68 to 71 wk of age had lower egg-specific gravity than hens fed diets based on finely ground wheat. Hafeez et al. (2015) indicated that differences in albumen weight and shell quality due to particle size of the feed were in all cases of little practical interest. Most of these authors recorded that the proportion of shell, yolk, and albumen of the eggs were not affected by particle size. Consequently, the screen size use to grind the cereal can be modified according to specific requirements without any effect on egg quality.

1.2.3. Influence on gastrointestinal tract development and body measurements

The effects of particle size of the diet on GIT development were studied in experiment 2. None of the GIT traits was affected by the screen size, except gizzard weight that increased as the screen size used to grind the cereal increased. Röhe et al. (2014) observed that brown hens fed coarsely ground feeds showed heavier gizzards than hens fed finely ground diets at 23 wk of age. The authors have not found any report comparing the influence of particle size of corn, wheat, and barley on GIT development of brown-egg laying hens but these observations agree with data of numerous studies conducted in broilers (Nir et al., 1994a; Svihus et al., 2004; Amerah et al., 2007). The reason for the increase in gizzard development is not known. The gizzard grinds feed particles until reaching a consistent fine particle size, irrespective of the physical characteristics and particle size distribution of the diet (Hetland et al., 2004). Fine particles pass faster through the upper part of the GIT than coarse particles (Hetland et al., 2002) and consequently, gizzard size and function will increase with coarse grinding (Jiménez-Moreno et al., 2009b; Mateos et al., 2012). However, some authors showed that coarser particles can pass the gizzard at some extent and enter the small intestine, affecting negatively digestive efficiency of the bird (Lentle et al., 2006; Amerah et al., 2007). Probably, particle density might be as important as particle size in this respect.

BMI, body length, and tarsus length and diameter are used to estimate BW of broilers (Van Roover-Reijrink, 2013), pullets (Saldaña et al., 2015b), and laying hens (Guzmán et al., 2016). In the current research, none of these traits was affected by the screen size used to grind the cereal, results that are consistent with the lack of effect of the characteristics of the diets on BW gain. On the other hand, the significant positive relation detected between BW and BMI, body length, and tarsus length and diameter, confirm that these traits can be used at a certain extent, to estimate BW in hens.

1.2.4. Influence on preference behavior

The effects of the particle size of the diet on preference behavior of the hens were studied in experiment 3. We hypothesized that grinding size could affect preference behavior in laying hens with effects that could depend on the physico-chemical characteristics of the main cereal used. Also, it was hypothesized that differences in the nutritional composition of the coarse and the fine fractions of the diets, could affect the feeding behavior of the hens. Hens showed a significant preference for coarser particles, an effect that was more evident when the cereals were ground coarsely. In general, chickens are known to have a preference for larger feed particles (Schiffman, 1968) which is observed at all ages (Portella et al., 1988a). Also, it has been reported that particle size preference is thought to increase with age (Nir et al., 1994a). This may be related to the size of the birds 'gape' (width of the beak), that increases with age (Gentle, 1979). It is possible that the particle size be increase with age is of important interest, for optimal poultry performance. The information available on particle size preference by brown-egg laying hens is limited and contradictory. Schiffman (1968) suggested that birds have a pecking preference for textured feed over non-textured feed and that as they become older their preference for larger particles increases. Portella et al. (1988a) suggested that the preference for coarse particles of the laying hens might be altered not only by the average size but also by the proportion of very fine particles. These authors reported also that the disappearance of small particles was overwhelming when the feed had an excess of large particles (> 2.36 mm), but was less obvious as the experiment progressed and the percentage of coarse particle was reduced, consistent with the results of the current experiment. The data confirm that when allowed to choose, laying hens show a clear preference for coarse particles.

Also, a possible explanation for particle size preference could be that birds selected particles sizes in order to obtain a balanced diet. When the diet is deficient in a given nutrient, birds might choose to eat the sieving fraction that is richer in the deficient nutrient (Portella et al., 1988a). As a result, hen preference might depend not only on particle size but also on the physical distribution of the limiting nutrient or feed component within the sieving fractions. In the current experiment, hens did not show any preference for consuming those feed fractions with a greater proportion of CP, ash, or Ca content. In fact, Ca concentration in the different feed fractions was in relative terms, in-dependent of their size. Moreover, Ca content of the feed refusals increased with time showing a dislike of the hens for eating Ca in the early hours of the day. This observation deserves further attention because it might be related to differences in Ca requirements of the hens along the day and the timing at which the feed was available for consumption. Birds have a specific appetite for calcium, and thus, particle selection by the hens may be influenced by the requirement in this nutrient. It has been suggested that Ca and feed intake are regulated independently (Mongin and Sauveur 1979) and if given a choice, laying hens are able to choose between different feed sources to regulate their Ca intake (Holcombe et al. 1975). In this respect, Portella et al. (1988a) reported that birds tend to regulate their Ca intake more efficiently when fine particles were fed than when coarse particles are offered. When fed coarse diets, birds apparently ate large particles which contained less Ca while smaller particles with higher Ca content were less eagerly consumed. This may explain why in the current experiment the percentage of Ca was higher in the residual feed than in the original diets. Hughes et al. (1972) reported that laying hens tended to over-consume Ca late in the day, when most of the shell formation process occurs, but tended to under-consume Ca during the morning. In the evening, however, hens might try to balance Ca intake and Ca requirements, giving

preference to those particles rich in the deficient nutrient, regardless of feed coarseness, whereas an opposite pattern might occur during the morning. The data demonstrate that under the condition of the current research, hen preference for coarse particles was not modulated by the desire to meet any potential requirement for CP or Ca.

2. General conclusions

- Wheat and barley can be used in substitution of corn, at level of up to 45 % in diets for brown-egg laying hens provided that the feed is supplemented with adequate enzymes. Therefore, the use of one or other cereal will depend on their relative purchasing cost.
- Eggs were heavier in hens fed barley than in hens fed wheat or corn, probably because of differences in the level of supplemental fat, rather than because of differences in linoleic acid content.
- Type of cereal and the screen size used to grind the cereal did not affect any egg quality trait of the hens.
- Corn, wheat, and barley ground through a 6 to 12-mm screen can be used indistinctly in commercial diets for laying hens, without any effect on hen performance.
- An increase in screen size from (4 to 12 mm) improved gizzard development in hens at 49 wk of age.
- Hens show a preference behavior for coarse particles, with differences that are more pronounced when the feed is coarsely ground than when the feed is finely ground.

- The CP, ash, and Ca contents of the diet were not uniform and varied with the diameter of the screen used to grind the cereal and also with the sieving fractions (fine vs. coarse).
- Brown hens reduced Ca intake during the morning after the lights were switch on, resulting in residual feeds with greater concentration of ash and Ca than in the original diets, irrespective of the size of the feed fractions.
- Crude protein content of the different sieve fractions, did not affect hen preference behavior. In fact, hens did not show any preference for consuming extra amounts of protein during the early part of the day.
- The preference of hens for consuming coarse particle is not affected by the desire of the hens to ingest faulty nutrients to meet their nutrient requirements.

CHAPTER 6:
REFERENCES

REFERENCES

- Abdollahi, M. R., V. Ravindran, T. J. Wester, G. Ravindran, and D. V. Thomas. 2011. Influence of feed form and conditioning temperature on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. *Anim. Feed Sci. Technol.* 168:88-99.
- Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013a. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Anim. Feed Sci. Technol.* 179:1-23.
- Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013b. Influence of grain type and feed form on performance, apparent metabolisable energy and ileal digestibility of nitrogen, starch, fat, calcium and phosphorus in broiler starters. *Anim. Feed Sci. Technol.* 186:193-203.
- Abdollahi, M. R., Y. Duangnumsaeng, R. P. Kwakkel, S. Steinfeldt, S. M. Bootwalla, and V. Ravindran. 2016. Investigation of the interaction between separate calcium feeding and phytase supplementation on growth performance, calcium intake, nutrient digestibility and energy utilisation in broiler starters. *Anim. Feed Sci. Technol.* 219:48-58.
- Allen, J. and G. C. Perry 1977. Feeding patterns and food selection of caged birds. *Br. Vet. J.* 133:99 (Abstr.).
- Amerah, A.M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007. Feed particle size: implications on the digestion and performance of poultry. *World's Poult. Sci. J.* 63:439-451.
- Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development,

- and digesta parameters of broiler starters fed wheat- and corn- based diets. *Poult. Sci.* 87:2320-2328.
- AOAC International. 2005. *Official Methods of Analysis of the AOAC International*. 18th ed. AOAC Int., Gaithersburg, MD.
- 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, MO, pp. 461-462.
- Baker, S., and T. Herrman. 2002. Evaluating particle size. MF-2051 feed manufacturing, department of grain science and industry, Kansas State University. Manhattan, KS.
- Balnave, D. 1970. Essential fatty acids in poultry nutrition. *World's Poult. Sci. J.* 26:442-460.
- Balnave, D. 1971. Response of laying hens to dietary supplementation with energetically equivalent amounts of maize starch or maize oil. *J. Sci. Food Agric.* 22:125-128.
- Benabdeljelil, K. and M. I. Arbaoui. 1994. Effects of enzyme supplementation of barley-based diets on hen performance and egg quality. *Anim. Feed Sci. Technol.* 48:325-334.
- Berg, L. R., and G. E. Bearse. 1948. Studies of the granulation of chicken laying mashes. *Poult. Sci.* 27:653 (Abstr.).
- Berg, L. R. 1959. Enzyme supplementation of barley diets for laying hens. *Poult. Sci.* 38:1132-1139

- Boletín Oficial del Estado. 1995. Real Decreto 2257/1994 por el que se aprueba los métodos oficiales de análisis de piensos o alimentos para animales y sus primeras materias. BOE 52:7161-7237.
- Boletín Oficial del Estado. 2007. Ley 32/2007 de 7 de Noviembre para el cuidado de los animales, en su explotación, transporte, experimentación y sacrificio. BOE 268:45914-45920.
- Brufau, J., R. Cos, A. Pérez-Vendrell, and E. Esteve-García. 1994. Performance of laying hens as affected by the supplementation of a barley-based diet with a crude enzyme preparation from *Trichoderma viride*. *Can. J. Anim. Sci.* 74:129-133.
- Carré, B. 2000. Effet de la taille des particules alimentaires sur les processus digestifs chez les oiseaux d'élevage. *INRA Prod. Anim.* 13:131-136.
- Carré, B., N. Muley, J. Gomez, F. X. Oury, E. Laffite, D. Guillo, and C. Signoret. 2005. Soft wheat instead of hard wheat in pelleted diets results in high starch digestibility in broiler chickens. *Br. Poult. Sci.* 46:66-74.
- Chewning, C. G., C. R. Stark, and J. Brake. 2012. Effects of particle size and feed form on broiler performance. *J. Appl. Poult. Res.* 21:830-837.
- Çiftci, I., E. Yenice, and H. Eleroglu. 2003. Use of triticale alone and in combination with wheat or maize: effects of diet type and enzyme supplementation on hen performance, egg quality, organ weights, intestinal viscosity, and digestive system characteristics. *Anim. Feed Sci. Technol.* 105:149-161.
- Classen, H. L. 1996. Cereal grain starch and exogenous enzymes in poultry diets. *Anim. Feed Sci. Technol.* 62:21-27.
- Coon, C. N., I. Obi, and M. L. Hamre. 1988. Use of barley in laying hens diets. *Poult. Sci.* 67:1306-1313.

- Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. *Anim. Feed Sci. Technol.* 119:293-305.
- Cowieson, A. J., and O. Adeola. 2005. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. *Poult. Sci.* 84:126-141.
- Craig, L.W. and T. Goodman. 1993. Utilization of feed enzymes in laying hen rations. *J. Appl. Poult. Res.* 2:68-74.
- Davis, R. L., E. G. Hill, H. J. Sloan, and G. M. Briggs. 1951. Detrimental effect of corn of coarse particle size in rations for chicks. *Poult. Sci.* 30:325-328.
- Deaton, J. W., B. D. Lott, and J. D. Simmons. 1989. Hammer mill versus roller mill grinding of corn for commercial egg layer. *Poult. Sci.* 68:1342-1344.
- De Coca-Sinova, A. D., G. Valencia, E. Jiménez-Moreno, J. M. González-Alvarado, R. Lázaro, and G. G. Mateos. 2008. Apparent ileal digestibility of nitrogen, amino acids, and energy of soybean meals from different origins in broilers. *Poult. Sci.* 87:2613-2623.
- Douglas, J. H., T. W. Sullivan, P. L. Bond, F. J. Struwe, J. G. Baier, and L. G. Robeson. 1990. Influence of grinding, rolling, and pelleting on the nutritional value of grain sorghums and yellow corn for broilers. *Poult. Sci.* 69:2150-2156.
- Duke, G. E. 1992. Recent studies on regulation of gastric motility in turkeys. *Poult. Sci.* 81:1-8.
- Edney, M. J., G. L. Campbell, and H. L. Classen. 1989. The effect of β -glucanase supplementation on nutrient digestibility and growth in broilers given diets containing barley, oat groats or wheat. *Anim. Feed Sci. Technol.* 25:193-200.

- European Commission, 2017. EU market situation for eggs. Committee for the common organization of the agricultural markets. <http://ec.europa.eu/agriculture/markets/indexen.htm>. Accessed May 2017.
- FAO, 2016. Food Agriculture Organization of the United Nations. Food outlook. Biannual report on global food markets, issn 1560-8182 (online) <http://www.fao.org/>. Accessed May 2017.
- FEDNA (Fundación Española Desarrollo Nutrición Animal). 2008. Necesidades Nutricionales para Avicultura: Pollos de Carne y Aves de Puesta. In: R. Lázaro, and G. G. Mateos (Eds). Fund. Esp. Desarro. Nutr. Anim., Madrid, Spain.
- FEDNA (Fundación Española Desarrollo Nutrición Animal). 2010. Normas FEDNA para la Formulación de Piensos Compuestos. 3rd ed. In: C. de Blas, G. G. Mateos, and P. G. Rebollar (Eds). Fund. Esp. Desarro. Nutr. Anim., Madrid, Spain.
- Feedipedia. 2017. Animal Feed Resources Information System. Accessed July 2017. <http://www.feedipedia.org/>.
- Forbes, J. M., and F. S., Shariatmadari. 1994. Diet selection for protein by poultry. Word's Poult. Sci. J. 50:7-24.
- Francesch, M., A. M. Pérez-Vendrell, E. Esteve-García, and J. Brufau. 1995. Enzyme supplementation of a barley and sunflower-based diet on laying hen performance. J. Appl. Poult. Res. 4:32-40.
- Francesch, M., and L. Brufau. 2004. Nutritional factors affecting excreta/litter moisture and quality. World's Poult. Sci. J. 60:64-75.
- Francesch, M., J. Broza, and J. Brufau. 2005. Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus bioavailability in laying hens fed on maize- or barley based diets. Br. Poult. Sci. 46:340-348.

- Frikha, M., H.M. Safaa, M.P. Serrano, X. Arbe, and G.G. Mateos. 2009a. Influence of the main cereal and feed form of the diet on performance and digestive tract of brown-egg laying pullets. *Poult. Sci.* 88:994-1002.
- Frikha, M., H. M. Safaa, E. Jiménez-Moreno, R. Lázaro, M. Serrano, G. G. Mateos. 2009b. Influence of energy concentration and feed form of the diet on growth performance and digestive traits of brown egg-laying pullets from 1 to 120 days of age. *Anim. Feed Sci. Technol.* 153:292-302.
- Frikha, H., H. M. Safaa, M.P. Serrano, E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2011. Influence of the main cereal in the diet and particle size of the cereal on productive performance and digestive traits of brown-egg laying pullets. *Anim. Feed Sci. Technol.* 164:106-115.
- Gabriel, I., S. Mallet, M. Leconte, A. Travel, and J. P. Lalles. 2008. Effects of whole wheat feeding on the development of the digestive tract of broiler chickens. *Anim. Feed Sci. Technol.* 142:144-162.
- García, M., R. Lázaro, M. A. Latorre, M. I. Gracia, and G. G. Mateos. 2008. Influence of enzyme supplementation and heat processing of barley on digestive traits and productive performance of broilers. *Poult. Sci.* 87:940-948.
- Gentle, M. J. 1979. Sensory control of feed intake. In: *Food Intake Regulation in Poultry*, (K. N. Boorman and B. M. Freeman, eds.). Edinburg: Br. Poult. Sci. Ltd, pp. 259-273.
- González-Alvarado, J. M., E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. *Poult. Sci.* 86:1705-1715.

- González-Alvarado, J. M., E. Jiménez-Moreno, D. González-Sánchez, R. Lázaro, and G. G. Mateos. 2010. Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive performance and digestive traits of broilers from 1 to 42 days of age. *Anim. Feed Sci. Technol.* 162:37-46.
- Gracia, M. I., M. A. Latorre, M. García, R. Lázaro, and G. G. Mateos. 2003. Heat processing of barley and enzyme supplementation of diets for broilers. *Poult. Sci.* 82:1281-1291.
- Grobas, S., J. Méndez, C. de Blas, and G. G. Mateos. 1999a. Influence of dietary energy, supplemental fat and linoleic acid concentration on performance of laying hens at two ages. *Br. Poult. Sci.* 40:681-687.
- Grobas, S., G. G. Mateos, and J. Méndez. 1999b. Influence of dietary linoleic acid on production and egg weight of eggs and egg components in young brown hens. *J. Appl. Poult. Res.* 8:177-184.
- Grobas, S., J. Méndez, C. de Blas, and G. G. Mateos. 1999c. Laying hen productivity as affected by energy, supplemental fat, and linoleic acid concentration of the diet. *Poult. Sci.* 78:1542-1551.
- Gutiérrez-Alamo, A., P. Pérez de Ayala, M. W. A. Verstegen, L. A. Den Hartog, and M. J. Villamide. 2008. Variability in wheat: factors affecting its nutritional value. *World's Poult. Sci. J.* 64:20-39.
- Guzmán, P., B. Saldaña, M. V. Kimiaetalab, J. García, and G. G. Mateos. 2015. Inclusion of fiber in diets for brown-egg laying pullets: effects on growth performance and digestive tract traits from hatching to 17 weeks of age. *Poult. Sci.* 94:2722-2733.
- Guzmán, P., B. Saldaña, O. Bouali, L. Cámara, and G. G. Mateos. 2016. Effect of level of fiber of the rearing phase diets on egg production, digestive tract traits, and

- body measurements of brown egg-laying hens fed diets differing in energy concentration. *Poult. Sci.* 95:1836-1847.
- Hafeez, A., A. Mader, I. Röhe, I. Ruhnke, F. G. Boroojeni, M. S. Yousaf, K. Männer, and J. Zentek. 2015. The effect of milling methods, thermal treatment, and particle size of feed on exterior and interior egg quality in laying hens. *Eur. Poult. Sci.* 79: ISSN 1612-9199. doi:10.1399/eps.2015.76.
- Hamilton, R.M.G., and F. G. Proudfoot. 1995a. Ingredient particle size and feed texture effects on the performance of broiler chickens. *Anim. Feed Sci. Technol.* 51:203-210.
- Hamilton, R.M.G., and F. G. Proudfoot. 1995b. Effects of ingredient particle size and feed form on the performance of leghorn hens. *Can. J. Anim. Sci.* 75:109-114.
- Hartini, S., M. Choct, G. Hinch, A. Kocher, and J. V. Nolan. 2002. Effects of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of Isa brown laying hens. *J. Appl. Poult. Res.* 11:104-110.
- Herrera, J., B. Saldaña, P. Guzmán, L. Cámara, and G.G. Mateos. 2017. Influence of particle size of the main cereal of the diet on egg production, gastrointestinal tract traits, and body measurements of brown laying hens. *Poult. Sci.* 96:440-448.
- Herrera, J., B. Saldaña, P. Guzmán, M. A. Ibáñez, H. Mandalawi, L. Cámara, and G. G. Mateos. 2018. Particle size affects short term preference behavior of brown-egg laying hens fed diets based on corn or barley. *Poult. Sci.* (In press).
- Hetland, H., B. Svihus, and V. Olaisen. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *Br. Poult. Sci.* 43:416-423.

- Hetland H., B. Svihus, and Å. Krögdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *Br. Poult. Sci.* 44:275-282.
- Hetland, H., M. Choct, and B. Svihus. 2004. Role of insoluble non-starch polysaccharide in poultry nutrition. *World's Poult. Sci. J.* 60:415-422.
- Holcombe, D. J., D. A. Roland, and R. H. Harms. 1975. The ability of hens to adjust calcium intake when given a choice of diets containing two levels of calcium. *Poult. Sci.* 54:552-561.
- Hughes, B. O., and D. G. M. Wood-Gush. 1971. A specific appetite for calcium in domestic chickens. *Anim. Behav.* 19:490-499.
- Hughes, B. O., 1972. A circadian rhythm of calcium intake in the domestic fowl. *Br. Poult. Sci.* 13:485-493.
- ISA Brown. 1999. Energy levels and feed presentation for laying hens: effects on performance and intake. Institut de Selection Animale. B. V, Boxmeer, The Netherlands.
- ISA Brown, 2009. Nutrition Management Guide. Institut de Selection Animale. B.V. Boxmeer, The Netherlands.
- ISA Brown. 2010. Nutrition Management Guide. Isa Brown Commercial Layer. Institut de Selection Animale. B. V, Boxmeer, The Netherlands.
- Jacob, J. P., and A. J. Pescatore. 2012. Using barley in poultry diets-A review. *J. Appl. Poult. Res.* 21:915-940.
- Jamroz, D., J. Skokupinska, J. Orda, A. Wiliczekiewicz, and A. M. Klunter. 2001. Use of wheat, barley or triticale in feed for laying hens supplemented with carbohydrates derived from *Trichoderma longibrachiatum*. *J. Appl. Anim. Res.* 19:255-263.

- Jensen, L. S., J. B. Allred, R. E. Fry, and J. McGinnis. 1958. Evidence for an unidentified factor necessary for maximum egg weight in chickens. *J. Nutr.* 65:219-233.
- Jiménez-Moreno, E., J. M. González-Alvarado, A. de Coca-Sinova, R. Lázaro, and G. G. Mateos. 2009a. Effects of source of fiber on the development and pH of the gastrointestinal tract of broilers. *Anim. Feed Sci. Technol.* 154:93-101.
- Jiménez-Moreno, E., J. M. González-Alvarado, A. González-Serrano, R. Lázaro, and G. G. Mateos. 2009b. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. *Poult. Sci.* 88:2562-2574.
- Jiménez-Moreno, E., J. M. González-Alvarado, R. Lázaro, and G. G. Mateos. 2009c. Effects of type of cereal, heat processing of the cereal, and fiber inclusion in the diet on gizzard pH and nutrient utilization in broilers at different ages. *Poult. Sci.* 88:1925-1933.
- Jiménez-Moreno, E., J. M. González-Alvarado, D. González-Sánchez, R. Lázaro, G. G. Mateos. 2010. Effects of type and particle size of dietary fiber on growth performance and digestive traits of broilers from 1 to 21 days of age. *Poult. Sci.* 89: 2197-2212.
- Jiménez-Moreno, E., A. De Coca-Sinova, J. M. González-Alvarado, and G. G. Mateos. 2016. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth performance and water intake. *Poult. Sci.* 95:41-52.
- Jorgensen, H., X. Zhao, K. E. B. Knudsen, and B. O. Eggum. 1996. The influence of dietary fibre source and level on the development of gastrointestinal tract, digestibility and energy metabolism in broiler chickens. *Br. J. Nutr.* 75:379-395.

- Kim, S. M., M. B. Patel, S. J. Reddy, and J. McGinnis. 1976. Effects of different cereal grains in diets for laying hens on production parameters and liver fat content. *Poult. Sci.* 55:520-530.
- Koch, K. 1996. Hammermills and rollermills. MF-2048 feed manufacturing. Dept. Grain Sci. Ind. Kansas State University, Manhattan, KS.
- Koch, B. K. 2008. Feed Mill Efficiency. 16th Annual American Soybean Association. International Marketing, S.E.A. Feed Technology and Nutrition Workshop, Singapore, pp. 1-13.
- Lázaro, R., M. García, M. J. Aranibar, and G.G. Mateos. 2003a. Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens. *Br. Poult. Sci.* 44:256-265.
- Lázaro, R., M. García, P. Medel, and G.G. Mateos. 2003b. Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. *Poult. Sci.* 82:132-140.
- Lázaro, R., M. A. Latorre, P. Medel, M. I. Gracia, and G.G. Mateos. 2004. Feeding regimen and enzyme supplementation to rye-based diets for broilers. *Poult. Sci.* 83:152-160.
- Lentle, R. G., V. Ravindran, G. Ravindran, and D. V. Thomas. 2006. Influence of feed particle size on the efficiency of broiler chickens fed wheat-based diets. *Jpn. Poult. Sci.* 43:135-142.
- Lillie, R. J., and C. A. Denton. 1968. Evaluation of four cereal grain and three protein level combinations for layer performance. *Poult. Sci.* 47:1000-1004.
- Lohmann. 2014. Management Guide for Lohmann Brown-Classic. Lohmann Tierzucht. GMBH. Cuxhaven, Germany.

- Lohmann, 2016. Management Guide for Lohmann Brown-Classic. Lohmann Tierzucht. GMBH. Cuxhaven, Germany.
- Lott, B. D., E. J. Day, J. W. Deaton, and J. D. May. 1992. The effect of temperature, dietary energy level, and corn particle size on broiler performance. *Poult. Sci.* 71:618-624.
- MacIsaac, J. L., and D. M., Anderson. 2007. Effect of whole wheat, enzyme supplementation and grain texture on the production performance of laying hens. *Can. J. Anim. Sci.* 87:579-589.
- MAPAMA (Ministerio de Agricultura, Pesca, Alimentación y Medio Ambiente). 2017. <http://www.mapama.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/> . Accessed May 2017.
- Marquardt, R.R., D. Boros, W. Guenter, and G. Crow. 1994. The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a trichoderma reesei enzyme preparation. *Anim. Feed Sci. Technol.* 45:363-378.
- Mateos, G. G., and J. L. Sell. 1980. Influence of carbohydrate and supplemental fat source of the metabolizable energy of the diet. *Poult. Sci.* 59:2129-2135.
- Mateos, G. G., R. Lázaro, and M. I. Gracia. 2002. The feasibility of using nutritional modifications to replace drugs in poultry feeds. *J. Appl. Poult. Res.* 11:437-452.
- Mateos, G. G., E. Jiménez-Moreno, M. P. Serrano, and R. Lázaro. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *J. Appl. Poult. Res.* 21:156-174.
- Mateos, G. G., L. Cámara, G. Fondevila, and R. P. Lázaro. 2017a. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. *J. Appl. Poult. Res.* (in press).

- Mateos, G.G., L. Cámara, G. Fondevila, H. Mandalawi, J. Arch, and R. Lázaro. 2017b. Insoluble fiber for gut development, microbial management and performance. Poultry Beyond 2023 6th Broiler Nutritionist's Conference. R. Ravindran (ed). Queenstown, New Zealand.
- Mathlouthi, N., M. Larbier, M. A. Mohamed, and M. Lessire. 2002. Performance of laying hens fed wheat, wheat-barley or wheat-barley-wheat bran based diets supplemented with xylanase. *Can. J. Anim. Sci.* 82:193-199.
- Mendes, M., D. Ecmel, and E. Arslan. 2007. Profile analysis and growth curve for body mass index of broiler chickens reared under different feed restrictions in early age. *Arch. Tierz.* 50:403-411.
- Mirzaie, S., M. Zaghari, M. Aminzadeh, M. Shivazad, and G. G. Mateos. 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens. *Poult. Sci.* 91:413-425.
- Mongin, P., and B. Sauveur. 1974. Voluntary food and calcium intake by the laying hens. *Br. Poult. Sci.* 15:349-359.
- Mongin, P. and B. Sauveur. 1979. The specific calcium appetite of the domestic fowl. *Food Intake Regulation*, (K. N. Boorman and B. M. Freeman, eds.). Edinburgh: Br. Poult. Sci. Ltd, pp. 171-189.
- Moran, E. T. JR., X. Chen, and J. P. Blake. 1993. Comparison of broiler strain crosses developed in the US and UK using corn and wheat based feeds: live performance and processing of male for nine piece cuts. *J. Appl. Poult. Res.* 2:26-32.
- Nahashon, S. N., H. S. Nakaue, S. P. Snyder, and L. W. Mirosh. 1994. Performance of Single Comb White Leghorn layers fed corn-soybean meal and barley-corn-

- soybean meal diets supplemented with a direct-fed microbial. *Poult. Sci.* 73:1712-1723.
- Nir, I., R. Hillel, I. Ptichi, and G. Shefet. 1994a. Effect of grain particle size on performance. 2. Grain texture interaction. *Poult. Sci.* 73:781-791.
- Nir, I., G. Shefet, and Y. Aaroni. 1994b. Effect of particle size on performance. 1. Corn. *Poult. Sci.* 73:45-49.
- Nir, I., Hillel, R., Ptichi, I., and G. Shefet. 1995. Effect of particle size on performance. 3. Grinding pelleting interaction. *Poult. Sci.* 74:771-783.
- Nir, I., and I. Ptichi. 2001. Feed particle size and hardness: Influence on performance, nutritional, behavioral and metabolic aspects. In: *Proceedings of the 1st World Feed Conference, Utrecht, the Netherlands*, pp. 157-186.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Ouart, M. D., J. E. Marion, and R. H. Harms. 1986. Research notes: Influence of wheat particle size in diets of laying hens. *Poult. Sci.* 65:1015-1017.
- Ouhida, I., J. F. Perez, J. Gasa, and F. Puchal. 2000. Enzymes (β -glucanase and arabinoxylanase) and/or sepiolite supplementation and the nutritive value of maize-barley-wheat based diets for broiler chickens. *Br. Poult. Sci.* 41:617-624.
- Parera, N., R. P. Lázaro, M. P. Serrano, D. G. Valencia, and G. G. Mateos. 2010. Influence of the inclusion of cooked cereals and pea starch in diets based on soy or pea protein concentrate on nutrient digestibility and performance of young pigs. *J. Anim. Sci.* 88:671-679.
- Parsons, A. S., N. P. Buchanan, K. P. Blemings, M. E. Wilson, and J. S. Moritz. 2006. Effect of corn particle size and pellet texture on broiler performance in the growing phase. *J. Appl. Poult. Res.* 15:245-255.

- Pérez-Bonilla, A., M. Frikha, S. Mirzaie, J. García, and G. G. Mateos. 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.
- Pérez-Bonilla, A., S. Novoa, J. García, M. Mohiti-Asli, M. Frikha, and G. G. Mateos. 2012. Effects of energy concentration of the diet on productive performance and egg quality of brown egg-laying hens differing in initial body weight. *Poult. Sci.* 91:3156-3166.
- Pérez-Bonilla, A., M. Frikha, R. Lázaro, and G. G. Mateos. 2014. Type of grinding of the main cereal of the diet affects production of brown egg-laying hens. *Anim. Feed Sci. Technol.* 194:121-130.
- Péron, A., D. Bastianelli, F. X. Oury, J. Gomez, and B. Carré. 2005. Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet. *Br. Poult. Sci.* 46:223-230.
- Picard, M., J. P. Melcion, C. Bouchot, and J. M. Faure. 1997. Picorage et préhensibilité des particules alimentaires chez les volailles. *INRA Prod. Anim.* 10:403-414..
- Portella, F. J., L. J. Caston, and S. Leeson. 1988a. Apparent feed particle size preference by laying hens. *Can. J. Anim. Sci.* 68:915-922.
- Portella, F. J., L. J. Caston, and S. Leeson. 1988b. Apparent feed particle size preference by broilers. *Can. J. Anim. Sci.* 68:923-930.
- Pousga, S., H. Boly, and B. Ogle. 2005. Choice feeding of poultry: a review. *Liv. Res. Rural Develop.* Vol. 17, Article 45. Retrieved from <http://www.lrrd.org/lrrd17/4/pous17045.htm>.

- Ravindran, V., Y. B. Wu, D. G. Thomas, and C. H. Morel. 2006. Influence of whole wheat feeding on the development of gastrointestinal tract and performance of broiler chickens. *Aust. J. Agric. Res.* 57:21-26.
- Ravindran, V., P Tancharoenrat, F Zaefarian and G. Ravindran. 2016. Fats in poultry nutrition: digestive physiology and factors influencing their utilization. *Anim. Feed Sci. Technol.* 213:1-21.
- Reece, F. N., B. D. Lott, and J. W. Deaton. 1985. The effects of feed form, grinding method, energy level, and gender on broiler performance in a maderate (21°C) environment. *Poult. Sci.* 64:1834-1839.
- Röhe, I., I. Ruhnke, F. Knorr, A. Mader, F. G. Boroojeni, R. Lowe, and J. Zentek. 2014. Effects of grinding method, particle size, and physical form of the diet on gastrointestinal morphology and jejunal glucose transport in laying hens. *Poult. Sci.* 93:2060-2068.
- Ruiz, N., J. E. Marion, R. D. Miles, and R. B. Barnett. 1987. Nutritive value of new cultivars of triticale and wheat for broiler chick diets. *Poult. Sci.* 66:90-97.
- Ruhnke, I., I. Röhe, C. Krämer, F. G. Boroojeni, F. Knorr, A. Mader, E. Schulze, A. Hafeez, K. Neumann, R. Löwe, and J. Zentek. 2015. The effects of particle size, milling method, and thermal treatment of feed on performance, apparent ileal digestibility, and pH of the digesta in laying hens. *Poult. Sci.* 94:692-699.
- Sacranie, A., B. Svihus, V. Denstadli, B. Moen, P. A. Iji, and M. Choct. 2012. The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. *Poult. Sci.* 91:693-700.
- Safaa, H. M., M. P. Serrano, D. G. Valencia, X. Arbe, E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2008b. Effects of the levels of methionine, linoleic acid, and

- added fat in the diet of productive performance and egg quality of brown laying hens in the late phase of production. *Poult. Sci.* 87:1595-1602.
- Safaa, H. M., E. Jiménez-Moreno, D. G. Valencia, M. Frikha, M. P. Serrano, and G. G. Mateos. 2009. Effect of main cereal of the diet and particle size of the cereal on productive performance and egg quality of brown egg-laying hens in early phase of production. *Poult. Sci.* 88:608-614.
- Saki, A. A., S. Mirzayi, S. Ghazi, M. M. Moini, R. N. Harsini, M. Haghighat, and R., Mahdavi. 2010. Effect of various level of treated barley on small intestinal content viscosity, litter moisture, uric acid and broiler chicken performance. *J. Anim. Vet. Adv.* 9:2627-2632.
- Saldaña, B., P. Guzmán, H. M. Safaa, R. Harzalli, and G. G. Mateos. 2015a. Influence of the main cereal and feed form of the rearing phase diets on performance and digestive tract and body traits of brown-egg laying pullets from hatch to 17 weeks of age. *Poult. Sci.* 94:2650-2661.
- Saldaña, B., P. Guzmán, L. Cámara, J. García, and G. G. Mateos. 2015b. Feed form and energy concentration of the diet affect productive performance and digestive tract traits of brown-egg laying pullets from hatching to 17 weeks of age. *Poult. Sci.* 94:1879-1893.
- SAS Institute. 2004. *SAS/STAT User's Guide*. Version 9.1. SAS Inst. Inc., Cary, NC.
- Schiffman, H. R. 1968. Texture preference in the domestic chick. *J. Comp. Physiol. Psychol.* 66:540-541.
- Scragg, R. H., N. B. Logan, and N. Geddes. 1987. Response of egg weight to the inclusion of various fats in layer diets. *Br. Poult. Sci.* 28:15-21.

- Shutze, J. V., L. S. Jensen, and J. McGinnis. 1962. Accelerated increase in egg weight of young pullets fed practical diets supplemented with corn oil. *Poult. Sci.* 41:1846-1851.
- Singh, J., A. Dartois, and L. Kaur. 2010. Starch digestibility in food matrix: A review. *Trends Food Sci. Technol.* 21:168-180.
- Steenfeldt, S. 2001. The dietary effect of different wheat cultivars for broiler chickens. *Br. Poult. Sci.* 42:595-609.
- Svihus, B., and H. Hetland. 2001. Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. *Br. Poult. Sci.* 42:633-637.
- Svihus, B., E. Juvik, H. Hetland and Å. Krogdahl. 2004. Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. *Br. Poult. Sci.* 45:55-60.
- Svihus, B., A. K. Uhlen, and O. M. Harstad. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Anim. Feed Sci. Technol.* 122:303-320.
- Svihus, B., 2010. Challenging current poultry feeding dogmas by feed intake restriction and the use of coarse feed ingredients. 21st. Proc. of the Austr. Poult. Sci. Symp., pp 9–16, Sydney Australia.
- Svihus, B. 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. *World's Poult. Sci. J.* 67:207-224.
- Svihus, B. 2014. Starch digestion capacity of poultry. *Poult. Sci.* 93:2394–2399.
- Taylor, R. D., and G. P. D. Jones. 2004a. The incorporation of whole grain into pelleted broiler chicken diet. II. Gastrointestinal and digesta characteristics. *Br. Poult. Sci.* 37:237-246.

- Taylor, R. D., and G. P. D. Jones. 2004b. The influence of whole grain inclusion in pelleted broiler diets on proventricular dilatation and ascites mortality. *Br. Poult. Sci.* 37:247-254.
- Tester, R. F., X. Qi, and J. Karkalas. 2006. Hydrolysis of native starches with amylases. *Anim. Feed Sci. Technol.* 130:39-54.
- Thomas, D.V., and V. Ravindran. 2008. Effect of cereal type on the performance, gastrointestinal tract development and intestinal morphology of the newly hatched broiler chick. *J. Poult. Sci.* 45:46-50.
- Traineau, M., I. Bouvarel, C. Mulsant, L. Roffidal, C. Launay, and P. Lescoat. 2015. Modulation of energy and protein supplies in sequential feeding in laying hens. *Animal* 9:49-57.
- USDA-FAS (United States Department of Agriculture- Foreign Agricultural Service), 2015. Spain's Poultry Meat and Egg Production Report, Gain Report Number: SP1538. (USDA-FAS) <https://gain.fas.usda.gov/>.
- Van Roover-Reijrink, I. 2013. Incubation affects chick quality. *World Poult.* 29:22-23.
- Wahlström, A., R. Tauson, and K. Elwinger. 1999. Production and egg quality as influenced by mash or crumbled diets fed to laying hens in an aviary system. *Poult. Sci.* 78:1675-1680.
- Waldroup, P.W. 1997. Particle size reduction of cereal grains and its significance in Poultry Nutrition. Technical Bulletin PO34-1997, American Soybean Association, Singapore. 14 pp.
- Wang, Z. R., S. Y. Qiao, W. Q. Lu, and D. F. Li. 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poult. Sci.* 84:875-881.

- WPSA (World's Poultry Science Association). 1989. European Table of Energy Values for Poultry Feedstuffs, 3rd ed. Subcommittee of Energy of the Working Group no 2. Nutrition of the European Federation of Branches of the World's Poultry Science Association, Spelderholt Centre for Poultry Research, Beekbergen, The Netherlands.
- Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, and J. Brake. 2015. Effects of feed form and dietary coarse ground corn on broiler live performance, body weight uniformity, relative gizzard weight, excreta nitrogen, and particle size preference behaviors. *Poult. Sci.* 94:1549-1556.
- Yasar, S., and J. M. Forbes. 1999. Performance and gastro-intestinal response of broiler chickens fed on cereal grain based foods soaked in water. *Br. Poult. Sci.* 40:65-76.
- Yegani, M., and D. R. Korver. 2012. Review: Prediction of variation in energetic value of wheat for poultry. *Can. J. Anim. Sci.* 92:261-273.
- Yu, B., J. C. Hsu, and P. W. S. Chiou. 1998. Effects of β -glucanase supplementation of barley diets on growth performance of broilers. *Anim. Feed Sci. Technol.* 70:353-361.
- Zanella, I., N. K. Sakomura, F.G. Silversides, A. Figueirido, and M. Pack. 1999. Effect of enzyme supplementation of broiler diets based on maize and soybeans. *Poult. Sci.* 78: 561-568.
- Zimonja, O., A. Stevnebø, and B. Svihus. 2007. Nutritional value of diets for broiler chickens as affected by fat source, amylose level and diet processing. *Can. J. Anim. Sci.* 87:553-562.