UNIVERSIDAD POLITÉCNICA DE MADRID
ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA,
ALIMENTARIA Y DE BIOSISTEMAS

INFLUENCE OF FEED FORM AND NUTRITIONAL
CHARACTERISTICS OF THE DIETS ON PULLET
PERFORMANCE AND EGG-LAYING HEN
PRODUCTIVITY

TESIS DOCTORAL

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Ingeniero Agrónomo

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PhD Thesis

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CHARACTERISTICS OF THE DIETS ON PULLET
PERFORMANCE AND EGG-LAYING HEN
PRODUCTIVITY**

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A mi familia

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ABBREVIATION LIST

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CMD	Consumo medio diario	PR	Peso relativo
EMAn	Energía metabolizable aves, corregida por nitrógeno	PV	Peso vivo
GMD	Ganancia media diaria	TGI	Tracto gastrointestinal
IC	Índice de conversión		
ADFI	Average daily feed intake	HE	High energy content
ADG	Average daily gain	L	Linear effect
AMEn	Apparent metabolizable energy, N-corrected	LE	Low energy content
BMI	Body mass index	LNA	Linoleic acid
BW	Body weight	ME	Medium energy content
CP	Crude protein	NSP	Nonstarch polysaccharides
ECR	Energy conversion ratio	Q	Quadratic effect
EI	Energy intake	RL	Relative length
FCR	Feed conversion ratio	RW	Relative weight
FI	Feed intake	SI	Small intestine
GIT	Gastrointestinal tract	VHE	Very high energy content
GMD	Geometric mean diameter	VLE	Very low energy content
GSD	Geometric standard desviation (Log normal SD)		

RESUMEN

El objetivo actual en recría de pollitas rubias para producción de huevos consiste en lograr pesos vivos (**PV**) adecuados y uniformes, conforme a las guías de producción, y mejorar el desarrollo del tracto gastrointestinal (**TGI**) y del sistema óseo a fin de mejorar la productividad durante la fase puesta. Todo ello debe ser compatible con un coste ajustado de la alimentación. Estos objetivos se pueden alcanzar mediante modificaciones en las estrategias nutricionales utilizados. En la presente tesis se estudiaron los efectos de estrategias tales como el tipo de cereal base utilizado, el nivel energético del pienso y la forma de presentación del mismo, sobre los rendimientos productivos y el desarrollo del TGI y corporal de pollitas y gallinas ponedoras.

En el experimento 1 se estudió la influencia de la presentación y la concentración energética del pienso sobre los parámetros productivos y el desarrollo del TGI en pollitas rubias de 0 a 17 sem de edad. El diseño experimental fue completamente al azar con 10 tratamientos organizados de forma factorial, con 2 presentaciones del pienso (harina y miga) y 5 niveles de energía, que diferían en 50 kcal EMAn/kg en cada uno de los 3 periodos de la fase de recría. En el global de la prueba (0 a 17 sem de edad), la utilización de migas aumentó el consumo medio diario de pienso (**CMD**; 52,9 vs. 49,7 g; $P < 0,001$) y la ganancia media diaria de peso (**GMD**; 12,7 vs. 11,6 g; $P < 0,001$) y mejoró el índice de conversión (**IC**; 4,18 vs. 4,27; $P < 0,001$) de las aves. Al aumentar el contenido energético del pienso se redujo el CMD de forma lineal ($P < 0,001$) y se mejoró el IC de forma cuadrática ($P < 0,01$). El consumo energético (kcal EMAn/d) sin embargo, no se vio afectado. La uniformidad de los PV fue superior ($P < 0,05$) en las pollitas alimentadas con migas que en las alimentadas con harina pero no se vió afectada por el contenido energético del pienso ($P > 0,05$). A las 5, 10 y 17 sem de vida, el peso relativo (**PR**, % PV) del TGI y de la molleja y el contenido de digesta fresca en la misma fueron inferiores ($P < 0,05$ a $P < 0,001$) y el pH de la molleja fue

superior ($P < 0,05$ a $P < 0,001$) en pollitas alimentadas con migas. La concentración energética del pienso no afectó a ninguna de las otras variables digestivas estudiadas. En resumen, la alimentación con migas mejoró el crecimiento de las pollitas, redujo el PR del TGI y de la molleja, e incrementó el pH de la misma a todas las edades. Un incremento de la energía metabolizable del pienso mejoró el IC global. La utilización de migas y el incremento del contenido energético del pienso son estrategias nutricionales a utilizar cuando el objetivo es incrementar el PV sin afectar a la uniformidad de las pollitas al inicio de la puesta. Sin embargo, la utilización de migas podría reducir el desarrollo y peso de los órganos del TGI, lo que podría tener efectos negativos sobre el consumo de pienso en las fases iniciales del ciclo de puesta.

En el experimento 2 se estudió el efecto del cereal base y de la presentación del pienso de recría sobre los rendimientos productivos, las características del TGI y diversas mediciones corporales en pollitas rubias desde la eclosión hasta las 17 sem de vida. Se utilizó un diseño completamente al azar con 8 tratamientos que resultaron de la combinación de 2 cereales (maíz vs. trigo) y 4 programas de alimentación. Los programas de alimentación utilizados consistieron en alimentar de forma continua con harina o con migas de 0 a 17 sem de vida, o alimentar con migas de 0 a 5 o 0 a 10 sem de edad, seguido de harina hasta las 17 sem. Cada tratamiento se replicó 9 veces y la unidad experimental fue la jaula con 50 pollitas. Desde la eclosión hasta las 17 sem de vida, las pollitas alimentadas con maíz tuvieron una GMD similar pero peor IC ($P < 0,001$) que las pollitas alimentadas con trigo. Además, las pollitas que se alimentaron con migas durante toda la prueba (0 a 17 sem) tuvieron mejores GMD (12,3 vs. 11,5 g; $P < 0,001$) e IC (4,21 vs. 4,36; $P < 0,001$) que las pollitas alimentadas de continuo con harina, con las pollitas que cambiaron en algún momento del periodo de recría de migas a harina mostrando resultados intermedios. A las 17 sem de vida, el PR del TGI y de la

molleja fue superior en las pollitas alimentadas con maíz que en las pollitas alimentadas con trigo ($P < 0,01$) pero la longitud relativa (cm/kg PV) del intestino delgado, tarso y corporal (desde la punta del pico al final de la última falange) no se vieron afectados. Las pollitas alimentadas con migas durante toda la prueba presentaron mollejas de menor peso ($P < 0,001$) con un pH superior ($P < 0,001$) y tuvieron una menor longitud corporal ($P < 0,01$) que las pollitas alimentadas con harina de continuo, con las pollitas alimentadas con los otros 2 tratamientos mostrando resultados intermedios. En resumen, trigo y maíz pueden ser utilizados indistintamente en piensos para pollitas de recría sin efecto adverso alguno sobre los rendimientos productivos. La alimentación con migas mejoró los rendimientos productivos pero redujo el desarrollo de la molleja y del TGI. Los rendimientos productivos, el TGI y los parámetros anatómicos corporales se adaptaron rápidamente a los cambios en la presentación del pienso durante el periodo de recría.

En el experimento 3 se estudió la influencia de la presentación y la concentración energética del pienso de recría y la concentración energética del pienso de puesta, sobre los rendimientos productivos, el desarrollo del TGI y mediciones anatómicas corporales en gallinas ponedoras rubias. El experimento fue completamente al azar con 12 tratamientos estructurados factorialmente $(2 \times 3) \times 2$ con 2 formas de presentación del pienso (harina vs. migas) y 3 concentraciones de EMA_n (baja, media y alta) del pienso durante la fase de recría y 2 concentraciones de EMA_n (2.650 vs. 2.750 kcal/kg) de los piensos durante la fase de puesta. En cada uno de los 3 periodos de la fase de recría (0 a 5 sem, 5 a 10 sem y 10 a 17 sem de edad) y durante la fase de puesta (17 a 46 sem de edad), los piensos fueron formulados para tener una concentración similar en nutrientes esenciales por kcal de $EMAn$. Los resultados productivos en puesta se determinaron durante 7 periodos de 28 d cada uno y las variables del TGI se midieron

al final de la prueba. La presentación y el contenido energético de los piensos suministrados durante la fase de recría no afectaron a ninguno de los parámetros productivos, así como tampoco al desarrollo del TGI o a las mediciones corporales al final de la puesta. De 17 a 46 sem de edad, la ganancia de peso fue mayor ($P < 0,01$) en las gallinas alimentadas con harina durante la fase de recría pero el PV final no se vio afectado. Un incremento en el contenido energético del pienso de la fase de puesta de 2.650 a 2.750 kcal/kg redujo el CMD ($P < 0,001$), mejoró el IC ($P < 0,001$) y disminuyó el contenido fresco de la molleja ($P < 0,01$) a las 46 sem, pero no afectó a ningún otro de los parámetros estudiados. A las 46 sem de edad, el PV de las gallinas estuvo relacionado positivamente ($P < 0,001$) con la longitud corporal, la longitud y el diámetro del tarso y el índice de masa corporal. Los datos indican que ni la presentación del pienso de recría ni la concentración energética del mismo afectaron a la productividad en la fase de puesta. El incremento en el contenido de EMAn del pienso de las ponedoras de 2.650 a 2.750 kcal/kg redujo el CMD y mejoró el IC durante la fase de puesta pero no afectó a la producción global de huevos.

En resumen, la utilización de migas en el pienso mejoró los rendimientos productivos pero redujo el desarrollo del TGI en las pollitas. El cambio de presentación del pienso de migas a harina a diferentes edades provocó en todos los casos una disminución de los rendimientos productivos y una mejora del desarrollo del TGI en los siguientes periodos. La forma de presentación del pienso durante la recría tuvo poco efecto sobre la productividad y la morfología del TGI de la gallina durante la puesta. Los datos demuestran que pollitas y gallinas en puesta se adaptan rápidamente tanto en parámetros productivos como en parámetros de fisiología digestiva a cambios en la presentación del pienso. En consecuencia, el efecto de la presentación del pienso durante la recría podría ser menos relevante de lo que se cree. Por otro lado, un

incremento del contenido energético en el pienso de recría aumentó el CMD y mejoró el IC proporcionalmente, sin afectar a la uniformidad de los PV o al desarrollo del TGI. La concentración energética de los piensos de recría tuvieron poco efecto sobre la productividad de las aves en el periodo de puesta. Por tanto, el nivel de energía de los piensos de recría y puesta dependerá, dentro de ciertos límites, del coste relativo de los ingredientes disponibles. Por último, el cereal base del pienso no afectó ni al peso ni a la uniformidad de las pollitas y de hecho, el IC fue mejor en las aves que consumieron piensos basados en trigo, por lo que este cereal suplementado con enzimas adecuadas se puede utilizar a niveles de al menos 40%. Sin embargo, el TGI y la molleja presentaron un menor desarrollo anatómico y funcional en pollitas alimentadas con trigo que en pollitas alimentadas con maíz. Por tanto, los nutricionistas pueden adaptar las características de los piensos de recría y puesta, dentro de los rangos estudiados, según los costes relativos de las materias primas disponibles, sin efectos destacados sobre la productividad de las aves o la calidad de los huevos.

SUMMARY

The main objective in the production of commercial pullets for egg production is to increase body weight (**BW**) and uniformity and improve gastrointestinal tract (**GIT**) and bone development to improve subsequent hen production. These objectives can be achieved by using adequate nutritional strategies. In this thesis several of these strategies, namely feed form, energy concentration, and main cereal of the diets are discussed.

In experiment 1, the influence of feed form and energy concentration of the diet on growth performance and the development of the GIT was studied in brown-egg laying pullets. The experimental design was completely randomized with 10 diets organized as a 2 x 5 factorial with 2 feed forms (mash vs. crumbles) and 5 levels of energy that differed in 50 kcal AMEn/kg in the 3 feeding period considered (0 to 5, 5 to 10, and 10 to 17 wk of age). For the entire experiment (0 to 17 wk of age) feeding crumbles increased average daily feed intake (**ADFI**; 52.9 vs. 49.7 g; $P < 0.001$) and average daily gain (**ADG**; 12.7 vs. 11.6 g; $P < 0.001$) and improved feed conversion ratio (**FCR**; 4.18 vs. 4.27; $P < 0.001$) of the pullets. An increase in the energy content of the diets decreased ADFI linearly (**L**, $P < 0.001$) and improved FCR quadratically (**Q**, $P < 0.01$) but energy intake (**EI**, kcal AMEn/d) was not affected. BW uniformity was higher ($P < 0.05$) in pullets fed crumbles than in pullets fed mash but it was not affected ($P > 0.05$) by the energy content of the diet. At 5, 10, and 17 wk of age, the relative weight (**RW**, % BW) of the GIT and the gizzard, and the fresh gizzard digesta content were lower ($P < 0.05$ to $P < 0.001$) and the gizzard pH was higher ($P < 0.05$ to $P < 0.001$) in pullets fed crumbles than in pullets fed mash. Energy concentration of the diet did not affect any of the GIT variables studied. In summary, feeding crumbles improved pullet performance but reduced the RW of the GIT and gizzard and increased gizzard pH at all ages. An increase in the energy content of the diet improved FCR from 0 to 17

wk of age. The use of crumbles and the increase in the AMEn content of the diet might be used advantageously when the objective is to increase the BW of the pullets. However, crumbles affected the development and weight of the organs of the GIT, which might have negative effects on feed intake (**FI**) at the beginning of the egg laying cycle.

In experiment 2, the effects of the main cereal and feed form of the rearing phase diets on growth performance, GIT characteristics, and body traits were studied in brown-egg pullets from hatch to 17 wk of age. The experimental design was completely randomized with 8 dietary treatments that were a combination of 2 main cereals (corn vs. wheat) and 4 feeding programs. The feeding program consisted in feeding crumbles from 0 to 5, 0 to 10, or 0 to 17 wk of age followed by mash until 17 wk, or feeding mash continuously from 0 to 17 wk. Each treatment was replicated 9 times. From hatch to 17 wk of age, pullets fed corn had similar ADG but poorer FCR ($P < 0.001$) than pullets fed wheat. Also, pullets fed crumbles continuously (0 to 17 wk) had greater ADG (12.3 vs. 11.5 g; $P < 0.001$) and better FCR (4.21 vs. 4.36; $P < 0.001$) than pullets feed mash continuously, with pullets that were changed at any age of the rearing period from crumbles to mash feeding showing intermediate results. At 17 wk of age, the RW of the GIT and gizzard were greater in pullets fed corn than in pullets fed wheat ($P < 0.01$) but the relative length (**RL**; cm/kg full BW) of the small intestine (**SI**) and of the body and tarsus were not affected. Pullets fed crumbles continuously had lighter gizzards ($P < 0.001$), higher gizzard pH ($P < 0.001$), and were shorter in length ($P < 0.01$) than pullets fed mash continuously with pullets fed any of the other 2 treatments being intermediate. In summary, wheat can be used in substitution of corn in pullet diets without any adverse effect on growth performance. Feeding crumbles improved pullet performance but hindered gizzard and GIT development. Growth performance, GIT,

and body traits of the pullets re-adapted quickly to changes in feed form of the rearing diets.

In experiment 3, the influence of feed form and energy concentration of the rearing diets on production, GIT traits, and body measurements was studied in brown-egg laying hens. The experimental design was completely randomized with 12 treatments arranged as a (2 x 3) x 2 factorial with 2 feed forms (mash vs. crumbles) and 3 AME_n concentrations (low, medium, and high) of the rearing phase diets and 2 AME_n concentrations (2,650 vs. 2,750 kcal/kg) of the laying phase diets. Within each of the 3 feeding periods of the rearing phase (0 to 5 wk, 5 to 10 wk, and 10 to 17 wk of age) and during the laying phase (17 to 46 wk of age), diets had similar nutrient content per kcal of AME_n. Production was measured for seven periods of 28-d each and GIT and body measurements were determined at 46 wk of age. Feed form and energy content of the rearing phase diets did not affect hen performance, digestive tract traits, or body measurements of the eggs at 46 wk of age. From 17 to 46 wk of age BW gain was higher ($P < 0.01$) in hens fed mash during the rearing phase, although final BW was not affected. An increase in the energy content of the laying phase diet from 2,650 to 2,750 kcal/kg reduced ADFI ($P < 0.001$) and improved FCR ($P < 0.001$) and reduced gizzard contents ($P < 0.01$) at 46 wk but did not affect any of the other traits studied. At 46 wk of age, BW of the hens was positively ($P < 0.001$) correlated with body length, tarsus length and diameter, and body mass index (**BMI**; g BW/body length²). The data indicate that neither feed form nor energy concentration of the rearing diets affected subsequent hen performance. An increase in the AME_n content of the diets from 2,650 to 2,750 kcal/kg, decreased ADFI and improved FCR during the laying phase but did not affect hen production.

In summary, feeding crumbles to pullets improved growth performance but reduced the development of the GIT. Pullets changed from crumble to mash feeding at different ages responded always with a reduction in growth performance and an improvement in the development of the GIT in the subsequent period of the rearing phase. Feed form of the rearing phase diets had little effect on the performance and egg quality of the hens during the entire laying cycle or on the GIT traits and morphometric measurements of the hens at 46 wk of age. The data demonstrate that pullets and hens re-adapt quickly to changes in feed form with changes in GIT traits and growth performance or production. Consequently, the effects of ingredient composition and feed form of the rearing diets on subsequent pullet performance might be less relevant than currently accepted. On the other hand, an increase in the energy content of the rearing or laying diet decreased ADFI and improved FCR proportionally, without affecting BW uniformity or GIT development at any age. Energy concentration of the rearing phase diets had little effect on egg production or GIT development of the hens at the end of the laying cycle. Consequently, the recommended energy content of the pullet and hen diets will depend at a high extent on the relative cost of available ingredients. The main cereal of the diet did not affect ADG of the pullet from hatch to 17 wk of age and in fact FCR was improved with wheat feeding. Consequently, at least up to 40% wheat supplemented with adequate NSP enzymes, can be used in substitution of corn in these diets. However, pullets fed wheat had lighter GIT and gizzard and less gizzard content than pullets fed corn, a finding that suggested that under certain circumstances, the main cereal of the diet might affect indirectly subsequent hen performance. Consequently, nutritionists may adapt the characteristics of the rearing and laying phase diets, within the range of values studied, to the cost of available ingredients, without any detectable effect on hen production or egg quality.

CHAPTER 1: LITERATURE REVIEW AND OBJECTIVES

1. LITERATURE REVIEW

1.1. Introduction

Egg production in Spain represented almost 10.3% (690×10^3 t) of the total production of the European Union-28 (EU-28) in 2015. With 41 M hens placed in 1,194 layer farms. Spain was in 2015 one of the largest egg producer countries in the EU-28, with France, Germany, and Italy in the first positions (Figure 1). Approximately, 91.7% of the hens are housed in cages, 5.0% on floor, 2.8% free range, and 0.4% organic production. These percentages are changed very quickly. In fact, the number of hens in alternative production systems expected for 2018 will be 13% of total production.

Figure 1. Egg production ($\times 10^3$ t) in the EU (adapted from MAGRAMA, 2016).

The economic success of the egg industry depends at a great extent on egg mass produced by the hen during the entire laying period. To attain adequate performance and optimal economic output of a given flock, farmers should focus on reaching a high peak of production while maintain a good persistency during the egg cycle. Two points of interest are the percentage of large eggs early in the production cycle and the maintenance of egg shell quality with a low percentage of shell-less, fissured, and undegraded eggs, during the last part of the egg cycle. Egg size has important

connotations for the success of commercial egg operations in countries such as Spain in which consumers show a clear preference for large eggs (L and XL) and are willing to pay extra per dozen of these eggs (Grobas et al., 1999a; Pérez-Bonilla et al., 2012a,b). Therefore, the production of heavy eggs is of paramount importance for the industry. In this respect, producers tend to increase the duration of the egg laying cycle because egg size increases with the age of the hens, increasing the importance of keeping egg shell quality in old hens.

One of the most critical points to improve egg rate and maintain egg quality along the egg cycle is to insure an adequate management and maintain sound feeding and nutrition practices during the rearing phase. Body weight and uniformity of the flock are key factors in future hen production (Leeson et al., 1997; Gous and Cherry, 2004). Light hens at the onset of the laying period produce less eggs that are smaller during the whole cycle than heavy hens (Pérez-Bonilla et al., 2012a,b). BW and flock uniformity of the pullets at sexual maturity are positively correlated with high peaks, good persistency of egg production, and proportion of large eggs (Summers and Leeson, 1994).

There is a growing interest on the influence of different dietary factors on GIT development of the pullets and subsequent hen production. In this respect, González-Alvarado et al. (2008) suggested that heavy, more functional gizzards, might result in more intensive refluxes and better mixing of the digesta and endogenous enzymes in the GIT of the chicken. Consequently, a well-developed gizzard might increase nutrient digestibility and help to maintain a healthy microbial population in the GIT (Gabriel et al., 2008; Santos et al., 2008). Thus, potential benefits on pullet health and growth performance could be obtained adopting nutritional strategies that improve GIT development. Under all circumstances an important objective of rearing pullets and laying

hens is to maintain production costs under control. Feed account for 65 to 70% of the total cost of production in layer farms and therefore, the relative cost among raw materials (i.e., corn vs. weat vs. barley) is an important factor influencing overall profitability.

Numerous factors, such as feed form, energy concentration, and main cereal of the diet, affect GIT development (Frikha et al., 2009a; Frikha et al., 2011; Guzmán et al., 2015b) and productive performance (Leeson et al., 1997; Gous and Morris, 2001; Guzmán et al., 2015a) of the pullets during the rearing period. However, the information available on the influence of feed form and feeding program during the rearing period on the growth and development of the GIT both in pullets and laying hens is very limited. More information is needed to help nutritionists to formulate diets that allow a proper development of the GIT, and at the same time, maximize BW and uniformity of the pullets.

The present PhD thesis investigates the effects of key factors of the feeding program (feed form, energy concentration, and main cereal of the diet) on productive performance, BW uniformity, and GIT development on brown egg-laying pullets.

1.2. Feed form

The utilization of crumbles is an alternative to the use of mash diets to improve pullet performance during the rearing period. Pelleting agglomerates the small particles of the feed ingredients into larger particles by means of a mechanical process that combines moisture, steam, heat, and pressure (Falk, 1985). The process includes the grinding of the ingredients to reduce particle size, the addition of steam in the conditioner for a short period of time (1-2 min) at high temperature (80-85°C), followed by passing the mixture through the press die, which causes additional mechanical

pressure, and then the warm pellets are cooled to about 8°C above ambient temperature by using a stream of ambient air (Thomas et al., 1997; Abdollahi et al., 2013a).

Pelleting of the diet has been shown to improve growth rate and feed efficiency in broilers (Amerah et al., 2007a; Serrano et al., 2012, 2013) with most of the benefits caused by an increase in FI and a reduction in feed wastage. On the other hand, mash feeding might be more favorable for improving nutrient digestibility than pellet feeding because mash diets favours GIT development and gizzard function (Abdollahi et al., 2010; Svihus, 2011; Serrano et al., 2013).

1.2.1. Effect of feed form on bird performance

Pelleting is a common practice to maximize growth performance in broilers (Abdollahi et al., 2011; Serrano et al., 2013). Pelleting of the feed agglomerates the feed particles and reduces the percentages of fines and the natural selection of coarse particles by the bird (Abdollahi et al., 2013a). Moreover, the pelleting process increases bulk density and improve feed texture, facilitating feed prehension by the birds which may result in an increase in feed consumption. The rate of passage through the GIT is faster with pellets than with mash which in turn facilitates consumption and increases FI. As a result, ADG increases when the feed is pelleted (Amerah et al., 2007a; Serrano et al., 2012; Jiménez-Moreno et al., 2016). In broilers, feeding pellets increases ADG and reduced FCR compared with feeding mash (Reece et al., 1985; Douglas et al., 1990; Amerah et al., 2007a). Quentin et al. (2004) reported improved performance in broilers from 15 to 35 d of age when fed pellets compared to broilers fed the same diet ground through 0.5 mm diameter screen. In the research of Quentin et al. (2004), broiler fed pellets had 19% greater BW, 12% higher ADFI, and 12% better FCR than broilers fed the same diet after regrinding. Not much information is available on the effects of

pelleting on pullet growth. In general, the expected in proportion is lower in pullets than in broilers and decreases as the bird ages. Gous and Morris (2001) observed that pullets fed crumbles from 1 to 4 wk of age and then pellets from 5 to 20 wk, consumed 1.63% less feed but were 6.25% heavier than pullets fed mash. Guzmán et al. (2015a) reported also 1.86% lower ADFI but 6.60% higher ADG in pullets from 0 to 5 wk of age when fed crumbles than when fed mash.

Pelleting improves often the AMEn content of the diet (Skinner-Noble et al., 2005; Serrano et al., 2013). The heat, moisture, and pressure applied during the pelleting process modifies the physical structure of the ingredients by reducing particle size and releasing the encapsulated lipid fractions within the cells increasing nutrient digestibility (Lemme et al., 2006; Abdollahi et al., 2011; Medic et al., 2014). Adeyemi et al. (2008) reported that ether extract retention increased from 60 to 72% when the feed was pelleted. Zelenka (2003) and Zang et al. (2009) reported higher digestibility of crude fat and an increase in the AMEn of the diet in broilers fed pellets than in broilers fed mash. On the other hand, the finer particles of pelleted feeds are retained for less time in the gizzard than the coarser particles of the mash feeds and consequently, gizzard function and the intensity of the reverse peristaltic movements in the GIT might be reduced (Nir et al., 1994a,b; Jiménez-Moreno et al., 2009a,b). In this respect, Abdollahi et al. (2011) reported higher apparent ileal digestibility of starch at 21 d in broiler fed wheat based diets in mash form than in pellet form. Both effects, better access to nutrients but lower retention time in the gizzard, might counteract each other and result in different final effect of pelleting on nutrient retention depending on the conditions of the experiment (Abdollahi et al., 2010; Mateos et al., 2012). In this respect, several authors (Patterson, 1989; Medel et al., 2004; Serrano et al., 2013; Jiménez-Moreno et al., 2016) indicated

that the improvement in FCR observed with pelleting might be more related to reduced feed wastage than to increased nutrient digestibility.

1.2.2. Effect of feed form on the development of the gastrointestinal tract

Feed form influences digestive organ development in broilers (Choi et al., 1986; Kilburn and Edwards, 2001; Mateos et al., 2002; Jiménez-Moreno et al., 2016). Coarser particles are retained for longer in the gizzard increasing the mechanical stimulation of this organ and improving GIT development, which in turn might improve nutrient digestibility compared with fine particles (Svihus and Hetland, 2001; Abdollahi et al., 2010; Svihus, 2011; Serrano et al., 2013). Ingredients to be pelleted are ground fine because fine particles improve pellet quality. Consequently, feeding pellets results usually in a significant reduction in the relative size of the gizzard in broilers (Nir et al., 1994; Engberg et al., 2002; Svihus et al., 2004; Serrano et al., 2013) and pullets (Guzmán et al., 2015a; Saldaña et al., 2015a). Abdollahi et al. (2011) reported that pellet feeding reduced the RW of the GIT. Moreover, Nir et al. (1995) found that pelleting reduced the RL of the jejunum and the ileum by 15%. In this respect, Frikha et al. (2009b) observed also lighter GIT at 17 wk of age in pullets that were fed pellets for the first 5 wk of life than in pullets that were fed mash. Serrano et al. (2013) reported lower gizzard pH in broilers fed a mash corn-soybean diets than in broilers fed the same diets in crumble or pellet form. The higher gizzard pH observed in broilers fed pellets might be related with the lower particle size and the higher ADFI of these birds, which reduced the retention of the feed and increase the rate of passage through the GIT. A low pH in the upper part of the GIT improved the solubility and absorption of mineral salts (Guinotte et al., 1995) and favoured pepsin activation which may contribute to improve mineral absorption and crude protein (CP) digestion. In addition, a reduction in pH might have an indirect potential benefit on GIT health, reducing the growth of the

pathogenic microflora existing in large intestine (Engberg et al., 2002). In fact, the incidence of wet litter and *Escherichia coli* and *Salmonella* spp growth in the GIT, increase when the diets are pelleted (Engberg et al., 2002; Huang et al., 2006).

1.3. Energy concentration

Energy is often the most expensive nutrient of poultry diet. In fact, lipid sources such as soybean oil, acidulated vegetable soapstocks, and lard, are some of the most expensive raw materials used in poultry diets. Energy concentration of the diet affects FI and growth performance of broilers (Brickett et al., 2007), pullets (Frikha et al., 2009a; Guzmán et al., 2015a, Saldaña et al., 2015a), and laying hens (Grobass et al., 1999a; Pérez-Bonilla et al. 2012a). On the other hand, when the energy concentration of the diet is reduced, fat content decreases and fiber content increases, changes in diet composition that affect in different ways, GIT development and growth performance of the birds.

1.3.1. Effect of the energy concentration of the diet on bird performance

Energy is the most important variable affecting FI and animal production. Birds eat to satisfy their energy requirements and therefore, voluntary FI will decrease as the energy content of the diet increases (Hill et al., 1956; Leeson et al., 1996; Veldkamp et al., 2005). However, the adjustment to regulate FI with changes in energy concentration of the diet is not perfect and pullets tend to over consume energy when fed crumbles or high energy diets (Frikha et al., 2009b; Serrano et al., 2012; Guzmán et al., 2015a). High energy diets contain more fat and are more palatable than low energy diets which might favor EI of the birds (Grobass et al., 1999b; Frikha et al., 2009a). Moreover, supplemental fat may reduce digesta transit time and improve the utilization of other components of the diet, as demonstrated by Mateos and Sell (1980, 1981a). In contrast,

if the diet is diluted excessively, birds might not be able to consume enough feed to maintain EI constant, leading to a reduction in productive performance (Nielsen, 2004; Pérez-Bonilla et al., 2012a). In this respect, Latshaw (2008) observed that not only energy content, but also diet composition, had significant effects on FI of broilers. In this research, birds ate approximately 10% more energy of a diet with 3,000 kcal AME/kg that contained 5% fat than of an isoenergetic diet without fat supplementation. In the same experiment, it was also observed that birds fed a diet with 4% added fiber ate approximately 20% less energy than those fed a similar diet with no fiber supplementation. Brickett et al. (2007) using diets varying in energy content from 2,800 to 3,100 kcal/kg, observed that BW of the broilers was not affected by energy concentration of the diet when the feeds were pelleted. However, when the diets were fed as mash, EI decreased linearly as the energy concentration of the diet decreased. These authors suggested that the effect of energy concentration of the diet on FI was less pronounced with pellets than with mash diets. Leeson et al. (1996) observed similar EI and ADG in 49 d-old broilers fed pelleted diets containing from 2,700 to 3,300 kcal AMEn/kg. However, when the birds received a fixed amount of feed per day, growth performance was reduced with the lower energy diets. In laying hens, Cherry et al. (1983) reported an initial increase in EI when the hens were fed a high energy diet whereas an opposite effect occurred when they were fed a low energy diet.

The effect of energy concentration of the diet on egg production is a subject of debate. Most published research on this subject reported an increase in egg weight with increases in the energy concentration of the diet (De Groote, 1972; Walker et al., 1991). Also, Mathlouthi et al. (2002a) reported that egg production in SCWL hens increased as the AMEn of the diet increased from 2,650 to 2,750 kcal/kg. Pérez-Bonilla et al. (2012a) observed that an increase in energy concentration of the laying hen diets from

2,650 to 2,950 kcal AMEn/kg increased egg production, egg mass, and energy efficiency. However, Grobas et al., (1999c) in brown hens fed diets varying from 2,680 to 2,810 kcal AMEn/kg, Harms et al. (2000) in brown and SCWL hens fed diets varying in AMEn from 2,500 to 3,100 kcal/kg, and Jalal et al. (2006, 2007) in SCWL hens fed diets varying from 2,800 to 2,900 kcal AMEn/kg did not detect any significant difference in egg production with changes in the energy content of the diet. The reasons for the discrepancies among authors in relation to the effects of an increase in energy content of the diet on egg production are not apparent but might be related to the level of fat and the linoleic acid (**LNA**) content of the control diet. Fats are used in poultry to increase the energy content of the diets, and usually results in an increase in EI, ADG, and egg size (Grobas et al., 2001; Bouvarel et al., 2010), probably because of a reduction in dust formation and an improved in palatability and FI. In addition, the efficacy in converting AMEn into NE is greater for fats than for the other components of the diet, resulting in more energy available for production.

1.3.2. Effect of the energy concentration of the diet on the development of the gastrointestinal tract

Changes in ingredient composition of the rearing phase diets affect in different ways the development and function of the GIT in poultry (Jiménez-Moreno et al., 2009a; Svihus et al. 2011) which in turn might affect FI and subsequent performance during the laying phase. A poor developed GIT might reduce FI, resulting in a decrease in BW of the pullets at 17 wk of age and a reduction in egg mash production during the first period of the laying phase. When the GIT is not well developed at 17 wk of age, pullets might not consume enough energy during the first weeks of the laying phase (Pérez-Bonilla et al., 2012b). On the other hand, the use of low vs. high energy diets during the laying phase might result in lower egg mass and egg weight, with the

negative effects being more pronounced in those hens that were lighter at the onset of the egg cycle. When the energy concentration of the diet is reduced, fiber content increases, which might benefit the development and function of the GIT (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009a; Sacranie et al., 2012). In broilers, González-Alvarado et al. (2007, 2008) observed that the inclusion of 3% oat hulls or 4% soy hulls in the diet increased the RW of the gizzard and of the GIT and reduced the RL of the SI. Jiménez-Moreno et al. (2009a) reported that the inclusion of fiber in the diet increased the retention time of the digesta in the upper part of the GIT (from crop to gizzard) and stimulated gizzard function and HCl production by the proventriculus. Scheideler et al. (1998) observed greater gizzard size at 17 wk of age in two strains of SCWL pullets fed diets with 10% oat hulls inclusion than in pullets fed a control corn-soybean meal diet. In this respect, Frikha et al. (2009a) reported that from 1 to 45 d of age, pullets fed low-energy diets had higher RW of the gizzard and of the GIT than pullets fed high-energy diets that were lower in crude fiber. Consequently, low AMEn diets might benefit the development of the GIT, because of the higher fiber content, and thus improve pullet performance.

1.4. Main cereal

Cereals are the most widely used energy sources in poultry feeds with the starch fraction providing the main part of the energy of the diet. In addition, cereals provide a high proportion of the AA required by the birds. The most common cereals used in poultry diets worldwide are corn (*Zea mays* L.) and soft wheat (*Triticum Aestivum* L.).

In Spain, the price of corn is usually higher than the price of wheat (Figure 2). However, under commercial conditions, many egg producers formulate diets for laying hens with a minimum level of corn to ensure a high FI and maximize egg size early in the production cycle, especially when the pullets were stimulated at young ages. The reasons for this practice are unknown but might be related to the better structure of the feed when coarse corn is included in the diet or to the more uniform nutritive value of corn (Frikha et al., 2009b). In this respect, corn has less CP (7.5% vs. 10.2%) and crude fiber (2.3% vs. 2.6%) and more starch (63.3% vs. 60.2%), ether extract (3.6% vs. 1.6%), and available energy (3,280 vs. 3,100 kcal AMEn/kg) than wheat (FEDNA, 2010). Also, corn has more LNA content (1.81% vs. 0.64%) than wheat, and an increase in LNA content might improve egg weight, especially in young hens under hot climate conditions in which FI is low (Jensen et al., 1958; Scragg et al., 1987; Grobas et al., 1999b). On the other hand, chemical composition of the wheat vary depending on factors such as cultivar used, agronomic practices, weather conditions, and length of storage period (Pirgozliev et al., 2003; Gutiérrez-Álamo et al., 2008; Frikha et al., 2011). Consequently, nutritionist should use different nutritive values of wheat depending on year crop and on the geographical region. The variation in the nutritive value of wheat in Spain with year crop and geographical region within Spain is shown in Tables 1 and 2, respectively. Furthermore, wheat contains a high and variable amount of nonstarch polysaccharides (**NSP**), including arabinoxylans and β -glucans, which are

known to increase digesta viscosity and to reduce productive performance in poultry (Lázaro et al., 2003a,b, García et al., 2008). Thus, the recommended level of inclusion of wheat in poultry diets depends on many factors such as the species considered, age, enzyme supplementation, and chemical characteristics of the batch including AMEn, CP, and NSP content (Classen, 1996; Gutiérrez-Álamo et al., 2008; Abdollahi et al., 2011).

Table 1. Variation of the nutritive value of wheat with year crop, Spain (adapted from NUTEGA, 2016)

Year	Moisture (%)	Crude protein (%)	Crude fiber (%)	Starch (%)
2013	10.3	11.0	3.33	59.6
2014	10.8	11.8	3.15	59.7
2015	9.7	12.6	3.20	59.0
2016	9.8	11.5	2.94	60.6

Table 2. Variation of the nutritive value of wheat according to the geographical region of production, Spain (adapted from NUTEGA, 2016)

Region	Moisture (%)	Crude protein (%)	Crude fiber (%)	Starch (%)
Castilla León	9.2	10.9	3.09	60.9
Castilla la Mancha	8.6	10.9	3.03	59.9
Aragón	10.7	11.3	2.83	60.8
Cataluña	10.5	12.0	2.85	60.5
Andalucía	10.1	14.0	2.76	61.1

1.4.1. Effect of the main cereal of the diet on bird performance

Several reports compared the inclusion of corn and wheat in the diet on productive performance of broilers (Ruiz et al., 1987; Mathlouthi et al., 2002b), pullets (Frikha et al., 2009b), and laying hens (Lázaro et al., 2003a; Safaa et al., 2009; Pérez-Bonilla et al., 2011). In general, these studies indicate that wheat is a good alternative to corn in all these species, provided that the diets are supplemented with adequate enzymes. In broilers, Mathlouthi et al. (2002b) reported similar performance when 60% of the corn was substituted by a combination of 40% wheat and 20% barley. Also, Ruiz et al. (1987) reported similar ADG and FCR in broilers when the corn was substituted by wheat in diets fed as mash. However, Crouch et al. (1997) compared corn and two varieties of wheat at 40% of inclusion in mash diets for broilers and found that ADG and FCR were impaired with one of the two wheats. In laying hens, Craig and Goodman (1993), Lázaro et al. (2003a), Safaa et al. (2009), and Pérez-Bonilla et al. (2011) reported similar hen production when wheat diets were supplemented with exogenous enzymes. Kim et al. (1976) reported that SCWL hens fed a diet based on corn ate more feed and produced heavier eggs than hens fed a diet based on wheat from 21 to 43 wk of age. In pullets, Frikha et al. (2009b) reported higher ADG in pullets fed corn than in pullets fed wheat, both diets being supplemented with enzymes. In contrast, Moran et al. (1993) observed better productive performance with wheat than with corn in Ross broilers fed pelleted feeds from 1 to 42 d of age. The reasons for these discrepancies are unknown but might be related to the energy value given to the 2 cereals as well as the use of different enzyme complexes and different criteria when estimating the increase in energy value of the cereals because of enzyme supplementation.

The information available on the effects of the main cereal of the diet on egg quality is scarce. In general, the inclusion of wheat increased the incidence of dirty eggs

compared with the inclusion of corn (Francesch et al., 1995; Lázaro et al. (2003a). However, Çiftci et al. (2003) and Safaa et al. (2009) reported that the substitution of corn by wheat in enzyme supplemented diets did not affect the percentage of dirty eggs in SCWL or brown egg-laying hens, respectively. Moreover, Jamroz et al. (2001) and Lázaro et al. (2003a) reported similar egg quality from hens fed wheat diets supplemented with enzymes.

1.4.2. Effect of the main cereal of the diet on the development of the gastrointestinal tract

Svihus (2011) recommended that at least 20% of the cereal particles of the diet should be larger than 1.5 - 2.0 mm in size or at least 30% of the diet particles should be larger than 1 mm to insure a good size and function of the GIT in broilers. In this respect, Dombrink-Kurtzman and Bietz (1993) and Dobraszczyk et al. (2002) reported that the endosperm of dented corn is harder and more difficult to grind than the endosperm of soft wheat. Consequently, corn particles might stay for longer than wheat particles in the gizzard, resulting in greater development and larger size of the muscular layers of this organ (Jiménez-Moreno et al., 2010; Svihus, 2011; Mateos et al., 2012). In fact, Abdollahi et al. (2013b) compared diets based corn or wheat fed as mash or pellets in broilers and reported heavier gizzards at 3 wk of age for the corn in both cases. Similar differences between corn and wheat diets have been reported by Nir et al. (1994a). These authors observed heavier RW of the gizzard in 21 d-old broilers fed corn than in those fed wheat. In this research, gizzard pH, however, was not affected by the main cereal of the diet at any age. In pullets, Frikha et al. (2009b) observed that at 6 wk of age the gizzard was lighter when fed wheat- than when fed corn diets. However, Amerah et al. (2008b) did not observe any difference in the RW of the gizzard of 21 d-

old broilers fed a corn or a wheat based diet, although the RL of the different segments of the GIT was shorter with wheat feeding.

2. OBJECTIVES

The main objectives of the current PhD thesis were:

1. To study the effects of feed form and energy concentration of the diet and their potential interactions, on growth performance and gastrointestinal tract development of brown-egg laying pullets from hatching to 17 wk of age.
2. To evaluate the effects of feeding crumbles for different periods of time, followed by feeding mash to 17 wk of age, on performance, gastrointestinal tract development, and body measurements of brown-egg laying pullets fed diets based on corn or wheat.
3. To determine the effects of feed form and energy concentration of the rearing phase diets on performance, egg quality, digestive tract traits, and body measurements of hens fed diets differing in energy concentration from 17 to 46 wk of age.

CHAPTER 2: FEED FORM AND ENERGY
CONCENTRATION OF THE DIET AFFECT GROWTH
PERFORMANCE AND DIGESTIVE TRACT TRAITS OF
BROWN-EGG LAYING PULLETS FROM HATCHING TO
17 WEEKS OF AGE

1. INTRODUCTION

Pelleting results usually in increases in FI and improvements of ADG and FCR in poultry (Abdollahi et al., 2013a; Guzmán et al., 2015a; Jiménez-Moreno et al., 2016). When the diets are fed as pellets, feed wastage is reduced (Serrano et al., 2013) and nutrient utilization is increased (Lemme et al., 2006; Abdollahi et al., 2011). Pelleting reduces the RW of the gizzard and increases gizzard pH in poultry (Preston et al., 2000; Abdollahi et al., 2011), effects that might be less pronounced in pullets because of their reduced growth rate and lower ingesta capacity (Frikha et al., 2009b; Guzmán et al., 2015a). However, the information available on the influence of feed form on growth performance and the development of the GIT in pullets is limited.

Energy concentration of the diet affects ADFI and growth performance of broilers (Brickett et al., 2007), pullets (Frikha et al., 2009a; Guzmán et al., 2015a), and laying hens (Grobas et al., 1999a; Pérez-Bonilla et al., 2012a). Birds eat to satisfy their energy requirements and therefore, voluntary FI decreases as the energy content of the diet increases (Leeson et al., 1996; Veldkamp et al., 2005). However, the adaptation of the digestive tract of the birds to regulate FI with changes in energy concentration of the diet might not be complete. For example, high energy diets contain more fat and are more palatable than low energy diets which may result in an increase in EI and ADG (Grobas et al., 1999b). Moreover, an increase in the fat content of the diet reduces digesta transit time and improves the utilization of other components of the diet (Mateos and Sell 1980, 1981a). In contrast, if the diet is diluted excessively, birds might not be able of maintaining their EI constant, resulting in poor performance (Nielsen, 2004; Pérez-Bonilla et al., 2012a). On the other hand, when the energy concentration of the diet is reduced, fiber content increases, which benefits the development and function of the GIT (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009a; Sacranie et al.,

2012). In this respect, Frikha et al. (2009a) reported that from 1 to 45 d of age, pullets fed low-energy diets had higher RW of the gizzard and GIT than pullets fed high-energy diets.

We hypothesized that pullets fed crumbles and high energy diets could show improved BW and feed efficiency but also poorer development of the GIT than pullets fed mash and low-energy diets. The objective of the study was to compare the effects of feed form and energy concentration of the diet and their potential interactions on growth performance and GIT development of brown-egg laying pullets from hatching to 17 wk of age.

2. MATERIALS AND METHODS

2.1. Husbandry, diets, and experimental design

The study 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).

Upon arrival to the study facility, pullets (1-dayold Lohmann Brown Classic) were weighed individually (36.7 ± 2.84 g) and allotted in groups of 50 birds into 60 cages (Facco S.A., Padova, Italy) provided with an open-trough feeder and 2 nipple drinkers. The average BW of the pullets was similar for all cages. Chicks were beak-trimmed at the hatchery and vaccinated against main diseases (Infectious Bronchitis Disease, Infectious Bursal Disease, Newcastle Disease, and *Salmonella* spp.) according to accepted commercial practices in Spain (Lohmann, 2013). Because of the dimensions ($80 \times 68 \times 40$ cm) of the cages, only 22 pullets chosen at random formed the study unit after 3 wk of age. The environmental conditions of the room were controlled automatically according to the age of the birds. The light program consisted on 22 h/d for the first wk of life and then, light was decreased 2 h/wk until reaching 12 h at 7 wk of age. From 7 to 17 wk of age, the light program was kept constant. Room temperature was maintained at $32 \pm 1.5^{\circ}\text{C}$ for the first 3 d of life and then, it was reduced gradually until reaching 21°C at 5 wk of age. Pullets had free access to feed and water throughout the trial.

The feeding program consisted in 3 types of diet that were supplied from 0 to 5, 5 to 10, and 10 to 17 wk of age. Within each feeding period, 5 diets [very low energy (VLE), low energy (LE), medium energy (ME), high energy (HE), and very high energy (VHE)] that differed in 50 kcal AMEn/kg but with similar indispensable amino acids

and nutrient content per unit energy, were manufactured. The AMEn of the diets varied from 2,850 to 3,050 kcal/kg from 0 to 5 wk, 2,700 to 2,900 kcal/kg from 5 to 10 wk, and 2,600 to 2,800 kcal/kg from 10 to 17 wk. For the manufacturing of the diets all the ingredients were ground with a hammer mill (Model MRA 220, Rosal S.A., 08130, Barcelona, Spain) fitted with a 2-mm (diets fed from 0 to 5 wk) or a 3-mm (diets fed from 5 to 17 wk of age) screen. All diets contained a commercial enzyme complex with xylanase and β -glucanase activities (Roxazyme, DSM S.A., Madrid, Spain). In the formulation of the diets it was assumed that the inclusion of the enzyme complex increased by 2% the AMEn content of the wheat (from 3,150 to 3,213 kcal/kg) and barley (from 2,800 to 2,856 kcal/kg) but had no effects on the energy content of the other ingredients (FEDNA, 2010). Within each feeding period, the 2 summit diets (VLE and VHE) were formulated according to the recommendations of FEDNA (2008) and the intermediate feeds resulted from the mixing in adequate portions of the 2 summit diets. Then, all feed batches (3 feeding periods and 5 diets/period) were divided into 2 portions; the first portion was used as such and the second portion was steam-conditioned at 72°C for 60 s, passed through a pellet press (Model PVR 180 2T, Mabrik, Barcelona, Spain) provided with a 60-mm thick die and a 3-mm screen, and crumbled. The ingredient composition and the calculated nutrient content (FEDNA, 2010) of the study diets used in the 3 feeding periods are shown in Tables 1 and 2, respectively.

The study design was completely randomized with 10 treatments in a factorial arrangement with 2 feed forms (crumbles vs. mash) and 5 energy levels (VLE, LE, ME, HE, and VHE). Each treatment was replicated 6 times and the study unit was the cage for all measurements.

2.2. Laboratory analysis

Representative samples of the diets were ground in 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 using a muffle furnace (Method 942.05), crude fiber by sequential extraction with diluted acid and alkali (Method 962.09), 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). Also, the dietary fiber content of the sunflower meal and soybean meal was determined (Megazyme International, Bray, Ireland) according to Method 991.43 of the AOAC International (2005). Gross energy of the diets was determined with an adiabatic bomb calorimeter (Model 1356, Parr Instrument Company, Moline, IL). The amino acid content of the 2 extreme diets (VLE and VHE) of each of the 3 feeding periods was determined by ion-exchange chromatography (Hewlett-Packard 1100, Waldbronn, Germany) after acid hydrolysis, as indicated by De Coca-Sinova et al. (2008). Tryptophan was not determined. The chemical analyses of the study diets of the 3 feeding periods (average of the 2 feed forms) are shown in Table 3.

Table 1. Ingredient composition (% as fed basis) of the experimental diets¹

	0 to 5 wk of age					5 to 10 wk of age					10 to 17 wk of age				
	VLE	LE	ME	HE	VHE	VLE	LE	ME	HE	VHE	VLE	LE	ME	HE	VHE
Corn	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	20.0	20.0	20.0	20.0	20.0
Wheat	19.2	18.3	17.4	16.6	15.7	-	2.5	5.0	7.5	10.0	31.5	33.6	35.7	37.8	40.0
Barley	-	-	-	-	-	28.0	25.6	23.3	20.9	18.5	10.0	10.1	10.3	10.4	10.5
Soybean meal, 45.5% CP	33.3	35.0	36.7	38.4	40.1	22.0	24.3	26.5	28.8	31.0	9.0	11.0	13.0	15.0	17.0
Sunflower meal, 28% CP	6.0	4.5	3.0	1.5	-	-	-	-	-	-	15.0	13.3	11.5	9.8	8.0
Sunflower meal, 32% CP	-	-	-	-	-	10.0	7.5	5.0	2.5	-	-	-	-	-	-
Bran	-	-	-	-	-	-	-	-	-	-	10.0	7.5	5.0	2.5	-
Soy oil	2.71	3.39	4.08	4.69	5.37	1.0	1.25	1.50	1.75	2.0	0.85	0.94	1.03	1.11	1.20
Dicalcium phosphate	2.07	2.07	2.07	2.06	2.06	1.53	1.55	1.57	1.58	1.60	1.50	1.51	1.51	1.52	1.53
Calcium carbonate	1.04	1.05	1.05	1.05	1.06	1.64	1.52	1.40	1.28	1.16	1.44	1.37	1.29	1.23	1.16
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.33	0.35	0.35	0.35	0.35	0.35
DL-methionine, 99%	0.13	0.14	0.15	0.15	0.16	0.11	0.12	0.13	0.14	0.15	0.05	0.05	0.06	0.06	0.06
<i>L</i> -threonine, 98%						0.08	0.08	0.07	0.07	0.06					
<i>L</i> -lysine·HCl, 78%						0.09	0.07	0.05	0.02	-	0.11	0.08	0.06	0.03	-
Vitamin-trace mineral premix ²	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

¹The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

²Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,500 IU; vitamin E (all-*rac*-tocopherol-acetate), 35 mg; vitamin B₁, 2 mg; vitamin B₂, 8 mg; vitamin B₆, 4 mg; vitamin B₁₂ (cyanocobalamin), 0.025 mg; vitamin K₃ (bisulphate menadione complex), 3mg; choline (choline chloride), 270 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; D-biotin, 0.15 mg; zinc (ZnO), 90 mg; manganese (MnO), 75 mg iron (FeCO₃), 60 mg; copper (CuSO₄ · 5H₂O), 8 mg; iodine (KI), 2 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; and Natuphos 5000 [300 FTU/kg 6-phytase (EC 3.1.3.26), 60 mg, supplied by Basf Española S.A, Tarragona, Spain].

Table 2. Calculated analyses¹ (% as fed basis) of the experimental diets²

	0 to 5 wk of age					5 to 10 wk of age					10 to 17 wk of age				
	VLE	LE	ME	HE	VHE	VLE	LE	ME	HE	VHE	VLE	LE	ME	HE	VHE
AME _n (kcal/kg)	2,850	2,900	2,950	3,000	3,050	2,700	2,750	2,800	2,850	2,900	2,600	2,650	2,700	2,750	2,800
Ether extract	5.1	5.7	6.4	7.0	7.7	3.4	3.7	3.9	4.2	4.4	3.1	3.1	3.2	3.3	3.3
Crude fiber	4.5	4.2	3.9	3.6	3.3	5.4	4.9	4.4	3.9	3.5	7.1	6.6	6.0	5.5	5.0
Dietary fiber	17.6	17.2	16.8	16.4	16.0	19.4	18.8	18.2	17.5	16.9	22.1	21.1	20.0	19.0	17.9
Crude protein	21.7	22.0	22.2	22.5	22.7	19.0	19.2	19.4	19.7	19.9	16.1	16.4	16.7	16.9	17.2
Digestible amino acid															
Arg	1.32	1.34	1.35	1.37	1.38	1.13	1.14	1.15	1.16	1.17	0.92	0.94	0.95	0.97	0.98
Ile	0.80	0.82	0.83	0.84	0.85	0.68	0.69	0.70	0.71	0.73	0.53	0.55	0.57	0.58	0.60
Lys	0.97	0.99	1.02	1.05	1.08	0.84	0.86	0.87	0.88	0.90	0.61	0.62	0.63	0.64	0.65
Met	0.43	0.44	0.44	0.45	0.46	0.39	0.39	0.40	0.41	0.41	0.29	0.30	0.30	0.31	0.31
Met + Cys	0.72	0.73	0.74	0.75	0.76	0.65	0.66	0.67	0.68	0.69	0.53	0.54	0.55	0.55	0.56
Thr	0.68	0.70	0.71	0.72	0.73	0.67	0.67	0.68	0.68	0.68	0.45	0.46	0.48	0.49	0.50
Trp	0.23	0.23	0.23	0.24	0.24	0.19	0.20	0.20	0.20	0.21	0.16	0.17	0.17	0.18	0.18
Val	0.89	0.90	0.91	0.92	0.93	0.78	0.79	0.80	0.81	0.82	0.63	0.64	0.65	0.67	0.68
Calcium	1.10	1.10	1.10	1.10	1.10	1.03	0.98	0.94	0.89	0.85	1.07	1.05	1.02	1.00	0.97
Digestible phosphorus	0.44	0.43	0.43	0.43	0.43	0.43	0.44	0.44	0.44	0.44	0.37	0.36	0.36	0.35	0.35

¹According to FEDNA (2010).²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

Table 3. Determined analyses¹ (% as fed basis) of the experimental diets²

	0 to 5 wk of age					5 to 10 wk of age					10 to 17 wk of age				
	VLE	LE	ME	HE	VHE	VLE	LE	ME	HE	VHE	VLE	LE	ME	HE	VHE
Dry matter	91.1	91.0	90.7	90.0	90.9	90.4	90.6	91.1	91.5	91.9	91.4	91.3	91.7	91.4	91.3
GE (kcal/kg)	4,160	4,175	4,181	4,230	4,286	3,962	3,993	3,997	3,993	4,054	4,016	4,023	3,958	4,054	4,001
Ether extract	5.6	6.1	6.4	7.2	7.5	3.5	3.8	4.2	3.9	4.6	3.0	3.2	3.0	3.3	3.1
Crude fiber	4.1	3.8	3.8	3.6	3.2	6.2	6.5	5.9	5.9	5.4	6.6	6.2	5.8	5.9	5.2
Crude protein	21.3	21.8	22.5	22.6	23.5	18.3	18.1	18.2	19.0	18.6	16.1	15.9	16.4	17.5	16.4
Arg	1.44	- ³	-	-	1.54	1.25	- ³	-	-	1.30	1.03	- ³	-	-	1.09
Ile	0.91	-	-	-	1.00	0.77	-	-	-	0.84	0.62	-	-	-	0.69
Lys	1.13	-	-	-	1.21	0.99	-	-	-	1.05	0.69	-	-	-	0.76
Met	0.46	-	-	-	0.52	0.42	-	-	-	0.46	0.31	-	-	-	0.34
Met + Cys	0.84	-	-	-	0.90	0.73	-	-	-	0.79	0.64	-	-	-	0.65
Thr	0.83	-	-	-	0.87	0.77	-	-	-	0.81	0.52	-	-	-	0.60
Val	1.00	-	-	-	1.07	0.92	-	-	-	0.95	0.74	-	-	-	0.80
Total ash	5.9	6.7	6.6	6.4	5.9	5.0	5.5	5.8	6.9	6.2	5.7	6.0	6.0	4.6	5.3

¹Data correspond to the average of the mash and crumble diets. The differences in values between the mash and crumble diets were within acceptable ranges

²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³Not determined.

Particle size distribution and mean particle size of the mash and crumble diets, expressed as geometric mean diameter (**GMD**), were determined by dried sieving as outlined by ASAE (1995) in 100-g samples using a shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 μm (Table 4). All the analyses were conducted in duplicate, except for the GMD of the diets that were determined in triplicate.

2.3. Measurements

Individual BW of the pullets and feed disappearance were recorded by replicate at 5, 10, and 17 wk of age. Any mortality was recorded and weighed as produced. From these data, ADFI, ADG, FCR, EI (kcal AMEn/d), energy conversion ratio (**ECR**; kcal AMEn/g ADG), and BW uniformity were determined by period and cumulatively. BW uniformity was determined by replicate as indicated by Peak et al. (2000). Briefly, the CV of the individual BW of the pullets of each cage was generated and this variable was used as an indirect measurement of BW uniformity.

For the determination of GIT traits, birds were fasted for 60 min after the corresponding growth performance control and then fed ad libitum for 3 h to maximize FI and digesta content. Then, 2 birds/replicate were randomly selected, weighed, and euthanized by CO₂ inhalation. The GIT (from the post-crop esophagus to the cloaca, including digesta content, liver, pancreas, and spleen) was removed aseptically and weighed. After GIT removal, the pullets were weighed again and carcass yield (% BW, including the head, neck, and feet) was calculated. Then, the liver and the full proventriculus and gizzard were carefully excised and weighed. The pH of the gizzard contents was measured in duplicate in situ 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 average value of the 2 measurements was used for

further analysis. Afterwards, the proventriculus and gizzard were emptied from any digesta, cleaned, dried with desiccant paper, and weighed again. The fresh digesta content of the 2 organs was calculated as the difference between the full and empty organ weight and expressed relative (i.e., percent) to full organ weight. In addition, the length of the SI (duodenum, jejunum, and ileum) from the gizzard to ileocecal junction, and of the 2 ceca (from the ostium to the tip of the ceca) were measured on a glass surface using a flexible tape with a precision of 1 mm, and expressed relative to full BW (RL, cm/kg BW). Body length of the pullet, from the tip of the beak to the end of longest phalanx, was also measured in extended birds. Finally, the length and diameter (in the middle point of the bone) of the tarsus were measured using a digital caliper, and expressed as cm/kg BW.

Table 4. Particle size distribution and geometric mean diameter (GMD, μm) of the experimental diets¹

	VLE		LE		ME		HE		VHE	
	Mash	Crumble	Mash	Crumble	Mash	Crumble	Mash	Crumble	Mash	Crumble
From 0 to 5 wk of age										
Sieve diameter ² (μm)										
2,500	12.0	9.3	9.7	10.0	11.3	6.9	10.9	4.6	10.3	4.8
1,250	27.3	65.4	30.0	57.8	28.7	54.8	27.9	50.6	26.0	50.1
630	31.7	19.3	32.7	20.9	33.2	24.3	33.6	28.7	35.4	28.6
315	18.4	4.0	17.7	8.2	17.4	9.9	18.0	11.2	17.7	11.3
160	10.2	2.2	9.6	2.8	8.6	3.8	8.9	4.5	10.8	4.8
GMD $\pm$ GSD ³	950 $\pm$ 2.2	1,519 $\pm$ 1.7	951 $\pm$ 2.1	1,382 $\pm$ 1.9	957 $\pm$ 2.1	1,250 $\pm$ 1.8	938 $\pm$ 2.1	1,158 $\pm$ 1.9	952 $\pm$ 2.2	1,157 $\pm$ 1.9
From 5 to 10 wk of age										
Sieve diameter (μm)										
2,500	7.1	21.1	4.6	14.8	10.7	13.8	11.3	13.9	15.2	11.6
1,250	47.4	61.6	50.6	57.2	49.9	57.7	50.5	62.3	35.1	58.3
630	27.9	13.6	28.7	19.6	24.2	19.5	23.4	17.4	27.3	20.4
315	12.0	2.6	11.2	5.9	9.8	6.0	9.4	4.3	12.4	6.5
160	5.7	1.0	4.5	2.2	5.2	2.6	5.1	1.9	9.4	3.0
GMD $\pm$ GSD	1,172 $\pm$ 2.0	1,760 $\pm$ 1.7	1,158 $\pm$ 1.9	1,496 $\pm$ 1.8	1,267 $\pm$ 2.0	1,475 $\pm$ 1.8	1,294 $\pm$ 2.0	1,563 $\pm$ 1.7	1,112 $\pm$ 2.3	1,424 $\pm$ 1.84
From 10 to 17 wk of age										
Sieve diameter (μm)										
2,500	7.7	35.4	4.6	28.9	8.5	31.1	11.2	28.4	14.8	17.2
1,250	44.4	52.1	50.6	52.4	48.2	54.8	50.5	58.2	37.2	63.4
630	27.8	10.2	28.7	11.7	25.5	10.2	23.4	9.5	24.8	14.1
315	13.1	1.6	11.2	4.4	11.6	2.3	9.9	2.4	12.7	3.6
160	6.7	0.6	4.5	2.3	6.0	1.5	4.9	1.2	9.9	1.4
GMD $\pm$ GSD	1,127 $\pm$ 2.1	2,027 $\pm$ 1.7	1,158 $\pm$ 1.9	1,772 $\pm$ 1.9	1,193 $\pm$ 2.0	1,913 $\pm$ 1.7	1,302 $\pm$ 2.0	1,885 $\pm$ 1.7	1,112 $\pm$ 2.3	1,660 $\pm$ 1.7

¹The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; VHE, and very high energy).

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

³GMD = Geometric mean diameter; GSD = Log normal SD.

2.4. Statistical analysis

Data on growth performance, BW uniformity, GIT traits, and body measurements were analyzed as a completely randomized design with feed form and energy concentration of the diet as main effects using the GLM procedure of SAS (SAS Institute, 2004). In addition, treatment sum of squares for the level of energy was partitioned into linear (L) and quadratic (Q) effects. When significant differences among treatments were observed, means were separated using the Tukey test. All differences were considered significant at $P < 0.05$.

3. RESULTS

The physico-chemical characteristics of the study diets of the 3 feeding periods are shown in Tables 1-3, and their GMD is shown in Table 4. The GMD of the mash diets were not affected by the energy content of the feed but that of the crumble diets decreased as the energy content increased. The determined dietary fiber content of the sunflower meal and the soybean meal used were 46.8 and 26.2%, respectively.

3.1. Growth performance and body weight uniformity

Mortality was 2.7% and was not related to treatment (data not shown). Most of the mortality (2.6%) occurred during the first week life.

3.1.1. Feed form

From 0 to 17 wk of age, pullets fed crumbles had greater ADFI (52.9 vs. 49.7 g; $P < 0.001$) and ADG (12.7 vs. 11.6 g; $P < 0.001$) and better FCR (4.18 vs. 4.27; $P < 0.001$) than pullets fed mash (Table 5). Consequently, EI was greater (146 vs. 137 kcal AMEn/d; $P < 0.001$) and ECR was better (11.3 vs. 11.7 kcal AMEn/g; $P < 0.001$) with crumbles (Table 6). Similar results were observed in each of the 3 feeding periods, except from 0 to 5 wk, a period in which ADFI was lower ($P < 0.01$) in pullets fed crumbles than in pullets fed mash. Crumble feeding improved BW uniformity, consequently, with differences being significant at 10 ($P < 0.001$) and 17 ($P < 0.05$) wk of age (Table 7).

3.1.2. Energy concentration of the diet

From hatching to 17 wk of age, ADFI decreased (L; $P < 0.001$) but ADG (L; $P = 0.054$) and FCR (Q; $P < 0.01$) improved as the energy content of the diet increased. No interactions between feed form and energy concentration of the diet were observed for

ADG in any of the 3 feeding periods considered but some interactions were detected for ADFI and FCR. For example, from 0 to 10 wk of age, ADFI decreased and FCR improved as the energy content of the diet increased in pullets fed crumbles but no differences were detected in pullets fed mash ($P < 0.001$ and $P < 0.01$ for the interactions from 0 to 5 wk and $P < 0.001$ for the interactions from 5 to 10 wk of age for ADFI and FCR, respectively) (Figure 1). Consequently, EI increased as the AMEn of the diet increased in pullets fed mash but not in pullets fed crumbles ($P < 0.001$ and $P < 0.01$ for the interaction from 0 to 5 and 5 to 10 wk of age, respectively). BW uniformity was not affected by the energy content of the diet at any age.

Table 5. Influence of feed form and energy content (kcal AME_n /kg) of the diet on pullet performance from hatchery to 17 weeks of age¹

Treatment	0 to 5 week			5 to 10 week			10 to 17 week			0 to 17 week		
	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR
Feed form												
Mash	21.1 ^a	9.1 ^b	2.31 ^a	51.1 ^b	14.6 ^b	3.50 ^a	69.2 ^b	11.3 ^b	6.11	49.7 ^b	11.6 ^b	4.27 ^a
Crumbles	20.7 ^b	9.7 ^a	2.13 ^b	54.0 ^a	15.8 ^a	3.42 ^b	75.2 ^a	12.5 ^a	6.00	52.9 ^a	12.7 ^a	4.18 ^b
AME _n content ²												
VLE	21.2 ^a	9.4	2.26 ^a	54.2 ^a	14.9	3.63 ^a	75.6 ^a	11.9	6.37 ^a	53.3 ^a	12.0	4.43 ^a
LE	21.0 ^{ab}	9.4	2.24 ^{ab}	53.5 ^a	15.1	3.54 ^a	73.3 ^{ab}	11.9	6.18 ^{ab}	52.1 ^{ab}	12.1	4.31 ^b
ME	21.0 ^{ab}	9.4	2.25 ^a	52.7 ^{ab}	15.4	3.42 ^b	70.8 ^{bc}	11.8	6.00 ^{bc}	50.8 ^{bc}	12.1	4.19 ^c
HE	20.8 ^{ab}	9.5	2.19 ^{bc}	51.7 ^{bc}	15.3	3.37 ^b	71.1 ^{bc}	12.0	5.92 ^c	50.6 ^c	12.2	4.13 ^{cd}
VHE	20.6 ^b	9.5	2.17 ^c	50.8 ^c	15.2	3.34 ^b	70.0 ^c	12.1	5.82 ^c	49.8 ^c	12.2	4.08 ^d
SD ³	0.53	0.26	0.045	1.36	0.62	0.089	2.47	0.64	0.246	1.25	0.31	0.062
<i>P</i> -value												
Feed form	0.009	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.072	<.001	<.001	<.001
AME _n content ⁶												
L	0.002	0.328	<.001	<.001	0.112	<.001	<.001	0.409	<.001	<.001	0.154	<.001
Q	0.454	0.812	0.185	0.624	0.142	0.057	0.054	0.629	0.277	0.180	0.863	0.005

¹Only main effects are shown. When a significant interaction was detected (ADFI and FCR from 0 to 5 wk and from 5 to 10 wk of age) the data are shown in Figure 1.

²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³30 replicates for feed form and 12 replicates for AME_n content of the diet.

⁴L = linear effect of energy content of the diet; Q = quadratic effect of energy content of the diet.

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

Table 6. Influence of feed form and energy content (kcal AME_n/kg) of the diet on energy intake (EI, kcal AME_n/d) and energy conversion ratio² (ECR, kcal AME_n/g) of the pullets from hatchery to 17 weeks of age¹

Treatment	0 to 5 week		5 to 10 week		10 to 17 week		0 to 17 week	
	EI	ECR	EI	ECR	EI	ECR	EI	ECR
Feed form								
Mash	62.3 ^a	6.82 ^a	143 ^b	9.81 ^a	187 ^b	16.5 ^a	137 ^b	11.7 ^a
Crumbles	61.1 ^b	6.29 ^b	151 ^a	9.57 ^b	203 ^a	16.2 ^b	146 ^a	11.3 ^b
AME _n content ³								
VLE	60.4 ^b	6.44 ^a	146	9.81	197	16.6	142	11.6
LE	61.0 ^{ab}	6.48 ^{ab}	147	9.74	194	16.4	141	11.5
ME	62.1 ^{ab}	6.64 ^a	148	9.59	191	16.2	140	11.4
HE	62.3 ^{ab}	6.59 ^{ab}	147	9.62	196	16.3	142	11.5
VHE	62.7 ^a	6.63 ^b	147	9.69	196	16.3	142	11.5
SD ⁴	1.55	0.131	3.79	0.250	6.62	0.662	3.41	0.282
P-value								
Feed form	0.005	<.001	<.001	<.001	<.001	0.075	<.001	<.001
AMEn content ⁵								
L	<.001	<.001	0.537	0.109	0.991	0.237	0.450	0.369
Q	0.411	0.121	0.502	0.089	0.071	0.350	0.263	0.214

¹Only main effects are shown. When a significant interaction was detected (EI and ECR from 0 to 5 wk and from 5 to 10 wk of age) the data are shown in Fig 1.

²Energy conversion ratio was obtained from the calculated AME_n intake and ADG of the pullets shown in Table 6.

³The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

⁴30 replicates for feed form and 12 replicates for AME_n content of the diet.

⁵L = linear effect of energy content of the diet; ⁶Q = quadratic effect of energy content of the diet.

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

Table 7. Influence of feed form and energy content (kcal AME_n /kg) of the diet on BW uniformity¹ of the pullets

Treatment	5 week	10 week	17 week
Feed form			
Mash	8.57	8.61 ^a	9.25 ^a
Crumbles	7.98	7.27 ^b	7.97 ^b
AME _n content ²			
VLE	8.65	7.75	9.00
LE	8.02	7.71	8.12
ME	8.44	8.28	8.57
HE	7.71	7.97	8.43
VHE	8.58	7.99	8.95
SD ³	1.473	1.282	2.111
<i>P</i> -value ⁴			
Feed form	0.123	<.001	0.022
AME _n content ⁵			
L	0.744	0.502	0.921
Q	0.236	0.570	0.330

¹ Evaluated as the CV (%) of the individual BW of the pullets from each pen (Peak et al., 2000).

² The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³ 30 replicates for feed form and 12 replicates for AME_n content of the diet.

⁴ The interactions between feed form and AME_n content of the diet were not significant ($P > 0.05$).

⁵ L = linear effect of energy content of the diet; Q = quadratic effect of energy content of the diet.

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

Figure 1. Interactions between feed form and energy content¹ (kcal AME_n/kg) of the diet on ADFI, FCR, energy intake (EI, kcal AME_n/d), and energy conversion ratio (ECR, kcal AME_n/g ADG) of the pullets from 0 to 5 wk (A) and 5 to 10 wk (B) of age

A.

SEM (n=6) = 0.22; P < 0.001

SEM (n=6) = 0.018; P = 0.003

SEM (n=6) = 0.63; P < 0.001

SEM (n=6) = 0.053; P < 0.001

B.

SEM (n=6) = 0.56; P = 0.006

SEM (n=6) = 0.036; P < 0.001

SEM (n=6) = 1.55; P = 0.009

SEM (n=6) = 0.10; P < 0.001

■ Mash □ Crumbles

¹The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

a-b-c-d-e Means without a common superscript differ significantly.

3.2. Gastrointestinal tract traits and body measurements

3.2.1. Feed form

Feed form affected all GIT and body traits measured. At 5, 10, and 17 wk of age, carcass yield was higher ($P < 0.001$) in pullets fed crumbles than in pullets fed mash (Table 8). Liver weight was greater in pullets fed mash than in pullets fed crumbles at 5 wk of age ($P < 0.01$) but no effects were observed after this age. The fresh content of the proventriculus was greater ($P < 0.001$) in pullets fed crumbles than in pullets fed mash at all ages (Table 9). Also, the RW of the proventriculus was consistently greater ($P < 0.001$) in pullets fed crumbles than in pullets fed mash, but the differences were significant only at 17 wk of age. On the other hand, the full gizzard was lighter ($P < 0.001$) at all ages in pullets fed crumbles (Table 10) whereas gizzard digesta content was higher ($P < 0.05$ at 5 wk, $P = 0.06$ at 10 wk, and $P < 0.001$ at 17 wk of age) and gizzard pH was lower ($P < 0.05$ at 5 wk and $P < 0.001$ at 10 and 17 wk of age) in pullets fed mash. Pullets fed mash had longer SI at 5 wk of age ($P < 0.01$) and longer ceca at all ages ($P < 0.05$) than pullets fed crumbles (Table 11).

Body length ($P < 0.01$) and tarsus length ($P < 0.01$ at 5 wk and $P < 0.001$ at 10 and 17 wk of age) were shorter at all ages in pullets fed crumbles than in pullets fed mash (Table 12). At 10 wk ($P < 0.01$) and 17 wk ($P < 0.05$) age, tarsus diameter was greater in pullets fed mash than in pullets fed crumbles.

3.2.2. Energy concentration of the diet

Energy concentration of the diet did not affect any GIT or body trait at any age.

Table 8. Influence of feed form and energy content (kcal AME_n /kg) of the diet on carcass yield¹ and relative weight of the liver (% BW) of the pullets

Treatment	5 week			10 week			17 week		
	BW (g)	Carcass yield	Liver	BW (g)	Carcass yield	Liver	BW (g)	Carcass yield	Liver
Feed form									
Mash	371	81.7 ^b	3.69 ^a	911	85.4 ^b	2.70	1,496	88.5 ^b	2.10
Crumbles	396	83.3 ^a	3.50 ^b	967	87.1 ^a	2.67	1,606	89.4 ^a	2.14
AME _n content ²									
VLE	372	82.2	3.55	956	86.2	2.66	1,552	88.6	2.18
LE	388	82.6	3.69	938	86.2	2.73	1,549	89.2	2.07
ME	385	82.9	3.58	938	86.2	2.73	1,552	88.9	2.10
HE	384	82.5	3.60	916	86.2	2.70	1,596	88.9	2.05
VHE	386	82.4	3.55	945	86.5	2.65	1,505	89.3	2.20
SD ³		0.83	0.250		1.01	0.204		1.04	0.255
<i>P</i> -value ⁴									
Feed form		<.001	0.006		<.001	0.602		0.001	0.566
AME _n content ⁵									
L		0.525	0.647		0.483	0.873		0.195	0.955
Q		0.060	0.342		0.513	0.247		0.888	0.117

¹Calculated as a percentage of the empty BW (including the head, neck, and feet and without GIT and digesta contents) and BW.

²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³30 and 12 replicates of 2 birds each, for feed form and AME_n content of the diet, respectively.

⁴The interactions between feed form and AME_n content of the diet were not significant ($P > 0.05$).

⁵ L = linear effect of energy content of the diet; Q = quadratic effect of energy content of the diet.

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

Table 9. Influence of feed form and energy content (kcal AME_n /kg) of the diet on the relative weight (RW, % BW¹) and digesta content of the proventriculus (% full organ weight) of the pullets

Treatment	5 week		10 week		17 week	
	RW	Content	RW	Content	RW	Content
Feed form						
Mash	0.79	6.13 ^b	0.58	6.2 ^b	0.54 ^b	6.4 ^b
Crumbles	0.82	13.38 ^a	0.62	12.3 ^a	0.65 ^a	11.4 ^a
AME _n content ²						
VLE	0.77	7.77	0.58	10.1	0.57	10.2
LE	0.80	9.68	0.63	10.6	0.59	7.8
ME	0.80	9.18	0.61	8.3	0.58	8.9
HE	0.82	11.24	0.60	8.3	0.63	8.9
VHE	0.82	10.88	0.60	9.0	0.60	8.7
SD ³	0.090	4.77	0.075	4.58	0.083	4.28
<i>P</i> -value ⁴						
Feed form	0.242	<.001	0.062	<.001	<.001	<.001
AME _n content ⁵						
L	0.133	0.079	0.984	0.348	0.262	0.592
Q	0.719	0.701	0.378	0.767	0.611	0.481

¹The BW of the pullets are shown in Table 9.

²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³30 and 12 replicates of 2 birds each, for feed form and AME_n content of the diet, respectively.

⁴The interactions between feed form and AME_n content of the diet were not significant ($P > 0.05$).

⁵L = linear effect of energy content of the diet; Q = quadratic effect of energy content of the diet.

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

Table 10. Influence of feed form and energy content (kcal AME_n /kg) of the diet on the relative weight (RW; % BW¹), digesta content (% full organ weight), and pH of the gizzard of the pullets

Treatment	5 week			10 week			17 week		
	RW	Content	pH	RW	Content	pH	RW	Content	pH
Feed form									
Mash	5.08 ^a	31.5 ^a	2.97 ^b	4.60 ^a	28.4	2.72 ^b	3.65 ^a	27.1 ^a	3.26 ^b
Crumbles	3.79 ^b	29.1 ^b	3.17 ^a	3.26 ^b	24.5	3.41 ^a	2.38 ^b	12.1 ^b	4.03 ^a
AME _n content ²									
VLE	4.55	30.3	3.19	3.94	26.9	2.94	3.14	22.1	3.57
LE	4.58	32.0	3.05	4.06	28.2	2.98	2.91	18.8	3.75
ME	4.32	31.0	2.95	3.96	26.0	3.19	3.05	19.9	3.61
HE	4.38	30.2	3.09	3.96	28.0	3.05	3.03	18.9	3.67
VHE	4.35	28.1	3.07	3.73	23.2	3.17	2.95	18.5	3.63
SD ³	0.500	4.66	0.356	0.519	7.854	0.402	0.332	5.20	0.357
P-value ⁴									
Feed form	<.001	0.048	0.026	<.001	0.060	<.001	<.001	<.001	<.001
AME _n content ⁵									
L	0.192	0.145	0.619	0.276	0.298	0.152	0.404	0.138	0.917
Q	0.718	0.162	0.231	0.284	0.355	0.708	0.733	0.522	0.548

¹The BW of the pullets are shown in Table 9.

²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³30 and 12 replicates of 2 birds each, for feed form and AME_n content of the diet, respectively.

⁴The interactions between feed form and AME_n content of the diet were not significant ($P > 0.05$).

⁵L = linear effect of energy content of the diet; Q = quadratic effect of energy content of the diet.

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

Table 11. Influence of feed form and energy content (kcal AME_n /kg) of the diet on the relative length (cm/kg BW¹) of the small intestine and ceca of the pullets

Treatment	5 week		10 week		17 week	
	Small intestine	Ceca	Small intestine	Ceca	Small intestine	Ceca
Feed form						
Mash	276 ^a	56.2 ^a	139	31.8 ^a	87.0	20.4 ^a
Crumbles	262 ^b	52.8 ^b	135	29.8 ^b	84.3	19.4 ^b
AME _n content ²						
VLE	269	54.2	133	30.0	86.4	20.1
LE	258	54.2	148	31.5	85.4	19.8
ME	269	54.2	133	31.4	86.1	19.9
HE	273	53.9	135	30.5	85.0	19.8
VHE	275	56.0	136	30.5	85.3	19.9
SD ³	17.8	4.50	15.3	3.41	5.48	1.54
<i>P</i> -value ⁴						
Feed form	0.005	0.005	0.491	0.032	0.065	0.021
AME _n content ⁵						
L	0.110	0.438	0.626	0.977	0.631	0.733
Q	0.339	0.419	0.617	0.324	0.878	0.684

¹The BW of the pullets are shown in Table 9.

²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³30 and 12 replicates of 2 birds each, for feed form and AME_n content of the diet, respectively.

⁴The interactions between feed form and AME_n content of the diet were not significant ($P > 0.05$).

⁵L = linear effect of energy content of the diet; Q = quadratic effect of energy content of the diet.

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

Table 12. Influence of feed form and energy content (kcal AME_n /kg) of the diet on relative body length (cm/kg BW¹) and tarsus length (L) and diameter (D) of the pullets

Treatment	5 week			10 week			17 week		
	Body	Tarsus (L)	Tarsus (D)	Body	Tarsus (L)	Tarsus (D)	Body	Tarsus (L)	Tarsus (D)
Feed form									
Mash	109 ^a	17.5 ^a	1.75	63.8 ^a	7.85 ^a	0.91 ^a	44.8 ^a	5.20 ^a	0.67 ^a
Crumbles	103 ^b	16.8 ^b	1.75	61.3 ^b	7.53 ^b	0.87 ^b	41.6 ^b	4.75 ^b	0.63 ^b
AME _n content ²									
VLE	108	17.4	1.77	62.1	7.59	0.88	43.1	4.96	0.64
LE	105	17.1	1.77	62.6	7.70	0.90	43.2	5.10	0.66
ME	105	17.0	1.75	62.1	7.73	0.88	43.0	4.87	0.63
HE	106	17.2	1.74	63.8	7.83	0.91	41.7	4.87	0.64
VHE	104	17.1	1.72	62.0	7.60	0.88	45.0	5.08	0.67
SD ³	5.64	0.85	0.105	3.14	0.372	0.044	3.07	0.392	0.081
P-value ⁴									
Feed form	<.001	0.005	0.225	0.003	0.001	0.009	<.001	<.001	0.035
AME _n content ⁵									
L	0.164	0.586	0.666	0.741	0.679	0.837	0.430	0.853	0.789
Q	0.637	0.354	0.827	0.482	0.107	0.446	0.115	0.349	0.509

¹The BW of the pullets are shown in Table 9.

²The AME_n content varied in 50 kcal/kg between diets (VLE, very low energy; LE, low energy; ME, medium energy; HE, high energy; and VHE, very high energy).

³30 and 12 replicates of 2 birds each, for feed form and AME_n content of the diet, respectively.

⁴The interactions between feed form and AME_n content of the diet were not significant ($P > 0.05$).

⁵L = linear effect of energy content of the diet; Q = quadratic effect of energy content of the diet.

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

4. DISCUSSION

The GMD of the starter diets (0 to 5 wk) was lower than that of the 5 to 10 and 10 to 17 wk of age diets, consistent with the reduced size of the screen used for grinding the pre-starter diets. An increase in energy content did not affect the GMD of the mash diets but decreased that of the crumble diets. The ingredient composition of the diets varied as the energy concentration of the diet increased. In this respect, the high energy diets contained more soy oil than the low-energy diets, and an increase in fat content may result in a loss of pellet quality, with higher proportion of fines and reduced GMD (Briggs et al., 1999). The dietary fiber content of the sunflower meal and soybean meal batches used was similar to values reported by Bach Knudsen (1997).

4.1. Growth performance and body weight uniformity

4.1.1. Feed form

From hatching to 17 wk of age, pullets fed crumbles had greater ADFI and ADG and better FCR than pullets fed mash, consistent with most research published in broilers (Serrano et al., 2012; Abdollahi et al., 2013a; Jiménez-Moreno et al., 2016) and pullets (Frikha et al., 2009b; Guzmán et al., 2015a). Poultry require more time and energy for compacting feed particles when the diets are presented as mash than when presented as crumbles (Jensen et al., 1962; Savory and Hetherington, 1997). Consequently, pullets fed mash might consume less feed than pullets fed crumbles. In addition, the heat and pressure applied during the pelleting process might modify the structure of the starch and the protein fraction of the feed (Gracia et al., 2009; Zimonja and Svihus, 2009), increasing energy (Jiménez-Moreno et al., 2009b) and CP (Lacassagne et al., 1988) digestibility. Moreover, fine grinding and pelleting disrupt the outer coat of the seed and fracture the endosperm of the grain, releasing the intracellular

oil contained in the spherosomes of the corn and facilitating the access of digestive enzymes to nutrients (Calet, 1965; Mateos et al., 2002). On the other hand, crumbling reduces the particle size of the feed (Guillou and Landeau, 2000; Amerah et al., 2008a) and a reduction in size increases rate of feed passage through the upper part of the GIT and increase voluntary FI (Hamm and Stephenson, 1959; Mateos et al., 2012).

From hatching to 5 wk of age, pullets fed crumbles ate less feed than those fed mash whereas an opposite effect was observed from 5 to 17 wk of age. However, ADG was greater and FCR was better in pullets fed crumble for all periods. Guzmán et al. (2015a) reported also higher ADG but lower ADFI in pullets from 0 to 5 wk of age when fed crumbles than when fed mash. Probably, during the first period of life feed wastage was higher with mash than with crumbles but the differences disappeared with age. In this respect, Serrano et al. (2013) reported that the differences in feed wastage between crumbles and mash tended to decrease with age, consistent with the results of the current study.

BW uniformity was better in pullets fed crumbles than in pullets fed mash at all ages, with differences being significant at 10 and 17 wk of age. These results agree with data of Guzmán et al. (2015a) who reported a trend for better BW uniformity with crumbles at 5 wk of age. Similarly, Brickett et al. (2007) reported an improvement in BW uniformity of 35-day-old broilers with pellet feeding.

4.1.2. Energy concentration of the diet

Energy concentration of the diet affected pullet performance in different ways. From hatching to 17 wk of age, ADFI decreased with increases in energy content of the diet but EI was not affected. Birds have the ability to regulate voluntary FI to satisfy their energy requirements and therefore, ADFI decreases and FCR improves as the energy concentration of the diet increases. In the current study, the decrease in FI and the improvement in FCR with increases in dietary energy concentration were evident from 0 to 5 and 5 to 10 wk of age in pullets fed crumbles but not in pullets fed mash. In both feeding periods, EI increased as the energy content of the mash diets increased but no changes occurred in pullets fed crumbles. The differences observed between feed forms with increases in dietary energy content, might have been due to higher wastage with mash feeding (Guzmán et al., 2015a). An increase in feed wastage with the mash diets might have counteracted the beneficial effect of an increase in dietary energy on EI. Probably, young pullets have less ability to increase voluntary FI when low-energy, bulkier diets are fed in mash form, as has been shown in broilers by Scott (2002) and Brickett et al. (2007). After 10 wk of age, however, the GIT of the pullets is well-developed with sufficient capacity to increase voluntary FI when fed low-energy diets.

BW uniformity was not affected by energy concentration of the diet at any age, in agreement with data in broilers (Brickett et al., 2007) and pullets (Frikha et al., 2009a; Guzmán et al., 2015a). The data indicate that when the main objective is to increase flock uniformity, pullets respond better to changes in feed form than to increases in energy content of the diet.

4.2. Gastrointestinal tract traits and body measurements

4.2.1. Feed form

Diet structure has a strong influence on the anatomy and physiology of the GIT of broilers (Mateos et al., 2012) and pullets (Frikha et al., 2009b). At 17 wk of age, pullets fed crumbles had lighter gizzards and GIT and heavier proventriculi than pullets fed mash, consistent with data of Choi et al. (1986) and Nir et al. (1994a, b) in broilers. Jiménez-Moreno et al. (2010) and Abdollahi et al. (2011) reported that a decrease in particle size, as occurred when the diets were pelleted, led to faster rate of passage, with the digesta remaining for less time in the gizzard, which in turn reduced the development of the muscular layers of this organ. The RW of the full proventriculus was higher in pullets fed crumbles than in pullets fed mash, consistent with the increase in digesta content observed. As a result, carcass yield was higher in pullets fed crumbles than in pullets fed mash.

The RW of the empty proventriculus was higher in pullets fed crumbles than in pullets fed mash (data not shown), suggesting that crumbles cause a hypertrophy of this organ (Mateos et al., 2012). In this respect, Svihus (2011) indicated that an excess of digesta might result in higher incidence of ruptures of the walls of the proventriculus at the slaughterhouse and an increase in contamination of the carcasses. Crumbles reduced gizzard digesta content at all ages and gizzard pH, results that agree with data of Engberg et al. (2002) and Serrano et al. (2013) in broilers and Frikha et al. (2009b) in pullets. In contrast, Dahlke et al. (2003) did not observe any effect of pelleting on gizzard pH in 42-day-old broilers.

Feeding crumbles decreased the RL of the SI and ceca at all ages, consistent with data of Frikha et al. (2009b) in 6-week-old pullets. In broilers, Nir et al. (1994a)

reported that pelleting reduced the RL of the jejunum and ileum, in agreement with the results of the current research. Amerah et al. (2007a) suggested that a decrease in the RL of the GIT was positively correlated with the growth of the chicks, consistent with the results reported herein.

Body length and tarsus length have been used as indirect measurements to predict chick growth (Senar and Pascual, 1997; Mendes et al., 2008). In the current study, pullets fed mash were longer and had longer and wider tarsus than pullets fed crumbles. The authors have not found any research on the effects of feed form on these variables in pullets to compare with the results reported herein. Zuidhof (2005) indicated that because of differences in the allometric growth of tissues and body organs, carcass yield increases as the BW of the bird increases. Interestingly, and in contrast with the relative values reported, absolute values for body length and tarsus length were higher for pullets fed crumbles than for pullets fed mash (data not shown). Consequently, data on weight and length of the different organs of the bird, as a consequence of feeding crumbles or mash, must be taken with caution.

4.2.2. Energy concentration of the diet

The RW of the liver was not affected by the energy content of the diet at any age. In contrast, Scott (2002) reported lighter livers in 34-day-old broilers as the AMEn content of the diet increased. The reason for the discrepancy is not known but differences in diet composition (level of fat vs. level of starch and fiber) and on the capacity for FI between pullets and broilers, might explain the lack of agreement among researches.

Energy concentration of the diet did not affect the RW of the full proventriculus or gizzard at any age, in agreement with data of Keshavarz (1998). In contrast, Frikha et

al. (2009a) observed that an increase in the AMEn concentration of the diet from 2,880 to 3,025 kcal/kg reduced gizzard weight of the pullets at 45 d of age. High-energy diets contain less fiber than low-energy diets and a reduction in fiber content might reduce digestive organ size (Hetland and Svihus, 2001). In the current study, however, the crude fiber content of the lowest- and highest-energy diets did not vary much (i.e., from 4.1 to 3.2% crude fiber from 0 to 5 wk of age). Probably, the differences in insoluble fiber content among diets were too small to produce any marked difference in digestive organ size of the pullets.

In summary, feeding crumbles to pullets increased ADFI and ADG and improved FCR and BW uniformity from 0 to 17 wk of age but reduced the development and relative weight of the different organs of the GIT, including the gizzard. An increase in the energy content of the diet decreased ADFI and improved FCR but did not affect BW uniformity or GIT development at any age. Crumbles might be preferred to mash feeds when the main objective is to improve growth and BW uniformity of the pullets. Crumble feeding, however, might reduce FI early in the production cycle, because of a poor development of the GIT, reducing egg weight during the laying phase. On the other hand, the recommended energy content of the pullet diets will depend on feed form and the relative cost of available ingredients.

**CHAPTER 3: 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**

1. INTRODUCTION

Dent corn (*Zea mays* L.) and soft wheat (*Triticum aestivum* L.) are the most common cereals used in poultry diets. Because of differences in growing and storage conditions, and NSP content, the nutritive value of wheat is more variable than that of corn (Kim et al., 1976; Mollah et al., 1983; Gutiérrez-Álamo et al., 2008) and consequently its use in diets of young birds is limited (FEDNA, 2010). In addition, corn has on average less CP (7.7 vs. 10.2%) but more energy (3,260 vs. 3,100 kcal AMEn/kg) than wheat (FEDNA, 2010). Several researches have compared diets based on corn or wheat in broilers (Crouch et al., 1997; Mathlouthi et al., 2002b; Masey-O'Neill et al., 2014), pullets (Frikha et al., 2009b, 2011), and laying hens (Lázaro et al., 2003a; Safaa et al., 2009; Pérez-Bonilla et al., 2011). In most cases, the authors concluded that wheat can be used in substitution of corn without any effect on performance, provided that the feed is supplemented with NSP enzymes. However, Frikha et al. (2009b) reported a 1.5% reduction in ADG in pullets fed wheat with NSP enzymes as compared with pullets fed corn from 0 to 17 wk of age, although no differences in FCR were detected. Also, these authors observed that at 6 wk of age the gizzard was lighter in pullets fed wheat than in pullets fed corn.

Hens that are light at the initiation of the laying period produce less eggs that are smaller during the whole cycle than hens that are heavy (Leeson et al., 1997; Pérez-Bonilla et al., 2012a,b). The use of crumbles during the rearing phase might be a sound nutritional strategy for improving BW of the hens at the onset of egg production (Frikha et al., 2009a; Saldaña et al., 2015a). Under commercial conditions, broilers are frequently fed crumbles for the first 2 to 3 wk of life and then pellets until slaughter, a practice that results usually in improved ADG and FCR (Amerah et al., 2007b; Abdollahi et al., 2013a; Serrano et al., 2013). Similar results, although of less

magnitude, have been reported in pullets (Guzmán et al., 2015a; Saldaña et al., 2015a). Frikha et al. (2009a) observed that ADFI and ADG were greater in pullets fed pellets from 0 to 6 wk and then mash to 17 wk of age, than in pullets fed mash continuously from hatch to 17 wk of age. Serrano et al. (2012) showed that some of the benefits of feeding crumbles to broilers during the prestarter phase disappeared when the broilers were fed a pelleted diet until slaughter. The authors have not found any report on the effects of the main cereal of the diet and the duration of feeding crumbles, on productive performance and gastrointestinal tract development of brown-egg pullets during the entire rearing period.

We hypothesize that pullets could respond similarly, independent of feed form, to the feeding of diets based on corn or wheat supplemented with adequate NSP enzymes. Also, pullets would quickly adapt their gastrointestinal tract and modify productive performance accordingly, when switched from crumbles to mash feeds. The aim of this research was to evaluate the effects of feeding crumbles for different periods of time, followed by feeding mash to 17 wk of age, on performance, gastrointestinal tract development, and body measurements of brown-egg laying pullets fed diets based on corn or wheat.

2. MATERIALS AND METHODS

2.1. Husbandry

The experimental procedures used in this research 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).

In total, 3,600 one-day-old Lohmann Brown Classic pullets were used in this experiment. On arrival at the experimental farm, pullets were weighed individually and distributed at random into groups of 50 into 72 cages. Because of the dimensions (80 cm × 68 cm × 40 cm) and equipment of the cages, 27 to 28 pullets of each cage (depending on previous mortality) chosen at random, were discarded at 3 wk of age. Consequently, only 22 pullets formed the experimental unit after 3 wk of age. Pullets were beak-trimmed at 8 d of age, vaccinated against the main diseases (Infectious Bronchitis Disease, Marek Disease, Infectious Bursal Disease, Newcastle Disease, and *Salmonella* spp.), and managed according to accepted commercial practices (Lohmann, 2013). The environmental conditions during the experiment were controlled automatically according to the age of the birds. Room temperature was maintained at 32°C during the first 3 d of life and then was reduced gradually until reaching 24°C at 6 wk of age. The light program consisted of 24 h of light for the first week, and then light was decreased 2 h per week until reaching 12 h at 7 wk of age. From 7 wk of age to the end of the experiment, pullets were exposed to a constant light period of 12 h.

2.2. Feeding program, diets, and experiment design

The feeding program consisted of 3 diets supplied from 0 to 5 wk, 5 to 10 wk, and 10 to 17 wk of age. Within each period, the diets had similar nutrient content (FEDNA, 2010) and met or exceeded the nutritional recommendations of FEDNA (2008) for pullets (Table 1). All diets were supplemented with a commercial enzyme complex with xylanase and β -glucanase activity (Roxazyme, DSM S.A., Madrid, Spain). In the formulation of the diets, it was assumed that the inclusion of the NSP enzyme complex increased the energy content of the wheat by 2% (from 3,100 to 3,162 kcal AMEn/kg) but that the energy content of all the other ingredients were not affected (FEDNA, 2010). For the manufacturing of the diets, the cereals were ground to pass through a 4 or 5 mm screen for feeds used from 0 to 5 wk and from 5 to 17 wk of age, respectively. Within each of the 3 feeding periods, feed batches were divided into 2 portions; the first portion was used as it was, and the second portion was steam-conditioned at 72°C for 60 s, pelleted using a 3 mm screen (Model PVR 180 2T, Mabrik, Barcelona, Spain), and crumbled.

The experimental design was completely randomized with 8 treatments arranged as a 2×4 factorial with 2 cereals (corn vs. wheat) and 4 feeding programs that consisted in feeding crumbles or mash continuously from hatch to 17 wk of age, or changing the feed form from crumbles to mash at 5 or 10 wk of age.

2.3. Laboratory analyses

Representative samples of the diets were ground in a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) fitted with a 1 mm screen and analyzed for moisture by oven-drying (method 930.01), ash by a muffle furnace (method 942.05), and nitrogen by combustion (method 990.03) using Leco equipment (model FP- 528, Leco Corporation,

St. Joseph, MI) as described by AOAC International (2005). Gross energy was measured using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL). Ether extract was analyzed after 3 N HCl acid hydrolysis (method 159 Am 5–04) as indicated by AOCS (2004) using an Ankom XT10 Extraction system 160 (Ankom Technology Corp. Macedon, NY). Calcium and P were determined by spectrophotometry (methods 968.08 and 965.17) as described by AOAC International (2005). Particle size distribution and the GMD of the mash and crumble diets were determined in 3 subsamples of 100 g each using a shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 µm, as indicated by ASAE (1995). All of the analyses were conducted in duplicate, except for the GMD of the diets which were determined in triplicate. The determined chemical analysis and the GMD of the experimental diets are shown in Table 2.

2.4. Growth performance

Individual BW of the pullets and feed disappearance per replicate were measured at 5, 10, and 17 wk of age, and mortality was recorded and weighed as produced. These data were used to calculate ADG, ADFI, FCR, and BW uniformity by period, and for the entire experiment. Body weight uniformity was determined as indicated by Peak et al. (2000). Briefly, the CV of the individual BW within each cage was generated and this variable was used to estimate BW uniformity of the pullets at each particular age.

Table 1. Ingredient composition and calculated nutritive value of the experimental diets
(% as fed basis)

Item	1-5 wk		5-10 wk		10-17 wk	
	Corn	Wheat	Corn	Wheat	Corn	Wheat
Ingredient						
Dent corn	40.0	-	40.0	-	40.0	-
Soft wheat	-	40.0	-	40.0	-	40.0
Soybean meal (46% CP)	34.2	31.7	18.5	15.6	12.3	9.3
Barley	13.0	17.0	24.1	27.0	20.0	22.8
Wheat middling	-	-	-	-	12.0	12.0
Sunflower meal (28% CP)	5.97	4.54	12.70	12.80	11.52	11.64
Soybean oil	2.71	2.92	1.00	0.90	1.00	1.00
Dicalcium phosphate	2.07	2.04	1.21	1.15	0.88	0.70
Calcium carbonate	1.04	0.82	1.40	1.40	1.36	1.56
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
L-Lys-HCL (78%)	-	-	0.13	0.20	0.03	0.08
DL-Met (99%)	0.16	0.13	0.11	0.10	0.06	0.07
Vitamin and mineral premix ¹	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis²						
AME _n (kcal/kg)	2,790	2,790	2,700	2,700	2,650	2,650
Crude fiber	4.7	5.3	6.2	6.4	6.3	6.6
CP	21.5	21.8	18.0	18.0	16.0	16.0
Ether extract	5.2	4.6	3.5	2.7	3.7	2.9
Total ash	6.2	6.2	5.4	5.4	5.4	5.4
Digestible AA						
Arg	1.30	1.34	1.05	1.05	0.92	0.89
Lys	0.97	0.97	0.79	0.79	0.60	0.60
Met	0.45	0.43	0.37	0.35	0.30	0.30
Met + Cys	0.74	0.74	0.62	0.62	0.53	0.53
Thr	0.68	0.68	0.54	0.53	0.47	0.45
Trp	0.22	0.24	0.18	0.19	0.16	0.17
Calcium	0.95	0.95	0.90	0.90	0.85	0.85
Phosphorus	0.78	0.80	0.63	0.63	0.62	0.60
Digestible phosphorus	0.45	0.45	0.42	0.42	0.40	0.40

¹Provided the following (per kilogram of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (all-rac-tocopherol-acetate), 30 mg; vitamin K3 (bisulphatemenadione complex), 3 mg; riboflavin, 8 mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (dicalcium pantothenate), 15 mg; folic acid, 1.5 mg; D-biotin, 0.2 mg; thiamin (thiamine-mononitrate), 2 mg; vitamin B₁₂ (cyanocobalamin), 25 µg; Se (Na₂SeO₃) 0.2 mg; I (KI), 2 mg; Cu (CuSO₄ 5H₂O), 8 mg; Fe (FeCO₃), 60 mg; Mn (MnO), 70 mg; Zn (ZnO), 80 mg. Roxazyme [1,600 IU endo-1,4-β-glucanase (EC 3.2.1.4), 5,200 IU endo-1,3(4)-β-glucanase (EC 3.2.1.6), and 5,200 IU endo-1,4-β-xylanase (EC 3.2.1.8)], 200 mg; Natuphos 5000 [400 FTU/kg 6-phytase (EC 3.1.3.26) Basf Española S.A, Tarragona, Spain], 80 mg.

²According to FEDNA (2010)

Table 2. Chemical analyses (% as-fed basis), particle size distribution (%), and geometric mean diameter (GMD, μm) of the experimental diets¹

Item	0-5 wk				5-10 wk				10-17 wk			
	Corn		Wheat		Corn		Wheat		Corn		Wheat	
	Crumble	Mash	Crumble	Mash	Crumble	Mash	Crumble	Mash	Crumble	Mash	Crumble	Mash
GE ² (kcal/kg)	4,000	3,970	4,075	3,995	3,931	3,931	3,881	3,890	3,952	4,038	3,951	4,015
DM	91.7	91.0	91.4	91.1	91.7	91.7	91.2	91.2	90.8	91.6	90.7	91.2
CP	20.5	20.4	20.6	20.7	17.8	18.1	17.4	17.5	15.4	15.7	15.9	16.2
Ether extract	5.1	5.3	5.0	5.2	3.6	3.6	2.8	3.1	3.4	3.6	3.0	3.3
Total ash	6.7	6.6	6.0	6.5	5.9	6.1	6.5	6.3	6.2	6.3	6.4	6.7
Calcium	1.0	1.1	1.0	1.0	0.9	0.9	1.0	1.1	1.0	1.1	0.9	0.9
Phosphorus	0.83	0.87	0.83	0.79	0.72	0.70	0.71	0.65	0.68	0.65	0.66	0.68
Particle size ³												
>2,500	9.3	7.6	10.4	6.7	22.8	23.0	28.6	16.2	17.8	23.1	20.2	19.0
1,250	51.3	27.7	53.9	29.6	61.2	30.2	51.5	39.0	65.2	29.0	57.9	36.7
630	26.2	23.0	21.6	21.6	12.4	26.1	13.2	26.5	14.6	26.4	15.5	24.0
315	8.7	16.5	6.6	27.7	2.3	10.9	3.2	11.1	1.7	13.5	3.4	11.5
160	2.8	10.1	6.0	11.4	0.5	7.4	2.0	5.0	0.5	7.5	1.1	6.0
<80	1.5	2.1	1.3	2.8	0.3	2.1	1.0	2.0	0.3	1.4	1.2	2.0
GMD $\pm$ GSD ⁴	1,255 $\pm$ 2.0	735 $\pm$ 2.6	1,295 $\pm$ 2.0	799 $\pm$ 2.3	1,792 $\pm$ 1.7	1,204 $\pm$ 2.4	1,741 $\pm$ 2.0	1,204 $\pm$ 2.2	1,734 $\pm$ 1.6	1,191 $\pm$ 2.4	1,645 $\pm$ 1.9	1,199 $\pm$ 2.3

¹Analyzed in duplicate samples.²Gross energy.³The percentage of particles bigger than 5,000 μm or smaller than 40 μm was negligible for all diets.⁴GSD=Log normal SD.

2.5. Gastrointestinal tract traits and body and tarsus length

At each of the 3 growth performance control periods (5, 10, and 17 wk of age), 2 birds per replicate were randomly selected, weighed individually, and slaughtered by CO₂ asphyxiation. The GIT, from the beginning of the proventriculus to the cloaca, including the digesta content, and the spleen, liver, and pancreas, were removed and weighed, and the GIT weight expressed as RW. Then, the gizzard was excised and the pH of its contents was measured in situ 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 full gizzard was weighed, emptied of any digesta content, cleaned, dried with desiccant paper, and weighed again, and the gizzard content was estimated by difference. The weight of the full gizzard was expressed relative to BW and that of the digesta content relative to full organ weight. The length of the duodenum (from the gizzard to the pancreo-biliary duct), jejunum (from the pancreobiliary ducts to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileo-cecal junction), SI (duodenum, jejunum, and ileum), and the 2 ceca (from the ostium to the tip of the right and left ceca) were also determined, and expressed relative to BW (RL, cm/kg BW).

Before removing the GIT, the length of the pullets, from the tip of the beak to the end of the longest phalanx, was measured in extended birds using a flexible tape with a precision of 1 mm, and expressed relative to BW. Tarsus length was also determined using a digital caliper and expressed relative to BW.

2.6. Statistical analysis

Data were analyzed with a completely randomized design with 8 treatments arranged as a 2 × 4 factorial with 2 main cereals, and 4 feeding programs, that differed

in the form of the feeds used during the 3 periods of the rearing phase. Main effects and their interactions were analyzed using the GLM procedure of SAS (SAS Institute, 2004). The experimental unit was the cage for all traits. Results in the tables are presented as least-square means and differences were considered significant at $P < 0.05$. When the model was significant, treatment means were separated using the Tukey test.

3. RESULTS

The chemical analyses of the experimental diets are shown in Table 2. The GMD of the corn and wheat mash diets was lower from 0 to 5 wk (735 and 799 μm) than from 5 to 10 wk (1,204 and 1,204 μm) or from 10 to 17 wk (1,118 and 1,199 μm) of age. Also, the GMD of the corn and wheat diets in crumble form was lower from 0 to 5 wk (1,255 and 1,295 μm) than from 5 to 10 wk (1,792 and 1,741 μm) or from 10 to 17 wk (1,734 and 1,645 μm) of age.

3.1. Growth performance

From 0 to 10 wk of age, no interactions between main cereal and feed form of the diet were detected for any trait. However, from 10 to 17 wk of age, ADFI was similar in pullets fed diets based on corn or wheat in crumbles continuously (0 to 17 wk) but not in pullets that received the diets in mash form in any of the previous feeding periods ($P < 0.01$ for the interaction; Figure 1A). From hatch to 17 wk of age, pullets fed corn had greater ADFI than pullets fed wheat (51.2 vs. 50.1 g; $P < 0.001$) with most of the differences observed after 5 wk of age (57.4 vs. 56.5 g from 5 to 10 wk; $P < 0.01$ and 69.6 vs. 67.6 g from 10 to 17 wk; $P < 0.001$) (Table 3). Consequently, from hatch to 17 wk of age, pullets fed wheat had better FCR than pullets fed corn (4.25 vs. 4.33; $P < 0.001$) with most of the benefits observed from 0 to 5 wk of age (2.09 vs. 2.21, respectively; $P < 0.001$). ADG, however, was not affected by the main cereal of the diet. BW uniformity was not affected by the main cereal of the diet at any age (Table 4).

Figure 1: Interaction between the main cereal and feed form of the diet on (a) ADFI from 10 to 17 wk of age ($P = 0.007$ for the interaction and $SD = 1.66$) and (b) ADFI from 0 to 17 wk of age ($P = 0.028$ for the interaction and $SD = 0.92$)

	CCC ¹	CCM ²	CMM ³	MMM ⁴
Corn	70.6 ^{ab}	68.3 ^{bc}	69.4 ^{ab}	69.9 ^{ab}
Wheat	70.8 ^a	66.6 ^c	66.6 ^c	66.3 ^c

	CCC	CCM	CMM	MMM
Corn	52.0 ^a	51.0 ^{abc}	50.5 ^{bcd}	51.4 ^{ab}
Wheat	51.9 ^{ab}	50.0 ^{cd}	49.2 ^d	49.4 ^d

¹CCC = crumbles from 0 to 17 wk.

²CCM = crumbles from 0 to 10 wk followed by mash to 17 wk.

³CMM = crumbles from 0 to 5 wk followed by mash to 17 wk.

⁴MMM = mash from 0 to 17 wk.

^{a-b-c-d-e} Means without a common superscript differ significantly.

From hatch to 17 wk of age, ADG was greater in pullets fed crumbles continuously than in pullets fed any of the other treatments (12.3, 11.8, 11.6, and 11.5 g for pullets fed crumbles from 0 to 17 wk, switched from crumbles to mash at 10 or 5 wk of age, or fed mash continuously; $P < 0.001$). Consequently, FCR was better in pullets fed crumbles continuously than in pullets fed mash continuously, with pullets fed the other 2 treatments being intermediate (4.21, 4.36, 4.29, and 4.30, respectively; $P < 0.001$). In all periods considered (0 to 5 wk, 5 to 10 wk, and 10 to 17 wk of age), crumble feeding improved ADG ($P < 0.001$) and FCR ($P < 0.001$) as compared with mash feeding. From 5 to 10 wk of age, pullets that were switched from crumbles to mash feeding at 5 wk of age had similar ADFI, ADG, and FCR to pullets that were fed

mash continuously from 0 to 10 wk of age. Similarly, from 10 to 17 wk of age, pullets switched from crumbles to mash diets at 10 wk of age had similar ADFI, ADG, and FCR to pullets switched to mash at 5 wk of age. At 5 wk of age, pullets fed crumbles tended to have better BW uniformity than pullets fed mash (9.8 vs. 11.1%; $P = 0.06$) but no differences were detected at 10 and 17 wk (Table 4).

3.2. Gastrointestinal tract traits

No interactions between main cereal and feed form of the diets were detected for any of the GIT traits measured at any age and therefore only main effects are presented. At 5 and 10 wk of age, the RW of the GIT was not affected by the main cereal of the diet but at 17 wk of age, the GIT was heavier (12.2 vs. 11.7%; $P < 0.001$) in pullets fed corn than in pullets fed wheat (Table 5). The RW of the gizzard was higher at all ages in pullets fed corn compared to pullets fed wheat, with differences being significant at 5 wk (4.9 vs. 4.6%; $P < 0.01$) and 17 wk (4.1 vs. 3.7%; $P < 0.001$) of age (Table 6). Also, the digesta content of the gizzard at 17 wk of age, was greater (26.3 vs. 24.5%; $P < 0.05$) in pullets fed corn than in pullets fed wheat. However, gizzard pH and the RL of the SI and the ceca were not affected by the main cereal of the diet at any age (Tables 6 and 7).

Feed form affected the RW of the GIT and gizzard at all ages (Tables 5 and 6, respectively). At 17 wk of age, pullets fed mash continuously had heavier GIT (12.7 vs. 11.0%; $P < 0.001$) and gizzards (4.4 vs. 2.9%; $P < 0.001$) than pullets fed crumbles continuously, with pullets fed the other 2 treatments being intermediate. Similar results were observed at 5 and 10 wk of age. When pullets were switched from crumble to mash feeding at 5 or 10 wk of age, the RW of the GIT and gizzard increased. In fact, at 10 and 17 wk of age, the RW of both organs were similar for all the pullets that were fed mash feeds in any of the previous periods (0 to 5 wk, 5 to 10 wk, or 0 to 10 wk of

age). At 17 wk of age, the RW of the GIT and gizzard of pullets switched from crumbles to mash feeding at 5 or 10 wk of age, were intermediate between those of pullets that were fed crumbles or mash continuously ($P < 0.001$). Also at this age, the digesta content of the gizzard was lower in pullets fed crumbles continuously than in pullets that were fed mash in any of the 3 feeding periods considered (17.3, 27.8, 29.0, and 27.6% for pullets fed crumbles from 0 to 17 wk, 0 to 10 wk, and 0 to 5 wk of age, or fed mash continuously; $P < 0.001$). Similar results, with lower gizzard digesta content for pullets fed crumbles than for pullets fed mash, were observed at 5 and 10 wk of age. When pullets were switched from crumbles to mash feeds at any age, gizzard digesta content increased to levels similar to those of the pullets fed mash continuously (Table 6). Gizzard pH responded quickly to changes in feed form; pullets switched from crumbles to mash feeding at 5 or 10 wk of age showed in the next control (10 or 17 wk of age, respectively) a pH similar to that of pullets that were fed mash continuously. In fact, at 17 wk of age, gizzard pH was higher in pullets fed crumbles from 0 to 17 wk than in pullets that received mash in any of the 3 feeding periods considered (3.88, 3.26, 3.11, and 3.17, for pullets fed crumbles from 0 to 17 wk, 0 to 10 wk, or 0 to 5 wk of age, or fed mash continuously; $P < 0.001$).

The SI and the ceca were longer in pullets fed mash than in pullets fed crumbles at any age but the differences tended to be significant only for the SI at 5 wk (323 vs. 317 cm/kg BW; $P = 0.065$) and 10 wk (126 vs. 124 cm/kg BW; $P = 0.095$) of age (Table 7). Most of the differences in the lengths of the SI were detected for the duodenum and jejunum. The RL of the SI and ceca re-adapted quickly to changes in feed form, following a similar pattern to those described for the RW of the GIT and the gizzard.

3.3. Body and tarsus length

No interactions between main cereal and feed form of the diet were detected for body length or tarsus length at any age and therefore only main effects are presented (Table 8). Pullets fed corn were longer at all ages than pullets fed wheat, with the differences tending to be significant at 5 wk (118.4 vs. 115.4 cm/kg BW; $P = 0.06$) and 17 wk (48.9 vs. 48.1 cm/kg BW; $P = 0.08$) of age. Tarsus length was also longer at all ages in pullets fed corn than in pullets fed wheat, but the differences were not significant.

At 17 wk of age, pullets fed crumbles continuously were shorter ($P < 0.01$) and had a shorter tarsus ($P < 0.05$) than pullets fed mash continuously. Similar results were observed at 5 and 10 wk of age. Body and tarsus length responded quickly to changes in feed form, with increases in RL as the pullets were switched from crumbles to mash feed, following a similar pattern to those described for the RW of the GIT and the gizzard and for the RL of the SI and ceca.

Table 3. Influence of the main cereal and feed form of the diet on growth performance of brown-egg pullets from 0 to 17 wk of age¹

Treatment	0-5 wk			5-10 wk			10-17 wk			0-17 wk		
	ADFI (g)	ADG (g)	FCR ⁴	ADFI	ADG	FCR	ADFI	ADG	FCR	ADFI	ADG	FCR
Cereal												
Corn	19.3	8.77 ^b	2.21 ^a	57.4 ^a	16.7	3.45 ^a	69.6 ^a	10.5 ^a	6.62	51.2 ^a	11.8	4.33 ^a
Wheat	19.2	9.20 ^a	2.09 ^b	56.5 ^b	16.7	3.39 ^b	67.6 ^b	10.2 ^b	6.67	50.1 ^b	11.8	4.25 ^b
Feeding program ²												
CCC	19.1 ^b	9.18 ^a	2.09 ^b	58.3 ^a	17.3 ^a	3.38 ^b	70.7 ^a	11.0 ^a	6.41 ^b	51.9 ^a	12.3 ^a	4.21 ^c
CCM	18.9 ^b	9.12 ^a	2.08 ^b	58.4 ^a	17.2 ^a	3.39 ^b	67.4 ^b	9.7 ^c	6.96 ^a	50.5 ^b	11.8 ^b	4.30 ^{ab}
CMM	18.8 ^b	9.19 ^a	2.05 ^b	55.5 ^b	16.1 ^b	3.45 ^a	68.0 ^b	10.2 ^{bc}	6.68 ^{ab}	49.9 ^b	11.6 ^b	4.29 ^b
MMM	20.2 ^a	8.46 ^b	2.39 ^a	55.7 ^b	16.1 ^b	3.45 ^a	68.1 ^b	10.5 ^b	6.52 ^b	50.4 ^b	11.5 ^b	4.36 ^a
SD ³	0.55	0.293	0.089	1.29	0.47	0.071	1.66	0.56	0.337	0.92	0.28	0.083
<i>P</i> -value												
Cereal	0.39	<.001	<.001	0.006	0.66	<.001	<.001	0.006	0.50	<.001	0.84	<.001
Feeding program	<.001	<.001	<.001	<.001	<.001	0.001	<.001	<.001	<.001	<.001	<.001	<.001
Cereal x feeding program	0.71	0.54	0.14	0.74	0.93	0.74	0.007	0.34	0.96	0.028	0.68	0.36

¹Only main effects are shown. The significant interaction detected (for ADFI from 10 to 17 wk and from 0 to 17 wk of age) are shown in Figure 1.

²CCC = crumbles from 0 to 17 wk; CCM = crumbles from 0 to 10 wk followed by mash to 17 wk; CMM = crumbles from 0 to 5 wk followed by mash to 17 wk; MMM = mash from 0 to 17 wk.

³36 replicates for the main cereal, 18 replicates for feed form, and 9 replicates for the interaction, with of 17, 15, or 13 pullets each from 0 to 5 wk, 5 to 10 wk, and 10 to 17 wk of age, respectively.

⁴Feed conversion ratio.

^{a,b}Means with different superscripts in the same column within each main effect or the interaction are significantly different ($P < 0.05$).

Table 4. Influence of the main cereal and feed form of the diet on BW uniformity of the pullets¹

Treatment	5 wk	10 wk	17 wk
Cereal			
Corn	10.1	7.4	6.3
Wheat	10.1	7.3	6.3
Feeding program ²			
CCC	9.8	7.6	6.4
CCM	9.8	7.2	6.1
CMM	9.8	6.8	6.4
MMM	11.1	7.8	6.4
SD ³	1.80	1.44	1.38
<i>P</i> -value			
Cereal	0.89	0.79	0.89
Feeding program	0.059	0.18	0.89

¹Uniformity presented as the CV of pullets BW per replicate (Peak et al., 2000).

²CCC = crumbles from 0 to 17 wk; CCM = crumbles from 0 to 10 wk followed by mash to 17 wk; CMM = crumbles from 0 to 5 wk followed by mash to 17 wk; MMM = mash from 0 to 17 wk.

³36 replicates for the main cereal, 18 replicates for feed form, and 9 replicates for the interaction, with of 17, 15, or 13 pullets each from 0 to 5 wk, 5 to 10 wk, and 10 to 17 wk of age, respectively.

⁴The interactions between cereal and feed form were not significant ($P > 0.05$).

^{a,b}Means with different superscripts in the same column within each main effect are significantly different ($P < 0.05$).

Table 5. Influence of the main cereal and feed form of the diet on the BW (g) and the relative weight (% BW) of the gastrointestinal tract (GIT) of the pullets

Treatment	5 wk		10 wk		17 wk	
	BW	GIT ¹	BW	GIT	BW	GIT
Cereal						
Corn	329	21.2	930	14.7	1,314	12.2 ^a
Wheat	335	20.9	947	14.8	1,339	11.7 ^b
Feeding program ²						
CCC	337 ^a	20.5 ^b	968 ^a	14.0 ^b	1,374 ^a	11.0 ^c
CCM	344 ^a	20.5 ^b	969 ^a	14.0 ^b	1,321 ^{ab}	11.9 ^b
CMM	333 ^{ab}	21.0 ^{ab}	909 ^b	15.3 ^a	1,307 ^b	12.3 ^{ab}
MMM	314 ^b	22.2 ^a	907 ^b	15.7 ^a	1,304 ^b	12.7 ^a
SD ³	22.5	1.44	54.0	0.81	61.50	0.66
<i>P</i> -value						
Cereal	0.25	0.32	0.17	0.26	0.092	0.002
Feeding program	0.010	0.010	0.001	0.001	0.003	0.001

^{a,b}Means with different superscripts in the same column within each main effect are significantly different ($P < 0.05$).

¹Weight of the digestive tract (from the beginning of the proventriculus to cloaca), including digesta contents, spleen, gizzard, liver, and pancreas.

²CCC = crumbles from 0 to 17 wk; CCM = crumbles from 0 to 10 wk followed by mash to 17 wk; CMM = crumbles from 0 to 5 wk followed by mash to 17 wk; MMM = mash from 0 to 17 wk.

³36 replicates for the main cereal, 18 replicates for feed form, and 9 replicates for the interaction, with of 2 pullets each.

⁴The interactions between cereal and feed form were not significant ($P > 0.05$).

Table 6. Influence of the main cereal and feed form of the diet on the relative weight (% BW) of the full gizzard, gizzard digest content (% full gizzard weight), and gizzard pH of the pullets¹

Treatment	5 wk			10 wk			17 wk		
	Weight	Content	pH	Weight	Content	pH	Weight	Content	pH
Cereal									
Corn	4.9 ^a	30.9	3.33	4.0	25.3	3.27	4.1 ^a	26.3 ^a	3.31
Wheat	4.6 ^b	29.3	3.21	3.9	25.4	3.20	3.7 ^b	24.5 ^b	3.40
Feeding program ²									
CCC	4.3 ^b	29.2 ^b	3.43 ^a	3.1 ^b	20.0 ^b	3.84 ^a	2.9 ^c	17.3 ^b	3.88 ^a
CCM	4.3 ^b	28.7 ^b	3.35 ^a	3.0 ^b	18.4 ^b	3.71 ^a	3.9 ^b	27.8 ^a	3.26 ^b
CMM	4.6 ^b	28.4 ^b	3.38 ^a	4.8 ^a	31.1 ^a	2.71 ^b	4.3 ^a	29.0 ^a	3.11 ^b
MMM	5.8 ^a	34.1 ^a	2.92 ^b	5.0 ^a	31.9 ^a	2.68 ^b	4.4 ^a	27.6 ^a	3.17 ^b
SD ³	0.54	5.10	0.345	0.45	4.14	0.381	0.36	3.57	0.468
<i>P</i> -value									
Cereal	0.008	0.20	0.16	0.34	0.86	0.47	0.001	0.034	0.39
Feeding program	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001

^{a,b}Means with different superscripts in the same column within each main effect are significantly different ($P < 0.05$).

¹The BW of the pullets is shown in Table 5.

²CCC = crumbles from 0 to 17 wk; CCM = crumbles from 0 to 10 wk followed by mash to 17 wk; CMM = crumbles from 0 to 5 wk followed by mash to 17 wk; MMM = mash from 0 to 17 wk.

³36 replicates for the main cereal, 18 replicates for feed form, and 9 replicates for the interaction, with of 2 pullets each.

⁴The interactions between cereal and feed form were not significant ($P > 0.05$).

Table 7. Influence of the main cereal and feed form of the diet on the relative length (cm/ kg BW) of the organs of the gastrointestinal tract of the pullets¹

Treatment	5 wk					10 wk					17 wk				
	Duod ²	Jejun ³	Ileum	SI ⁴	Ceca	Duod	Jejun	Ileum	SI	Ceca	Duod	Jejun	Ileum	SI	Ceca
Cereal															
Corn	30.6	161	132	323	33.4	11.3	64.3	50.7	126	13.7	7.9	46.8	37.9	93	12.7
Wheat	30.5	159	128	317	33.1	11.1	63.3	49.9	124	13.5	7.6	47.1	37.7	92	12.8
Feeding program ⁵															
CCC	30.8 ^{ab}	157	128	315	32.9	10.7 ^b	61.6 ^b	49.0	121	13.2	7.5	44.7 ^b	36.7	89	12.6
CCM	29.2 ^b	155	127	311	32.4	10.9 ^{ab}	62.1 ^{ab}	50.2	123	13.4	7.8	46.9 ^{ab}	37.5	92	12.6
CMM	30.0 ^{ab}	159	130	319	33.1	11.7 ^b	65.6 ^{ab}	50.3	128	13.9	7.9	47.5 ^{ab}	38.2	94	12.8
MMM	32.3 ^a	168	136	336	34.6	11.5 ^{ab}	66.0 ^a	51.7	129	13.9	7.7	48.7 ^a	38.8	95	13.1
SD ⁶	3.00	15.6	13.9	31.2	3.21	1.02	4.98	5.16	9.9	1.14	0.90	4.05	4.11	8.1	1.05
<i>P</i> -value ⁷															
Cereal	0.98	0.61	0.22	0.42	0.70	0.23	0.36	0.54	0.37	0.44	0.28	0.76	0.78	0.91	0.63
Feeding program	0.024	0.066	0.24	0.095	0.21	0.007	0.013	0.49	0.065	0.17	0.63	0.038	0.44	0.13	0.51

¹The BW of the pullets is shown in Table 5.

^{a,b}Means with different superscripts in the same column within each main effect are significantly different ($P < 0.05$).

²Duodenum.

³Jejunum.

⁴Small intestine (duodenum, jejunum, and ileum).

⁵CCC = crumbles from 0 to 17 wk; CCM = crumbles from 0 to 10 wk followed by mash to 17 wk; CMM = crumbles from 0 to 5 wk followed by mash to 17 wk; MMM = mash from 0 to 17 wk.

⁶36 replicates for the main cereal, 18 replicates for feed form, and 9 replicates for the interaction, with of 2 pullets each.

⁷The interactions between cereal and feed form were not significant ($P > 0.05$).

Table 8. Influence of the main cereal and feed form of the diet on the relative length (cm/ kg BW) of the tarsus and the corporal length of the pullets¹

Treatment	5 wk		10 wk		17 wk	
	Tarsus ²	Pullet ³	Tarsus	Pullet	Tarsus	Pullet
Cereal						
Corn	18.5	118.4	7.6	62.4	6.5	48.9
Wheat	18.1	115.4	7.5	61.5	6.4	48.1
Feeding program ⁵						
CCC	17.9 ^b	115.6 ^b	7.4 ^b	60.5 ^b	6.2 ^b	47.0 ^b
CCM	17.9 ^b	114.2 ^b	7.4 ^b	60.7 ^b	6.4 ^{ab}	48.9 ^a
CMM	18.3 ^{ab}	116.1 ^{ab}	7.7 ^{ab}	63.2 ^a	6.5 ^{ab}	49.0 ^a
MMM	19.0 ^a	121.7 ^a	7.7 ^a	63.4 ^a	6.5 ^a	49.1 ^a
SD ⁵	1.05	6.48	0.39	2.73	0.36	1.74
P-value ⁶						
Cereal	0.11	0.056	0.16	0.18	0.87	0.076
Feeding program	0.008	0.005	0.004	0.010	0.020	0.009

^{a,b}Means with different superscripts in the same column within each main effect are significantly different ($P < 0.05$).

¹The BW of the pullets is shown in Table 5.

²Measured with a caliper above the spur.

³Measured from the tip of the beak to the end of the longest phalanx.

⁴CCC = crumbles from 0 to 17 wk; CCM = crumbles from 0 to 10 wk followed by mash to 17 wk; CMM = crumbles from 0 to 5 wk followed by mash to 17 wk; MMM = mash from 0 to 17 wk.

⁵36 replicates for the main cereal, 18 replicates for feed form, and 9 replicates for the interaction, with of 2 pullets each.

⁶The interactions between cereal and feed form were not not significant ($P > 0.05$).

4. DISCUSSION

Within each feeding period, the gross energy, CP, ether extract, and ash content of the mash and crumbles diets were similar to calculated values, indicating that the ingredients were mixed correctly. The GMD of the diets fed from 0 to 5 wk of age was smaller than that of the diets fed from 5 to 10 wk or 10 to 17 wk of age, consistent with the smaller size of the screen used to grind the cereals for the starter feeds. Within each feeding period, the GMD was not affected by the main cereal of the diet.

4.1. Growth performance

From 0 to 17 wk of age, FI was reduced, and FCR was improved, in pullets fed wheat as compared with pullets fed corn but ADG was not affected. Published data comparing wheat and corn in poultry diets, however, are conflicting with reports showing better (Frikha et al., 2009b; Kiarie et al., 2014), similar (Frikha et al., 2011; Pérez-Bonilla et al., 2011), or reduced (Mathlouthi et al., 2002b; Amerah et al., 2008b; Abdollahi et al., 2013b) performance with wheat. The reasons for the discrepancies are unknown but might be related to the variability in the physico-chemical characteristics of the cereals (Gutiérrez-Alamo et al., 2008; Pérez-Bonilla et al., 2011). Wheat in Spain is produced on non-irrigated land and consequently, its chemical composition and nutritive values vary depending on climate conditions. In addition, wheat contains a high and variable amount of pentosans and β -glucans, which increase digesta viscosity and reduces productive performance in poultry (Lázaro et al., 2003a; Mirzaie et al., 2012). NSP enzymes reduce digesta viscosity and improve nutrient digestibility in birds fed diets with a high NSP content (Lázaro et al., 2003b, 2004; García et al., 2008). Consequently, differences in performance between birds fed diets based on corn or wheat might be reduced, or even disappear, when the feeds are supplemented with adequate enzymes. Moreover, in the current research, the NSP enzyme supplementation

increased the AMEn content of the wheat by 2% (FEDNA, 2010). It is probable the enzymes used in the current research increased the utilization of the energy contained in the wheat more than expected (Lázaro et al., 2003a). However, the DM content of the corn is variable depending on the drying process, which will affect its AMEn content. Consequently, the effects of the main cereal of the diet on ADFI and FCR will depend on the source and characteristics of the particular batch of grain, as well as on the type of NSP enzyme complex used.

From hatch to 5 wk of age, pullets fed crumbles had lower ADFI than pullets fed mash, but an opposite effect was observed from 5 to 17 wk of age. The reason for the contrasting effects on ADFI between crumbles and mash during these 2 periods is not known. Feed wastage could not be measured in this research and consequently, feed refusal was considered to be a part of voluntary FI. It is probable that feed wastage was higher in pullets fed mash than in pullets fed crumbles during the early stages of life, but not thereafter, as has been reported in broilers by Serrano et al. (2013) and Abdollahi et al. (2013a). This suggestion is consistent with the greater BW and better FCR recorded in the starter period for pullets fed crumbles as compared with pullets fed mash, in spite of the reduced “apparent” ADFI.

The beneficial effects of feeding crumbles or pellets on growth performance are well documented in broilers (Abdollahi et al., 2013b; Jiménez-Moreno et al., 2016) but not in pullets. In the current research, pullets fed crumbles showed a 6.6% greater ADG and 11.5% better FCR from 0 to 5 wk than pullets fed mash. From 0 to 17 wk of age, the advantages of feeding crumbles continuously over feeding mash continuously were reduced (3.0% for ADG and 3.4% for FCR) but still of considerable practical interest. Guzmán et al. (2015a) reported that pullets fed crumbles from hatching to 5 wk of age had 6.5% greater ADG and 8.2% better FCR than pullets fed mash, consistent with the

data reported herein. Frikha et al. (2009a) reported 2.8% heavier weights at 6 wk of age in pullets fed 2 mm pellets compared to pullets fed mash, but in this research no differences were detected for FCR. Gous and Morris (2001) reported that pullets fed crumbles from 0 to 4 wk of age, and then pellets from 5 to 20 wk, had 6% better ADG and 7.5% better FCR from 0 to 20 wk of age than pullets fed mash continuously. Saldaña et al. (2015a) reported that pullets fed crumbles had 9.1% higher ADG and 2.4% better FCR than pullets fed mash from hatching to 17 wk of age. In general, differences in growth reported in the literature between birds fed crumbles and mash diets are of a higher magnitude in broilers than in pullets, especially during the first 3 wk of life. For example, Serrano et al. (2013) reported 17.2% greater ADG and 4.8% better FCR with broilers fed crumbles from 1 to 25 d of age than for those fed mash diets. Also, Jiménez-Moreno et al. (2016) observed 32.6% greater ADG and 2.9% better FCR with pellets than with mash feeds from 1 to 21 d of age. Similar improvements in ADG and FCR with crumbles have been reported in broilers by Cerrate et al. (2009). Broilers are more voracious than pullets and therefore a reduction in feed particle size, as occurs when the feed is pelleted, may result in higher increases in voluntary FI and growth.

In the current research, ADFI was reduced any time pullets were switched from crumbles to mash feeding. In fact, pullets fed crumbles from 0 to 5 wk or from 0 to 10 wk of age had similar ADFI, ADG, and FCR in the following feeding period (5 to 10 wk or 10 to 17 wk of age, respectively) than pullets fed mash continuously. Consequently, from hatch to 17 wk of age, FCR was better in pullets fed crumbles continuously than in pullets fed mash continuously, with pullets switched from crumbles to mash feeds at 5 or 10 wk of age showing intermediate results. The data reported herein indicate that the growth pattern of the pullets re-adapts quickly to

changes in feed form, as has been suggested in broilers by Mateos et al. (2012) and Svihus (2014).

Heat processing modifies the structure of the starch, CP, and fiber fraction of the cereal, facilitating nutrient digestibility and broiler growth (Gracia et al., 2003; Abdollahi et al., 2013a). However, the temperature used for pelleting the feeds in the current experiment was low (72°C for 60 s) and consequently, the heat and friction applied during the process should not have affected to a high degree the nutrient digestibility and FCR. However, feed particles are finer in pelleted than in mash feeds, and fine particles facilitate the contact and access of endogenous enzymes to nutrients improving FCR. Moreover, crumbling improves the texture and density of the feed, which might increase voluntary FI and reduce feed wastage.

Body weight uniformity was not affected by the main cereal or feed form of the diets at 10 or 17 wk of age, but at 5 wk, pullets fed crumbles tended ($P = 0.06$) to be more uniform, in agreement with the data of Guzmán et al. (2015a). Frikha et al. (2009a), however, did not detect any effect of feed form (2 mm pellets vs. mash diets) on BW uniformity at 6 wk of age.

4.2. Gastrointestinal tract traits

The information available on the effects of the main cereal of the diet on GIT and gizzard weights in pullets is very scarce. In the current research, pullets fed corn had heavier GIT and gizzard at 17 wk of age than pullets fed wheat. Similar results were observed at 5 and 10 wk, but at these ages, the differences observed were not significant in most cases. Frikha et al. (2009b) reported also heavier gizzards at 6 wk of age in pullets fed corn than in pullets fed wheat, but in this research, GIT weight was not affected. In broilers, Abdollahi et al. (2013b) compared corn and wheat in diets fed as

mash or pellets and reported heavier gizzards at 3 wk of age for the corn in both cases. Similar differences between corn and wheat diets have been reported by Nir et al. (1994a) with mash diets, and by Amerah et al. (2008) with pelleted diets. The heavier GIT and gizzards in broilers fed corn than in broilers fed wheat might be related to differences in the physico-chemical characteristics of the 2 cereals. In this respect, Dombrink-Kurtzman and Bietz (1993) and Dobraszczyk et al. (2002) reported that the endosperm of dent corn is harder and more difficult to grind than the endosperm of soft wheat. Consequently, corn particles might stay for longer in the gizzard than wheat particles, resulting in greater development and larger size of the muscular layers of this organ (Jiménez-Moreno et al., 2010; Svihus, 2011; Mateos et al., 2012). In fact, in the current research, gizzard digesta content was higher in birds fed corn than in birds fed wheat at all ages (although the differences were significant only at 17 wk of age), data that are consistent with the harder endosperm of the corn. In addition, the proportion of coarse particles ($> 2,500 \mu\text{m}$) was higher for the corn than for the wheat diets, which may also increase the content and weight of the gizzard (Hetland et al., 2002; Svihus and Hetland, 2002). Gizzard pH, however, was not affected by the main cereal of the diet at any age, in agreement with data of Nir et al. (1994a) in 3-week-old broilers fed mash diets.

Type of cereal did not affect the RL of the SI or the ceca at any age, consistent with the data of Frikha et al. (2009b) in 6-week-old pullets. In contrast, Abdollahi et al. (2013b) reported that the length of the SI and ceca of 3-week-old broilers increased with wheat feeding. The reasons for the discrepancies are not known but might be related to the different physico-chemical characteristics of the grains used by the authors. In this respect, Amerah et al. (2008b) reported longer SI and ceca in 3-week-old broilers fed

corn when the diets were finely ground whereas no differences were detected when the diets were coarsely ground.

Feed form affected the development of the different segments of the GIT in different ways. Crumbling reduced the RW of the GIT and the gizzard and its content at all ages. The reduction in the RW of the gizzard with pellets has been well documented in broilers (Nir et al., 1994b; Amerah et al., 2007b; Abdollahi et al., 2011) but the information available for pullets is scarce. Frikha et al. (2009a) reported that feeding pellets reduced the RW of the GIT and gizzard in pullets at 6 wk of age as compared with feeding mash, data that agree with the results reported herein. Pelleting reduces feed particle size and increases transit time throughout the upper part of the GIT which may result in less digesta content and lighter gizzards as compared with mash feeding (Mateos et al., 2012; Mateos et al., 2014). Crumbling increased gizzard pH at all ages, results that agree with the data of Frikha et al. (2009b) in pullets and Serrano et al. (2013) in broilers. In contrast, Nir et al. (1995) did not observe any difference in gizzard pH in 3- or 6-week-old broilers fed pelleted or mash diets, although in this research sorghum was used as the main cereal of the diet.

Feeding crumbles reduced the RL of the SI and the ceca at all ages but the differences were not significant in most cases. Frikha et al. (2009a) reported also that feeding pellets from hatch to 6 wk of age, and then mash from 6 wk to 17 wk of age, reduced the length of the SI and ceca in pullets at 6 and 17 wk of age, although the differences detected for the ceca were not significant at 17 wk. Amerah et al. (2007a) and Abdollahi et al. (2011) reported shorter SI and ceca in 3-week-old broilers fed pellets rather than mash diets, in agreement with the results reported herein for pullets.

The size of the organs of the GIT of the pullets readapted quickly any time the form of the feed was switched from crumbles to mash. For example, pullets that were

switched from crumbles to a mash diet at 5 wk had similar RW of the GIT at 10 wk and 17 wk of age compared to pullets that were fed mash continuously. A similar re-adaptation pattern was observed for all the other variables studied (digesta content, weight, and pH of the gizzard, and RL of SI and ceca) at 17 wk of age when the pullets were switched from crumbles to mash at 10 wk. We have not found any report on the effects of changing feed form from crumbles to mash with time on the development of the different organs of the GIT in any poultry species. In the current experiment, the RW of the GIT and gizzard, the RW of the gizzard content, and the RL of the SI increased, and gizzard pH decreased, any time pullets were switched from crumble to mash feeding. The data reported herein indicate that the GIT of the pullets responds quickly to changes in diet form, suggesting that the digestive tract re-adapts as needed, to maintain optimal bird performance (Svihus, 2014). The data show also that crumbling affects negatively the development of the GIT, whether the pullets are fed a corn or a wheat based diet.

4.3. Body and tarsus length

Body length and tarsus length are useful criteria to evaluate body size and to predict future performance of serin (Senar and Pascual, 1997), sparrows (Cleasby et al., 2011), broilers (Mendes et al., 2007; Van RooverReijrink, 2013), and pullets (Lamazares et al., 2006; Itza et al., 2011). In the current experiment, the RL of the pullets decreased with age and 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 pullet or tarsus length to compare with the results reported herein.

Pullets fed crumbles continuously were shorter and had shorter tarsus than pullets fed mash continuously at all ages. At 10 wk of age, pullet and tarsus length were

similar for pullets that were fed mash from 5 to 10 wk of age, irrespective of the form of the feed supplied from 0 to 5 wk of age. The data indicate that changes in feed form produce a quick re-adaptation of pullet body traits, following a similar pattern to that described for GIT measurements.

In summary, the main cereal of the diet did not affect ADG of the pullet from hatch to 17 wk of age and in fact, FCR was improved with wheat feeding. Consequently, up to 40% wheat supplemented with adequate NSP enzymes, can be used in substitution of corn in these diets. However, pullets fed wheat had lighter GIT and gizzard and less gizzard content than pullets fed corn, a finding that suggested that under certain circumstances, the main cereal of the diet might affect indirectly subsequent hen performance. Crumble feeding improved growth performance of the pullets but reduced the RW of the gizzard and increased gizzard pH. Pullets changed from crumble to mash feeding at different ages always respond with a reduction in growth performance, an increase in RW, and an improvement in the development of the GIT and the gizzard in the subsequent period of the rearing phase. The data demonstrate that pullets re-adapt quickly to changes in feed form with changes in GIT traits, body and tarsus length, and growth. Consequently, the effects of ingredient composition and feed form of the rearing diets on subsequent pullet performance might be less relevant than currently accepted. Further research is needed to determine how these changes may affect hen production during the subsequent laying period.

**CHAPTER 4: FEED FORM AND ENERGY
CONCENTRATION OF THE REARING DIETS DO NOT
AFFECT PRODUCTION, DIGESTIVE TRACT
DEVELOPMENT, OR BODY MEASUREMENTS OF
BROWN-EGG LAYING HENS FROM 17 TO 46 WK OF
AGE**

1. INTRODUCTION

Light hens produce less eggs at the start of the laying period and lay smaller eggs during the laying cycle than heavy hens (Leeson et al., 1997; Pérez-Bonilla et al., 2012a), resulting in a reduction in flock profitability (Pérez-Bonilla et al., 2012b). Two potential nutritional strategies used for increasing the initial BW of the hens at the onset of egg production are the feeding of crumbled diets (Frikha et al., 2009b; Gous and Morris, 2001; Saldaña et al., 2015b) and the use of high energy diets (Frikha et al., 2009a; Saldaña et al., 2015a) during the rearing phase. However, Nir et al. (1994b) and Serrano et al. (2013) in broilers and Guzmán et al. (2015a) and Saldaña et al. (2015a, b) in pullets, have shown that feeding crumbles or pellets reduced the relative weight (g/kg BW) of the gizzard and the development of the GIT as compared with feeding mash. Also, when the energy concentration of the diet increases, fiber content decreases, which also reduces GIT development (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009a; Sacranie et al., 2012). A poorly developed GIT affects FI and might result in pullets not consuming enough energy to meet their requirements for optimal egg production during the first weeks of the laying phase (Pérez-Bonilla et al., 2012b; Guzmán et al., 2016). Consequently, the effects of greater BW but reduced GIT capacity at 17 wk of age, because of feeding a high energy diet in crumble form during the rearing phase, might counteract each other and the final effect on egg production and egg size might depend on other nutritional and management practices.

Laying hens eat to satisfy their energy requirements and therefore, voluntary FI decreases as the energy content of the diet increases (Leeson et al., 1997). However, low energy diets might result in hens consuming less feed than required to maximize egg production, especially in light birds at the onset of egg production (Pérez-Bonilla et al.,

2012a). Moreover, high energy diets contain usually increased amounts of supplemental fat which may result in improved feed efficiency and an increase in egg size (Mateos and Sell, 1981b; Grobas et al., 1999c).

Body measurements, such as body length, BMI, and tarsus length and diameter, are useful criteria to predict body size and future production of laying hens (Ojedapo et al., 2012; Guzmán et al., 2016). However, the information available on the effects of the characteristics of the rearing and laying hen diets on these variables are limited.

The hypothesis of this research was that pullets fed high energy diets in crumble form during the rearing phase could have greater BW but less developed GIT at the start of the laying phase than pullets fed low energy diets in mash form, which could result in similar productivity during the laying phase. Also, feeding low energy diets during the laying phase could result in reduced egg mass production and egg weight, effects that could be more pronounced in those hens that were lighter or had a poor developed GIT at the onset of the egg production cycle. This research evaluated the effects of feed form and energy concentration of the rearing phase diets on performance, egg quality, digestive tract traits and body measurements of hens fed diets differing in energy concentration from 17 to 46 wk of age.

2. MATERIALS AND METHODS

The procedures described in this research 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).

2.1. Husbandry, diets and experiment design

Details on bird management and feeding program from hatching to 17 wk of age have been reported by Saldaña et al. (2015a). Briefly, one-day-old Lohmann Brown Classic pullets were placed in an environmentally controlled barn and allotted in groups of 50 into 36 cages with similar initial average BW. The feeding program consisted of three periods (0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age) in which the energy concentrations of the diets varied in 100 kcal/kg (from 2,850 to 3,050, 2,700 to 2,900, and 2,650 to 2,850 kcal AME_n/kg for the three feeding periods, respectively). During the rearing phase, the treatments consisted in six diets arranged as a 2 x 3 factorial with two feed forms (crumbles vs. mash) and three energy concentrations (LE, low energy, ME, medium energy; HE, high energy). Each treatment was replicated six times. All diets had similar amino acids and nutrient content per unit of energy. The ingredient composition and the calculated and determined nutritive value of the rearing phase diets are shown in Table 1.

Table 1. Ingredient composition and calculated and determined analyses (% as fed basis) of the rearing phase diets¹

Ingredient	0 to 5 week			5 to 10 week			10 to 17 week		
	LE	ME	HE	LE	ME	HE	LE	ME	HE
Corn	35.0	35.0	35.0	35.0	35.0	35.0	20.0	20.0	20.0
Wheat	19.2	17.4	15.7	-	5.0	10.0	31.5	35.7	40.0
Barley	-	-	-	28.0	23.3	18.5	10.0	10.3	10.5
Soybean meal, 45.5% CP	33.3	36.7	40.1	22.0	26.5	31.0	9.0	13.0	17.0
Sunflower meal, 32% CP	-	-	-	10.0	5.0	-	-	-	-
Sunflower meal, 28% CP	6.0	3.0	-	-	-	-	15.0	11.5	8.0
Bran	-	-	-	-	-	-	10.0	5.0	-
Soy oil	2.71	4.08	5.37	1.00	1.50	2.00	0.85	1.03	1.2
Dicalcium phosphate	2.07	2.07	2.06	1.53	1.57	1.60	1.50	1.51	1.53
Calcium carbonate	1.04	1.05	1.06	1.64	1.40	1.16	1.44	1.29	1.16
Sodium chloride	0.35	0.35	0.35	0.35	0.34	0.33	0.35	0.35	0.35
<i>DL</i> -methionine, 99%	0.13	0.15	0.16	0.11	0.13	0.15	0.05	0.06	0.06
<i>L</i> -threonine, 98%	-	-	-	0.08	0.07	0.06	-	-	-
<i>L</i> -lysine·HCl, 78%	-	-	-	0.09	0.05	-	0.11	0.06	-
Premix ²	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calculated analyses ³									
AME _n (kcal/kg)	2,850	2,950	3,050	2,700	2,800	2,900	2,650	2,750	2,850
Neutral detergent fiber	11.1	10.0	8.9	13.6	12.0	10.4	18.0	15.5	13.0
Crude protein	21.7	22.2	22.7	19.0	19.4	19.9	16.1	16.7	17.2
Digestible amino acid									
Lys	0.97	1.02	1.08	0.84	0.87	0.90	0.61	0.63	0.65
Met + Cys	0.72	0.74	0.76	0.65	0.67	0.69	0.53	0.55	0.56
Thr	0.68	0.71	0.73	0.67	0.68	0.68	0.45	0.48	0.50
Calcium	1.10	1.10	1.10	1.03	0.94	0.85	1.07	1.02	0.97
Digestible phosphorus	0.44	0.43	0.43	0.43	0.44	0.44	0.37	0.36	0.35
Determined analyses ⁴									
Dry matter	91.1	90.7	90.9	90.4	91.1	91.9	91.4	91.7	91.3
GE (kcal/kg)	4,160	4,181	4,286	3,862	3,997	4,054	4,016	3,958	4,001
Ether extract	5.6	6.4	7.5	3.5	4.2	4.6	3.0	3.0	3.1
Crude fiber	4.1	3.8	3.2	6.2	5.9	5.4	6.6	5.8	5.2
Crude protein ⁷	21.3	22.5	23.5	18.3	18.2	18.6	16.1	16.4	16.4
Lys	1.13	-	1.21	0.99	-	1.05	0.69	-	0.76
Met + Cys	0.84	-	0.90	0.73	-	0.79	0.64	-	0.65
Thr	0.83	-	0.87	0.77	-	0.81	0.52	-	0.60
Total ash	5.9	-	5.9	5.0	-	6.2	5.7	-	5.3

¹LE = 2,850, 2,700, and 2,650 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; ME = 2,950, 2,800, and 2,750 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; HE = 3,050, 2,900, and 2,850 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively.

²Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,500 IU; vitamin E (all-*rac*-tocopherol-acetate), 35 mg; vitamin B₁, 2 mg; vitamin B₂, 8

mg; vitamin B₆, 4 mg; vitamin B₁₂ (cyanocobalamin), 0.025 mg; vitamin K₃ (bisulphatemenadione complex), 3 mg; choline (choline chloride), 270 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; D-biotin, 0.15 mg; zinc (ZnO), 90 mg; manganese (MnO), 75 mg; iron (FeCO₃), 60 mg; copper (CuSO₄ • 5H₂O), 8 mg; iodine (KI), 2 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; and Natuphos 5000 [300 FTU/kg 6-phytase (EC 3.1.3.26), 60 mg, supplied by Basf Española S.A, Tarragona, Spain].

³According to FEDNA (2010).

⁴Data correspond to the average of the mash and crumble diets. The differences in values between the mash and crumble diets were within acceptable ranges.

⁵Total amino acids was not determined for the ME diets.

At 17 wk of age, pullets from each of the six previous rearing treatments (LE, ME and HE in mash or crumble form) were pooled and then, distributed in groups of nine birds into ten enriched cages (40 cm × 80 cm × 68 cm; Facco S.A., Padova, Italy). Half of the cages of each of the six groups received a low energy layer diet (2,650 kcal AME_n/kg) and the other half a high energy layer diet (2,750 kcal AME_n/kg). The two layer diets had the same nutritive value per unit of energy (FEDNA, 2010). Feed in mash form and water were provided for ad libitum consumption. The light program consisted of 15 h for the first week of the experiment and 16 h thereafter. Room temperature was recorded daily throughout the experiment with a maximum of 27 ± 3°C (July, second period of the experiment) and a minimum of 23 ± 3°C (January, last period of the experiment). The experiment lasted for seven periods of four week each, with the exception of the first period that lasted five wk (17 to 22 wk). The ingredient composition and the calculated and determined analyses of the laying phase diets are shown in Table 2.

The experiment was conducted as a completely randomized design with 12 treatments in a factorial arrangement with two feed forms (mash vs. crumbles) and three energy concentrations (LE, ME and HE) during the rearing phase and two diets that differed in AME_n content (low and high) during the laying phase. Each of the 12

treatments was replicated five times and the experimental unit was an enriched cage with nine hens.

2.2. Measurements

Feed disappearance and egg production by replicate were recorded by period as well as for the entire experiment. Hens were weighed by replicate at the beginning of the experiment and at the end of each of the seven experimental periods. Any mortality was recorded and weighed as produced. Egg production data are presented as number of eggs per hen per day. Egg size was estimated by weighing all eggs produced the last two days of each week. The average value of the four weeks (or five wk for the first period) was used to estimate egg weight by period and used for further analysis. From these data, ADFI, egg production, egg weight, egg mass, FCR per kilogram of eggs and per dozen of eggs and ADG were calculated by period and for the entire experiment.

Table 2. Ingredient composition and calculated and determined analyses (g/kg as fed basis) of the laying phase diets

Ingredient	2,650 kcal/kg	2,750 kcal/kg
Wheat	400	400
Corn	200	170
Soybean meal, 47% CP	171	180
Sunflower meal, 34% CP	103	110
Sunflower oil soapstocks	14.5	33.9
Dicalcium phosphate	10.8	11.8
Calcium carbonate	82.4	85.0
Sodium chloride	2.8	3.0
<i>DL</i> -methionine, 99%	1.1	1.3
Sepiolite ¹	9.4	-
Vitamin and mineral premix ²	5.0	5.0
Calculated analysis ³		
DM	894	898
AME _n , kcal/kg	2,650	2,750
CP	174	180
Crude fiber	44	46
Neutral detergent fiber	111	114
Digestible amino acid		
Lys	6.8	7.1
Met	3.7	4.0
Met + Cys	6.3	6.6
Thr	5.3	5.5
Trp	1.8	1.9
Calcium	37.5	38.5
Total phosphorus	6.1	6.4
Digestible phosphorus	3.5	3.7
Determined analyses ⁴		
Gross energy kcal/kg	3,848	3,872
DM	900	895
CP	182	185
Total amino acid		
Lys	8.2	8.4
Met	4.1	4.3
Met + Cys	7.4	7.5
Thr	6.6	6.7
Trp	2.1	2.2
Ether extract	54	68
Linoleic acid	19	27
Ash	130	133
GMD $\pm$ GSD ⁵	1,112 $\pm$ 2.06	1,132 $\pm$ 2.10

¹A complex magnesium silicate clay incorporated as an inert material.

²Provided 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.

³According to FEDNA (2010).

⁴Determined analyses were analyzed in duplicate except geometric mean diameter that was determined in triplicate.

⁵Geometric mean diameter $\pm$ Log normal SD.

All eggs produced throughout the experiment were classified and the number of dirty, broken and shell-less eggs, was recorded by replicate. 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 and shell color, Haugh units, shell strength and shell thickness, were measured in 12 eggs collected randomly from each replicate the last two days of each of the seven experimental periods. Haugh units and yolk color (Roche color fan) were measured in fresh eggs using a multitester equipment (QCMSsystem, Technical Services and Supplies, Dunnington, York, UK) as indicated by Pérez-Bonilla et al. (2012a). Shell color was evaluated 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. Egg shell strength, expressed in g/cm², was evaluated using a press meter (Egg Force Reader, SANOVO Technology A/S, Odense, Denmark) as indicated by Safaa et al. (2008). 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 three measurements of each of the 12 eggs was used for further analyses.

At 46 wk of age, after the corresponding hen production control, two hens per replicate were randomly selected, weighed individually and euthanized by CO₂ inhalation. The full digestive tract, from the post-crop esophagus to the cloaca, including digesta content, liver, pancreas and spleen, was removed and weighed. Then, the full proventriculus and gizzard, and the liver were excised and weighed. In addition, the gizzard was emptied from any digest content, cleaned, dried with desiccant paper and weighed again. The weight of the organs was expressed relative (g/kg) to live BW

and the weight of the gizzard contents was expressed as a percentage of the full organ weight. Gizzard pH was measured in situ in duplicate in all these hens 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. (2009b). 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 of the two ceca (from the ostium to the tip of the right and left ceca) was also measured. The length of the SI was determined by adding the length of the duodenum, jejunum and ileum. In addition, the length of the hens from the tip of the beak to the end of the longest phalanx was measured on a glass surface at 17 and 46 wk of age in extended birds, using a flexible tape with a precision of one mm. From these measurements, BMI was estimated by dividing the BW (g) of the hen by the corporal length² (cm) as indicated by Mendes et al. (2007). Also, the length and diameter of the tarsus of these hens were measured with the aid of a digital caliper and expressed as cm per kg of BW. The average value of the traits measured in the two hens was used for further statistical analysis.

2.3. Laboratory analysis

Representative samples of the laying hen diets were ground in a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) equipped with a one-mm screen and analysed 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 was determined after 3N HCl acid hydrolysis (Boletín Oficial del Estado, 1995) and gross energy was determined using an adiabatic bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL, USA). The amino acid content was analyzed by

chromatography (Hewlett-Packard 1100, Waldbronn, Germany) in samples ground at 0.5 mm as described by De Coca-Sinova et al (2008) and the LNA content as indicated by Grobas et al. (1999c). Particle size distribution and mean particle size of the diets, expressed as GMD, were determined in a 100 g sample using a shaker (Retsch, Stuttgart, Germany) provided with eight sieves ranging in mesh from 5,000 to 40 µm as indicated by the ASAE (1995).

2.4. Statistical analysis

The normal distribution of residuals and variance homogeneity of the data were tested by the UNIVARIATE procedure and the Levene's Test, respectively (SAS Institute, 2004). The effects of dietary treatment (feed form and AMEn content of the rearing phase diet and AMEn content of the laying phase diet) and their interactions on hen production were analyzed as repeated measures using the MIXED. Data on GIT development and body measurements were analyzed using the GLM procedure of SAS (SAS Institute, 2004). When significant differences among treatments were detected, means were separated using the Tukey test. In addition, the Pearson correlation analyses (SAS Institute, 2004) was used to study the relation between BW of the hens and body length, BMI and tarsus length and diameter at 46 wk of age. Differences were considered significant at $P < 0.05$.

3. RESULTS

3.1. Hen performance and egg quality

Details on pullet performance from hatching to 17 wk of age have been reported by Saldaña et al. (2015a). Briefly, feeding crumbles increased ADFI (52.9 vs. 49.7 g; $P<0.001$) and ADG (12.7 vs. 11.6 g; $P<0.001$) and improved FCR (4.18 vs. 4.27; $P<0.001$) of the pullets. Consequently, BW at 17 wk of age was higher in pullets that were fed crumbles than in pullets that were fed mash (1,554 vs. 1,432 g; $P<0.001$). An increase in the energy content of the rearing diets decreased ADFI linearly ($P<0.001$) and improved FCR quadratically ($P<0.01$) but EI was not affected.

Mortality during the laying phase was 1.8% and was not related to treatment (data not shown). No interactions among main effects on hen productivity were detected and therefore, only main effects are presented (Table 3). From 17 to 46 wk of age, type of diet fed during the rearing phase did not affect any of the productive traits studied, except ADG that was higher (386 vs. 282 g; $P < 0.001$) for hens that were fed mash during the rearing phase than for hens that were fed crumbles (Figs. 1 and 2). Most of the differences in ADG between the two groups of hens occurred from 17 to 22 wk of age and in fact, no significant differences were detected after this age. As a result, BW of the hens at 46 wk of age was similar for all hens, irrespective of the feed form or energy concentration of the rearing phase diets.

An increase in the energy content of the laying phase diets reduced ADFI (113 vs. 109 g; $P < 0.001$) and improved FCR per kg (1.98 vs. 1.92; $P < 0.001$) and per dozen (1.44 vs. 1.41; $P < 0.005$) of eggs but did not affect any of the other traits studied. The effects of energy concentration of the diet on hen productivity by period are shown in Fig. 3. Egg quality (percentage of dirty eggs, Haugh units, yolk color, shell color, shell

strength and shell thickness) was not affected by the type of diet used during the rearing or the laying phases (Table 4).

3.2. Digestive traits and body measurements

Details on the effects of the characteristics of the rearing phase diet on GIT traits and body measurements of the pullets have been reported elsewhere (Saldaña et al., 2015a). Briefly, the relative weight of the GIT and the gizzard and gizzard digesta content was lower ($P < 0.05$ to $P < 0.001$) and gizzard pH was higher ($P < 0.05$ to $P < 0.001$) in pullets fed crumbles than in pullets fed mash at all ages (5, 10 and 17 wk of age). Body and tarsus length was shorter ($P < 0.01$) at all ages in pullets fed crumbles than in pullets fed mash. Energy concentration of the diet did not affect any of the GIT traits or body measurements evaluated.

During the laying period, no interactions between the characteristics of the rearing phase diets and the energy content of the laying phase diets were detected for any of the traits studied and therefore, only main effects are presented (Tables 5 and 6). At 46 wk of age, gizzard content was higher in hens fed the low energy layer diet than in hens fed the high energy layer diet (281 vs. 261 g/kg; $P < 0.01$). The RW of the full proventriculus, full and empty gizzard and the liver, as well as the pH of the gizzard contents and the length of the SI and cecum, were not affected by the energy content of the layer diet. Similarly, body length, BMI and tarsus length and diameter of the hens were not affected by dietary treatment (Table 7). Hen BW showed a positive correlation ($P < 0.001$) with body length, BMI and tarsus length and diameter (Table 8)

Table 3. Effects of feed form and energy concentration of the rearing diets and energy concentration (AME_n /kg) of the laying hen diet on hen production from 17 to 46 wk of age

	Egg production ¹	ADFI (g/hen)	Egg weight (g)	Egg mass (g/d)	FCR		BW (g)		ADG (g)
					(kg:kg)	(kg:dozen)	17 wk	46 wk	
Feed form, rearing phase									
Mash	0.936	111	61.0	57.0	1.95	1.43	1,432 ^b	1,816	386 ^a
Crumbles	0.930	110	60.9	56.6	1.95	1.42	1,554 ^a	1,836	282 ^b
Energy level, rearing phase ²									
LE	0.942	111	61.1	57.2	1.94	1.42	1,486	1,833	347
ME	0.927	111	60.8	56.3	1.96	1.43	1,487	1,802	316
HE	0.930	110	61.0	56.7	1.96	1.43	1,506	1,842	340
Energy level, laying phase ³									
Low	0.938	113 ^a	60.9	57.0	1.98 ^a	1.44 ^a	1,494	1,815	321
High	0.928	109 ^b	61.0	56.6	1.92 ^b	1.41 ^b	1,492	1,837	347
SD ⁴	0.0298	3.07	0.948	1.55	0.0047	0.0047	37.6	60.4	58.5
P-value ⁵									
Rearing phase									
Feed form	0.380	0.423	0.738	0.325	0.721	0.840	0.001	0.210	0.002
Energy level	0.296	0.613	0.743	0.109	0.199	0.532	0.174	0.104	0.338
Laying phase									
Energy level	0.198	0.001	0.797	0.476	0.001	0.005	0.848	0.174	0.102

¹Eggs/hen per day.²LE = 2,850, 2,700, and 2,650 kcal AME_n /kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; ME = 2,950, 2,800, and 2,750 kcal AME_n /kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; HE = 3,050, 2,900, and 2,850 kcal AME_n /kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively.³Low = 2,650 kcal AME_n /kg; High = 2,750 kcal AME_n /kg.⁴30 replicates for feed for in the rearing phase, 20 replicates for energy level in the rearing phase, and 30 replicates for energy level in the laying phase.⁵The interactions among main effects were not significant ($P > 0.05$).^{a,b}Means with different superscript within each main effect are significantly different ($P < 0.05$).

Figure 1. Effect of feed form of the rearing pullet diets on egg production (A), feed intake (B), egg weight (C), feed conversion ratio (D) and ADG (E) of the hens from 17 to 46 wk of age^a

^aThe effect of the period was significant ($P < 0.005$) for all the variables studied.

^bStandard error of the mean ($n=30$).

^{NS} $P > 0.05$; ^{***} $P < 0.005$.

Figure 2. Effect of energy content of the rearing diets^a on egg production (A), feed intake (B), egg weight (C), feed conversion ratio (D) and ADG (E) of the hens from 17 to 46 wk of age^b

^aLow: 2,850, 2,700, and 2,650 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; medium: 2,950, 2,800, and 2,750 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; high: 3,050, 2,900, and 2,850 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively.

^bThe effect of the period was significant ($P < 0.005$) for all the variables studied.

^cStandard error of the mean ($n=20$).

^{NS} $P > 0.05$.

Figure 3. Effect of energy concentration (AME_n/kg) on of the laying hen diets^a on egg production (A), feed intake (B), egg weight (C), feed conversion ratio (D) and ADG (E) from 17 to 46 wk of age^b

^aLow 2,650 kcal AME_n/kg ; high: 2,750 kcal AME_n/kg .

^bThe effect of the period was significant ($P < 0.005$) for all the variables studied.

^cStandard error of the mean ($n=30$).

^{NS} $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

Table 4. Effects of feed form and energy concentration of the rearing diets and energy concentration (AME_n/kg) of the laying hen diets on egg quality from 17 to 46 wk of age

	Dirty eggs (%)	Broken (%)	Shell-less (%)	Haugh units	Shell color ¹			Yolk color ²	Shell strength (g/cm ²)	Shell thickness (mm)
					L	a	b			
Feed form, rearing phase										
Mash	1.47	0.51	0.22	92.1	61.2	15.5	32.1	10.3	4,939	0.38
Crumbles	1.29	0.46	0.18	92.0	61.2	15.5	32.1	10.2	4,898	0.38
Energy level, rearing phase ³										
LE	1.19	0.49	0.18	91.8	61.2	15.6	32.3	10.3	4,992	0.38
ME	1.46	0.54	0.17	92.1	61.5	15.3	31.9	10.3	4,846	0.38
HE	1.47	0.46	0.19	92.3	61.1	15.6	32.4	10.3	4,916	0.38
Energy level, laying phase ⁴										
Low	1.39	0.44	0.17	92.1	61.0	15.6	32.1	10.3	4,915	0.38
High	1.36	0.52	0.23	91.9	61.4	15.4	32.1	10.3	4,925	0.38
SD ⁵	0.528	0.109	0.028	0.931	0.860	0.898	0.520	0.411	216	0.0071
P-value ⁶										
Rearing phase										
Feed form	0.272	0.414	0.347	0.833	0.896	0.695	0.590	0.322	0.560	0.543
Energy level	0.193	0.291	0.100	0.158	0.249	0.265	0.124	0.793	0.170	0.399
Laying phase										
Energy level	0.876	0.175	0.144	0.629	0.112	0.151	0.688	0.794	0.867	0.320

¹Hunter color values, L* (lightness), a* (green to red) and b* (blue to yellow).²Measured with roche color fan.³LE = 2,850, 2,700, and 2,650 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; ME = 2,950, 2,800, and 2,750 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; HE = 3,050, 2,900, and 2,850 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively.⁴Low = 2,650 kcal AME_n/kg; High = 2,750 kcal AME_n/kg.⁵30 replicates for feed for in the rearing phase, 20 replicates for energy level in the rearing phase, and 30 replicates for energy level in the laying phase.⁶The interactions among main effects were not significant (P > 0.05).

Table 5. Effects of feed form and energy concentration of the rearing diets and energy concentration of the laying hen diets on the relative weight (g/kg BW) of the full gastrointestinal tract (GIT) and of the digestive organs of the hens at 46 wk of age

	BW (g)	GIT (g/kg BW)	Liver (g/kg BW)	Proventriculus (g/kg BW)	Gizzard			pH
					Full (g/kg BW)	Empty (g/kg BW)	Content (g/kg organ W)	
Feed form, rearing phase								
Mash	1,838	121	25.9	5.0	25.8	18.8	272	3.96
Crumbles	1,858	119	25.1	5.3	25.1	18.1	269	3.88
Energy level, rearing phase ¹								
LE	1,850	120	25.2	4.9	25.5	18.3	273	3.85
ME	1,844	120	25.8	5.4	25.4	18.7	274	3.91
HE	1,838	121	25.5	5.1	25.4	18.6	267	4.01
Energy level, laying phase ²								
Low	1,849	120	25.7	5.1	25.6	18.3	281 ^a	3.97
High	1,848	120	25.3	5.1	25.3	18.7	261 ^b	3.88
SD ³	79.4	4.502	2.91	0.668	2.24	1.79	14.3	0.335
<i>P</i> -value ⁴								
Rearing phase								
Feed form		0.234	0.307	0.157	0.995	0.885	0.780	0.429
Energy level		0.965	0.812	0.180	0.109	0.156	0.825	0.370
Laying phase								
Energy level		0.545	0.636	0.962	0.724	0.313	0.026	0.311

¹LE = 2,850, 2,700, and 2,650 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; ME = 2,950, 2,800, and 2,750 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; HE = 3,050, 2,900, and 2,850 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively.

²Low = 2,650 kcal AME_n/kg; High = 2,750 kcal AME_n/kg.

³30 replicates for feed for in the rearing phase, 20 replicates for energy level in the rearing phase, and 30 replicates for energy level in the laying phase.

⁴The interactions among main effects were not significant ($P > 0.05$).

^{a,b}Means with different superscript within each main effect are significantly different ($P < 0.05$).

Table 6. Effects of feed form and energy concentration of the rearing diets and energy concentration (AME_n/kg) of the laying hen diets on the relative length (cm/kg BW^1) of the gastrointestinal tract at 46 wk of age

	Duodenum	Jejunum	Ileum	SI ²	Cecum
Feed form, rearing phase					
Mash	14.0	41.8	38.4	94.2	24.4
Crumbles	13.8	42.0	37.8	93.6	23.8
Energy level, rearing phase ³					
LE	13.7	41.1	38.0	92.7	23.5
ME	14.5	42.8	38.5	95.8	24.5
HE	13.7	41.7	37.9	93.3	24.3
Energy level, laying phase ⁴					
Low	14.1	41.8	37.8	93.7	23.8
High	13.8	42.0	38.4	94.2	24.4
SD ⁵	1.79	3.42	2.68	6.69	2.91
P-value ⁶					
Rearing phase					
Feed form	0.654	0.822	0.405	0.729	0.408
Energy level	0.201	0.285	0.734	0.290	0.530
Laying phase					
Energy level	0.489	0.810	0.399	0.776	0.455

¹The BW of the hens is shown in Table 5.

²Small intestine (duodenum, jejunum and ileum).

³LE = 2,850, 2,700, and 2,650 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; ME = 2,950, 2,800, and 2,750 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; HE = 3,050, 2,900, and 2,850 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively.

⁴Low = 2,650 kcal AME_n/kg ; High = 2,750 kcal AME_n/kg .

⁵30 replicates for feed for in the rearing phase, 20 replicates for energy level in the rearing phase, and 30 replicates for energy level in the laying phase.

⁶The interactions among main effects were not significant ($P > 0.05$).

Table 7. Effects of feed form and energy content of the rearing diets and energy concentration (AME_n/kg) of the laying hen diets on body measurements at 46 wk of age

	Hen length (cm/BW ¹)	BMI ² (g/cm ²)	Tarsus (cm/BW)	
			Length	Diameter
Feed form, rearing phase				
Mash	36.0	0.43	5.54	0.71
Crumbles	35.6	0.44	5.46	0.72
Energy level, rearing phase ³				
LE	35.7	0.45	5.46	0.71
ME	35.8	0.43	5.46	0.71
HE	36.0	0.43	5.57	0.72
Energy level, laying phase ⁴				
Low	35.8	0.44	5.53	0.71
High	35.8	0.43	5.47	0.72
SD ⁵	1.68	0.022	0.291	0.022
<i>P</i> -value ⁶				
Rearing phase				
Feed form	0.347	0.193	0.353	0.339
Energy level	0.822	0.928	0.361	0.301
Laying phase				
Energy level	0.976	0.384	0.360	0.309

¹The BW of the hens is shown in Table 5.

²Body mass index.

³LE = 2,850, 2,700, and 2,650 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; ME = 2,950, 2,800, and 2,750 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively; HE = 3,050, 2,900, and 2,850 kcal AME_n/kg from 0 to 5 wk, 5 to 10 wk and 10 to 17 wk of age, respectively.

⁴Low = 2,650 kcal AME_n/kg; High = 2,750 kcal AME_n/kg.

⁵30 replicates for feed for in the rearing phase, 20 replicates for energy level in the rearing phase, and 30 replicates for energy level in the laying phase.

⁶The interactions among main effects were not significant ($P > 0.05$).

Table 8. Correlations between full BW¹ and body length, tarsus length and diameter and body mass index (BMI)² of the hens at 46 wk of age

	BW			
	Body length (cm)	BMI (g/cm ²)	Tarsus(cm)	
			Length	Diameter
r	0.394	0.637	0.414	0.351
P-value	< 0.001	< 0.001	< 0.001	< 0.001

¹The BW of the hens is shown in Table 5.

²Body length, BMI and tarsus length and diameter of the hens are shown in Table 7.

4. DISCUSSION

4.1. Hen performance

4.1.1. Influence of the characteristics of the rearingp phase pullet diets.

In general, pullets that are heavier at 17 wk of age anticipate sexual maturity and egg production and lay larger eggs than pullets that are lighter (Renema et al., 1999a; Pérez-Bonilla et al., 2012b). In this respect, Summers et al. (1987) and Renema et al. (1999b) reported a positive relation between BW of the hens and the maturation of the reproductive traits and the onset of egg production. In the current research, hens that received crumbles during the rearing phase were heavier at 17 wk of age and started egg production sooner (data not shown) and laid more eggs (0.913 vs. 0.898) that were heavier (56.2 vs. 55.4 g) from 17 to 22 wk of age than hens that were fed mash, although the differences did not reach significance. Moreover, no differences in egg production or egg weight among treatments were observed after this age. On the other hand, hens fed mash during the rearing phase were lighter at 17 wk of age than hens fed crumbles but they gained weight more rapidly and in fact, no differences in BW were detected at 22 wk of age. Consequently, the benefits of feeding crumbles to pullets on subsequent egg production might not be as relevant as expected and in most cases the potential benefits might be limited to the first few weeks of the laying period.

The authors have not found any published research on the effects of energy concentration of the pullet diets on subsequent hen performance. Usually, an increase in dietary energy results in heavier pullets that produce more egg mass and lay heavier eggs during the laying period (Pérez-Bonilla et al., 2012a). In the current research, BW at 17 wk of age was greater in pullets that were fed crumbles during the rearing phase than in pullets that were fed mash (1,554 vs. 1,432 g) but after 22 wk of age, BW was

not affected by the energy content of the rearing phase diets. The results reported herein indicate that a variation in the energy concentration of the rearing diets of ± 100 kcal AME_n/kg did not have any effect on subsequent hen performance. The data suggest that nutritionists might modify the energy level of the rearing phase diets, within the indicated limits, according to the relative prices of available ingredients.

4.1.2. Influence of the energy concentration of the laying hen phase diets.

From 17 to 46 wk of age, hens fed the high energy diet consumed 3.7% less feed and had 3.1% better FCR than hens fed the low energy diet but egg production was not affected, consistent with data of Grobas et al. (2001) and Safaa et al. (2009). The decrease in ADFI observed in hens fed the high energy diet was expected because poultry eat to satisfy their energy requirements, provided that the capacity of the GIT is not compromised (Hill et al., 1956; Leeson et al., 1997). Egg production and egg size were not affected by the energy content of the laying diet, consistent with data of Grobas et al. (1999b) in brown hens fed diets varying in AME_n from 2,680 to 2,810 kcal. In contrast, Mathlouthi et al. (2002a) reported higher egg mass production in hens fed diets with 2,750 kcal/AME than in hens fed diets with 2,650 kcal AME_n/kg. Similarly, Pérez-Bonilla et al. (2012a) reported higher egg production with increases in the AME_n content of the diet from 2,650 to 2,850 kcal/kg. The reason for the discrepancy among researches on the effects of energy concentration of the diet on egg production and egg mass, is not known but might be related to the strain of hens used as well as on the nutritional characteristics of the experimental diets. For example, in the experiment of Mathlouthi et al. (2002a) Single Comb White Leghorn hens were used and most of the difference in egg mass reported was caused by the lower weight of the eggs produced by hens fed the low energy diet (53.7 vs. 56.0 g). The authors did not give any reason for the difference in egg size observed but the LNA content of the low

and high energy diets used was 10.7 and 12.7 g/kg, respectively. The NRC (1994) recommended a minimum daily intake of 1.20 g LNA per day to maximize egg size in brown-egg laying hens. Probably, the low LNA intake was limiting egg mass production in hens fed the low energy diet, penalizing feed efficiency. In contrast, in the current research LNA intake was 2.94 and 2.15g/d for the high and low energy diets, respectively, values that were above requirements for both groups of hens (Grobas et al., 2001; Bouvarel et al., 2010). Also, in the research of Pérez-Bonilla et al. (2012a), in which egg production increased with increases in the energy content of the diet, an increase in energy concentration of 7.5% (2,650 to 2,850 kcal AME_n/kg) decreased ADFI by 3.5%, which resulted in a 3.9% increase in EI and in a subsequent improvement in egg production. In the current experiment, however, an increase of 100 kcal AME_n/kg diet (a 3.8% increase) resulted in a similar increase in EI and consequently, no changes in hen performance between the two groups of hens were expected.

An increase in dietary energy is generally attained by increasing the amount of supplemental fat. Mateos and Sell (1980, 1981b) showed that supplemental fat reduced the rate of passage of the digesta, facilitating the contact between nutrients and enzymes and improving the utilization of the non-fiber components of the diet. Moreover, when the lipid content of the diet increases, the level of LNA content also increases. Both effects, reduced rate of passage and increased LNA content, might result in an increase in egg size and an improvement in FCR (Grobas et al., 1999a). In the current experiment, the low and high energy diets contained 54 and 68 g/kg ether extract and 19 and 27 g/kg LNA, respectively. Probably, the small difference in ether extract content of the 2 diets did not result in any visible effect on egg weight. On the other hand, the LNA content of the low energy diet (1.9%) met already the requirement of the hens to

maximize egg weight (Grobas et al., 1999c; Safaa et al., 2009). The data suggest that laying hens, in the range of energy concentration studied (2,650 to 2,750 kcal AME_n/kg), and provided that the requirements in other essential nutrients (indispensable amino acids, LNA, and fat content) are satisfied, regulate ADFI precisely according to energy requirements.

4.2. Egg quality

Egg quality was not affected by feed form of the rearing phase diets or by the energy concentration of the rearing and laying phase diets. The authors have not found any published research on the effects of the characteristics of the rearing diets on egg quality to compare with the results reported herein. In regard to dietary energy, Grobas et al. (1999b) and Pérez-Bonilla et al. (2012a) observed similar egg quality (dirty, broken, shell-less, Haugh units, yolk pigmentation and yolk to albumen ratio) for hens fed diets containing 2,650 or 2,750 kcal AME_n/kg, in agreement with the results reported herein. However, Zimmermann and Andrews (1987) and Wu et al. (2005) observed a reduction in albumen quality and Junqueira et al. (2006) a decrease in the percentage of shell-less eggs with increases in the energy concentration of the diet. Factors such as strain and age of the hens, as well as the ingredient composition of the diet, might affect the response of egg quality traits to variations in energy content of the diet. For example, Zimmermann and Andrews (1987) and Junqueira et al. (2006) used Leghorn hens in the second cycle of egg production and Wu et al. (2005) reported that the decrease in AME_n of the diet reduced yolk size, which in turn could affect egg size and other egg quality traits.

4.3. Digestive traits and body measurements

The interest on the effects of the ingredient composition and physico-chemical characteristics of the diets on the anatomy and physiology of the GIT in poultry has increased in the last decade but most available data have been obtained in broilers (Mateos et al., 2002, 2012; Serrano et al., 2012; Jiménez-Moreno et al., 2016) and pullets (Saldaña et al., 2015b), with little information in laying hens. In fact, we have not found any published report on the effects of feed form and energy content of the rearing phase diets on digestive tract traits of the hens at the end of the laying period. In the current research, neither feed form nor energy content of the rearing phase diets affected the development and function of the different organs of the GIT of the hens at 46 wk of age. Moreover, the energy content of the layer diet had little effect on the development of the GIT at this age. In fact, the only difference observed was for gizzard digesta content that was greater in hens fed the low energy diet. In this respect, FI was higher in hens fed the low energy diet than in hens fed the high energy diets and consequently, an increase in the digesta content of the gizzard was expected. In addition, fiber content was higher in the low- than in the high energy diet, which may result in an increase in digesta content, as has been demonstrated in broilers by González-Alvarado et al. (2008). The results show that feed form and energy concentration of the rearing phase diets and energy concentration of the laying phase diets, had little effect on subsequent hen performance or on the characteristics of the digestive tract at the end of the laying cycle.

Body length, BMI and tarsus measurements are used to evaluate body composition and size and to predict future performance of broilers (Mendes et al., 2007; Van Roover-Reijrink, 2013), pullets (Itza et al., 2011; Guzmán et al., 2015b) and laying hens (Ojedapo et al., 2012; Guzmán et al., 2016). Body length, BMI and length and

diameter of the tarsus were not affected by dietary treatment, indicating that neither the characteristics of the rearing phase diets nor the energy content of the laying phase diets affected body size of the mature hens. However, a significant relationship between BW of the hens and body length, BMI and tarsus length and diameter was detected, consistent with the results of Guzmán et al. (2015b).

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

1. GENERAL DISCUSSION

Proper management, adequate feeding program, and sound nutrition practices during the rearing phase are key factors to improve future egg production and egg quality in laying hen operations. High peak production and persistency of the egg cycle, together with a high proportion of large eggs, correlate well with BW and uniformity of the pullets at the end of the rearing period (Summers and Leeson, 1994). Nutrition of the pullets and feeding practices plays important roles for reaching these objectives. However, the information available on the influence of feed form, energy concentration, and main cereal of the diet on growth performance and GIT development of the pullets is very limited.

1.1. Feed form

1.1.1. Effect of feed form on bird performance

The beneficial effects of feeding crumbles or pellets on growth performance are well documented in broilers (Abdollahi et al., 2013b; Jiménez-Moreno et al., 2016) but the information available in pullets is limited. In experiments 1 and 2, pullets fed crumbles from hatch to 5 wk of age, had lower ADFI than pullets fed mash, but an opposite effect was observed from 5 to 17 wk of age. However, ADG was greater and FCR better in pullets fed crumbles in both experiments. The reason for the contrasting effects on ADFI between crumbles and mash diets in these 2 periods is not known. Guzmán et al. (2015a) reported also 6.5% greater ADG, 2.0% lower ADFI, and 8.2% better FCR in pullets from 0 to 5 wk of age with crumbles than with mash. In the research reported herein, feed wastage could not be measured and consequently, feed refusal was considered a part of voluntary FI. Possibly, during the early stages of life,

feed wastage was higher in pullets fed mash than in pullets fed crumbles, but not thereafter, as has been reported in broilers by Serrano et al. (2013) and Abdollahi et al. (2013a). This suggestion is consistent with the greater BW and better FCR recorded in the starter period for pullets fed crumbles as compared with pullets fed mash, in spite of the reduced “apparent” ADFI.

In experiment 1, pullets fed crumbles showed 6.4% greater ADFI, 9.1% higher ADG, and 2.4% better FCR than pullets fed mash from hatching to 17 wk of age. In experiment 2, the advantages of feeding crumbles compared to mash were 7.0% for ADFI, 3.0% for ADG and 3.4% for FCR. Poultry require more time and spend more energy in the procurement of feed when fed mash than when fed crumbles (Jensen et al., 1962; Savory and Hetherington, 1997). Consequently, pullets fed mash might consume less feed and grow less than pullets fed crumbles. In addition, the heat and pressure applied during the pelleting process modifies the structure of the starch, protein, and fiber fractions of the feed (Gracia et al., 2009; Zimonja and Svihus, 2009), increasing energy (Jiménez-Moreno et al., 2009b) and CP (Lacassagne et al., 1988) digestibility. Moreover, feed particle size is reduced with pelleting which causes greater disruption on the outer coat of the seed and fracture the endosperm of the grain, facilitating the access of digestive enzymes to nutrients (Calet, 1965; Mateos et al., 2002; García-Rebollar et al., 2016) and releasing the intracellular oil contained in the spherosomes of the corn and soybeans, and improving FCR. On the other hand, crumbling reduces the particle size of the feed (Guillou and Landeau, 2000; Amerah et al., 2008a) and a reduction in size might increase rate of feed passage through the upper part of the GIT and increase voluntary FI (Hamm and Stephenson, 1959; Mateos et al., 2012). Moreover, crumbling improves the texture and density of the feed, which might increase voluntary FI and reduce feed wastage. The data on growth and feed efficiency reported

are consistent with most research published comparing feed forms in broilers (Serrano et al., 2012; Abdollahi et al., 2013a; Jiménez-Moreno et al., 2016) and pullets (Frikha et al., 2009b; Guzmán et al., 2015a).

The differences in growth reported in the literature between birds fed crumbles and mash diets are of a different magnitude in broilers than in pullets. In this respect, Gous and Morris (2001) reported that pullets fed crumbles from 0 to 4 wk of age and then pellets from 5 to 20 wk, had 6.3% higher ADG and 7.5% better FCR from 0 to 20 wk of age than pullets fed mash continuously. Guzmán et al. (2015a) reported also 6.6% higher ADG and 9.7% better FCR in pullets from 0 to 5 wk of age when fed crumbles than when fed mash. In broilers, Serrano et al. (2013) reported 17.2% greater ADG and 4.8% better FCR when fed crumbles from 1 to 25 d of age than when fed mash. Also, Jiménez-Moreno et al. (2016) observed 32.6% greater ADG and 2.9% better FCR with pellets than with mash feeds in broilers from 1 to 21 d of age. Similar improvements in ADG and FCR with crumbles have been reported by Cerrate et al. (2009). Broilers are more voracious than pullets and therefore, a reduction in particle size, as occurs when the feed is pelleted, may result in greater increases in voluntary FI and growth compared to pullets.

In experiment 1, BW uniformity was consequently better in pullets fed crumbles than in pullets fed mash, with differences that were significant at 10 and 17 wk of age. However, in experiment 2, uniformity was not affected by feed form of the diets in this period. Although, at 5 wk of age, pullets fed crumbles tended ($P = 0.06$) to be more uniform than pullets fed mash. These results agree with data of Guzmán et al. (2015a) who reported a trend for better BW uniformity in pullets fed crumbles at 5 wk of age. Similarly, Brickett et al. (2007) reported an improvement in BW uniformity of 35-day-

old broilers with pellet feeding. Frikha et al. (2009a), however, did not detect any effect of feed form (2 mm Ø pellets vs. mash diets) on BW uniformity at 6 wk of age.

In experiment 2, in which the birds were changed from crumbles to mash feeding at different ages, ADFI was reduced any time pullets were switched from crumbles to mash. In fact, pullets fed crumbles from 0 to 5 wk or from 0 to 10 wk of age had similar ADFI, ADG, and FCR in the following feeding period (5 to 10 wk or 10 to 17 wk of age, respectively) than pullets fed mash continuously. Consequently, from hatch to 17 wk of age, FCR was better in pullets fed crumbles continuously than in pullets fed mash continuously, with pullets switched from crumbles to mash feeds at 5 or 10 wk of age showing intermediate results. In experiment 3, birds were fed mash or crumbles during the rearing period followed by mash during the laying period. Pullets fed crumbles during the rearing phase were heavier at 17 wk of age, started egg production sooner, and laid 1.7% more eggs that were 1.4% heavier from 17 to 22 wk of age than pullets that were fed mash, although the differences did not reach significance. In general, pullets that are heavier at the start of the laying cycle anticipate sexual maturity and egg production and lay larger eggs than pullets that are lighter (Renema et al., 1999a; Pérez-Bonilla et al., 2012b). In this respect, Summers et al. (1987) and Renema et al. (1999b) reported a positive relation between BW of the hens and the maturation of the reproductive traits and the onset of egg production. However, no differences in egg production or egg weight among treatments were observed after this first period of the laying cycle. On the other hand, hens fed mash during the rearing phase were lighter at 17 wk of age than hens fed crumbles but gained weight more rapidly and in fact, no differences in BW were detected at 22 wk of age. The data reported herein indicate that the growth pattern of pullets and hens re-adapts quickly to changes in feed form, as has been suggested in broilers by Mateos et al. (2012) and

Svihus (2014). Consequently, the potential advantages of feeding crumbles to pullets on subsequent egg production might not be as relevant as expected and in most cases the benefits might be limited to the first few weeks of the laying period.

1.1.2. Effect of feed form on the development of the gastrointestinal tract

The interest to study the effects of ingredient composition and physico-chemical characteristics of the diets on the anatomy and physiology of the GIT in poultry has increased in the last decade but most research has been conducted with broilers (Mateos et al., 2002, 2012; Serrano et al., 2012; Jiménez-Moreno et al., 2016) with little information available for pullets (Frikha et al., 2009b) and laying hens.

In experiments 1 and 2, feed form affected the development of the segments of the GIT in a similar way. Pullets fed crumbles had lighter gizzards and GIT than pullets fed mash, consistent with data of Choi et al. (1986) and Nir et al. (1994a, b) in broilers. The reduction in gizzard weight with pellets has been well documented in broilers (Nir et al., 1994b; Amerah et al., 2007b; Abdollahi et al., 2011). Frikha et al. (2009a) reported that feeding pellets reduced the RW of the GIT and gizzard in pullets at 6 wk of age compared with feeding mash, data that agree with the results reported herein. Pelleting reduces feed particle size and increases transit time throughout the upper part of the GIT, which may reduce the development of the muscular layers of the gizzard and its content compared with mash feeding (Mateos et al., 2012; Mateos et al., 2014). Crumbling increased gizzard pH at all ages in the two experiments, results that agree with the data of Frikha et al. (2009b) in pullets and Engberg et al. (2002) and Serrano et al. (2013) in broilers. In contrast, Nir et al. (1995) and Dahlke et al. (2003) did not observe any difference in gizzard pH in broilers fed pellets or mash diets, although in this research sorghum was used as the main cereal of the diet. Also, the proventriculus

was heavier in pullets fed crumbles than in pullets fed mash in both experiments, suggesting a hypertrophy of this organ (Mateos et al., 2012). In this respect, Svihus (2011) indicated that crumbles increases digesta flow to the proventriculus and suggested that an excess of digesta could result in an increase in the incidence of ruptures of the walls of the proventriculus carcasses contamination at the slaughterhouse. Also, in both experiments, feeding crumbles decreased the RL of the SI and ceca, consistent with data of Frikha et al. (2009a,b) in 6 wk-old pullets. In broilers, Nir et al. (1994a) reported also that pelleting reduced the RL of the jejunum and ileum, in agreement with the results of the current research. Moreover, Amerah et al. (2007a) and Abdollahi et al. (2011) reported shorter SI and ceca in 3 wk-old broilers fed pellets compared to broilers fed mash, in agreement with the results reported herein.

In experiment 2, body length and tarsus length were used as indirect measurements to predict chick growth (Senar and Pascual, 1997; Mendes et al., 2008). Pullets fed mash were longer and had longer and wider tarsus than pullets fed crumbles. The authors have not found any research on the effects of feed form on these variables to compare with the results reported herein for pullets. Zuidhof (2005) indicated that because of differences in the allometric growth of tissues and body organs, carcass yield increases as the BW of the bird increases. Interestingly, and in contrast with the values reported in relative terms, body length and tarsus length were higher in absolute values in pullets fed crumbles than in pullets fed mash. Consequently, data on weight and length of the different organs of the bird, as a consequence of feeding crumbles or mash, must be taken with caution. Pullets fed crumbles continuously were shorter and had shorter tarsus than pullets fed mash continuously at all ages.

The size of the organs of the GIT of the pullets readapted quickly any time the form of the feed was switched from crumbles to mash, as shown in experiments 2 and 3.

For example, in experiment 2 pullets that were switched from crumble to mash diets at 5 wk had similar RW of the GIT at 10 and 17 wk of age compared to pullets that were fed mash continuously. A similar re-adaptation pattern was observed for all the other variables studied (digesta content, weight, and pH of the gizzard and RL of the SI and ceca) at 17 wk of age when the pullets were switched from crumbles to mash at 10 wk. Similarly, in experiment 3, feed form of the rearing phase diets did not affect the development and function of the different organs of the GIT of the hens at 46 wk of age. As reported for the GIT, body parameters also re-adapted quickly any time feed form was switched from crumbles to mash in experiment 2. At 10 wk of age, body and tarsus length were similar for pullets that were fed mash from 5 to 10 wk of age, irrespective of the form of the feed supplied from 0 to 5 wk of age. We have not found any report on the effects of changing feed form from crumbles to mash with time on the development of the different organs of the GIT or body traits in any poultry species. The data reported herein indicate that the GIT of the pullets and hens responds quickly to changes in diet form, suggesting that the digestive tract re-adapts quickly as needed to maintain optimal bird performance (Svihus, 2014).

1.2. Energy concentration

1.2.1. Effect of energy concentration of the diet on bird performance

The effects of energy concentration of the diet on pullet performance and subsequent laying hen production was studied in two experiments. In experiment 1, ADFI from hatching to 17 wk of age decreased with increases in the energy content of the diet but EI was not affected. Similarly in experiment 3, conducted with laying hens from 17 to 46 wk of age, hens fed the high energy diet consumed 3.7% less feed and had 3.1% better FCR than hens fed the low energy diet but egg production was not affected. Similar data were reported by Grobas et al. (2001) and Safaa et al. (2009). The decrease in ADFI observed in pullets and hens fed the high energy diet was expected because birds regulate voluntary FI to satisfy their energy requirements. Consequently, ADFI decreased and FCR improved as the energy concentration of the diet increased (Hill et al., 1956; Leeson et al., 1997).

The authors have not found any published research on the effects of energy concentration of the pullet rearing diets on subsequent hen performance. Usually, an increase in dietary energy results in heavier pullets at the start of the laying period. Consequently, these hens produce more egg mass and lay heavier eggs during the laying period (Pérez-Bonilla et al., 2012a). In experiment 3, BW of the pullets at 17 wk of age was not affected by the energy concentration of the rearing diets and consequently, once the hens received a common diet, no differences in BW of the hens were observed.

In experiment 3, egg production and egg size were not affected by the energy content of the layer diet, consistent with data of Grobas et al. (1999b) in brown hens fed diets varying in AME_n from 2,680 to 2,810 kcal. In contrast, Mathlouthi et al. (2002a) reported higher egg mass production in hens fed diets with 2,750 kcal/AME than in

hens fed diets with 2,650 kcal AME_n/kg. Similarly, Pérez-Bonilla et al. (2012a) observed higher egg production when the AME_n content of the diet increased from 2,650 to 2,850 kcal/kg. The reason for the discrepancy among those researches on the effects of energy concentration of the diet on egg production and egg mass is not known but might be related to the strain of hens used as well as on the nutritional characteristics of the experimental diets. For example, in the experiment of Mathlouthi et al. (2002a) Single Comb White Leghorn hens were used and most of the difference in egg mass reported was caused by the lower weight of the eggs produced by hens fed the low energy diet (53.7 vs. 56.0 g). The authors did not give any reason for the difference in egg size observed but the LNA content of the low and high energy diets used was 10.7 and 12.7 g/kg, respectively. The NRC (1994) recommended a minimum daily intake of 1.20 g LNA per day to maximize egg size in brown-egg laying hens. Probably, the low LNA intake of the hens limited egg mass production in those birds fed the low energy diet, penalizing feed efficiency per kg of eggs. In contrast, in experiment 3 of the current thesis, LNA intake was 2.94 and 2.15 g/d for the high and low energy diets respectively, values that were above requirements for both groups of hens (Grobass et al., 2001; Bouvarel et al., 2010). In the research of Pérez-Bonilla et al. (2012a), in which egg production increased with increases in the energy content of the diet, an increase in energy concentration of 7.5% (2,650 to 2,850 kcal AME_n/kg) decreased ADFI by 3.5%, which resulted in a 3.9% improvement in EI and in a subsequent improvement in egg production. In the current experiment, however, an increase of 100 kcal AME_n/kg diet (a 3.8% increase) resulted in a similar increase in EI and consequently, no changes in hen performance between the two groups of hens were expected.

An increase in dietary energy is generally attained by increasing the amount of supplemental fat. Mateos and Sell (1980, 1981b) showed that supplemental fat reduced

the rate of passage of the digesta through the GIT, facilitating the contact between nutrients and enzymes and improving the utilization of the non-fiber components of the diet. Moreover, when the lipid content of the diet increases, the level of LNA content also increases. Both effects, reduced rate of passage and increased LNA content, might result in an increase in egg size and an improvement in FCR (Grobas et al., 1999a). In experiment 3, the low and high energy diets contained 54 and 68 g/kg ether extract and 19 and 27 g/kg LNA, respectively. Probably, the small difference in ether extract content of the 2 diets did not result in any visible effect on egg weight. On the other hand, the LNA content of the low energy diet (1.9%) was enough to meet the requirement of the hens to maximize egg weight (Grobas et al., 1999c; Safaa et al., 2009). The data suggest that laying hens, in the range of energy concentration studied (2,650 to 2,750 kcal AME_n/kg), and provided that the requirements in other essential nutrients (indispensable amino acids, LNA, and fat content) are satisfied, regulate ADFI precisely according to energy requirements.

Egg quality was not affected by the energy concentration of the rearing or the laying phase diets. The authors have not found any published research on the effects of the energy content of the rearing diets on egg quality to compare with the results reported herein. Grobas et al. (1999b) and Pérez-Bonilla et al. (2012a) observed similar egg quality for hens fed diets containing 2,650 or 2,750 kcal AME_n/kg, in agreement with the results reported herein. However, Zimmermann and Andrews (1987) observed a reduction in albumen quality and Junqueira et al. (2006) a decrease in the percentage of shell-less eggs with increases in the energy concentration of the diet. Factors such as strain and age of the hens, as well as the ingredient composition of the diet, might affect the response of egg quality traits to variations in energy content of the diet. For

example, Zimmermann and Andrews (1987) and Junqueira et al. (2006) used Leghorn hens in the second cycle of egg production.

The results reported herein indicate that a variation in the energy concentration of the rearing diets of ± 50 kcal AME_n/kg did not have any effect on subsequent hen performance. Also, a variation in the energy concentration of the laying diets of ± 100 kcal AME_n/kg did not affect hen performance. The data suggest that nutritionists might modify the energy level of the diets, within the indicated limits, according to the relative prices of available ingredients.

1.2.2. Effect of energy concentration of the diet on the development of the gastrointestinal tract

The energy content of the rearing or of the layer diet had little effect on the development and function of the GIT of the pullets at 17 wk (experiment 1) or of the hens at 46 wk of age (experiment 3). In fact, the only variable affected by the energy content of the layer diet was the digesta content of the gizzard that was greater in hens fed the low energy diet. In this respect, FI was higher in hens fed the low energy diet than in hens fed the high energy diets and consequently, an increase in the digesta content of the gizzard was expected. In addition, fiber content was higher in the low- than in the high energy diet, which may result in an increase in digesta content, as has been demonstrated in broilers by González-Alvarado et al. (2008). However, Frikha et al. (2009a) observed that an increase in the AMEn concentration of the diet from 2,880 to 3,025 kcal/kg reduced gizzard weight of the pullets at 45 d of age. The reason for the discrepancy among researches is not known but differences in diet composition (level of fat vs. level of starch and fiber) and on the capacity for FI between pullets and broilers, might explain the lack of agreement reported. High-energy diets contain less fiber than low-energy diets and a reduction in fiber content might reduce digestive organ size

(Hetland and Svihus, 2001). Probably, the differences in insoluble fiber content among the diets used in the current research were too small to produce any marked difference in digestive organ size of the birds.

1.3. Main cereal

1.3.1. Effect of the main cereal of the diet on bird performance

The effects of the main cereal of the diet on bird performance was studied in experiment 2. In this experiment, FI was reduced and FCR was improved from 0 to 17 wk of age in pullets fed wheat as compared with pullets fed corn but ADG and BW uniformity were not affected. Published data comparing diets based on wheat or corn in poultry are conflicting, with reports showing better (Frikha et al., 2009b; Kiarie et al., 2014), similar (Frikha et al., 2011; Pérez-Bonilla et al., 2011), or reduced (Mathlouthi et al., 2002b; Amerah et al., 2008b; Abdollahi et al., 2013b) performance with wheat. The reasons for the discrepancies are unknown but might be related to the variability in the physico-chemical characteristics of the cereals (Gutiérrez-Alamo et al., 2008; Pérez-Bonilla et al., 2011). In many countries, wheat is produced in low rain, non-irrigated areas and consequently, its chemical composition and nutritive values vary depending on climate conditions. In addition, wheat contains a high and variable amount of pentosans and β -glucans, which increase digesta viscosity and might reduce productive performance in poultry (Lázaro et al., 2003a; Mirzaie et al., 2012). Enzyme supplementation might reduce digesta viscosity caused by the presence of NSP and improve nutrient digestibility in birds fed diets with a high wheat content (Lázaro et al., 2003b, 2004; García et al., 2008). Consequently, differences in performance between birds fed diets based on corn or wheat might be reduced, or even disappear, when the feeds are supplemented with adequate enzymes. Moreover, in the current research, enzyme supplementation was expected to increase the AMEn content of the wheat by

2% (FEDNA, 2010). It is probable that the enzymes used in the current research increased the utilization of the energy more than expected (Lázaro et al., 2003a). Consequently, the effects of the main cereal of the diet on ADFI and FCR will depend on the source and characteristics of the particular batch of grain, as well as the type of NSP enzyme complex used.

1.3.2. Effect of the main cereal of the diet on the development of the gastrointestinal tract

The information available on the effects of the main cereal of the diet on GIT and gizzard weights in pullets is very scarce. In experiment 2, pullets fed corn had heavier GIT and gizzard at 17 wk of age than pullets fed wheat. Similar results were observed at 5 and 10 wk but at these ages the differences observed were not significant in most cases. Frikha et al. (2009b) reported also heavier gizzards at 6 wk of age in pullets fed corn than in pullets fed wheat but GIT weight was not affected. Similar differences between diets based on corn or wheat were reported by Nir et al. (1994a) with mash diets and by Amerah et al. (2008) with pelleted diets. Abdollahi et al. (2013b) compared corn and wheat diets fed as mash or pellets and reported heavier gizzards at 3 wk of age for the corn in both cases. The heavier GIT and gizzards in broilers fed corn compared with broilers fed wheat might respond to differences in the physico-chemical characteristics of the two cereals. In this respect, Dombrink-Kurtzman and Bietz (1993) and Dobraszczyk et al. (2002) reported that the endosperm of dent corn is harder and more difficult to grind than the endosperm of soft wheat. Consequently, corn particles might stay for longer in the gizzard than wheat particles, resulting in greater development and larger size of the muscular layers (Jiménez-Moreno et al., 2010; Svihus, 2011; Mateos et al., 2012). In fact, in the current research, gizzard digesta content was higher in birds fed corn than in birds fed wheat at all ages

(although only at 17 wk of age the differences reported were significant), data that are consistent with the harder endosperm of the corn. In addition, the proportion of coarse particles ($> 2,500\ \mu\text{m}$) was higher for the corn than for the wheat diets, which may also increase the weight and contents of the gizzard (Hetland et al., 2002; Svihus and Hetland, 2002). Gizzard pH, however, was not affected by the main cereal of the diet at any age, in agreement with data of Nir et al. (1994a) in 3-wk-old broilers fed mash diets.

Type of cereal did not affect the RL of the SI or the ceca at any age, consistent with data of Frikha et al. (2009b) in 6-wk-old pullets. In contrast, Abdollahi et al. (2013b) reported that the length of the SI and ceca of 3-week-old broilers increased with wheat feeding. The reasons for the discrepancies are not known but might be related to the physico-chemical characteristics of the grains used by the authors. In this respect, Amerah et al. (2008b) reported longer SI and ceca in 3-wk-old broilers fed corn when the diets were finely ground whereas no differences were detected when the diets were coarsely ground.

The RL of the pullets decreased with age and 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 pullet or tarsus length to compare with the results reported herein.

2. CONCLUSIONS

The general conclusions of the present thesis are:

- Feeding crumbles to pullets increased ADFI and ADG and improved FCR and BW uniformity from 0 to 17 wk of age.
- Compared to mash diets feeding crumbles reduced the relative weight of the different organs of the GIT and increased gizzard pH at all ages.
- Pullets that were changed from crumble to mash feeding responded always in the subsequent period, independent of age, with a reduction in growth performance.
- When pullets were changed from crumble to mash diet, independent of age, the RW of the GIT, included the gizzard, was increased.
- An increase in the energy content of the diet 1) decreased ADFI and improved FCR but did not affect BW uniformity.
- GIT development was not affected by the energy content of the diet.
- The main cereal (wheat vs. corn) of the diet did not affect ADG of the pullet from hatch to 17 wk of age and in fact, wheat feeding improved FCR.
- Pullets fed wheat had lighter GIT and gizzard and less gizzard contents than pullets fed corn.

Based on the results of the current research, the following nutritional strategies are recommended for commercial pullets for egg production:

- Feed diets in crumble form when the main objective is to improve growth rate and BW uniformity of the pullets. Feeding mash diets might

be more beneficial when the main objective is to improve GIT development and function of the pullets.

- The effects of feed form of the diet on subsequent phase, including egg production might be less relevant than currently accepted.
- The energy content of the diet either for pullets or laying hens might be modified within a wide range of values, depending on the relative cost of available ingredients.
- At least 40% wheat can be used in substitution of corn in pullet diets supplemented with adequate NSP enzymes.

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