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INFLUENCE OF SOME NUTRITIONAL FACTORS ON PRODUCTIVE PERFORMANCE AND DIGESTIVE TRACT TRAITS IN COMMERCIAL BROWN-EGG LAYING PULLETS

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بسم الله الرحمن الرحيم

وقل اعملوا
فسيري الله عملكم
ورسوله والمؤمنون
وستردون إلى عالم
الغيب والشهادة
فينبئكم بما كنتم
تعملون

(أية 105 سورة التوبة)

صدق الله العظيم

He aprendido que la vida es dura,
pero yo lo soy más!

He aprendido que las oportunidades no han de perderse nunca.
Las que dejas marchar, no vuelven.

He aprendido que cuando siembras rencor y amargura
la felicidad se va a otra parte.

He aprendido que siempre has de usar palabras buenas,
porque de las malas quizás mañana te tengas que desdecir.

He aprendido que una sonrisa es un método económico
de mejorar tu vida.

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pero siempre puedo hacer algo por sentirme mejor.

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y no pensar sólo en la meta.

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ABBREVIATION KEYS

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ºC: degree Celsius

*: $P<0.05$

**: $P<0.01$

***: $P<0.001$

%: percentage

µm: micro meter

ADFI: average daily feed intake

AMEn: apparent metabolizable energy, nitrogen-corrected

BW: body weight

BWG: body weight gain

Ca: calcium

cm: centimeter

CP: crude protein

Cys: cysteine

DM: dry mater

d: day

EE: ether extract

FCR: feed conversion ratio

GIT: gastro intestinal tract

GMD: geometric mean diameter

GSD: geometric standard deviation

GLM: general lineal model

g: gram

h: hour

HIG: high

HCl: hydrochloridric acid

IU: international unit

kg: kilogram

LOW: low

Lys: lysine

m: meter

mm: millimeter

MET: methionine (Met)

MED: medium

mg: miligram

min: minute

MJ: mega joule

mL: milliliter

MPS: mean particle size

N: nitrogen

N: normality

n: number of replicates per treatment

NS: not significant difference (P>0.10)

P: probability

P: phosphorus

ppm: parts per million

RL: relative length (cm/kg BW)

RW: relative weight (g/kg BW)

s: second

SI: small intestine

SD: standard deviation

SCWL: single comb white leghorn

SEM: standard error of the mean

Thr: threonine

Trp: tryptophan

vs.: versus

wk: week

CHAPTER 1: ABSTRACT

ABSTRACT

The general objective of this Master Thesis was to study the influence of some nutritional factors that might affect the productive performance and the development of the gastro intestinal tract (GIT) of commercial brown egg-laying pullets from 1 to 120 d of age. In this respect, the influence of cereal type, energy level, and feed form of the diet were studied.

In experiment 1, the influence of the main cereal and feed form of the diet on performance and digestive tract traits was studied in 576 brown-egg laying pullets from 1 to 120 d of age. From 1 to 45 d of age, 4 diets arranged factorially with 2 cereals (corn vs. wheat) and 2 feed forms (mash vs. pellets) were used. Each treatment was replicated 6 times (24 pullets per replicate). From 46 to 120 d of age all diets were offered in mash form and therefore, the only difference among diets was the cereal used. Cumulatively, pullets fed the corn diets had higher body weight (BW) gain ($P < 0.05$) but similar feed conversion ratio (FCR) than pullets fed the wheat diets. From 1 to 45 d of age, pullets fed pellets consumed more feed ($P < 0.001$) and had higher BW gain ($P < 0.001$) than those fed mash. Most of the beneficial effects of pelleting on productive performance were still evident at 120 d of age. At 45 d of age, gizzard relative weight (RW; g/kg BW) was higher ($P < 0.01$) in pullets fed corn than in pullets fed wheat diets. Feeding pellets reduced the RW of the digestive tract and the gizzard ($P < 0.001$) as well as the relative length (RL; cm/kg BW) of the small intestine ($P < 0.01$) at both ages. The pH of gizzard contents at 120 d of age was not affected by cereal but was lower in pullets that were fed mash from 1 to 45 d of age ($P < 0.01$).We conclude that wheat can be used in substitution of corn in pullet diets with only a slight reduction in BW gain and that

feeding pellets from 1 to 45 d of age increased BW gain and pH of the gizzard, and reduced the RW of the gizzard and the RL of the GIT at 120 d of age.

In experiment 2, a total of 1,152 one-day-old Hy-Line Brown pullets were used to study the influence of the energy content of the diet and feed form on productive performance and several digestive tract traits. From 1 to 45 d of age, there were 6 diets arranged factorially with 3 concentrations of Apparent Metabolizable Energy nitrogen corrected (AMEn; low: 11.66, medium: 12.05 and high: 12.66 MJ/kg of diet) and 2 feed forms (mash vs. pellets). From 45 to 120 d all diets were fed in mash form and therefore, the only difference among treatments was the energy content of the diets. Each of the 6 treatments was replicated 8 times and the experimental unit was formed by 24 pullets. Cumulatively, BW gain and FCR improved as the AMEn of the diet increased ($P < 0.001$). Pullets fed pellets from 1 to 45 d of age had higher feed intake and BW gain ($P < 0.001$) in this period and higher BW gain ($P < 0.01$) cumulatively, than pullets fed mash. At 45 d of age, the relative weight (RW; g/kg BW) of all the segments of the GIT was lower for pullets fed the high- than for pullets fed the medium- or low- energy diets. At 120 d of age the RW of the gizzard was higher ($P < 0.01$) for pullets fed the low energy diets than for pullets fed the other diets. The RL of the GIT was not affected by the energy content of the diet. Feeding pellets reduced the RW of the proventriculus ($P < 0.05$), the gizzard ($P < 0.001$), and the GIT ($P < 0.001$), and the RL of the small intestine ($P < 0.05$) and the ceaca ($P < 0.001$) at 45 d of age. The effects of feeding pellets on RW of gizzard and proventriculus were still evident at 120 d of age. We concluded that feeding pellets from 1 to 45 d of age improved feed intake and BW of pullets at 120 d of age and that an increase in the energy content of the diet

increased pullet performance at all ages but reduced the RW of the proventriculus and gizzard.

We conclude that corn and wheat can be used indistinctly in diets for pullets from 1 to 120 d of age with only a slight reduction in BW when wheat is used. Pelleting of the feeds from 1 to 45 d of age and the use of high density energy diets are recommended in order to achieve adequate target BW at 120 d of age. However, pelleting of the feed and the use of high AMEn diets might jeopardize the development of the GIT especially that of the gizzard, which might affect feed intake of laying hens especially early in the production cycle.

RESUMEN

RESUMEN

El objetivo general de esta Tesis de Máster fue estudiar la influencia de diversos factores nutricionales que afectan a los parámetros productivos y al desarrollo del tracto digestivo de pollitas rubias para producción de huevos comerciales. Para alcanzar estos objetivos se realizaron dos experimentos donde se estudiaron el cereal utilizado, el nivel de energía de los piensos y la presentación del mismo.

En el experimento 1 se estudió la influencia del cereal y la presentación del pienso sobre los parámetros productivos y las características del tracto digestivo en 576 pollitas rubias de 1 a 120 d de edad. De 1 a 45 d de la edad, se utilizaron 4 piensos experimentales organizados de forma factorial con 2 cereales (maíz vs. trigo) y 2 presentaciones del pienso (harina vs. gránulo). Cada tratamiento se replicó 6 veces (24 pollitas por réplica). De 46 a 120 d de edad todas las dietas se ofrecieron en harina. Por tanto, la única diferencia entre tratamientos en esta fase de la cría fue el cereal utilizado. De 1 a 120 d de edad, las pollitas que recibieron los piensos basados en maíz tuvieron una ganancia de peso vivo (PV) superior ($P < 0,05$) que las pollitas que recibieron los piensos basados en trigo pero el índice de conversión fue similar. De 1 a 45 d de edad, las pollitas alimentadas con piensos en gránulo consumieron más alimento ($P < 0,001$) y tuvieron una ganancia de peso superior ($P < 0,001$) que las pollitas alimentadas con piensos en harina. La mayor parte de los efectos beneficiosos de la granulación sobre los parámetros productivos fueron todavía evidentes a 120 d de edad. A los 45 d de edad, el peso relativo de la molleja (PR; g/kg PV) fue superior ($P < 0,01$) en pollitas alimentadas con piensos en base a maíz que en pollitas alimentadas con piensos en base a trigo. La alimentación en gránulo redujo el PR del tracto gastro intestinal y de la

molleja (*P* < 0,001), así como la longitud relativa (LR; cm/kg PV) del intestino delgado (*P* < 0.01) a ambas edades. El pH del contenido de la molleja a 120 d de edad no se vio afectado por el tipo de cereal utilizado pero fue inferior en las pollitas que recibieron el pienso en harina de 1 a 45 d de la edad (*P* < 0,01). Concluimos que el trigo puede substituir al maíz en piensos para pollitas si se acepta una ligera reducción en la ganancia de peso. Así mismo, la alimentación en gránulo de 1 a 45 d de edad aumentó la ganancia de peso a 45 d y al final de la prueba, así como el pH de la molleja a 120 d de edad. La presentación del pienso en gránulo redujo el peso relativo de la molleja y de la longitud del tracto gastro intestinal a 120 d de edad.

En el experimento 2 se utilizaron un total de 1.152 pollitas rubias Hy-Line de 1 d de edad y se estudió la influencia del nivel de energía de la dieta y la presentación del pienso sobre los parámetros productivos y las características del tracto digestivo. De 1 a 45 d de edad hubo 6 piensos organizados de forma factorial con 3 concentraciones energéticas (baja: 11,44; media: 12,05 y alta: 12,66 MJ/kg de pienso) y 2 presentaciones del pienso (harina vs. gránulo). De 45 a 120 d todos los piensos experimentales se suministraron en forma de harina y por tanto, la única diferencia entre tratamientos fue el nivel de energía metabolizable aparente corregida por nitrógeno (AMEn) utilizado. Cada uno de los 6 tratamientos se replicó 8 veces y la unidad experimental estuvo formada por 24 pollitas. De 1 a120 d de edad, la ganancia de peso y el índice de conversión mejoraron a medida que aumentó la AMEn de la dieta (*P* < 0,001). Las pollitas alimentadas con gránulo de 1 a 45 d de edad tuvieron un consumo de pienso y una ganancia de peso superiores (*P* < 0,001) en este período que las alimentadas con harina. También de ganancia de peso fue mayor (*P* < 0,01) en el global de la prueba para las pollitas alimentadas con piensos en gránulo. A los 45 d de edad, el PR de todos los segmentos del tracto digestivo estudiados fue inferior para las pollitas alimentadas con piensos de alta energía que para las pollitas alimentadas con piensos de media o baja energía. A los 120 d de edad, el PR de la molleja fue superior ($P < 0{,}01$) para las pollitas alimentadas con piensos de baja energía que para los pollitas alimentadas con los otros piensos. La LR del tracto digestivo no se vio afectada por el nivel de energía de los piensos. La alimentación en gránulo redujo el PR del proventrículo ($P < 0{,}05$), de la molleja ($P < 0{,}001$) y del tracto digestivo ($P < 0.001$), así como la LR del intestino delgado ($P < 0{,}05$) y de los ciegos ($P < 0{,}001$) a 45 d de edad. Los efectos de la alimentación en gránulo sobre el PR de la molleja y del proventrículo fueron todavía evidentes a los 120 d de edad a pesar de que las pollitas solo recibieron los piensos en gránulo durante los primeros 45 d de vida. En base de estos resultados concluimos que la alimentación con gránulo de 1 a 45 d de edad mejora el consumo diario de pienso y el peso de las pollitas a 120 d de edad. Un aumento del nivel de energía de 12,0 a 12,7 MJ/kg de la dieta mejora los parámetros productivos de de 1 a 120 d de edad pero reduce el tamaño del proventrículo y de la molleja.

Concluimos que el maíz y el trigo se pueden utilizar indistintamente en las dietas para pollitas de 1 a 120 d de edad con una ligera disminución del peso vivo con el uso del trigo y que la granulación del pienso suministrado de 1 a 45 d de edad y el uso de dietas de alta densidad energética permiten alcanzar mejores pesos vivos a los 120 d de edad. Sin embargo, la granulación del pienso y el uso de dietas de alta AMEn (pobres en fibra bruta) pueden reducir el desarrollo del tracto digestivo especialmente de la molleja, lo que puede perjudicar el consumo diario de pienso de las gallinas ponedoras especialmente durante el inicio del periodo de puesta.

RÉSUMÉ

Résumé

L'objectif général de cette Thèse Master était d'étudier l'influence de divers facteurs nutritionnels qui pourraient affecter les performances productives et le développement du tube digestif des poulettes brunes pour la production d'œufs commerciaux. Pour atteindre ces objectifs, deux expériences ont été effectuées et les facteurs étudiés étaient le type de céréale, le niveau énergétique de l'aliment et sa forme de présentation.

Dans l'expérience 1 l'influence du type de céréale base et la forme de présentation de l'aliment sur les performances productives et le développement du tube digestif ont été étudiés sur 576 poussins ponte de souche brune depuis 1 jusqu'à 120 j d'âge. Depuis 1 jusqu'à 45j d'âge, 4 aliments expérimentaux distribuillés factoriellement avec 2 céréales (maïs contre blé) et 2 formes de présentation de l'aliment (farine contre granulé) ont été employés. Chaque traitement a été répété 6 fois (24 poulettes par répétition). Depuis 46 jusqu'à 120 j d'âge tous les régimes ont été offerts sous la forme farineuse et la seule différence entre les régimes était la céréale de base utilisée. Cumulativement, les poulettes alimentées avec des régimes à base de maïs ont eu un gain de poids plus élevé ($P < 0,05$) mais un indice de conversion semblable à ceux des poulettes alimentées avec des régimes à base de blé. Depuis 1 jusqu'à 45 j d'âge, les poulettes alimentées à base de granulé ont consommé plus d'aliment ($P < 0,001$) et ont eu un gain de poids plus élevé ($P < 0,001$) que les poulettes alimentées à base de farine. La plupart des effets bénéfiques de la granulation sur les performances productives étaient encore évidents à 120 j d'âge. À 45 j d'âge, le poids du gésier (g/kg de poids vif) était plus élevé ($P < 0,01$) chez les poulettes alimentées avec des régimes à base de maïs que chez les poulettes alimentées avec des régimes à base de blé. L'utilisation de

l'aliment granulé réduit le poids relatif du tube digestif et du gésier (*P* < 0,001) aussi bien que la longueur (cm/kg de poids vif) de l'intestin grêle (*P* < 0,01) aux deux âges. Le pH du contenu de gésier à 120 j d'âge n'a pas été affecté par la céréale de base utilisée mais il était inférieur chez les poulettes alimentées à base de farine depuis 1 jusqu'à 45 j d'âge (*P* < 0,01). Nous concluons que le blé peut substituer le maïs dans les régimes de poulette avec une légère réduction du gain de poids vif. Aussi, l'utilisation de l'aliment granulé depuis 1 jusqu'à 45j d'âge a augmenté le gain de poids vif et le pH du gésier à 120 j d'âge. La présentation de l'aliment granulé a réduit le poids relatif du gésier et la longueur du tube digestif à 120 j d'âge.

Dans l'expérience 2, un total de 1.152 poussins ponte d'un jour d'âge de la souche Hy-Line brune ont été employées pour étudier l'influence du niveau énergétique du régime et la forme de présentation de l'aliment sur les performances productives et le développement du tube digestif. Depuis 1 jusqu'à 45 j d'âge, il y avait 6 régimes distribuillés factoriellement avec 3 niveaux (bas: 11,66; moyen: 12,05 et haut: 12,66 MJ/kg d'aliment) d'énergie métabolisable apparente corrigé pour l'azote (AMEn) et 2 formes de présentation d'aliment (farine et granulé). Depuis 45 jusqu'à 120 j d'âge tous les régimes ont été offert sous forme farineuse, donc la seule différence était le niveau énergétique de l'aliment. Chacun des 6 traitements a été répété 8 fois et l'unité expérimentale a été constituée avec 24 poulettes. Durant l'expérience entière, le gain de poids vif et l'indice de conversion se sont améliorés à mesure que l'AMEn du régime augmentait (*P* < 0,001). Les poulettes alimentées à base de granulé depuis 1 jusqu'à 45 j d'âge ont eu une consommation d'aliment et un gain de poids plus élevés (*P* < 0,001) durant cette période et un gain de poids cumulatif supérieur (*P* < 0,01) que les poulettes recevant l'aliment farineux. À 45 j d'âge, le poids relatif (g/kg de poids vif) de tous les segments de tube digestif était inférieur pour les poulettes recevant l'aliment à haut

niveau énergétique que pour les poulettes recevant les aliments de moyen ou bas niveau énergétique. À 120 j d'âge le poids relatif du gésier était supérieur ($P < 0,01$) chez les poulettes alimentées avec des régimes de faible énergie que pour les poulettes alimentées avec les autres régimes (moyen et haut). La longueur relative (cm/kg de poids vif) du tube digestif n'a pas été affectée par la teneur en énergie du régime. L’utilisation de l’aliment granulé a réduit le poids relatif du proventricul ($P < 0,05$), du gésier ($P < 0,001$) et du tube digestif ($P < 0,001$), ainsi que la longueur relative de l’intestin grêle ($P < 0,05$) et des ceaca ($P < 0,001$) à 45 j d'âge. Les effets d’utilisation de l’aliment granulé sur le pois du gésier et du proventricul étaient encore évidents à 120 j d'âge malgré que les poulettes ont reçu l’aliment granulé seulement durant les premiers 45 j d’âge. Nous avons conclu que l’utilisation de l’aliment granulé depuis 1 jusqu’à 45 j d’âge a amélioré la consommation d'aliment et le poids des poulettes à 120 j d'âge. Une augmentation du niveau énergétique du régime améliore les performances productives des poulettes à tous les âges mais elle réduit le poids du proventricul et du gésier.

Nous concluons que le maïs et le blé peuvent être employés indistinctement dans les régimes pour des poulettes de 1 à 120 j d’âge avec une legère diminution du poids vif avec l’utilisation du blé et que l’utilisation de l’aliment granulé de 1 à 45 j d’'âge et du régime à haute densité énergétique sont fortement recommandés afin d’atteindre le poids vifs adéquat à 120 j d’âge, ciblé par le guide d’élevage. Cependant, la granulation de l’aliment et l'utilisation des régimes de haute densité énergétique pourraient réduire le développement du tube digestif, particulièrement celui du gésier, ce qui pourraient compromettre la consommation d'aliment par la poule pondeuse especialement au début du cycle de ponte.

CHAPTER 2:

LITERATURE REVIEW AND OBJECTIVES

1. General comments

The present Master Thesis has been carried out based on a CDTI research project (Ministerio de Ciencia e Innovacion, Madrid) of Cantos Blancos S.A. Cantos Blancos S. A. belongs to the Guillen group and it is the largest egg producing company of Spain with more than 5 millions hens.

Spanish consumers had a preference for heavy eggs for which they are willing to pay an extra price. In consequence, egg producers need to obtain a high percentage of eggs with a minimum weight of at least 63 g. The obtention of large eggs is a challenge for the first part of the laying cycle. Main objectives of egg producer to attain better economic results of the flock are next:

- ✓ Increase the percentage of large eggs early in the lay production cycle
- ✓ Increase feed intake and body weight (**BW**) of pullets and avoid poor uniformity of the flock at the beginning of the egg production cycle, especially under hot weather conditions.
- ✓ Maintain egg shell quality with a low percentage of shell-less, fissured, and broken eggs, especially at the end of laying cycle.

In order to increase egg size, without jeopardizing other egg quality characteristics, the following management and nutritional strategies can be used:

- ✓ Reach the desired target BW for the pullets prior to transference to the laying house at 18 wk of age.
- ✓ Use of brown-egg laying strains that have a higher BW and thus, produce heavier eggs at the start of the laying period.

- ✓ Implement an adequate light program for pullets. It consists in maintaining a constant light period until pullets reach an acceptable adult BW. (> 1600 g at 18 wk of age for brown-egg pullet).
- ✓ Increase the length of the laying cycle. Older hens produce heavier eggs than young hens. However, egg shell quality will be impaired if the egg production cycle is too long.
- ✓ Modify feed quality by increasing the apparent metabolisable energy nitrogen corrected (**AMEn**), the fat and the methionine content of the diets, while maintaining the ratio of AMEn with Ca, P, and others nutrient levels.
- ✓ Improve the structure (particle size) and the form (crumble, pellet, coarse mash) of the feeds. Feed particle size and feed form are especially important during the first stage of the rearing period as well as in early laying hen cycle.

One of the main objectives of this project was to study how to improve pullet productive performance (feed consumption, BW gain (**BWG**), and BW uniformity) during the rearing phase. In general, heavier pullets are better adapted to resist to the stresses related to the manipulation of birds during transference of the pullets to production house. Also, heavier pullets produce more eggs and have bigger egg size at the beginning of the egg production cycle with a better feed consumption than light pullets.

2. Literature review

2.1. Introduction

Spain is one of the most important egg producers in Europe with more than 40 millions of industrial laying hens, and more than 883 thousand tones of egg produced per year (FAO Stat, 2007). Moreover, Spain exported in 2006, 10 to 15% of the total egg production to other European countries. Egg consumption in Spain is estimated to be more than 220 eggs / person / per year, equivalent to 14 kg / person / year (FAO Stat, 2007). Egg size is especially important in Spain because consumers show a clear preference for large eggs (Grobas et al., 1999). Therefore, the production of big size eggs is of paramount importance for the industry.

The profitability of the egg industry depends mostly of 3 factors; number of eggs/ hen housed, quality and size of the eggs produced, and percentage of eggs that reach the table of the final consumer. To improve egg rate and the quality of the eggs, one of the most critical points is the management and the nutrition of the pullets during the rearing phase. Pullets should reach sexual maturity with a BW and uniformity as recommended by the genetic companies that market them. An adequate BW is well correlated with the rate of production and the percentage of large eggs (Summers and Leeson, 1994). In fact, one of the main challenges in rearing pullets is to produce birds with good feed intake at 18 wks of age. A high feed intake during the rearing phase will results in a well developed digestive tract that will allow to fulfill the nutritional requirements of the pullets, especially in the critical period of the onset of lay production. Most of the producers follow the recommendation of the genetic companies (Management guides) for pullet rearing to satisfy nutritional requirement and to meet productive targets. However, the information available on the nutritional requirements of pullets and the influence of the feeding program on the development of the digestive tract is very limited. Therefore, more information is needed to help the nutritionists to formulate diets that maximize feed intake and BWG of the pullets and that at the same time improve the development of the gastrointestinal tract (**GIT**) and the uniformity of the flock.

2.2. Effect of cereal type on productive performance

Cereals are the most widely used energy sources in poultry feeds, and the majority of the energy is derived from the starch fraction. In addition, cereals provide also a part of the protein and amino acids required by the birds. Starch utilization by poultry is affected by the cereal used because the structure of the starch varies with the source considered. Starch digestion depends on factors such as the soluble cell-wall polysaccharide content, the nature of grain starch, the presence of anti-nutritional factors in the grain, and the digestive capacity of the animal (Classen, 1996). The most common cereals produced in Europe and used in poultry diets are corn (*Zea mays L.*) and wheat (*Triticum L.*). Corn has less protein (7.7 vs. 13.8%) and fiber (2.5 vs. 2.9%) and more starch (63.4 vs. 56%) and AMEn (3,260 vs. 3,050 kcal/kg) than wheat (Fundación Española Desarrollo Nutrición Animal, 2003). Part of the wheat crop (more than 45% of 651 million tons production in 2008) is not used in the manufacturing of products for human consumption, and it is derived to animal feeding and other commercial purposes (International Grain Council, 2009). The inclusion level of wheat in poultry diets depends on many factors such as the species considered, the age, and the nutritive value, including AMEn, crude protein (**CP**), and non starch polysaccharide (**NSP**). Several reports have compared these two cereals in the diet on productive performance of broilers (Ruiz et al., 1987; Mathlouthi et al., 2002) and laying hens

(Lázaro et al., 2003; Safaa et al., 2009). In general, these studies suggest that wheat is a good alternative to corn in these species. For example, Ruiz et al. (1987) reported similar BWG and feed conversion ratio (**FCR**) in broilers fed mash when corn was substituted by wheat. However, Crouch et al. (1997) compared corn and two varieties of wheat at 40% of inclusion in mash diets for broilers and found that BWG and FCR were impaired with one of the two wheats. In this report, the addition of enzymes to the low quality wheat diet equalized broiler performance to that of the corn diet. 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. Gutiérrez-Alamo et al., (2008) indicate that the nutritive value of wheat for poultry might vary depending on several factors such as the cultivar used, the characteristics and NSP content of the grain, the form of the diet, and the type of birds used. In laying hens, Lillie and Denton (1968) indicated that Single Comb White Leghorn (**SCWL**) hens had similar productive performance when fed diets based on corn or wheat. Similarly, Liebert et al. (2005) and Safaa et al. (2009) observed that performance was not affected when corn was substituted by wheat as the main ingredient in diets of Lohmann Brown hens respectively from 22 to 61 wk of age and from 20 to 48 wk of age. Lázaro et al. (2003) found that the substitution of corn by wheat did not affect average daily feed intake (**ADFI**), hen productivity, or egg quality of SCWL hens from 20 to 44 wk of age. Similarly, Çiftci et al. (2003) observed that the substitution of 30% corn by wheat in diets for SCWL hens did not affect productivity from 27 to 43 wk of age. In contrast, Kim et al. (1976) found that SCWL hens fed a diet based on corn ate more feed and produced bigger eggs than hens fed a diet based on wheat from 21 to 43 wk of age. However, the information available comparing the effects of corn and wheat on productive performance of pullets is scarce.

2.3. Effect of energy concentration of the diet on productive performance

Poultry tend to eat to satisfy their energy requirements. Therefore, they regulate ADFI depending on the AMEn concentration of the diet. Thus, they increase the ADFI of the low AMEn diet. Latshaw (2008) observed that diet composition has a significant effect on the energy intake of broilers; birds ate approximately 10% more energy from diets containing 12.80 MJ/kg, when 5% fat was added, than when the diet had no added fat. In contrast, broilers fed a diet with 4% added fiber, ate approximately 20% less energy than those fed a similar diet with no added fiber. Summers et al. (1987) observed that an increase from 10.44 to 12.45 MJ/kg in the AMEn of the diet of SCWL pullets from 1 to 16 wk of age reduced ADFI by 12%. Also, Keshavarz and Nakajima (1995) observed that increasing the AMEn concentration of the diet of SCWL pullets from 10.88 to 12.97 MJ/kg from 14 to 18 wk of age reduced ADFI by 2% and FCR by 9.7%. In broilers, the effect of energy concentration (11.70 to 12.96 MJ/kg) on feed intake was less pronounced when birds were fed mash as compared to birds fed pellets. Moreover, Keshavarz (1998) reported that an increase in the AMEn concentration of the diet from 11.78 to 12.70 MJ/kg for the last 10 wk of the rearing period, improved performance of SCWL pullets. In contrast, Summers and Leeson (1993) reported that a 10% increase in the AMEn content of the diet of SCWL from 16 to 20 wk of age did not affect BW at 20 wk of age. The authors indicated that in order to be effective in improving BW, the increase in AMEn of the diet should be done early in the rearing period. It is believed that poultry try to satisfy their energy requirement. Leeson et al. (1996) observed similar BWG in broilers fed diets containing from 11.3 to 13.8 MJ AMEn/kg.

Under commercial conditions, energy consumption is the main factor influencing BWG of pullets (Summers et al., 1987) and it has been suggested that an increase in AMEn concentration of the diet increase energy intake by poultry, especially when the increase in AMEn is obtained by addition of fat. In this respect, Cherry et al. (1983) reported that chickens fed high energy diets initially increased ADFI whereas the opposite effect occurred with low energy diets. In fact, Leeson et al. (1993) indicated that pullets fed a diet containing 12.67 MJ AMEn/kg consumed 6% more energy than pullets fed a diet containing 11.53 MJ AMEn/kg.

2.4. Effect of pelleting on productive performance

Pelleting consists in the application of heat and vapor to the mash feed inside the conditioner which leads to a mild cooking of the diet. The steam is applied for a short period of time and with a temperature of no more than 80ºC to avoid inactivation of the enzymes and vitamins added to the diet. When a feed is pelleted, a reduction in the size of mash particles is required to improve the quality of the feed. Therefore, the process minimizes the differences in the initial particle size of the ingredients. After grinding, the feed passes through a pellet press provided with a die of variable diameter and thickness depending on the target species. During the pelleting process, the feed is steam-heated to soften the feed particles and then, it is pressed causing an additional mechanical pressure (Engberg et al. 2002, Svihus et al 2004). Grinding, steam and pressure applied to the meal help to agglomerate the particles of the diet with a concomitant improvement in bulk density and feed texture which in turn will facilitate feed intake. In addition, pelleted feeds have the advantage of better uniformity which reduces the natural selection of the feed particles by the animal.

In broilers, feeding pellets increases BWG and reduced FCR as compared to feeding mash (Reece et al., 1985; Douglas et al., 1990; Amerah et al., 2007). Brikett et al. (2007) using diets varying from 11.70 to 12.96 MJ/kg, observed that BW of broilers was not affected by the energy concentration of the diets when the feeds were pelleted but was decreased linearly when the low energy diets were fed as mash. Also, in this trial, FCR decreased with increasing energy concentration but feed form did not affected feed efficiency. The information available on the influence of feed form on pullet performance is scarce and contradictory. 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 2% less feed but were 6% heavier as compared to pullets fed mash. However, pelleting the diet from 5 to 20 wk of age is not a common commercial practice in pullet feeding. Deaton et al. (1988) reported that pullets reared under moderate temperature regimen (21ºC) had similar ADFI but higher BWG with pelleted than with mash feeds from 12 to 20 wk of age. However, under hot weather conditions (24-35ºC) no effects were observed. In contrast, Leeson and Summers (1984) indicated that crumbling of rearing diets of pullets has no effect on BW at maturity or on subsequent egg production. However, in this report pullets fed crumbles consumed more feed from hatching to 10 wk of age than pullets fed mash.

2.5. Effect of cereal type on digestive tract traits

There is a growing interest to study the influence of diet form and composition on the development of the GIT of chickens **(**Ravindran et al., 2006; Jiménez-Moreno et al., 2008; Gabriel et al., 2008). In broilers, the growth of the organs of the GIT and the production of hydrochloric acid and endogenous digestive enzymes vary depending on diet composition and feed form (Engberg et al., 2002; Gonzalez-Alvarado et al., 2007). Nir et al. (1994b) using a corn and sorghum based diet observed that gizzard weight of the broilers was not affected by the cereal used but was reduced by pelleting. However, Amerah et al. (2008) reported that 21 d-old broilers had heavier gizzards when fed coarse corn-based diets as compared to fine ground diets. However, no difference in gizzard weight was observed between coarse and fine particle size diets in birds fed wheat. The relative length of GIT organs was shorter in birds fed wheat based diets than in those fed the corn based diets (Amerah et al. 2008). These authors observed that the length of the GIT was shorter with coarse corn than with fine corn, but no differences were observed for the wheat diets. Unfortunately, no data is available comparing the influence of cereal on the size and length of the GIT in pullets.

2.6. Effect of energy concentration of the diet on digestive tract traits

We have not found any report in the literature, conducted with laying pullets, on the effects of energy concentration of the diet on BW uniformity and the development of the different segments of the GIT. However, when the AMEn of the diet is decreased, the crude fiber content of the diet increases. Thus, most of the effect of increasing the energy concentration of the diet on GIT development might be related to the concomitant decrease in crude fiber content.

In broilers, González-Alvarado et al. (2007, 2008) observed that the inclusion of 3 to 4% of oat hulls or soy hulls increased the relative weight (**RW**, g/kg BW) of the gizzard and of the GIT and reduced digesta pH and the relative length (**RL**, cm/kg BW) of the small intestine (**SI**). Working with two different strains of SCWL pullet, Scheideler et al. (1998) observed that the addition of 10% of oat hulls reduced pullet BWG and impaired FCR at 17 wk of age as compared to a corn-soybean meal diet. Also, the gizzard size was higher in pullets fed the fiber containing diet than in pullets fed the corn-soybean meal diet.

2.7. Effect of pelleting on digestive tract traits

The gizzard is a muscular organ that reduces the particle size of ingested feeds by applying a high mechanical grinding pressure. Hetland et al. (2004) observed that the gizzard has a remarkable ability to grind all the constituents of the feed to a consistently fine size, regardless of the original particle size of the feed. The rate passage of digesta through the gizzard is slower with coarse particles than with fine particles because coarse particles need a longer time to be grinded, witch in turn increase the length of exposition of nutrient to digestive enzymes, which, may improve nutrient digestibility (Carré, 2000). In contrast, fine grinding tends to have negative effects on gizzard size and gut functioning because the gizzard will be lighter and relatively under-developed and the proventriculus will become enlarged (Taylor and Jones, 2004). Under these conditions (fine grinding of the feed), the gizzard might not function as a grinding organ and will allow freely the pass of the feed to the distal part of the GIT. In addition, gizzard action increases the peristaltic movements of the GIT and the gastro-duodenal refluxes, improving the mixing of the nutrients containing in the feed with digestive enzymes (Duke, 1992). Also, a well-developed gizzard influences hydrochloridric acid and pancreatic enzyme secretions.

Feed form influences organ development and nutrient digestibility in broilers (Choi et al., 1986; Kilburn and Edwards, 2001; Mateos et al., 2002). Different authors (Choi et al., 1986, Nir et al., 1994a,b; Corchero et al., 2008) observed that feeding crumbles or pellets to broilers reduced the RW of the gizzard as compared to feeding mash. Amerah et al. (2007) suggest that pelleting decrease the need of grinding of the feed by the gizzard. Also, Nir et al. (1995) found that pelleting reduced the RL of the jejunum and the ileum by 15% and Nir et al. (1994b) observed that feeding crumbles or pellets to broilers reduced gizzard weight with respect to feeding mash.

Unfortunately, the effects of pelleting the feeds on the development of the GIT of pullets have not been studied and to our knowledge no research is available in this respect.

3. Objectives

The general aim of this Master Thesis was to study the influence of some nutritional factors that influence the productive performance and GIT development of commercial brown egg-laying pullets. In this respect, the influence of cereal type, energy concentration, and feed form of the diets were studied in two experiments. In experiment 1 (chapter 3) we evaluated the influence of the diet form fed to the pullets from 1 to 45 d of age and the main cereal of the diets fed from 1 to 120 d of age, on productive performance and development of the GIT of brown-egg laying pullets. In experiment 2 (chapter 4), we evaluated the influence of the energy concentration and feed form of the diet on productive performance and development of the GIT of brown-egg laying pullets at the same ages.

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CHAPTER 3:

EXPERIMENT 1: INFLUENCE OF THE MAIN CEREAL AND FEED FORM OF THE DIET ON PERFORMANCE AND DIGESTIVE TRAITS OF BROWN-EGG LAYING PULLETS

Poultry Science 88:994-1002

1. INTRODUCTION

Corn and wheat are widely used as energy sources in poultry feeds. Several reports have compared the effects of including these two cereals in the diet on productive performance of broilers (Ruiz et al., 1987; Mathlouthi et al., 2002) and laying hens (Lázaro et al., 2003; Safaa et al., 2009). Ruiz et al. (1987) reported similar BWG and FCR in broilers fed mash when corn was substituted by wheat. Crouch et al. (1997) compared corn and two varieties of wheat at 40% of inclusion in mash diets for broilers and found that BWG and FCR were impaired with one of the two wheats. In this report, the inclusion of enzymes to the low quality wheat diet equalized broiler performance to that of the corn diet. 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. These observations indicate that the nutritive value of wheat for poultry might vary depending on factors such as the cultivar used, the characteristics and nonstarch polysaccharide content of the grain, the form of the diet, and the type of birds used (Gutiérrez-Alamo et al., 2008). In laying hens, most reports indicate that wheat can be used successfully as the main ingredient of the diet. Lázaro et al. (2003) observed similar egg production, ADFI and FCR when corn was substituted by wheat in the diet of SCWL hens from 20 to 44 wk of age. Similar results were reported by Liebert et al. (2005) and Safaa et al. (2009) in brown-egg laying hens. However, the authors have not found any information comparing the effects of corn and wheat on productive performance of pullets.

In broilers, feeding pellets increased BWG and reduced FCR compared with feeding mash (Reece et al., 1985; Douglas et al., 1990; Amerah et al., 2007). In contrast to broilers, the information available on the influence of feed form on pullet performance is scarce and contradictory. Gous and Morris (2001) found that pullets fed crumbles from 1 to 4 wk of age and then pellets from 5 to 20 wk, consumed 2% less feed but were 6% heavier than pullets fed mash. However, pelleting the diet from 5 to 20 wk of age is not a common commercial practice in pullet feeding. Deaton et al. (1988) reported that pullets reared under moderate temperature regimen (21ºC) had similar ADFI but higher BWG with pelleted than with mash feeds from 12 to 20 wk of age. However, under hot weather conditions (24-35ºC) no effects were observed.

There is a growing interest on the influence of the diet on the development of the GIT of chickens (Ravindran et al., 2006; Jiménez-Moreno et al., 2008; Gabriel et al., 2008). The growth of the organs of the GIT and the production of hydrochloric acid and endogenous digestive enzymes might be affected by diet composition and feed form (Engberg et al., 2002; Gonzalez-Alvarado et al., 2007). In broilers, Choi et al. (1986) and Nir et al. (1994a,b) observed that feeding crumbles or pellets reduced the RW of the gizzard compared with feeding mash. In addition, Nir et al. (1994a) reported that pelleting reduced the RL of the jejunum and ileum, and Amerah et al. (2007) indicated that the decrease in length of the GIT with pelleting was associated with an improvement in broiler performance. The aim of this research was to evaluate the influence of feed form from 1 to 45 d of age and of the main cereal of the diet from 1 to 120 d of age on productive performance and development of the GIT of brown-egg laying pullets.

2. MATERIALS AND METHODS

2.1. Husbandry and Experimental Design

The experimental procedures used in this research were approved by the Animal Ethics Committee of Universidad Politécnica de Madrid and were in compliance with the Spanish guidelines for the care and use of animals in research (Boletín Oficial Estado, 2005).

A total of 576 day-old Hy-Line Brown pullets with an initial BW of 36.8 ± 2.4 g was obtained from a commercial hatchery (Avigan Terralta, Tarragona, Spain) and used in this experiment. On arrival at the experimental farm, pullets were placed in a windowless environmentally controlled room, weighed individually, and stratified by BW into three groups of 192 pullets each. Twenty four uniform groups of 24 pullets each (8 from each BW group) were formed and placed in 48 cages (12 pullets per cage). The experimental unit was formed by 24 pullets housed in groups of 12 in two adjacent cages, and 6 replicates were randomly assigned to each of the 4 experimental feeding programs. Each cage (0.50 x 0.76 m; Zucami, Pamplona, Spain) was provided with an open through feeder and two low pressure nipple drinkers. Room temperature was maintained at 32ºC during the first 3 d of life and then, the temperature was reduced gradually until reaching 21ºC at 5 wk of age. The pullets were kept on a 23 h/d light program for the first wk of life and then, light was decreased 2 h per wk until reaching 12 h at 7 wk of age. Birds were beak-trimmed at 12 d of age and vaccinated against main diseases (Infectious Bronchitis Disease, Infectious Bursal Disease, Newcastle Disease, Laryngotracheitis, Mycoplasma, and *Salmonella* spp.) according to accepted commercial practices.

2.2. Feeding Program and Experimental Diets

The feeding program consisted of 3 feeds that were supplied from 1 to 45 d, 46 to 85 d, and 86 to 120 d of age. Within each age period, diets were formulated to have similar nutrient content (Fundación Española Desarrollo Nutrición Animal, 2003) and met or exceeded the nutritional recommendations of NRC (1994) for pullets (Table 1). From 1 to 45 d of age the diets contained 50% of either corn or wheat and were fed as mash or pellets (2-mm diameter). From 46 to 120 d of age, all diets were fed as mash, and therefore, the only difference among treatments was the main cereal of the diet. Pullets had free access to feed and water throughout the trial. All diets incorporated a phytase (Natuphos, Basf Española, S.A., Tarragona, Spain) and a mixture of β-glucanases and xylanases (Roxazyme, DSM, S.A., Madrid, Spain) at the levels recommended by the supplier.

Table 1. Ingredient composition and calculated nutritive value of the experimental diets (%, as-fed basis, unless stated otherwise)

	1 to 45 d[1]		46 to 85 d		86 to 120 d	
	Corn	Wheat	Corn	Wheat	Corn	Wheat
Ingredient						
Corn[2]	50.00	--	50.00	--	50.00	--
Wheat[3]	--	50.00	--	50.00	--	50.00
Barley	13.27	19.52	17.06	23.37	16.58	22.66
Rice bran	--	--	--	--	4.00	4.00
Soybean meal, 44% CP	31.20	25.35	17.97	12.12	13.67	8.09
Sunflower meal, 32% CP	--	--	10.00	10.00	10.36	10.31
Soybean oil	1.87	1.34	1.40	0.88	0.74	0.19
Dicalcium phosphate	0.77	0.65	0.52	0.37	0.37	0.25
Calcium carbonate	1.83	1.92	2.00	2.10	2.52	2.61
Sodium chloride	0.35	0.35	0.33	0.33	0.32	0.32
Vitamin, mineral, and enzyme premix[5]	0.40	0.40	0.40	0.40	0.40	0.40
Met hydroxy analogue, 88%	0.20	0.22	0.17	0.15	0.03	0.05
L-Lys-HCl, 78%	0.08	0.17	0.15	0.23	0.01	0.08
L-Thr, 98%	0.03	0.08	--	0.05	--	0.04
Sepiolite[4]	--	--	--	--	1.00	1.00
Calculated analysis						
AME_n, kcal/kg	2,880	2,880	2,800	2,800	2,750	2,750
Crude fiber	3.6	3.7	5.3	5.4	5.4	5.5
Neutral detergent fiber	10.2	12.0	13.2	15.0	13.4	15.2
Digestible Lys	0.93	0.93	0.76	0.76	0.58	0.58
Digestible Met	0.45	0.45	0.41	0.38	0.28	0.28
Digestible Met + Cys	0.72	0.75	0.66	0.66	0.51	0.54
Digestible Thr	0.63	0.63	0.51	0.51	0.47	0.47
Digestible Trp	0.19	0.20	0.16	0.17	0.15	0.16

[1]Diets were offered either in mash or in pellet form.

[2]Determined chemical composition (n=3): 87.9% DM, 7.3% CP, 3.9% EE, and 1.2% total ash.

[3]Determined chemical composition (n=3): 90.0% DM, 11.2 % CP, 2.5% EE, and 1.6% total ash.

[4]A complex magnesium silicate clay.

[5]Provided the following (per kg of diet): vitamin A (*trans*-retinyl acetate), 9,000 IU; vitamin D_3 (cholecalciferol), 2,600 IU; vitamin E (DL-α-tocopheryl acetate), 16 mg; vitamin B_1, 1.6 mg; vitamin B_2, 6.5 mg; vitamin B_6, 2.2 mg; vitamin B_{12} (cyanocobalamin), 0.015 mg; vitamin K_3, 2.5 mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (D-calcium pantothenate), 10 mg; folic acid, 0.6

mg; D-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron ($FeSO_4$ H_2O), 40 mg; copper ($CuSO_4$ $5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na_2SeO_3), 0.3 mg; Roxazyme, 200 mg [1,600 U Endo-1,4-β-glucanase (EC 3.2.1.4) , 3,600 U Endo-1,3(4)-β-glucanase (EC 3.2.1.6), and 5,200 U Endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM, S.A., Madrid, Spain; Natuphos 5000, 80 mg (400 FTU phytase) supplied by BASF Española, S.A., Tarragona, Spain.

A batch of dent corn and a batch of soft wheat were obtained from a commercial supplier (Esasa, Valladolid, Spain), ground through a hammer mill fitted with a 6-mm screen and used in the formulation of the mash feeds. To prepare the pellet diets used from 1 to 45 d of age, an aliquot part of the mash diet (based on either corn or wheat) was reground with the same hammer mill fitted with a 2-mm screen. The diets were then steam-conditioned at 72ºC for 60 s and passed through a pellet press (Model 508-150, Mabrik, Barberá del Valles, Barcelona, Spain) provided with a 32-mm thick die and a 2-mm screen.

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 the oven-drying method (930.01), ash by a muffle furnace (942.05), nitrogen by combustion (990.03) using a LECO equipment (model FP-528, LECO Corporation, St. Joseph, MI), and Ca and P by spectrophotometry (968.08 and 965.17, respectively) as described by AOAC International (2000). Ether extract was determined by Soxhlet fat analysis after 3 N HCl acid hydrolysis (method 4. b) as indicated by Boletín Oficial Estado (1995) and gross energy was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL). The chemical analysis of the experimental diets is shown in Table 2.

The mean particle size of the mash diets, expressed as geometric mean diameter (**GMD**), was determined in a 100 g sample using a shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 µm. The method outlined by American Society of Agricultural Engineers (1995) was used. For the two pelleted diets used from 1 to 45 d of age, particle size distribution was determined after the mash mixture was re-ground but before pelleting. The durability of the pellet was calculated as the proportion of the feed sample (300 g) that did not pass through a 140-µm sieve after 10 min in the pellet tester (Mabrik, Barberá del Vallés, Barcelona, Spain) at a rotation speed of 50 rpm. The percentage of fines was determined by hand-sieving a 100 g feed sample through a 140-µm sieve for 3 min. In addition, the length of the pellets was determined with a digital micrometer (model IP 65, Mitutoyo, Kawasaki, Japan) in a 10 g sample. All analyses were determined in triplicate.

Table 2. Determined chemical analysis (%, as-fed basis, unless stated otherwise), particle size distribution (%), and geometric mean diameter ± geometric standard deviation (GMD ± GSD, µm) of the experimental diets [1]

	1 to 45 d				46 to 85 d		86 to 120 d	
	Corn		Wheat		Corn	Wheat	Corn	Wheat
Item	Mash	Pellet	Mash	Pellet	Mash		Mash	
Chemical analyses								
Gross energy, kcal/kg	3,994	4,007	3,987	4,020	3,957	3,962	3,836	3,887
DM	88.37	88.72	89.82	89.72	89.17	91.12	88.99	90.37
CP	19.79	19.80	20.06	20.22	17.21	17.30	15.27	16.14
Ether extract	4.95	5.20	3.70	3.95	4.50	2.90	3.80	2.80
Total ash	4.75	4.85	5.00	4.77	5.08	4.83	6.10	5.87
Ca	0.93	0.92	0.96	0.90	0.95	1.01	1.13	1.10
P	0.61	0.58	0.60	0.56	0.52	0.50	0.50	0.50
Particle size distribution[2], %								
2,500	5.44	0.30	5.53	0.20	7.09	6.18	7.85	5.87
1,250	26.79	5.65	27.14	7.52	33.56	31.42	33.95	23.26
630	38.53	46.20	34.04	42.13	31.61	31.20	29.78	36.88
315	22.54	40.42	21.37	34.84	17.40	19.05	18.91	22.10
160	6.18	7.37	9.57	14.95	8.98	9.42	8.78	9.82
80	0.52	0.07	2.26	0.36	1.36	2.74	0.73	2.08
GMD ± GSD[3]	899 ± 2.0	634 ± 1.7	837 ± 2.2	597 ± 1.8	942 ± 2.2	875 ± 2.2	960 ± 2.2	814 ± 2.2

[1]Analyzed in triplicate samples.

[2]Sieve diameter, µm. The percentages of particles smaller than 40 µm and bigger than 5000 µm were negligible for all diets.

[3]GSD = Log normal SD.

2.4. Productive Performance

Body weight and feed consumption were recorded by replicate at 45, 85, and 120 d of age. Feed wastage was observed to be negligible and was not measured. From these data, BWG, ADFI, and FCR corrected for mortality were determined by period and cumulatively. In addition, the pullets of one of the two cages that formed the experimental unit (12 birds at 45 d and 10 birds at 85 d and 120 d of age) were weighed individually and BW uniformity was assessed by calculating the percentage of pullets of each replicate that were within ± 1.25 SD of the mean average BW. The 1.25 SD range was selected to fit commercial target for BW homogeneity of the flock (80% of pullets within ± 10% of the average BW; Hy-Line Brown, 2008).

2.5. Development of the Gastrointestinal Tract

At 45 d of age, two birds per replicate were randomly selected, weighed individually, and euthanized by CO_2 inhalation. The digestive tract (from the beginning of the proventriculus to the cloaca, including digesta content) and the liver and the pancreas, were removed aseptically and weighed. Then, the proventriculus and the gizzard were excised, emptied from any digesta, cleaned, dried with desiccant paper, and weighed. The weight of the total digestive tract, including the weight of the liver and the pancreas, and that of the empty organs were expressed relative to live BW (RW, g/kg BW). In addition, the remainder of the GIT were emptied from any digesta content by gently squeezing, and the length of the duodenum (from gizzard to pancreo-biliary ducts), jejunum (from pancreo-biliary ducts to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileo-cecal junction), and of the two ceca (from the ostium to the tip of the right and left ceca) was measured on a glass surface using a flexible tape with a precision of 1 mm, and expressed relative to live BW (RL, cm/kg BW). At 120 d of age, two pullets from each replicate were euthanized. The procedures used and measures taken were similar to those indicated at 45 d of age. In addition, the pH of the gizzard content was measured in duplicate at this age using a digital pH meter fitted with a fine tip glass electrode (model 507, Crison Instruments S.A., Barcelona, Spain). The average value of the two measurements was used for statistical analysis.

2.6. Statistical Analysis

The experimental design was completely randomized with 4 treatments arranged factorially with cereal, feed form of the diet, and the interaction as main effects. The experimental unit consisted of a group of 24 pullets. The data were analyzed using the GLM Procedure of SAS software (SAS Institute, 1990). When the model was significant, treatment means were separated using the Tukey's test. Differences between treatment means were considered significant at $P < 0.05$. Results in tables are presented as means.

3. RESULTS

The GMD of the feeds was consistently higher for the corn- than for the wheat diets (Table 2). From 1 to 45 d of age, the GMD of the feeds was higher for the mash- than for the pellet diets (868 vs. 616 μm). The length (3.9 vs. 4.3 mm) and durability (98.2 vs. 97.2%) of the pellets were similar, and the percentage of fines was slightly lower (1.7 vs. 3.3%) for the wheat- than for the corn diet.

3.1. Productive Performance

Mortality was low (2.4%) and not related to treatment. Most of the mortality (87%) occurred during the first wk of life (data not shown). From 1 to 45 d of age, the main cereal of the diet did not affect productive performance of the pullets but those fed pellets consumed more feed and had higher BWG ($P < 0.001$) than those fed mash (Table 3). From 46 to 85 d of age, pullets that were fed pellets previously, had higher BWG ($P < 0.05$) than pullets that were fed mash. From 46 to 120 d of age, no differences in productive performance were observed among treatments (data not shown). From hatching to 120 d of age, pullets fed the corn diets had higher BWG ($P < 0.05$) than pullets fed the wheat diets but no differences were observed for ADFI or FCR (Table 3). In addition, BWG ($P < 0.001$) and ADFI ($P < 0.05$) were higher at this age for pullets that were fed pellets from 1 to 45 d of age than for pullets that were fed mash. Pullet uniformity was not affected by dietary treatment at any age (Table 4).

3.2. Development of the Gastrointestinal Tract

At 45 d of age, pullets fed the corn diets had heavier gizzards than those fed the wheat diets ($P < 0.01$) but no differences were detected at 120 d (Tables 5 and 6, respectively). No other effects of the main cereal of the diet were observed. Feed form affected the relative weight and relative length of the different segments of the GIT at both ages. At 45 d of age, the digestive tract and the gizzard were heavier ($P < 0.001$) in pullets fed mash than in pullets fed pellets (Table 5). In addition, the small intestine ($P < 0.01$) and the ceca ($P < 0.05$) were longer in pullets fed mash than in those fed pellets.

Most of the differences in length of the SI were observed for the jejunum ($P < 0.05$) and the ileum ($P < 0.001$). An interaction between type of cereal and feed form was detected for the relative weight of the gizzard and the RL of the SI and the ileum ($P < 0.01$). The gizzard was heavier with the corn than with the wheat diet in pullets fed pellets but no difference was observed in pullets fed mash. In contrast, the SI and the ileum were shorter with pellet-than with mash feeds in pullets fed the wheat diet but no differences were observed in pullets fed the corn diet (P < 0.01).

At 120 d of age, the gizzard was heavier ($P < 0.01$) in pullets that were fed mash from 1 to 45 d than in those that were fed pellets (Table 6). Similarly, the SI ($P < 0.01$), jejunum, and ileum ($P < 0.05$) were shorter, and the gizzard pH was higher ($P < 0.01$) at this age in pullets previously fed pellets. No interactions between main cereal and form of the diet were observed at this age.

Table 3. Influence of the main cereal and feed form of the diet on productive performance of pullets from 1 to 120 days of age

	1 to 45 d			46 to 85 d			86 to 120 d			1 to 120 d		
	BWG[1]	ADFI[2]	FCR[3]	BWG	ADFI	FCR	BWG	ADFI	FCR	BWG	ADFI	FCR
Cereal												
Corn	10.66	26.53	2.49	16.81	65.96	3.93	9.29	74.89	8.07	12.29	53.90	4.39
Wheat	10.51	26.40	2.51	16.67	65.64	3.94	9.03	74.40	8.24	12.11	53.61	4.43
Feed form												
Mash	10.28	25.69	2.50	16.55	64.79	3.92	9.15	73.45	8.03	12.02	52.78	4.39
Pellet	10.89	27.24	2.50	16.93	66.81	3.95	9.17	75.84	8.28	12.37	54.73	4.42
SEM[4] (n=12)	0.069	0.253	0.023	0.115	0.926	0.043	0.108	1.061	0.091	0.048	0.664	0.044
Effect[5]	Probability											
Cereal	0.13	0.72	0.48	0.40	0.81	0.86	0.10	0.75	0.20	*	0.76	0.52
Feed form	***	***	0.84	*	0.14	0.62	0.92	0.13	0.07	***	*	0.63

[1]BW gain, g/d.

[2]Average daily feed intake, g.

[3]Feed conversion ratio, g/g.

[4]SEM (12 replicates of 24 pullets from 1 to 45 d of age and of 22 pullets from 46 to 120 d of age per each main effect).

[5]The interactions were not significant ($P > 0.05$).

* $P < 0.05$; *** $P < 0.001$.

Table 4. Influence of the main cereal and feed form of the diet on BW uniformity[1] of pullets at 45, 85, and 120 days of age

	45 d	85 d	120 d
Cereal			
Corn	85.4	80.0	81.7
Wheat	81.2	82.5	83.3
Feed form			
Mash	81.9	79.2	82.5
Pellet	84.7	83.3	82.5
SEM[2] (n=12)	2.50	2.80	3.56
Effect[3]		Probability	
Cereal	0.25	0.54	0.74
Feed form	0.44	0.31	1.00

[1]Percentage of pullets with a BW within the mean average ± 1.25 SD range.

[2]SEM (12 replicates of 12 pullets at 45 d of age and of 10 pullets at 85 d and 120 d of age per each main effect).

[3]The interactions were not significant (*P* > 0.05)

Table 5. Influence of the main cereal and feed form of the diet on digestive tract plus digesta content (g/kg BW) and relative weight (g/kg BW) and length (cm/kg BW) of the gastrointestinal tract segments of pullets at 45 d of age

		Relative weight			Relative length				
Cereal	Feed form	Digestive tract[1]	Proventriculus	Gizzard	Small intestine	Duodenum	Jejunum	Ileum	Ceca
Corn	Mash	144.3	6.2	41.9[a]	248.4[ab]	42.3	108.9	97.1[a]	25.1
	Pellet	132.3	6.2	31.9[b]	244.5[ab]	40.6	107.7	96.2[a]	21.9
Wheat	Mash	147.7	6.0	41.5[a]	261.7[a]	42.3	115.3	104.0[a]	25.0
	Pellet	126.0	5.9	22.5[c]	230.5[b]	39.1	104.2	87.2[b]	20.7
SEM[2] (n= 6)		3.23	0.19	1.53	4.61	1.46	2.84	2.06	1.61
Main effects									
Cereal									
Corn		138.3	6.2	37.4	246.4	41.5	108.3	96.6	23.5
Wheat		136.8	5.9	31.1	246.1	40.7	109.7	95.6	22.8
Feed form									
Mash		146.0	6.1	41.9	255.0	42.3	112.1	100.6	25.0
Pellet		129.2	6.1	27.2	237.5	39.8	106.0	91.7	21.3
SEM (n= 12)		2.28	0.13	1.08	3.26	1.03	2.01	1.46	1.14
Effect		Probability							
Cereal		0.65	0.18	**	0.94	0.60	0.62	0.63	0.66
Feed form		***	0.82	***	**	0.10	*	***	*
Cereal x feed form		0.15	0.84	**	**	0.61	0.10	**	0.74

[1]Includes the weights of the digestive tract (from the beginning of the proventriculus to cloaca, with digesta content), the liver, and the pancreas.

[2]SEM (6 replicates of 2 pullets per each treatment).

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 6. Influence of the main cereal and feed form of the diet on digestive tract plus digesta content (g/kg BW) and relative weight (g/kg BW) and length (cm/kg BW) of the gastrointestinal tract segments and on gizzard pH of pullets at 120 d of age

	Relative weight			Relative length					
Main effects	Digestive tract[1]	Proventriculus	Gizzard	Small intestine	Duodenum	Jejunum	Ileum	Ceca	Gizzard pH
Cereal									
Corn	118.2	3.2	21.6	107.1	18.4	46.0	42.6	17.8	3.61
Wheat	119.0	3.2	20.9	109.2	18.3	47.4	43.6	19.0	3.84
Feed form									
Mash	120.6	3.5	23.1	111.7	18.8	48.5	44.4	18.8	3.46
Pellet	116.6	2.9	19.4	104.7	18.0	44.9	41.8	18.1	3.99
SEM[2] (n=12)[2]	3.12	0.11	0.76	0.39	0.91	0.72	1.57	0.41	0.114
Effect[3]	Probability								
Cereal	0.87	0.67	0.48	0.35	0.80	0.31	0.37	0.06	0.17
Feed form	0.37	**	**	**	0.16	*	*	0.21	**

[1]Includes the weights of the digestive tract (from the beginning of the proventriculus to cloaca, with digesta content), the liver, and the pancreas.

[2]SEM (12 replicates of 2 per each main effect).

[3]The interactions were not significant ($P > 0.05$).

*$P < 0.05$; ** $P < 0.01$.

4. DISCUSSION

4.1. Productive Performance

Feeding the corn diets increased BWG from 1 to 120 d of age with respect to feeding the wheat diets. However, no effects were observed for ADFI, FCR, or BW uniformity. The authors have not found any research comparing productive performance of pullets fed diets based on either corn or wheat. In broilers, Ruiz et al. (1987) found similar BWG and FCR between corn and wheat diets from 1 to 21 d of age. Moreover, Mathlouthi et al. (2002) reported same performance when 60 % corn of a broiler diet was substituted by a mixture of 40 % wheat and 20 % barley supplemented with enzymes. Similarly, Lázaro et al. (2003) and Safaa et al. (2009) did not observe any effect on performance of laying hens from 20 to 48 wk of age when 50% corn was substituted by wheat.

From 1 to 45 d of age, BWG and ADFI were higher for pullets fed pellets than for pullets fed mash, results that agree with data from previous studies conducted with broilers (Quentin et al., 2004; Amerah et al., 2007; Corchero et al., 2008). Birds consumed more of a pellet- than of a mash diet, probably because pelleting improved the texture of the feed. During the process of pelleting, steam and mechanical pressure are applied to the mash to agglomerate the feed particles. Consequently, pelleting increases bulk density and facilitates feed intake. Also, rate of feed passage in the upper GIT was accelerated with pellets (Sibbald, 1979) resulting in faster gastric emptying and thus, in higher feed consumption (Svihus and Hetland, 2001).

Average BW and BW uniformity at the onset of egg production are useful criteria to evaluate future productive performance of pullets (ISA Brown, 2007; Hy-

Line Brown, 2008). Bish et al. (1985) found that SCWL pullets weighing 1,377 g at 20 wk of age had higher ADFI and egg weight but worse FCR than pullets weighing 1,131 g. Similarly, Leeson et al. (1997) indicated that heavier pullets at 18 wk of age consumed more feed, matured faster, and produced more egg mass to 70 wk of age than lighter pullets. In this respect, Deaton et al. (1988) reported that BWG increased in three commercial-type pullets from 12 to 20 wk of age when the diet was pelleted. Therefore, feeding pellets from 1 to 45 d of age might benefit pullet growth under commercial conditions.

In the current experiment, feeding pellets did not improve FCR, which disagrees with most published reports conducted with broilers (Douglas et al., 1990; Quentin et al., 2004; Amerah et al., 2007). Moreover, Gous and Morris (2001) indicated that feeding crumbles to pullets from 1 to 4 wk, and then pellets from 5 to 20 wk of age, improved FCR with respect to pullets fed mash, a finding that also disagrees with our results. Jensen et al. (1962) and Nir et al. (1994a) reported that energy requirement for maintenance was lower in broilers fed pellets than in those fed mash because of less physical activity and less time spent in eating. In addition, Hamilton and Proudfoot (1995) and Wahlström et al. (1999) reported that pelleting improved nutrient digestibility in laying hens. Friction applied during pelleting might disrupt cell walls and release part of the intracellular fat contained in the oil bodies of corn and other ingredients, thereby increasing energy utilization (Huang, 1992). Consequently, FCR should improve with pellet feeding. However, several authors observed little effect of pelleting of the diet (Brickett et al., 2007) or heat processing the cereal portion of the diet (García et al., 2008; Gracia et al., 2009) on FCR of broilers, results that agree with the data of current research. The reason for the discrepancy is not known but the higher ADFI and poorer FCR observed by some authors with mash diets might be due, at least in part, to an increase in feed wastage. Medel et al. (2004) in piglets, and Corchero et al. (2008) in broilers, indicated that the observed improvement in FCR with pelleting was mostly due to a reduction in feed wastage. In the current trial, pullet uniformity was not affected by dietary treatment. No data are available in this respect with pullets, but in broilers Brickett et al. (2007) reported greater flock uniformity at 35 d of age with pellet- than with mash feeds.

4.2. Development of the Gastrointestinal Tract

At 45 d of age, pullets fed the corn diets had higher relative weight of the gizzard than pullets fed the wheat diets, a finding that agree with data of Amerah et al. (2008) in broilers. The higher weight of the gizzard in pullets fed corn might be related to differences in the hardness of the endosperm, that is harder and more difficult to grind for corn than for wheat (Dombrink-Kurtzman and Bietz. 1993; Dobraszczyk et al., 2002). Consequently, the muscular layers will be more developed with corn- than with wheat diets. In addition, the average percentage of very fine particles (160 μm or less) was higher for the wheat- than for the corn diets. Finer particles passed faster through the gizzard than coarser particles (Hetland et al., 2002; Svihus and Hetland, 2002), and consequently, the relative weight of the gizzard will be reduced when wheat replaced corn. In the current experiment, most of the differences in gizzard weight between the corn- and the wheat diets were observed in the pelleted feeds. Also, the length of the SI and ileum were reduced when the wheat based diet was pelleted but no effects were observed with the corn diet. The reason for the interactions in organ development between cereal and feed form is not known but the data of the current trial indicated that pelleting might have a negative effect on the development of the GIT of pullets fed wheat but not in pullets fed corn. In this respect, visual observations (Mateos, G.G. Unpublished data) detected the presence of more digesta, 6 h after slaughter, in the gizzard of pullets fed a corn-pelleted diet than in those fed a wheat-pelleted diet.

At 45 d of age, the relative weight of the gizzard was higher for pullets fed mash than for pullets fed pellets, a finding that is consistent with the lower GMD and faster transit time of the pellets (Sibbald, 1979) and agrees with data of Huang et al. (2006) and Jiménez-Moreno et al. (2008) in broilers. In the current experiment, the relative weight of the gizzard was 53% greater in pullets fed mash than in pullets fed pellets, an increase that was of similar magnitude than the 47% observed by Frikha (2008) in pullets and the 52% observed by Amerah et al. (2007) in broilers, but higher than the 17% increase observed by Scott and McCann (2008) in laying hens. Mateos et al. (2002) and González-Alvarado et al. (2008) suggested that a more functional gizzard might result in more reflux and better mixing of the digesta and endogenous enzymes in the GIT. Therefore, a well developed gizzard might increase nutrient digestibility and help to maintain a healthy microbiota population in the GIT (Gabriel et al., 2008; Santos et al., 2008).

At 120 d of age, gizzard pH was not affected by type of cereal, in agreement with data of Hetland et al. (2003) who found similar pH values in 24-d-old broilers fed wheat-, oat- or barley-diets. On the other hand, gizzard pH was lower for pullets that were fed mash than for pullets that were fed pellets, results that disagree with data of Dahlke et al. (2003) in broilers that did not observe any effect on gizzard pH when diets based on corn, varying in GMD from 340 µm to 1,120 µm, were pelleted. To our knowledge, this is the first report indicating that feeding pellets during the first period of life (1 to 45 d) reduced the size of the organs of the GIT and increased gizzard pH at

120 d of age. The data emphasize the importance of diet form during the first stage of pullet growth on the subsequent development of the GIT.

5. CONCLUSIONS

In conclusion, the substitution of corn by wheat in the diet resulted in a slight reduction in the BW of pullets at 120 d of age but FCR and BW uniformity were not affected. Pelleting the diet from 1 to 45 d of age improved growth performance at this age, an effect that was maintained at 120 d of age. However, feeding pellets reduced the relative weight and pH of the gizzard, and the relative length of the digestive tract at 120 d of age, effects that might influence feed intake and nutrient utilization of hens at the onset of the egg production cycle.

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CHAPTER 4:

EXPERIMENT 2: INFLUENCE OF ENERGY CONCENTRATION AND FEED FORM OF THE DIET ON GROWTH PERFORMANCE AND DIGESTIVE TRAITS OF BROWN-EGG LAYING PULLETS FROM 1 TO 120 DAYS OF AGE

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1. INTRODUCTION

Two major factors affecting productive performance of laying hens are BW and uniformity at the onset of the egg-laying cycle (Akanbi and Goodman, 1982; Bish et al, 1985). Summers and Leeson (1983) reported that BW of pullets at 18 weeks (wk) of age was the most important factor influencing early egg size. Consequently, a main objective for rearing pullets is to obtain flocks with desirable BW and uniformity at a target age (Hy-Line Brown, 2008). Several nutritional strategies have been recommended to improve BWG and uniformity of pullets, including the increase in the AMEn concentration of the diet and feeding pellets instead of mash. Under commercial conditions, energy consumption is the main factor influencing BW gain of pullets (Summers et al., 1987). Cherry et al. (1983) reported that chickens fed high energy diets initially increased their ADFI whereas the opposite effect occurred with low energy diets. In fact, Leeson et al. (1993) indicated that pullets fed a diet containing a 12.67 MJ AMEn/kg consumed 6% more energy than pullets fed a diet containing 11.53 MJ AMEn/kg.

The scientific information available on the influence of feed form on performance of pullets is limited. In SCWL pullets, Deaton et al. (1988) reported that pelleting increased BWG from 12 to 20 wk of age but that ADFI was not affected. Gous and Morris (2001) found that pullets fed crumbles from 1 to 4 wk and then pellets from 5 to 20 wk of age were 6% heavier and consumed 2% less feed than pullets fed mash. Frikha et al. (2009) observed that pullets fed pelleted diets based on maize or wheat from 1 to 45 d of age, had higher ADFI and BWG but similar FCR than pullets fed mash at both 45 and 120 d of life. In contrast, Leeson and Summers (1984) indicated that crumbling of rearing diets had no effect on BW of pullets at maturity or on subsequent egg production. However, in this report birds fed crumbles consumed more feed from hatching to 10 wk of age.

Feed form influences organ development and nutrient digestibility in broilers (Choi et al., 1986; Kilburn and Edwards, 2001; Mateos et al., 2002). Nir et al. (1995) found that pelleting reduced by 15% the RL of the jejunum and the ileum, and Nir et al. (1994) and Corchero et al. (2008) observed that feeding crumbles or pellets to broilers reduced gizzard weight with respect to feeding mash. Recently, Frikha et al. (2009) found that gizzard RW was lighter, and the SI shorter, in pullets fed pellets than in pullets fed mash. The authors have not found any report in the literature, conducted with laying pullets, on the effects of energy concentration of the diet on BW uniformity and the development of the different segments of the GIT. This study aimed to evaluate the influence of energy concentration and feed form of the diet on growth performance and development of the GIT of Hy-Line Brown pullets from 1 to 120 d of age.

2. MATERIAL AND METHODS

2.1. Husbandry and experimental design

All experimental 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 Estado, 2005).

A total of 1,152 one-day-old Hy-Line Brown pullets with an initial BW of 36.8 ± 2.45 g were obtained from a commercial hatchery (Avigan Terralta, Tarragona, Spain) and used in this experiment. On arrival to the experimental farm, the pullets were placed in a windowless environmentally controlled room with free access to feed and water. Room temperature was maintained at 32ºC during the first 3 d of life and then, the temperature was reduced gradually until reaching 21ºC at 5 wk. The pullets were kept on a 23 hours/d light program for the first wk of life and then, light was decreased 2 h/wk until reaching 12 hours of light at 7 wk of age. The birds were weighed individually at 1 d of age and stratified by BW into 4 groups of 288 pullets each. Forty-eight uniform groups of 24 pullets each (6 from each BW group) were formed and two adjacent cages (12 pullets each) constituted the experimental unit. Each cage (0.50m x 0.76m, Zucami, Pamplona, Spain) was provided with an open trough feeder and 2 low pressure nipple drinkers. Eight replicates (24 pullets each) were randomly assigned to each of the six experimental feeding programs. All the pullets were debeaked at 12 d of age and were vaccinated against main diseases (infectious bronchitis disease, infectious bursal disease, Newcastle disease, and *Salmonella spp*.) according to accepted commercial practices.

2.2. Feeding program and experimental diets

The feeding program consisted of three feeds that were supplied from 1 to 45 d (starter), 46-85 d (grower) and 86-120 d of age (developer). From 1 to 45 d of age, the main difference among diets was the energy concentration (LOW, MED and HIG) and the form (mash and 2-mm pellets) of the feed. From 45 to 120 d of age all diets were fed as mash, and therefore, the only difference among treatments was the energy concentration of the diets. The AMEn (MJ/kg) concentration of the MED diets was 12.05, 11.72 and 11.55 for starter, grower and developer feeds, respectively. Diets of the LOW and HIG feeding programs had 5% less or more AMEn than diets from the MED program for each of the three periods considered. Within each period, all diets had similar nutrient content per MJ of AMEn (Fundación Española Desarrollo Nutrición Animal, 2003) and met or exceeded the nutritional recommendations of the NRC (1994) for pullets. All the diets were based on cereals (corn, wheat and barley) and soya bean meal and sunflower meal were the main protein sources used (Table 1). The cereals used in the mash diets were hammer milled (Model CH-9240, Bühler AG, Uzwil, Switzerland) to pass through a 6-mm screen. To prepare the pelleted feeds, an aliquot part of the three mash diets used from 1 to 45 d of age was reground using the same hammer mill provided with a 2-mm screen, steam-conditioned at 72ºC for 60 seconds, and passed through a pellet press (Model 508-150, Mabrik, Barbera del Valles, Barcelona, Spain) provided with a die ring with a 32-mm thickness and a 2-mm screen.

2.3. Laboratory analyses

Representative samples of the diets were ground in a laboratory mill (Model Z-I, Retsch Stuttgart, Germany) provided with a 1-mm screen and analysed for moisture by the oven-drying method (930.01), total ash by a muffle furnace (942.05), nitrogen by combustion (990.03) using a LECO analyser (Model FP-528, LECO, St. Joseph, MI), and Ca and P by spectrophotometry (968.08 and 965.17) as described by AOAC International (2000). Ether extract was determined by Soxhlet analysis (method 4. B) after 3 N HCl acid hydrolysis (Boletín Oficial Estado, 1995) and gross energy was determined using an adiabatic bomb calorimeter (Model 356, Parr Instrument Company, Moline, IL). The calculated and determined analyses of the experimental diets are shown in Table 2. In addition, pellet quality of the starter diets (durability, percentage of fines, and length of the pellets) was measured as indicated by Frikha et al. (2009).

Table 1. Ingredient composition of the experimental diets (g/kg, as-fed basis unless otherwise indicated)

	1-45 days[a]			46-85 days			86-120 days		
Ingredient	Low	Medium	High	Low	Medium	High	Low	Medium	High
Corn	374.9	317.5	259.8	192.9	146.0	98.7	91.0	54.4	17.8
Wheat	--	150.0	300.0	58.7	270.0	481.5	89.9	310.0	530.0
Barley	300.0	150.0	--	481.5	270.0	58.7	530.0	310.0	89.9
Rice bran	--	--	--	--	--	--	40.0	40.0	40.0
Soya bean meal, 440 g CP[b]/kg	286.8	320.5	354.5	130.1	159.2	187.6	100.1	118.4	137.0
Soya bean oil	--	23.1	46.2	--	18.2	36.4	0.9	17.7	34.5
Sunflower meal, 320 g CP/kg	--	--	--	100	100.0	100.0	100.0	100.0	100.0
Methionine-OH, 880 g/kg	1.9	2.0	2.1	1.4	1.4	1.5	0.4	0.5	0.5
L-Lysine-HCl, 780 g/kg	0.6	0.4	0.1	1.7	1.5	1.3	--	--	--
L-Threonine, 980 g	1.1	1.1	1.2	--	0.2	0.5	--	0.1	0.2
Sepiolite[c]	--	--	--	--	--	--	14.0	14.0	14.0
Dicalcium phosphate	6.0	7.1	8.3	2.8	3.8	5.0	2.1	3.0	3.9
Calcium carbonate	21.9	21.2	20.3	24.1	22.6	21.3	24.8	24.8	24.7
Sodium chloride	3.2	3.3	3.5	3.2	3.3	3.5	3.2	3.3	3.5
Vitamin and mineral premix[d]	3.6	3.8	4.0	3.6	3.8	4.0	3.6	3.8	4.0

[a] Diets were offered in mash or pellet form according to treatment.

[b] Crude protein.

[c] Complex magnesium silicate clay.

[d] Supplied per kg of diet: vitamin A (*trans*-retinyl acetate), 9,000 IU; vitamin D_3 (cholecalciferol), 2,600 IU; vitamin E (DL-α-tocopheryl acetate), 16 mg; vitamin B_1, 1.6 mg; vitamin B_2, 6.5 mg; vitamin B_6, 2.2 mg; vitamin B_{12} (cyanocobalamin), 0.015 mg; vitamin K_3, 2.5 mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (D-calcium pantothenate), 10 mg; folic acid, 0.6 mg; D-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron ($FeSO_4$ H_2O), 40 mg; copper ($CuSO_4$ $5H_2O$), 7 mg; iodine $[Ca(IO_3)_2]$, 0.7 mg; selenium (Na_2SeO_3), 0.3 mg; Roxazyme (1,600 U Endo-1.4-β-glucanase; 3,600 U Endo-1.3(4) -β-glucanase, and 5,200 U Endo-1.4-β-xylanase), 200 mg

supplied by DSM, S.A., Madrid, Spain; Natuphos 5000 (300 FTU/Kg), 60 mg supplied by BASF Española, S.A., Tarragona, Spain.

Table 2. Composition of the experimental diets (g/kg, as-fed basis unless otherwise indicated)

	1 - 45 days						46 - 85 days			86 - 120 days		
	Pellets			Mash			Mash			Mash		
Item	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Calculated analysis[a]												
AMEn (MJ/kg)	11.44	12.05	12.66	11.44	12.05	12.66	11.11	11.71	12.32	10.96	11.55	12.13
Crude fibre	39.0	36.9	34.8	39.0	36.9	34.8	58.0	54.8	51.7	58.7	55.1	51.5
Digestible lysine	8.7	9.2	9.7	8.7	9.2	9.7	7.2	7.6	8.0	5.5	5.8	6.1
Digestible methionine	4.3	4.5	4.7	4.3	4.5	4.7	3.7	3.9	4.0	2.7	2.9	3.0
Digestible threonine	6.9	7.3	7.7	6.9	7.3	7.7	4.7	5.0	5.3	4.5	4.7	5.0
Digestible tryptophan	1.9	2.0	2.2	1.9	2.0	2.2	1.6	1.7	1.9	1.5	1.6	1.7
Available phosphorus	3.9	4.2	4.5	3.9	4.2	4.5	3.4	3.7	4.1	3.4	3.6	3.9
Determined analysis[b]												
Gross energy (MJ/kg)	16.26	16.77	17.31	15.94	16.72	17.50	16.29	16.48	17.07	15.85	16.60	16.71
Dry matter	888	891	893	883	885	892	899	902	906	904	904	922
Crude protein	206	212	214	203	210	212	167	175	178	166	170	180
Ether extract	40	54	74	41	53	75	32	44	67	38	59	69
Total ash	51	57	59	55	56	57	49	54	58	62	63	68
Calcium	8.9	8.0	8.5	9.3	8.7	8.5	11.0	10.6	10.8	11.8	10.8	11.8
Total phosphorus	6.1	6.3	7.1	6.8	6.6	7.1	5.8	5.7	6.6	6.1	5.8	5.8

[a] According to Fundación Española Desarrollo Nutrición Animal (2003).

[b] Analysed in triplicate samples.

2.4. Productive performance

Body weight and feed consumption were recorded by replicate at 45, 85 and 120 d of age. Feed wastage was observed to be negligible and was not measured. Mortality was recorded daily. From these data, BWG, ADFI and FCR were determined by period and cumulatively. In addition, all the pullets (12 birds at 45 d of age and 10 birds at 85 d and 120 d of age) of 1 of the 2 cages of each experimental unit were weighed individually at the same control days and BW uniformity was assessed by calculating the percentage of birds that were within ±1.25 SD of the average BW. The 1.25 SD range was selected to fit the commercial management target for BW homogeneity of pullet flocks (80% of birds within ±10% of the average BW of pullets; Hy-Line Brown, 2008).

2.5. Gastrointestinal tract development

At 45 d of age, after the productive performance measurements, two birds were randomly selected from each replicate, weighed individually, and euthanized by CO_2 inhalation. The digestive tract (from the beginning of the proventriculus to the cloaca with content) together with the liver and the pancreas was removed aseptically and weighed. Then, the proventriculus and the gizzard were excised, cleaned from digesta, dried with desiccant paper, and weighed. The weight of the digestive tract, including the digesta content, the liver and the pancreas, and that of the empty organs was expressed relative to live BW (RW, g/kg BW). The length of the duodenum, defined as the region from the pyloric junction to the distal-most point of insertion of the duodenal mesentery, jejunum (from the distal-most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), ileum (from the junction with Meckel's diverticulum to ileocecal junction), and the total length of the two ceca (from the ostium to the tip of the right and left ceca) was measured on a glass surface using a flexible tape with a precision of 1 mm and expressed relative to live BW (RL, cm/kg BW).

At 120 d of age, two extra pullets from each replicate chosen at random were euthanized. The procedures used and the measures taken were similar to those indicated at 45 d of age. In addition, the pH of the gizzard content was also measured in duplicate at this age using a digital pH meter (Crison Instruments S.A., Barcelona, Spain) fitted with a fine tip glass electrode (Model 507, Crison Instruments S.A., Barcelona, Spain). The average value of the two measurements was used for statistical analysis.

2.6. Statistical analysis

The experimental design was completely randomized with six treatments arranged factorially with energy concentration (LOW, MED and HIG) and feed form (mash and 2-mm pellet) as main effects. The data on performance, BW uniformity and GIT traits were analysed using the GLM procedure of SAS software (SAS Institute, 1990). When the model was significant, treatment means were separated using the Tukey's test. Differences between treatment means were considered significant at $P<0.05$. Results in tables are represented as means.

3. RESULTS

The determined chemical analyses of the experimental diets were close to expected values (Table 2). Pellet length (average of 4.13mm), pellet durability index (average of 97.3%) and proportion of fines (3.6%) were similar for all diets, irrespective of energy content (data not shown).

3.1. Productive performance

No interaction between energy concentration and feed form of the diet was detected for any trait studied. Therefore, only main effects are presented. Mortality was low (1.75%) and not related to treatment. Most of the mortality (87.5%) occurred during the first wk of life (data not shown). From 1 to 45 of age and cumulatively (1-120 d) ADFI was reduced and BWG was increased (P<0.001) when the AMEn of the diet increased (Table 3). Consequently, FCR was improved (P<0.001) with increases in the energy concentration of the diet. From 1 to 45 d of age, pullets fed pellets had higher ADFI and BWG (P<0.001) than pullets fed mash and the difference in BWG was maintained at the end of the experiment (P<0.01). Body weight uniformity was not affected by dietary treatment (Table 4).

Table 3. Influence of metabolizable energy concentration (AMEn) and feed form (FF) of the diet on BW gain (BWG, g/d), average daily feed intake (ADFI, g/d) and feed conversion ratio (FCR) of pullets

	1 - 45 days			46 - 85 days			86 - 120 days			1 - 120 days		
Treatment	BWG	ADFI	FCR	BWG	ADFI	FCR	BWG	ADFI	FCR	BWG	ADFI	FCR
AMEn[a]												
Low	10.5[b]	27.8[a]	2.65[a]	15.9[c]	70.4[a]	4.42[a]	8.8	76.3[a]	8.69[a]	11.8[c]	56.3[a]	4.77[a]
Medium	10.8[a]	27.5[a]	2.54[b]	16.6[b]	68.4[a]	4.13[b]	8.7	73.2[b]	8.41[a]	12.1[b]	54.5[b]	4.51[b]
High	10.9[a]	26.5[b]	2.44[c]	17.1[a]	65.7[b]	3.85[c]	8.9	69.4[c]	7.87[b]	12.3[a]	52.2[c]	4.23[c]
S.E.M.[b]	0.06	0.15	0.017	0.11	0.62	0.027	0.13	0.80	0.105	0.05	0.45	0.028
FF												
Mash	10.6	26.9	2.55	16.5	67.5	4.11	8.8	72.6	8.27	12.0	53.9	4.50
Pellet	10.9	27.7	2.54	16.6	68.8	4.15	8.8	73.3	8.37	12.2	54.8	4.51
S.E.M.[c]	0.05	0.12	0.014	0.09	0.51	0.022	0.11	0.65	0.086	0.04	0.37	0.023
Effect[d]	Probability											
AMEn	***	***	***	***	***	***	0.78	***	***	***	***	***
FF	***	***	0.72	0.26	0.09	0.26	0.91	0.49	0.47	**	0.09	0.71

**$P < 0.01$; ***$P < 0.001$.

[a-c] Mean values within a column and main effects not sharing a common superscript are different ($P<0.05$).

[a] AMEn was 11.44, 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium and high energy diets, respectively.

[b] Standard error of the mean (16 replicates of 24 pullets from 1 to 45 d of age and 22 pullets from 46 to 120 d of age).

[c] Standard error of the mean (24 replicates of 24 pullets from 1 to 45 d of age and 22 pullets from 46 to 120 d of age).

[d] The interaction between AMEn concentration and feed form was not significant ($P>0.05$).

Table 4. Influence of metabolizable energy concentration (AMEn) and feed form (FF) of the diet on BW uniformity of pullets at 45, 85 and 120 days of age[a]

Treatment	45 days	85 days	120 days
AMEn[b]			
Low	0.828	0.788	0.800
Medium	0.792	0.763	0.781
High	0.797	0.763	0.794
S.E.M.[c]	0.0314	0.0405	0.0370
FF			
Mash	0.813	0.746	0.775
Pellet	0.799	0.796	0.808
S.E.M.[d]	0.0257	0.0331	0.0302
Effect[e]	Probability		
AMEn	0.68	0.88	0.94
FF	0.70	0.29	0.44

[a] Proportion of pullets with a BW within the average ±1.25 SD range.

[b] AMEn was 11.44, 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d, and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium and high energy diets, respectively.

[c] Standard error of the mean (16 replicates of 12 pullets from 1 to 45 d of age and 10 pullets from 46 to 120 d of age).

[d] Standard error of the mean (24 replicates of 12 pullets from 1 to 45 d of age and 10 pullets from 46 to 120 d of age).

[e] The interaction between AMEn concentration and feed form was not significant (P>0.05).

3.2. Gastrointestinal tract development

No interactions between AMEn concentration of the diet and feed form were detected for any of the traits studied. Therefore, only main effects are presented. At 45 d of age, pullets fed the HIG diets had lower RW of the digestive tract (*P*<0.001), gizzard (*P*<0.001) and proventriculus (*P*<0.05) than pullets fed the LOW and MED diets (Table 5). However, the RL of duodenum, jejunum, ileum and ceca was not affected by the energy content of the diet. At 120 d of age, the only difference observed was for RW of the gizzard that was higher (*P*<0.01) in pullets fed the LOW diet than in those fed the HIG and MED diets (Table 6).

Feeding pellets reduced the RW of the gizzard (P<0.001), proventriculus (*P*<0.05) and digestive tract (*P*<0.001) at 45 d of age. Also, the RL of the SI (*P*<0.05), jejunum (*P*<0.05), ileum (*P*<0.01) and ceca (*P*<0.001) was reduced at this age. However, at 120 d of age, the only differences observed were for the RW of gizzard (*P*<0.001) and proventriculus (*P*<0.01) that were heavier for pullets previously fed mash than for those fed pellets. Gizzard pH at 120 d of age was not affected by diet.

Table 5. Influence of metabolizable energy concentration (AMEn) and feed form (FF) of the diet on relative weight (g/kg BW) and length (cm/kg BW) of the gastrointestinal tract in pullets at 45 days of age

	Relative weight			Relative length				
Treatment	Digestive tract[a]	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Small intestine
AMEn[b]								
Low	144.1[a]	6.2[ab]	36.7[a]	41.3	113.0	94.4	23.5	248.7
Medium	138.9[a]	6.3[a]	35.5[a]	40.6	110.8	91.5	23.1	242.8
High	129.2[b]	5.8[b]	28.9[b]	39.8	108.4	90.0	22.7	238.2
S.E.M.[c]	2.24	0.12	1.08	0.79	2.18	1.71	0.57	4.25
FF								
Mash	146.7	6.3	39.9	41.08	113.5	94.8	24.3	249.3
Pelleted	128.0	5.9	27.4	40.09	108.0	89.1	21.8	237.2
S.E.M.[d]	1.83	0.10	0.88	0.65	1.78	1.39	0.47	3.47
Effect[e]	Probability							
AMEn	***	*	***	0.40	0.34	0.20	0.63	0.23
FF	***	*	***	0.29	*	**	***	*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[a-c] Mean values within a column and main effects not sharing a common superscript are different (P<0.05).

[a] Weight of the digestive tract (from the beginning of the proventriculus to cloaca), including digesta content, the liver and the pancreas.

[b] AMEn was 11.44. 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium, and high energy diets, respectively.

[c] Standard error of the mean (16 replicates of 2 pullets each).

[d] Standard error of the mean (24 replicates of 2 pullets each).

[e] The interaction between AMEn concentration and feed form was not significant (*P*>0.05).

Table 6. Influence of metabolizable energy concentration (AMEn) and feed form (FF) of the diet on relative weight (g/kg BW) and length (cm/kg BW) of the gastrointestinal tract and gizzard pH in pullets at 120 days of age

Treatments	Relative weight			Relative length					Gizzard pH
	Digestive tract[a]	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Small intestine	
AMEn[b]									
Low	125.5	3.3	23.3[a]	18.3	45.8	43.9	18.5	107.9	3.82
Medium	121.7	3.2	21.1[b]	18.4	44.9	42.2	17.7	105.4	3.84
High	119.0	3.1	20.9[b]	18.8	46.4	43.2	18.4	108.3	3.89
S.E.M.[c]	2.61	0.09	0.53	0.41	0.91	0.92	0.33	1.97	0.100
FF									
Mash	125.1	3.4	22.9	18.6	45.9	43.8	18.5	108.4	3.79
Pellet	119.1	3.1	20.7	18.3	45.4	42.4	17.9	106.1	3.91
S.E.M.[d]	2.13	0.07	0.43	0.44	0.74	0.75	0.27	1.61	0.081
Effect[e]	Probability								
AMEn	0.22	0.17	**	0.67	0.50	0.44	0.21	0.54	0.87
FF	0.06	**	***	0.48	0.65	0.18	0.10	0.33	0.33

**$P < 0.01$; ***$P < 0.001$.

[a-c]Means within a column and main effects not sharing a common superscript are different (P<0.05).

[a] Weight of the digestive tract (from the beginning of the proventriculus to cloaca), included digesta content, the liver and the pancreas.

[b] AMEn was 11.44. 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium, and high energy diets, respectively.

[c] Standard error of the mean (16 replicates of 2 pullets each).

[d] Standard error of the mean (24 replicates of 2 pullets each).

[e] The interaction between AMEn concentration and feed form was not significant (*P*>0.05).

4. DISCUSSION

4.1. Productive performance

Body weight gain and FCR of pullets improved as the energy concentration of the diet increased, which agree with data of Summers et al. (1987) with increases in the AMEn of diets of SCWL pullets from 1 to 16 wk of age from 10.44 to 12.45 MJ/kg. Also, Keshavarz and Nakajima (1995) observed that increasing the AMEn concentration of the diet of SCWL pullets from 10.88 to 12.97 MJ/kg from 14 to 18 wk of age reduced ADFI and improved growth performance. Moreover, Keshavarz (1998) reported that an increase in the AMEn concentration of the diet from 11.78 to 12.70 MJ/kg for the last 10 wk of the rearing period, improved performance of SCWL pullets. In contrast, Summers and Leeson (1993) reported that a 10% increase in the AMEn concentration of the diet of SCWL from 16 to 20 wk of age did not affect BW at 20 wk of age. The authors indicated that in order to be effective in improving BW, the increase in AMEn of the diet should be done earlier in the rearing period. These results agree with the data of the current trial in which an increase in AMEn concentrationt of the diet from 10.96 to 12.13 MJ/kg fed to pullets from 12 to 17 wk improved FCR but not BWG.

Leeson et al. (1996) provided diets to broilers containing 11.3-13.8 MJ AMEn/kg and observed no change in growth rate. These authors concluded that the more recent genetic strains of broilers used by the industry possess a good ability to control feed intake based on their desire to normalize energy intake. However, in the current trial, pullets fed the lower energy diets had lower final weights than pullets fed the high energy diets. Probably, differences in the objectives for genetic improvement for broilers and pullets account for the differences detected between broilers and pullets.

In the current research, an increase in the AMEn concentration of the diet did not affect BW uniformity, which does not conform to data of Keshavarz (1998) who found that uniformity of SCWL pullets at 18 wk of age improved when the AMEn concentration of the diet fed from hatching to 18 wk of age was increased from 11.78 to 12.70 MJ/kg. Also, Brickett et al. (2007) found that BW uniformity of 35 d old broilers was improved when the AMEn concentration of the diet was increased from 11.71 to 12.97 MJ/kg.

Pelleting increased ADFI and BWG of pullets from 1 to 45 d of age in agreement with results of Corchero et al. (2008) in broilers from 1 to 42 d of age and consistent with data of Sibbald (1979) who observed that pelleting increased the rate of passage of the digesta in adult roosters. Consequently, feeding pellets should improve feed consumption in poultry. In addition, the application of steam and mechanical pressure to the meal to agglomerate feed particles improves bulk density and feed texture, which in turn might benefit feed intake. In the current experiment, pullets fed pellets from 1 to 45 d of age had higher BWG from 1 to 120 d of age than pullets fed mash, in agreement with data of Frikha et al. (2009) using the same strain of pullets. Consistent with these results, Gous and Morris (2001) found that pullets fed crumbles from 1 to 4 wk and then pellets from 5 to 20 wk of age were 6% heavier than pullets fed mash. Also, Deaton et al. (1988) reported that feeding pellets to pullets from 12 to 20 wk of age increased BWG, but in this research ADFI was not affected.

It has been reported that feeding pellets consistently improved FCR in broilers (Quentin et al., 2004; Amerah et al., 2007) and in pullets (Gous and Morris, 2001). Hamilton and Proudfoot (1995) indicated that the improvement in FCR with pelleting was a consequence of the increase in nutrient digestibility. In fact, Wahlström et al. (1999) observed that the digestibility of starch and fat in laying hens increased when the feed was crumbled. Pelleting might disrupt the structure of the cells' walls and starch granules of corn and other ingredients, releasing part of the intracellular fat contained in the oil bodies and facilitating the access of endogenous enzymes to nutrients. Consequently, energy utilization and FCR will be improved (Gracia et al., 2009). However, in the current experiment, feeding pellets did not affect FCR which agrees with data of Bolton (1960), Plavnik et al. (1997), and Brickett et al. (2007), that failed to find any significant advantage of feeding pellets on FCR or nutrient digestibility in broilers. Moreover, Svihus and Hetland (2001) found that pelleting increased ADFI but reduced nutrient digestibility, and García et al. (2008) observed that heat processing of the cereal did not affect organic matter digestibility or N retention in broilers diets. Feed wastage is higher with mash than with pelleted diets and non-recorded feed wastage might help to explain the higher ADFI and poorer FCR observed when mash diets are used. Our data support the hypothesis that pelleting of the diet has little effect on nutrient digestibility, and that most of the improvement in feed efficiency observed with pelleting by some authors is probably due to a reduction in feed wastage. In this respect, Medel et al. (2004) and Corchero et al. (2008) found that pelleting reduced feed wastage by 6.6% in piglets and by 8.5% in broilers from 1 to 14 d of age, respectively. In fact, Medel et al. (2004) indicated that the improvement in FCR observed with pellet feeding was due primarily to a reduction in feed wastage.

4.2. Gastrointestinal tract development

An increase in the AMEn concentration of the diet reduced the RW of the gizzard at 45 and 120 d of age without affecting the RL of the GIT. High energy diets contain less fibre and more fat than low energy diets. Summers and Leeson (1986) and González-Alvarado et al. (2007, 2008) found that a decrease in the fibre content of the diet reduced gizzard weight in broilers and in laying hens results that are consistent with the findings of the current research.

At 45 d of age, feeding pellets decreased the RW and the RL of all the segments of the GIT except the RL of the duodenum. However, at 120 d of age the only differences observed were for the RW of gizzard and proventriculus, that were higher for the mash than for the pelleted diets. The results agree with data of Frikha et al. (2009) who reported that feeding pellets from 1 to 45 d of age reduced the RW of the gizzard and proventriculus and also the RL of the jejunum and ileum in Hy-Line Brown pullets. In broilers, feeding crumbles or pellets consistently reduced the RW of the gizzard (Choi et al., 1986; Nir et al., 1994). Similar results have been reported by Scott and McCann (2008) in laying hens fed pellets from 30 to 36 wk of age. Moreover, Nir et al. (1995) found that pelleting reduced by 15% the RL of the jejunum and ileum of broilers, and Amerah et al. (2007) reported that the improvement in broiler performance observed with pelleting was associated with a decrease in the RL of the GIT. Gizzard pH at 120 d of age was not affected by diet, a finding that disagrees with data of Huang et al. (2006) and Corchero et al. (2008) that reported higher gizzard pH in broilers fed pellets than in broilers fed mash. However, in the current research pullets were fed a mash diet from 45 to 120 d of age which might have reduced the negative effects of pelleting on gizzard pH.

5. CONCLUSIONS

An increase in the energy concentration of the diet of Hy-Line Brown pullets improved pullet performance at all ages but had no effects on BW uniformity. Feeding pellets from 1 to 45 d of age improved BWG and ADFI at this age but FCR was not affected. The beneficial effects of feeding pellets from 1 to 45 d of age on BWG were maintained at 120 d of age. Increasing the AMEn concentration or pelleting of the diet fed from 1 to 45 d of age, reduced the RW of the gizzard at 120 d of age, a finding that has to be taken into account in pullet rearing because a poor development of the gizzard might affect productive performance at the onset of the egg-laying period.

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CHAPTER 5:

GENERAL CONCLUSIONS

Conclusions

The general conclusions of this Master Thesis are:

- *Wheat can be used successfully in substitution of corn at levels of up to 50% in diets for brown-egg laying pullets with only a slight reduction in body weight. Therefore, the cereal to use in the diet will depend mainly on the relative cost and availability at a given time.*

- *Brown egg-laying pullets eat more feed and achieve better body weight at 120 d of age when fed pellets than when fed mash diets. However, feed conversion ratio is not affected by feed form.*

- *An increase in the AMEn content of the diets used during the rearing period improves productive performance including body weight gain and feed conversion ratio.*

- *Feeding pellets, wheat, and high energy diets reduced the weight of the gizzard. Also, the relative weight and length of the different parts of the digestive tract were reduced when the diet was pelleted, but no effect was observed with corn or wheat diet.*

Therefore, the use of pelleted diets and high energy level diets for the first part of life might affect negatively feed intake and laying hen performance early in the production cycle.

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Influence of energy concentration and feed form of the diet on growth performance and digestive traits of brown egg-laying pullets from 1 to 120 days of age

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ABSTRACT

A total of 1152 one-day-old Hy-Line Brown pullets were used to study the influence of energy content of the diet and feed form on productive performance and digestive tract traits. From 1 to 45 days (d) of age, there were six diets arranged factorially with three Apparent Metabolizable Energy (AMEn) concentrations (low, medium and high) and two feed forms (mash and pellets). From 45 to 120 d all diets were fed in mash form and therefore, the only difference was the energy content. Each of the 6 treatments was replicated 8 times and the experimental unit was formed by 24 pullets housed in 2 adjacent cages. For the entire experiment, body weight (BW) gain and feed to gain ratio improved as the AMEn of the diet increased (P<0.001). Pullets fed pellets from 1 to 45 d of age had higher feed intake and BW gain (P<0.001) in this period and higher BW gain (P<0.01) cumulatively, than pullets fed mash. At 45 d of age, the relative weight (RW; g/kg BW) of all the segments of the gastrointestinal tract (GIT) was lower for pullets fed with the high- than for pullets fed the medium- or low-energy diets. At 120 d of age the RW of the gizzard was higher (P<0.01) for pullets fed the low energy diets than for pullets fed the other diets. The relative length (RL; cm/kg BW) of the GIT was not affected by the energy content of the diet. Feeding pellets reduced the RW of the proventriculus

Abbreviations: BW, body weight; BWG, body weight gain; AMEn, Apparent Metabolizable Energy nitrogen corrected; RW, relative weight; RL, relative length; d, day; wk, week; GIT, gastrointestinal tract; ADFI, average daily feed intake; SCWL, Single Comb White Leghorn; FCR, feed to gain ratio; SI, small intestine; LOW, low AMEn content; MED, medium AMEn content; HIG, high AMEn content.

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($P<0.05$), the gizzard ($P<0.001$) and the digestive tract ($P<0.001$), and the RL of the small intestine ($P<0.05$) and the ceca ($P<0.001$) at 45 d of age. The effects of feeding pellets on RW of gizzard and proventriculus were still evident at 120 d of age. We concluded that feeding pellets from 1 to 45 d of age improved feed intake and BW of pullets at 120 d of age, and that an increase in the energy content of the diet increased pullet performance at all ages but reduced the RW of the proventriculus and gizzard.

1. Introduction

Two major factors affecting productive performance of laying hens are body weight (BW) and uniformity at the onset of the egg-laying cycle (Akanbi and Goodman, 1982; Bish et al., 1985). Summers and Leeson (1983) reported that BW of pullets at 18 weeks (wk) of age was the most important factor influencing early egg size. Consequently, a main objective for rearing pullets is to obtain flocks with desirable BW and uniformity at a target age (Hy-Line Brown, 2008). Several nutritional strategies have been recommended to improve BW gain and uniformity of pullets, including the increase in the Apparent Metabolizable Energy (AMEn) content of the diet and feeding pellets instead of mash. Under commercial conditions, energy consumption is the main factor influencing BW gain of pullets (Summers et al., 1987). Cherry et al. (1983) reported that chickens fed high energy diets initially increased their average daily feed intake (ADFI) whereas the opposite effect occurred with low energy diets. In fact, Leeson et al. (1993) indicated that pullets fed a diet containing a 12.67 MJ AMEn/kg consumed 6% more energy than pullets fed a diet containing 11.53 MJ AMEn/kg.

The scientific information available on the influence of feed form on performance of pullets is limited. In Single Comb White Leghorn (SCWL) pullets, Deaton et al. (1988) reported that pelleting increased BW gain (BWG) from 12 to 20 wk of age but that ADFI was not affected. Gous and Morris (2001) found that pullets fed crumbles from 1 to 4 wk and then pellets from 5 to 20 wk of age were 6% heavier and consumed 2% less feed than pullets fed mash. Frikha et al. (2009) observed that pullets fed pelleted diets based on maize or wheat from 1 to 45 days (d) of age, had higher ADFI and BWG but similar feed conversion ratio (FCR) than pullets fed mash at both 45 and 120 d of life. In contrast, Leeson and Summers (1984) indicated that crumbling of rearing diets had no effect on BW of pullets at maturity or on subsequent egg production. However, in this report birds fed crumbles consumed more feed from hatching to 10 wk of age.

Feed form influences organ development and nutrient digestibility in broilers (Choi et al., 1986; Kilburn and Edwards, 2001; Mateos et al., 2002). Nir et al. (1995) found that pelleting reduced by 15% the relative length (RL; cm/kg BW) of the jejunum and the ileum, and Nir et al. (1994) and Corchero et al. (2008) observed that feeding crumbles or pellets to broilers reduced gizzard weight with respect to feeding mash. Recently, Frikha et al. (2009) found that gizzard RW was lighter, and the small intestine (SI) shorter, in pullets fed pellets than in pullets fed mash. The authors have not found any report in the literature, conducted with laying pullets, on the effects of energy concentration of the diet on BW uniformity and the development of the different segments of the gastrointestinal tract (GIT). This study aimed to evaluate the influence of energy concentration and feed form of the diet on growth performance and development of the GIT of Hy-Line Brown pullets from 1 to 120 d of age.

2. Materials and methods

2.1. Husbandry and experimental design

All experimental 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 Estado, 2005).

A total of 1152 one-day-old Hy-Line Brown pullets with an initial BW of 36.8 ± 2.45 g were obtained from a commercial hatchery (Avigan Terralta, Tarragona, Spain) and used in this experiment. On arrival to the experimental farm, the pullets were placed in a windowless environmentally controlled room with free access to feed and water. Room temperature was maintained at 32 °C during the first 3 d of life and then, the temperature was reduced gradually until reaching 21 °C at 5 wk. The pullets were kept on a 23 h/d light program for the first wk of life and then, light was decreased 2 h/wk until reaching 12 h of light at 7 wk of age. The birds were weighed individually at 1 day of age and stratified by BW into four groups of 288 pullets each. Forty-eight uniform groups of 24 pullets each (6 from each BW group) were formed and 2 adjacent cages (12 pullets each) constituted the experimental unit. Each cage (0.50 m × 0.76 m, Zucami, Pamplona, Spain) was provided with an open trough feeder and two low pressure nipple drinkers. Eight replicates (24 pullets each) were randomly assigned to each of the six experimental feeding programs. All the pullets were debeaked at 12 d of age and were vaccinated against main diseases (infectious bronchitis disease, infectious bursal disease, Newcastle disease, and *Salmonella spp*.) according to accepted commercial practices.

2.2. Feeding program and experimental diets

The feeding program consisted of three feeds that were supplied from 1 to 45 d (starter), 46–85 d (grower) and 86–120 d of age (developer). From 1 to 45 d of age, the main difference among diets was the energy concentration (LOW, MED and HIG) and the form (mash and 2-mm pellets) of the feed. From 45 to 120 d of age all diets were fed as mash, and therefore, the only difference among treatments was the energy concentration of the diets. The AMEn (MJ/kg) content of the MED diets was, 12.05, 11.72 and 11.55 for starter, grower and developer feeds, respectively. Diets of the LOW and HIG feeding programs had 5% less or more AMEn than diets from the MED program for each of the three periods considered. Within each period, all diets had similar nutrient content per MJ of AMEn (Fundación Española Desarrollo Nutrición Animal, 2003) and met or exceeded the nutritional recommendations of the NRC (1994) for pullets. All the diets were based on cereals (maize, wheat and barley) and soya bean meal and sunflower meal were the main protein sources used (Table 1). The cereals used in the

Table 1
Composition of the experimental diets (g/kg, as-fed basis unless otherwise indicated).

Ingredient	1–45 days[a]			46–85 days			86–120 days		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Maize	374.9	317.5	259.8	192.9	146.0	98.7	91.0	54.4	17.8
Wheat	–	150.0	300.0	58.7	270.0	481.5	89.9	310.0	530.0
Barley	300.0	150.0	–	481.5	270.0	58.7	530.0	310.0	89.9
Rice bran	–	–	–	–	–	–	40.0	40.0	40.0
Soya bean meal, 440 g CP[b]/kg	286.8	320.5	354.5	130.1	159.2	187.6	100.1	118.4	137.0
Soya bean oil	–	23.1	46.2	–	18.2	36.4	0.9	17.7	34.5
Sunflower meal, 320 g CP/kg	–	–	–	100	100.0	100.0	100.0	100.0	100.0
Methionine–OH, 880 g/kg	1.9	2.0	2.1	1.4	1.4	1.5	0.4	0.5	0.5
L-lysine–HCl, 780 g/kg	0.6	0.4	0.1	1.7	1.5	1.3	–	–	–
L-threonine, 980 g	1.1	1.1	1.2	–	0.2	0.5	–	0.1	0.2
Sepiolite[c]	–	–	–	–	–	–	14.0	14.0	14.0
Dicalcium phosphate	6.0	7.1	8.3	2.8	3.8	5.0	2.1	3.0	3.9
Calcium carbonate	21.9	21.2	20.3	24.1	22.6	21.3	24.8	24.8	24.7
Sodium chloride	3.2	3.3	3.5	3.2	3.3	3.5	3.2	3.3	3.5
Vitamin and mineral premix[d]	3.6	3.8	4.0	3.6	3.8	4.0	3.6	3.8	4.0

[a] Diets were offered in mash or pellet form according to treatment.

[b] Crude protein.

[c] Complex magnesium silicate clay.

[d] Supplied per kg of diet: vitamin A (*trans*-retinyl acetate), 9000 IU; vitamin D_3 (cholecalciferol), 2600 IU; vitamin E (DL-α-tocopheryl acetate), 16 mg; vitamin B_1, 1.6 mg; vitamin B_2, 6.5 mg; vitamin B_6, 2.2 mg; vitamin B_{12} (cyanocobalamin), 0.015 mg; vitamin K_3, 2.5 mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (D-calcium pantothenate), 10 mg; folic acid, 0.6 mg; D-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron ($FeSO_4 \cdot H_2O$), 40 mg; copper ($CuSO_4 \cdot 5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na_2SeO_3), 0.3 mg; Roxazyme (1600 U endo-1,4-β-glucanase, 3600 U endo-1,3(4)-β-glucanase, and 5200 U endo-1,4-β-xylanase), 200 mg supplied by DSM, S.A., Madrid, Spain; Natuphos 5000 (300 FTU/kg), 60 mg supplied by BASF Española, S.A., Tarragona, Spain.

mash diets were hammer milled (Model CH-9240, Bühler AG, Uzwil, Switzerland) to pass through a 6-mm screen. To prepare the pelleted feeds, an aliquot part of the three mash diets used from 1 to 45 d of age was reground using the same hammer mill provided with a 2-mm screen, steam-conditioned at 72 °C for 60 s, and passed through a pellet press (Model 508-150, Mabrik, Barbera del Valles, Barcelona, Spain) provided with a die ring with a 32-mm thickness and a 2-mm screen.

2.3. Laboratory analyses

Representative samples of the diets were ground in a laboratory mill (Model Z-I, Retsch Stuttgart, Germany) provided with a 1-mm screen and analysed for moisture by the oven-drying method (930.01), total ash by a muffle furnace (942.05), nitrogen by combustion (990.03) using a LECO analyser (Model FP-528, LECO, St. Joseph, MI), and Ca and P by spectrophotometry (968.08 and 965.17) as described by AOAC International (2000). Ether extract was determined by Soxhlet analysis (method 4. B) after 3N HCl acid hydrolysis (Boletín Oficial Estado, 1995) and gross energy was determined using an adiabatic bomb calorimeter (Model 356, Parr Instrument Company, Moline, IL). The calculated and determined analyses of the experimental diets are shown in Table 2. In addition, pellet quality of the starter diets (durability, percentage of fines, and length of the pellets) was measured as indicated by Frikha et al. (2009).

2.4. Productive performance

Body weight and feed consumption were recorded by replicate at 45, 85 and 120 d of age. Feed wastage was observed to be negligible and was not measured. Mortality was recorded daily. From these data, BWG, ADFI and FCR were determined by period and cumulatively. In addition, all the pullets (12 birds at 45 d of age and 10 birds at 85 d and 120 d of age) of 1 of the 2 cages of each experimental unit were weighed individually at the same control days and BW uniformity was assessed by calculating the percentage of birds that were within ±1.25 SD of the average BW. The 1.25 SD range was selected to fit the commercial management target for BW homogeneity of pullet flocks (80% of birds within ±10% of the average BW of pullets; Hy-Line Brown, 2008).

2.5. Gastrointestinal tract development

At 45 d of age, after the productive performance measurements, two birds were randomly selected from each replicate, weighed individually, and euthanized by CO_2 inhalation. The digestive tract (from the beginning of the proventriculus to the cloaca with content) together with the liver and the pancreas was removed aseptically and weighed. Then, the proventriculus and the gizzard were excised, cleaned from digesta, dried with desiccant paper, and weighed. The weight of the digestive tract, including the digesta content, the liver and the pancreas, and that of the empty organs was expressed relative to live BW (RW, g/kg BW). The length of the duodenum, defined as the region from the pyloric junction to the distal-most point of insertion of the duodenal mesentery, jejunum (from the distal-most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), ileum (from the junction with Meckel's diverticulum to ileocecal junction), and the total length of the two ceca (from the ostium to the tip of the right and left ceca) was measured on a glass surface using a flexible tape with a precision of 1 mm and expressed relative to live BW (RL, cm/kg BW).

At 120 d of age, two extra pullets from each replicate chosen at random were euthanized. The procedures used and the measures taken were similar to those indicated at 45 d of age. In addition, the pH of the gizzard content was also measured in duplicate at this age using a digital pH meter (Crison Instruments S.A., Barcelona, Spain) fitted with a fine tip glass electrode (Model 507, Crison Instruments S.A., Barcelona, Spain). The average value of the two measurements was used for statistical analysis.

2.6. Statistical analysis

The experimental design was completely randomized with six treatments arranged factorially with energy concentration (LOW, MED and HIG) and feed form (mash and 2-mm pellet) as main effects.

Table 2
Composition of the experimental diets (g/kg, as-fed basis unless otherwise indicated).

Item	1–45 days						46–85 days			86–120 days		
	Pellets			Mash			Mash			Mash		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Calculated analysis[a]												
AMEn (MJ/kg)	11.44	12.05	12.66	11.44	12.05	12.66	11.11	11.71	12.32	10.96	11.55	12.13
Crude fibre	39.0	36.9	34.8	39.0	36.9	34.8	58.0	54.8	51.7	58.7	55.1	51.5
Digestible lysine	8.7	9.2	9.7	8.7	9.2	9.7	7.2	7.6	8.0	5.5	5.8	6.1
Digestible methionine	4.3	4.5	4.7	4.3	4.5	4.7	3.7	3.9	4.0	2.7	2.9	3.0
Digestible threonine	6.9	7.3	7.7	6.9	7.3	7.7	4.7	5.0	5.3	4.5	4.7	5.0
Digestible tryptophan	1.9	2.0	2.2	1.9	2.0	2.2	1.6	1.7	1.9	1.5	1.6	1.7
Available phosphorus	3.9	4.2	4.5	3.9	4.2	4.5	3.4	3.7	4.1	3.4	3.6	3.9
Determined analysis[b]												
Gross energy (MJ/kg)	16.26	16.77	17.31	15.94	1 16.72	17.50	16.29	16.48	17.07	15.85	16.60	16.71
Dry matter	888	891	893	883	885	892	899	902	906	904	904	922
Crude protein	206	212	214	203	210	212	167	175	178	166	170	180
Ether extract	40	54	74	41	53	75	32	44	67	38	59	69
Total ash	51	57	59	55	56	57	49	54	58	62	63	68
Calcium	8.9	8.0	8.5	9.3	8.7	8.5	11.0	10.6	10.8	11.8	10.8	11.8
Total phosphorus	6.1	6.3	7.1	6.8	6.6	7.1	5.8	5.7	6.6	6.1	5.8	5.8

[a] According to Fundación Española Desarrollo Nutrición Animal (2003).
[b] Analysed in triplicate samples.

The data on performance, BW uniformity and GIT traits were analysed using the GLM procedure of SAS software (SAS Institute, 1990). When the model was significant, treatment means were separated using the Tukey's test. Differences between treatment means were considered significant at $P<0.05$. Results in tables are represented as means.

3. Results

The determined chemical analyses of the experimental diets were close to expected values (Table 2). Pellet length (average of 4.13 mm), pellet durability index (average of 97.3%) and proportion of fines (3.6%) were similar for all diets, irrespective of energy content (data not shown).

3.1. Productive performance

No interaction between energy concentration and feed form of the diet was detected for any trait studied. Therefore, only main effects are presented. Mortality was low (1.75%) and not related to treatment. Most of the mortality (87.5%) occurred during the first wk of life (data not shown). From 1 to 45 of age and cumulatively (1–120 d) ADFI was reduced and BWG was increased ($P<0.001$) when the AMEn of the diet increased (Table 3). Consequently, FCR was improved ($P<0.001$) with increases in the energy content of the diet. From 1 to 45 d of age, pullets fed pellets had higher ADFI and BWG ($P<0.001$) than pullets fed mash and the difference in BWG was maintained at the end of the experiment ($P<0.01$). Body weight uniformity was not affected by dietary treatment (Table 4).

3.2. Gastrointestinal tract development

No interactions between AMEn content of the diet and feed form were detected for any of the traits studied. Therefore, only main effects are presented. At 45 d of age, pullets fed the HIG diets had lower RW of the digestive tract ($P<0.001$), gizzard ($P<0.001$) and proventriculus ($P<0.05$) than pullets fed the LOW and MED diets (Table 5). However, the RL of duodenum, jejunum, ileum and ceca was not

Table 3
Influence of metabolizable energy content (AMEn) and feed form (FF) of the diet on BW gain (BWG, g/d), average daily feed intake (ADFI, g/d) and feed conversion ratio (FCR) of pullets.

Treatment	1–45 days			46–85 days			86–120 days			1–120 days		
	BWG	ADFI	FCR	BWG	ADFI	FCR	BWG	ADFI	FCR	BWG	ADFI	FCR
AMEn[a]												
Low	10.5^{b}	27.8^{a}	2.65^{a}	15.9^{c}	70.4^{a}	4.42^{a}	8.8	76.3^{a}	8.69^{a}	11.8^{c}	56.3^{a}	4.77^{a}
Medium	10.8^{a}	27.5^{a}	2.54^{b}	16.6^{b}	68.4^{a}	4.13^{b}	8.7	73.2^{b}	8.41^{a}	12.1^{b}	54.5^{b}	4.51^{b}
High	10.9^{a}	26.5^{b}	2.44^{c}	17.1^{a}	65.7^{b}	3.85^{c}	8.9	69.4^{c}	7.87^{b}	12.3^{a}	52.2^{c}	4.23^{c}
S.E.M.[b]	0.06	0.15	0.017	0.11	0.62	0.027	0.13	0.80	0.105	0.05	0.45	0.028
FF												
Mash	10.6	26.9	2.55	16.5	67.5	4.11	8.8	72.6	8.27	12.0	53.9	4.50
Pellet	10.9	27.7	2.54	16.6	68.8	4.15	8.8	73.3	8.37	12.2	54.8	4.51
S.E.M.[c]	0.05	0.12	0.014	0.09	0.51	0.022	0.11	0.65	0.086	0.04	0.37	0.023
Effect[d]												
AMEn	***	***	***	***	***	***	0.78	***	***	***	***	***
FF	***	***	0.72	0.26	0.09	0.26	0.91	0.49	0.47	**	0.09	0.71

(a–c) Mean values within a column and main effects not sharing a common superscript are different ($P<0.05$).

** $P<0.01$.

*** $P<0.001$.

[a] AMEn was 11.44, 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium and high energy diets, respectively.

[b] Standard error of the mean (16 replicates of 24 pullets from 1 to 45 d of age and 22 pullets from 46 to 120 d of age).

[c] Standard error of the mean (24 replicates of 24 pullets from 1 to 45 d of age and 22 pullets from 46 to 120 d of age).

[d] The interaction between AMEn concentration and feed form was not significant ($P>0.05$).

Table 4
Influence of metabolizable energy content (AMEn) and feed form (FF) of the diet on BW uniformity of pullets at 45, 85 and 120 days of age[a].

Treatment	45 days	85 days	120 days
AMEn[b]			
Low	0.828	0.788	0.800
Medium	0.792	0.763	0.781
High	0.797	0.763	0.794
S.E.M.[c]	0.0314	0.0405	0.0370
FF			
Mash	0.813	0.746	0.775
Pellet	0.799	0.796	0.808
S.E.M.[d]	0.0257	0.0331	0.0302
Effect[e]			
AMEn	0.68	0.88	0.94
FF	0.70	0.29	0.44

[a] Proportion of pullets with a BW within the average ±1.25 SD range.

[b] AMEn was 11.44, 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d, and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium and high energy diets, respectively.

[c] Standard error of the mean (16 replicates of 12 pullets from 1 to 45 d of age and 10 pullets from 46 to 120 d of age).

[d] Standard error of the mean (24 replicates of 12 pullets from 1 to 45 d of age and 10 pullets from 46 to 120 d of age).

[e] The interaction between AMEn concentration and feed form was not significant ($P>0.05$).

Table 5
Influence of metabolizable energy content (AMEn) and feed form (FF) of the diet on relative weight (g/kg BW) and length (cm/kg BW) of the gastrointestinal tract in pullets at 45 days of age.

Treatment	Relative weight			Relative length				
	Digestive tract[a]	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Small intestine
AMEn[b]								
Low	144.1[a]	6.2[a,b]	36.7[a]	41.3	113.0	94.4	23.5	248.7
Medium	138.9[a]	6.3[a]	35.5[a]	40.6	110.8	91.5	23.1	242.8
High	129.2[b]	5.8[b]	28.9[b]	39.8	108.4	90.0	22.7	238.2
S.E.M.[c]	2.24	0.12	1.08	0.79	2.18	1.71	0.57	4.25
FF								
Mash	146.7	6.3	39.9	41.08	113.5	94.8	24.3	249.3
Pelleted	128.0	5.9	27.4	40.09	108.0	89.1	21.8	237.2
S.E.M.[d]	1.83	0.10	0.88	0.65	1.78	1.39	0.47	3.47
Effect[e]								
AMEn	***	*	***	0.40	0.34	0.20	0.63	0.23
FF	***	*	***	0.29	*	**	***	*

(a–c) Mean values within a column and main effects not sharing a common superscript are different ($P<0.05$).

* $P<0.05$.

** $P<0.01$.

*** $P<0.001$.

[a] Weight of the digestive tract (from the beginning of the proventriculus to cloaca), including digesta content, the liver and the pancreas.

[b] AMEn was 11.44. 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium, and high energy diets, respectively.

[c] Standard error of the mean (16 replicates of 2 pullets each).

[d] Standard error of the mean (24 replicates of 2 pullets each).

[e] The interaction between AMEn concentration and feed form was not significant ($P>0.05$).

Table 6
Influence of metabolizable energy content (AMEn) and feed form (FF) of the diet on relative weight (g/kg BW) and length (cm/kg BW) of the gastrointestinal tract and gizzard pH in pullets at 120 days of age.

Treatments	Relative weight			Relative length					Gizzard pH
	Digestive tract[a]	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Small intestine	
AMEn[b]									
Low	125.5	3.3	23.3[a]	18.3	45.8	43.9	18.5	107.9	3.82
Medium	121.7	3.2	21.1[b]	18.4	44.9	42.2	17.7	105.4	3.84
High	119.0	3.1	20.9[b]	18.8	46.4	43.2	18.4	108.3	3.89
S.E.M.[c]	2.61	0.09	0.53	0.41	0.91	0.92	0.33	1.97	0.100
FF									
Mash	125.1	3.4	22.9	18.6	45.9	43.8	18.5	108.4	3.79
Pellet	119.1	3.1	20.7	18.3	45.4	42.4	17.9	106.1	3.91
S.E.M.[d]	2.13	0.07	0.43	0.44	0.74	0.75	0.27	1.61	0.081
Effect[e]									
AMEn	0.22	0.17	**	0.67	0.50	0.44	0.21	0.54	0.87
FF	0.06	**	***	0.48	0.65	0.18	0.10	0.33	0.33

(a–c) Means within a column and main effects not sharing a common superscript are different ($P<0.05$).

** $P<0.01$.

*** $P<0.001$.

[a] Weight of the digestive tract (from the beginning of the proventriculus to cloaca), included digesta content, the liver and the pancreas.

[b] AMEn was 11.44, 12.05 and 12.66 MJ/kg from 1 to 45 d, 11.11, 11.71 and 12.32 MJ/kg from 46 to 85 d and 10.96, 11.55 and 12.13 MJ/kg from 86 to 120 d of age for the low, medium, and high energy diets, respectively.

[c] Standard error of the mean (16 replicates of 2 pullets each).

[d] Standard error of the mean (24 replicates of 2 pullets each).

[e] The interaction between AMEn concentration and feed form was not significant ($P>0.05$).

affected by the energy content of the diet. At 120 d of age, the only difference observed was for RW of the gizzard that was higher ($P<0.01$) in pullets fed the LOW diet than in those fed the HIG and MED diets (Table 6).

Feeding pellets reduced the RW of the gizzard ($P<0.001$), proventriculus ($P<0.05$) and digestive tract ($P<0.001$) at 45 d of age. Also, the RL of the SI ($P<0.05$), jejunum ($P<0.05$), ileum ($P<0.01$) and ceca ($P<0.001$) was reduced at this age. However, at 120 d of age, the only differences observed were for the RW of gizzard ($P<0.001$) and proventriculus ($P<0.01$) that were heavier for pullets previously fed mash than for those fed pellets. Gizzard pH at 120 d of age was not affected by diet.

4. Discussion

4.1. Productive performance

Body weight gain and FCR of pullets improved as the energy concentration of the diet increased, which agree with data of Summers et al. (1987) with increases in the AMEn of diets of SCWL pullets from 1 to 16 wk of age from 10.44 to 12.45 MJ/kg. Also, Keshavarz and Nakajima (1995) observed that increasing the AMEn concentration of the diet of SCWL pullets from 10.88 to 12.97 MJ/kg from 14 to 18 wk of age reduced ADFI and improved growth performance. Moreover, Keshavarz (1998) reported that an increase in the AMEn concentration of the diet from 11.78 to 12.70 MJ/kg for the last 10 wk of the rearing period, improved performance of SCWL pullets. In contrast, Summers and Leeson (1993) reported that a 10% increase in the AMEn content of the diet of SCWL from 16 to 20 wk of age did not affect BW at 20 wk of age. The authors indicated that in order to be effective in improving BW, the increase in AMEn of the diet should be done earlier in the rearing period. These results agree with the data of the current trial in which an increase in AMEn content of the diet from 10.96 to 12.13 MJ/kg fed to pullets from 12 to 17 wk improved FCR but not BWG.

Leeson et al. (1996) provided diets to broilers containing 11.3–13.8 MJ AMEn/kg and observed no change in growth rate. These authors concluded that the more recent genetic strains of broilers used by the industry possess a good ability to control feed intake based on their desire to normalize energy intake. However, in the current trial, pullets fed the lower energy diets had lower final weights than pullets fed the high energy diets. Probably, differences in the objectives for genetic improvement for broilers and pullets account for the differences detected between broilers and pullets.

In the current research, an increase in the AMEn content of the diet did not affect BW uniformity, which does not conform to data of Keshavarz (1998) who found that uniformity of SCWL pullets at 18 wk of age improved when the AMEn content of the diet fed from hatching to 18 wk of age was increased from 11.78 to 12.70 MJ/kg. Also, Brickett et al. (2007) found that BW uniformity of 35 d old broilers was improved when the AMEn content of the diet was increased from 11.71 to 12.97 MJ/kg.

Pelleting increased ADFI and BWG of pullets from 1 to 45 d of age in agreement with results of Corchero et al. (2008) in broilers from 1 to 42 d of age and consistent with data of Sibbald (1979), who observed that pelleting increased the rate of passage of the digesta in adult roosters. Consequently, feeding pellets should improve feed consumption in poultry. In addition, the application of steam and mechanical pressure to the meal to agglomerate feed particles improves bulk density and feed texture, which in turn might benefit feed intake. In the current experiment, pullets fed pellets from 1 to 45 d of age had higher BWG from 1 to 120 d of age than pullets fed mash, in agreement with data of Frikha et al. (2009) using the same strain of pullets. Consistent with these results, Gous and Morris (2001) found that pullets fed crumbles from 1 to 4 wk and then pellets from 5 to 20 wk of age were 6% heavier than pullets fed mash. Also, Deaton et al. (1988) reported that feeding pellets to pullets from 12 to 20 wk of age increased BWG, but in this research ADFI was not affected.

It has been reported that feeding pellets consistently improved FCR in broilers (Quentin et al., 2004; Amerah et al., 2007) and in pullets (Gous and Morris, 2001). Hamilton and Proudfoot (1995) indicated that the improvement in FCR with pelleting was a consequence of the increase in nutrient digestibility. In fact, Wahlström et al. (1999) observed that the digestibility of starch and fat in laying hens increased when the feed was crumbled. Pelleting might disrupt the structure of the cells' walls and starch granules of maize and other ingredients, releasing part of the intracellular fat contained in the oil bodies and facilitating the access of endogenous enzymes to nutrients. Consequently, energy utilization and FCR will be improved (Gracia et al., 2009). However, in the current experiment, feeding pellets did not affect FCR which agrees with data of Bolton (1960), Plavnik et al. (1997), and Brickett et al. (2007), that failed to find any significant advantage of feeding pellets on FCR or nutrient digestibility in broilers. Moreover, Svihus and Hetland (2001) found that pelleting increased ADFI but reduced nutrient digestibility, and García et al. (2008) observed that heat processing of the cereal did not affect organic matter digestibility or N retention in broilers diets. Feed wastage is higher with mash than with pelleted diets and non-recorded feed wastage might help to explain the higher ADFI and poorer FCR observed when mash diets are used. Our data support the hypothesis that pelleting of the diet has little effect on nutrient digestibility, and that most of the improvement in feed efficiency observed with pelleting by some authors is probably due to a reduction in feed wastage. In this respect, Medel et al. (2004) and Corchero et al. (2008) found that pelleting reduced feed wastage by 6.6% in piglets and by 8.5% in broilers from 1 to 14 d of age, respectively. In fact, Medel et al. (2004) indicated that the improvement in FCR observed with pellet feeding was due primarily to a reduction in feed wastage.

4.2. Gastrointestinal tract development

An increase in the AMEn concentration of the diet reduced the RW of the gizzard at 45 and 120 d of age without affecting the RL of the GIT. High energy diets contain less fibre and more fat than low energy diets. Summers and Leeson (1986) and González-Alvarado et al. (2007, 2008) found that a decrease in the fibre content of the diet reduced gizzard weight in broilers and in laying hens results that are consistent with the findings of the current research.

At 45 d of age, feeding pellets decreased the RW and the RL of all the segments of the GIT except the RL of the duodenum. However, at 120 d of age the only differences observed were for the RW of gizzard and proventriculus, that were higher for the mash than for the pelleted diets. The results agree with data of Frikha et al. (2009) who reported that feeding pellets from 1 to 45 d of age reduced the RW of the gizzard and proventriculus and also the RL of the jejunum and ileum in Hy-Line Brown pullets. In broilers, feeding crumbles or pellets consistently reduced the RW of the gizzard (Choi et al., 1986; Nir et al., 1994). Similar results have been reported by Scott and McCann (2008) in laying hens fed pellets from 30 to 36 wk of age. Moreover, Nir et al. (1995) found that pelleting reduced by 15% the RL of the jejunum and ileum of broilers, and Amerah et al. (2007) reported that the improvement in broiler performance observed with pelleting was associated with a decrease in the RL of the GIT. Gizzard pH at 120 d of age was not affected by diet, a finding that disagrees with data of Huang et al. (2006) and Corchero et al. (2008) that reported higher gizzard pH in broilers fed pellets than in broilers fed mash. However, in the current research pullets were fed a mash diet from 45 to 120 d of age which might have reduced the negative effects of pelleting on gizzard pH.

5. Conclusions

An increase in the energy content of the diet of Hy-Line Brown pullets improved pullet performance at all ages but had no effects on BW uniformity. Feeding pellets from 1 to 45 d of age improved BWG and ADFI at this age but FCR was not affected. The beneficial effects of feeding pellets from 1 to 45 d of age on BWG were maintained at 120 d of age. Increasing the AMEn concentration or pelleting of the diet fed from 1 to 45 d of age, reduced the RW of the gizzard at 120 d of age, a finding that has to be taken into account in pullet rearing because a poor development of the gizzard might affect productive performance at the onset of the egg-laying period.

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