UNIVERSIDAD POLITÉCNICA DE MADRID
ESCUELA TÉCNICA SUPERIOR DE INGENIEROS AGRÓNOMOS

**INFLUENCE OF NUTRITIONAL STRATEGIES ON
GROWTH PERFORMANCE AND DIGESTIVE TRACT
TRAITS OF BROWN EGG LAYING PULLETS AND
SUBSEQUENT EFFECTS ON EGG PRODUCTION**

TESIS DOCTORAL

Pilar Guzmán Medina

Licenciada en Veterinaria

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PhD Thesis

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INDEX OF CONTENTS

ABBREVIATION LIST	9
SUMMARY	12
RESUMEN	17
CHAPTER 1: INTRODUCTION AND OBJECTIVES	23
CHAPTER 2: LITERATURE REVIEW	27
2.1. Dietary fiber	27
2.1.1. Definition and methods of analysis	27
2.1.2. Dietary fiber: chemical structure and associated functions	29
2.1.3. Fiber effects on the gastrointestinal tract of poultry	31
2.1.4. Fiber in poultry feeding	32
2.2. Feed form and particle size of the diet	35
2.3. Energy content of the diet	36
CHAPTER 3: PRODUCTIVE PERFORMANCE OF BROWN-EGG LAYING PULLETS FROM HATCHING TO 5 WEEKS OF AGE AS AFFECTED BY FIBER INCLUSION, FEED FORM, AND ENERGY CONCENTRATION OF THE DIET	38
3.1. Introduction	38
3.2. Materials and methods	39
3.2.1. Experiment 1	40
3.2.2. Experiment 2	47
3.2.3. Laboratory analysis	52
3.3. Results	53
3.3.1. Experiment 1	53
3.3.2. Experiment 2	58
3.3.2.1. <i>Feed form</i>	58
3.3.2.2. <i>Energy concentration</i>	62
3.4. Discussion	64
3.4.1. Experiment 1	64
3.4.2. Experiment 2	66
3.4.2.1. <i>Feed form</i>	66
3.4.2.2. <i>Energy concentration</i>	68
CHAPTER 4: INCLUSION OF FIBER IN DIETS FOR BROWN-EGG LAYING PULLETS: EFFECTS ON GROWTH PERFORMANCE AND DIGESTIVE TRACT TRAITS FROM HATCHING TO 17 WEEKS OF AGE	71
4.1. Introduction	71

4.2. Materials and methods	72
4.3. Results	82
4.3.1. <i>Growth performance</i>	82
4.3.2. <i>Gastrointestinal tract traits and body measurements</i>	87
4.4. Discussion	92
4.4.1. <i>Growth performance</i>	92
4.4.2. <i>Gastrointestinal tract traits and body measurements</i>	95
CHAPTER 5: EFFECT OF LEVEL OF FIBER OF THE REARING PHASE DIETS ON EGG PRODUCTION, DIGESTIVE TRACT TRAITS, AND BODY MEASUREMENTS OF BROWN EGG-LAYING HENS FED DIETS DIFFERING IN ENERGY CONCENTRATION	100
5.1. Introduction	100
5.2. Materials and methods	102
5.3. Results	109
5.3.1. <i>Hen production and egg quality traits</i>	109
5.3.2. <i>Digestive tract traits and body measurements</i>	116
5.4. Discussion	120
5.4.1. <i>Productive performance and egg quality</i>	120
5.4.2. <i>Digestive tract traits and body measurements</i>	122
CHAPTER 6: GENERAL DISCUSSION	126
CONCLUSIONS AND IMPLICATIONS	139
CONCLUSIONES E IMPLICACIONES	142
REFERENCES	145

ABBREVIATION LIST

AA	Amino acids
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
ADL	Acid detergent lignin
AME _n	Apparent metabolizable energy nitrogen corrected
BW	Body weight
BWG	Body weight gain
CEn	Conversión energética
CF	Crude fiber
CGI	Cascarilla de girasol
CMD	Consumo medio diario
CP	Crude protein
CV	Coefficient of variation
d	Day
DF	Dietary fiber
DM	Dry matter
ECR	Energy conversion ratio
EMA _n	Energía metabolizable aparente corregida por nitrógeno
EnI	Energy intake
FCR	Feed conversion ratio
FI	Feed intake
FTU	Phytase units
GE	Gross energy
GIT	Gastrointestinal tract
GLM	General linear model
GMD	Ganancia media diaria
GMD	Geometric mean diameter
GSD	Geometric standard deviation

HCl	Hydrochloric acid
IC	Índice de conversión
L	Linear
Log	Logarithm
NaOH	Sodium hydroxide
NDF	Neutral detergent fiber
OH	Oat hulls
<i>P</i>	Probability
PRE	Pulpa de remolacha
PV	Peso vivo
Q	Cuadratic
RL	Relative length
RW	Relative weight
SBP	Sugar beet pulp
Sd	Standard deviation
sem	Semanas
SFH	Sunflower hulls
SI	Small intestine
SWC	Swelling capacity
TGI	Tracto gastrointestinal
WHC	Water holding capacity
wk	Weeks
μEq	Microequivalent

SUMMARY

The thesis contributes to clarify current knowledge on pullet nutrition, a field with scarce available information. To achieve adequate egg production it is necessary that both pullets and hens are healthy and with a well developed gastrointestinal tract (GIT). The better the uniformity and body development of the pullet is the higher the hen productivity will be. With this in mind, three experiments were conducted with the following objectives: 1) study the effects of feed form and diet composition during the first stages of life on pullet growth and uniformity; 2) study the effects of two different types of fiber at two and levels of inclusion on pullet growth and GIT development for the entire rearing period; 3) analyse the influence of pullet diet during rearing (fiber inclusion) on subsequent egg production and GIT of the hens at the end of the egg cycle.

In experiment 1, the effects of fiber inclusion, feed form, and energy concentration of the diet on growth performance of pullets from hatching to 5 wk of age were studied in 2 trials. In trial 1, there was a control diet based on cereals and soybean meal and 6 extra diets that included 2 or 4% of cereal straw (straw), sugar beet pulp (SBP), or sunflower hulls (SFH) at the expense (wt/wt) of the whole control diet. Fiber inclusion increased ($P < 0.05$) average daily gain (ADG) and average daily feed intake (ADFI), and improved ($P < 0.05$) energy conversion ratio (ECR; kcal AME_n/g ADG). Body weight (BW) uniformity, however, was not affected by treatment. Pullets fed SFH tended to have higher ADG than pullets fed SBP ($P = 0.072$) with pullets fed straw being intermediate. Feed conversion ratio (FCR) was better ($P < 0.05$) with the inclusion of 2% than with the inclusion of 4% fiber. In trial 2, 10 diets were arranged as a 2x5 factorial with 2 feed forms (mash vs. crumbles) and 5 levels of AME_n (2,850, 2,900, 2,950, 3,000, and 3,050 kcal/kg). Pullets fed crumbles were heavier and had

better FCR than pullets fed mash ($P < 0.001$). An increase in the energy content of the crumble diets reduced ADFI and improved FCR linearly but no effects were detected with the mash diets ($P < 0.01$ and $P < 0.05$ for the interactions). Feeding crumbles tended to improve BW uniformity at 5 wk of age ($P = 0.077$) but no effects were detected with increases in energy concentration of the diet.

Experiment 2 investigated the effects of fiber inclusion in the diet on growth performance and digestive traits in pullets from hatching to 17 wk of age. The control diets of the 3 feeding periods (0 to 5 wk, 5 to 10 wk, and 10 to 17 wk) were based on corn and soybean meal and did not include any additional fiber source. The experimental diets included 2 or 4% of cereal straw or SBP at the expense (wt:wt) of the control diet. From 0 to 5 wk of age, fiber inclusion did not affect pullet performance. From hatch to 17 wk of age, the inclusion of straw had little effect on pullet performance but the inclusion of 4% SBP reduced ADG ($P < 0.05$) and worsened FCR ($P < 0.001$). Pullets fed straw had greater ADG ($P < 0.05$) and better ECR ($P < 0.01$) than pullets fed SBP. An increase in fiber from 2 to 4% worsened FCR ($P < 0.05$). Body weight uniformity was not affected by diet. Fiber inclusion increased the relative weight (% BW) of the gizzard at 5 wk ($P = 0.056$) and 10 wk ($P < 0.01$) of age but no differences were detected between fiber sources. At same ages, the relative length (cm/kg BW) of the pullets ($P = 0.058$ and $P < 0.01$, respectively) and tarsus ($P = 0.079$ and $P < 0.05$, respectively) was higher in pullets fed SBP than in pullets fed straw. Fiber inclusion, however, did not affect any of these traits at 17 wk of age.

Experiment 3 was designed to study the effects of the treatments used in experiment 2 (type and level of fiber) on egg production, GIT traits, and body measurements of brown egg-laying hens fed diets varying in energy concentration from 17 to 46 wk of age. The experiment was completely randomized with 10 treatments

arranged as a 5 x 2 factorial with 5 rearing phase diets and 2 laying phase diets. During the rearing phase, treatments consisted of a control diet based on cereals and soybean meal and 4 extra diets that resulted from including in this diet a combination of 2 fiber sources (cereal straw and SBP) at 2 levels (2 and 4%). During the laying phase, diets differed in energy content (2,650 vs. 2,750 kcal AME_n/kg) but had the same amino acid (AA) content per unit of energy. The rearing diet did not affect any production trait except egg production that was lower in birds that were fed SBP than in birds that were fed straw (91.6% and 94.1%, respectively; $P < 0.05$). Laying hens fed the high energy diet had lower feed intake ($P < 0.001$), better FCR ($P < 0.01$), and greater BW gain ($P < 0.05$) than hens fed the low energy diet but egg production and egg weight were not affected. At 46 wk of age, hen BW was positively related with body length ($r = 0.500$; $P < 0.01$), tarsus length ($r = 0.758$; $P < 0.001$), and body mass index ($r = 0.762$; $P < 0.001$) but no effects of type of diet on these traits were detected.

In summary:

- a) the response of pullets to increases in energy content of the diet depends on feed form with a decrease in feed intake when fed crumbles but no changes when fed mash. The interaction observed could be due to differences in feed wastage, accounted as consumed by the birds, between diets differing in feed form.
- b) Feeding crumbles might be preferred to feeding mash
- c) to pullets from hatching to 5 wk of age.
- d) The inclusion of moderate amounts of fiber at the expense (wt:wt) of the whole diet improved pullet performance from hatching to 5 wk of age. However, the inclusion of SBP reduced pullet growth at 17 wk of age, with effects being more pronounced at the higher level. Also, an increase in the level of straw from 2 to 4% worsened FCR but did not affect ADG.

- e) Moreover, the inclusion of up to 4% of a fiber source in the rearing diets did not affect GIT development of the hens but SBP reduced egg production.
- f) An increase in the energy content of the laying phase diet reduced ADFI and improved feed efficiency but did not affect any of the other traits studied.

RESUMEN

La presente tesis doctoral contribuye a aumentar el conocimiento sobre diversos factores nutricionales que influyen sobre el crecimiento y la calidad de pollitas de recría, un área de la producción de aves de puesta en la que la información disponible es escasa. Para conseguir índices de puesta adecuados es necesario que tanto la pollita como la ponedora adulta presenten uniformidad y desarrollo corporal óptimo. Cuanto mejor sea la uniformidad de las aves y su desarrollo en sus estadíos iniciales, mayor será su productividad durante la fase de puesta. En base a estos criterios, se llevaron a cabo tres experimentos con los siguientes objetivos: 1) estudiar el efecto de la presentación y composición del pienso sobre el desarrollo durante la primera fase de la recría de las aves; 2) estudiar el efecto de distintas fuentes de fibra a distintos niveles de inclusión sobre el crecimiento y el desarrollo del tracto gastrointestinal (TGI) durante la fase completa de la recría; 3) determinar si la inclusión de fibra adicional al pienso de las pollitas durante la fase de recría afecta a la productividad posterior de las aves y al desarrollo digestivo de las mismas.

El experimento 1 constó de 2 ensayos donde se estudiaron la influencia de la inclusión de fibra, la presentación del pienso y el nivel energético del mismo sobre el rendimiento productivo y la uniformidad del peso vivo (PV) de pollitas entre el nacimiento y las 5 sem de vida. En el ensayo 1 se dispuso de un pienso control basado en cereales y harina de soja y 6 piensos extras en los que se incluyó un 2 o un 4% de paja de cereales, pulpa de remolacha (PRE) o cascarilla de girasol (CGI) a expensas (kg:kg) del pienso control. En el global de la prueba (desde el nacimiento hasta las 5 sem de edad) la inclusión de fibra incrementó ($P < 0,05$) la ganancia media diaria (GMD) y el consumo medio diario (CMD) y mejoró ($P < 0,05$) la conversión energética (CEn; kcal EMA_n/g GMD) pero no afectó a la uniformidad del PV. Las pollitas

alimentadas con CGI tendieron a ser más pesadas que las pollitas alimentadas con PRE ($P = 0,072$) con las pollitas alimentadas con paja ocupando una situación intermedia. El índice de conversión (IC) mejoró ($P < 0,05$) más con la inclusión en el pienso de un 2% de fibra que con la inclusión de un 4%. En el ensayo 2 de este primer experimento, se dispuso de 10 tratamientos distribuidos de manera factorial (2×5) en base a la combinación de 2 presentaciones del pienso (harina vs. migas) y 5 niveles de EMA_n (2.850, 2.900, 2.950, 3.000 y 3.050 kcal/kg). Las pollitas que recibieron el pienso en forma de migas pesaron más y tuvieron mejor IC que las pollitas que lo recibieron en forma de harina ($P < 0,001$). El incremento de energía del pienso en migas se tradujo, de manera lineal, en una reducción del CMD y una mejora del IC. Sin embargo, no se vio efecto alguno sobre estos parámetros cuando el pienso se suministró en forma de harina ($P < 0,01$ y $P < 0,05$ para las interacciones). La uniformidad del PV de las pollitas a las 5 sem de edad tendió a mejorar con el pienso en migas ($P = 0,077$), pero no se vio afectado por el nivel energético del pienso.

En el experimento 2, se utilizaron pollitas de recría desde el nacimiento hasta las 17 sem de edad. El objetivo fue estudiar el efecto de la inclusión de fibra en el pienso sobre el rendimiento productivo y el desarrollo del TGI. La prueba constó de tres periodos (0 a 5, 5 a 10 y 10 a 17 sem de edad), en cada uno de los cuales el pienso control estaba basado en maíz y harina de soja, sin fibra adicional. Los piensos experimentales de cada periodo contenían un 2 o un 4% de paja de cereales o PRE a expensas (kg:kg) del pienso control. Entre las 0 y las 5 sem de edad, la inclusión de fibra no afectó al rendimiento productivo de las pollitas. En cambio, entre las 0 y las 17 sem de edad, la inclusión de un 4% de PRE redujo la GMD ($P < 0,05$) y empeoró el IC ($P < 0,001$). La inclusión de paja, sin embargo, tuvo poco efecto sobre el rendimiento productivo de las pollitas. Además, las pollitas alimentadas con el pienso que contenía paja tuvieron

mayores GMD ($P < 0,05$) y mejores CEn ($P < 0,01$) que las pollitas alimentadas con el pienso que contenía PRE. Un incremento del nivel de fibra de un 2 a un 4% empeoró el IC ($P < 0,05$). La uniformidad del PV no se vio afectada por ninguno de los tratamientos. En relación con el desarrollo digestivo, la inclusión de fibra aumentó el peso relativo (% PV) de la molleja a las 5 ($P = 0,056$) y a las 10 ($P < 0,01$) sem de edad pero no se detectaron diferencias entre ambas fuentes de fibra. A esas mismas edades, la longitud relativa (cm/kg PV) corporal ($P = 0,058$ y $P < 0,01$, respectivamente) y del tarso ($P = 0,079$ y $P < 0,05$, respectivamente) fue mayor en pollitas alimentadas con el pienso en base a PRE que en pollitas alimentadas con el pienso en base a paja de cereales. La inclusión de fibra, sin embargo, no tuvo efecto alguno sobre estos parámetros a 17 sem de edad.

El experimento 3 fue continuación del experimento 2 y tuvo como objetivo estudiar el efecto del tratamiento durante la fase de recría sobre la producción de huevos y la calidad de los mismos, el desarrollo del TGI y diversas medidas corporales en gallinas ponedoras alimentadas con piensos con distinta concentración energética entre las 17 y las 46 sem de edad. El experimento se realizó totalmente al azar, con 10 tratamientos organizados como un factorial 5 x 2, con 5 piensos en la fase de recría y 2 piensos en la fase de puesta. Durante la recría hubo un pienso control basado en cereales y harina de soja y 4 piensos extra que resultaron de sustituir (kg:kg) el pienso control por la combinación de 2 fuentes de fibra (paja de cereales y PRE) a 2 niveles (2 y 4%). En el periodo de puesta, los piensos diferían en el contenido de EMA_n (2.650 vs. 2.750 kcal/kg) pero el contenido en aminoácidos por unidad de energía fue similar. El pienso suministrado durante la recría no afectó a ninguno de los parámetros estudiados durante la fase de puesta, excepto la producción de huevos, que fue menor en las aves que habían sido alimentadas con PRE que en las alimentadas con paja (91,6% and 94,1%,

respectivamente; $P < 0,05$). Las gallinas que consumieron durante la fase de puesta el pienso alto en energía tuvieron un menor CMD ($P < 0,001$), mejor IC ($P < 0,01$) y mayor ganancia de PV ($P < 0,05$) que las gallinas que comieron el pienso bajo en energía. Sin embargo, el índice de puesta y el peso del huevo no se vieron afectados. A 46 sem de edad, el PV de las gallinas mostró una correlación positiva con la longitud corporal ($r = 0,500$; $P < 0,01$), la longitud del tarso ($r = 0,758$; $P < 0,001$) y el índice de masa corporal ($r = 0,762$; $P < 0,001$), aunque ninguno de estos parámetros se vio afectado por el tipo de pienso.

En resumen:

- a) la respuesta de las pollitas a un incremento del nivel energético del pienso dependió de la presentación del mismo, con una reducción del CMD cuando se suministró en forma de migas pero sin efecto cuando se suministró en forma de harina.
- b) La utilización de migas puede ser preferible a la utilización de harina pollitas desde el nacimiento hasta las 5 sem de vida.
- c) La inclusión de cantidades moderadas de fibra en el pienso mejoró el rendimiento productivo de 0 a 5 sem de vida. Sin embargo, la inclusión de PRE redujo el crecimiento a las 17 sem de edad, siendo este efecto más acentuado con el nivel de inclusión más alto. Un incremento del nivel de paja de un 2 a un 4% empeoró el IC pero no afectó a las GMD.
- d) La inclusión de hasta un 4% de fibra en el pienso de recría no afectó al desarrollo del TGI de las gallinas al final de la puesta. Sin embargo, la inclusión de PRE redujo la producción de huevos.

- e) Un incremento del nivel energético del pienso en la fase de puesta redujo el consumo y mejoró la eficiencia alimenticia, pero no afectó a ningún otro de los parámetros estudiados.

CHAPTER 1: INTRODUCTION AND OBJECTIVES

According to data offered by MAGRAMA (2015), Spain had more than 40 million hens destined to egg production, representing almost 11% (798 thousand tonnes) of the total European Union-28 (EU) production in 2014. Spain is the fourth biggest egg producer in the EU-28 after France, Germany, and Italy.

To improve the economies of the egg industry, hens should reach a high peak of production (over 94-95%) early in the laying phase, with a high persistency and good shell quality throughout the laying cycle. The potential for egg production depends primarily on the genetic background and the health status of the hens but also on management and feeding practices during the rearing and laying phases. Light pullets at the onset of the egg cycle produce fewer and smaller eggs throughout the whole laying phase than heavy pullets (Leeson et al., 1997; Pérez-Bonilla et al., 2012a,b). This issue is especially important in countries such as Spain in which consumers have a preference for large eggs. Consequently, the implementation of feeding strategies that favor pullet growth during the rearing phase should result in an improvement of the economics of egg production.

The interest in finding alternatives to in feed antibiotics to maintain and improve animal health has increased over the years. The use of antimicrobials in animal diets as “in feed” growth promoters was banned in the EU in 2006, to reduce the possibility of developing antibiotic resistance which may be transmitted from animals to humans (McDermott et al., 2002; Witte et al., 2002). Therefore, the egg sector needs to search for other options to avoid potential health disorders in poultry (Mateos et al., 2002). Most alternatives focused on the use of approved feed additives, such as organic acids, prebiotics, probiotics, and essential oils. Other potential alternatives consist in the implementation of modifications in the production systems and introducing changes in

the ingredient composition of the feed. Numerous studies have shown that poultry health and performance can be improved with the inclusion of moderate amounts of insoluble fiber (González-Alvarado et al., 2010; Jiménez-Moreno et al., 2011), the supplementation of the diet with adequate enzymes (Wyatt and Goodman, 1993; Lázaro et al., 2003), heat processing of the cereal (Fancher et al., 1996; Gracia et al., 2003), and changes in feed form (Saldaña et al., 2015a,b). On the other hand, current social and economic situation has pushed researches and poultry producers to look for cheaper alternatives to achieve optimum production at the least cost.

Energy, is probably the most expensive nutrient of poultry diet. An increase in the energy content of the diet decreases feed intake and vice versa (Leeson et al., 1996; Veldkamp et al., 2005; Ravindran et al., 2016). Also, the energy content of the diet, basically the use of additional fat or fiber, affects digesta transit time, nutrient utilization, and GIT function (Mateos and Sell 1980, 1981; Frikha et al., 2009a; Sacranie et al., 2012). Fibrous ingredients have been used primarily in ruminant feeds, but the interest in feeding fiber sources to non-ruminants has increased in the last years due to low cost, increased availability and positive effect on gut health and animal well-being (De Leeuw et al., 2008). A second alternative that can be used to improve bird performance, as previously indicated, consists in changing feed presentation. Pelleting of the diet has been shown to improve growth rate and feed efficiency in broilers (Amerah et al., 2007; Serrano et al., 2012, 2013). On the other hand, the use of mash diets reduces water intake and improves the structure and consistency of the excreta. Consequently, mash feeding might be more favourable for nutrient digestibility than pellet feeding because of an improvement in gizzard function (Abdollahi et al., 2010; Svihus, 2011; Serrano et al., 2013). Consequently, the choice of the most convenient

ingredients and of the energy level and feed form of the diet, depends not only on the objectives of the producers but also on the relative cost of available ingredients.

Numerous researchers have studied the effects of dietary fiber, energy concentration of the diet, and feed form on different aspects of broilers and laying hen production. However, the information available in pullets is scarce and contradictory. In fact, few papers have been published in the last 15 years on the effects of these key factors on pullet development and performance and subsequent egg production. Information on these areas of knowledge will help the nutritionists to formulate diets that improve gastrointestinal tract development and maximize performance of the pullets while maintaining an adequate health status and uniformity of the flock.

The main objectives of the current PhD thesis were:

1. To study the effects of DF, feed form, and energy concentration of the diet on productive performance and BW uniformity of pullets during the early stages of life (0 to 5 wk of age).
2. To determine the influence of different fiber sources at different levels of inclusion on productive performance, BW uniformity, and the development of the GIT on pullets during the entire rearing period (0 to 17 wk of age).
3. To determine the potential effects of feeding different fiber sources, varying in physicochemical properties, during the rearing phase on productivity and gastrointestinal tract traits of the hens during the subsequent laying cycle.

CHAPTER2: LITERATURE REVIEW

2.1. Dietary fiber

2.1.1. Definition and methods of analysis

The definition of the term dietary fiber (**DF**) has been for many years a question of debate. In 1953, Hipsley defined “dietary fiber” for the first time as the non-digestible constituents present in the cell wall of the plants. The definition included cellulose, hemicellulose, and lignin fractions. The definition was enlarged by including other minor components such as waxes, cutin, and suberin and DF was then defined as the plant components resistant to hydrolysis by alimentary enzymes (Trowell, 1972; Painter and Burkitt, 1971). The basic definition accepted throughout the world was broadened by Trowell et al. (1976) by adding all the indigestible polysaccharides (modified celluloses, mucilages, gums, oligosaccharides, and pectins). In 2009, the Codex Alimentarius Commission established a new and clear definition of DF based on the recommendations of the Codex Committee on Nutrition and Foods for Special Dietary Uses (2008): “dietary fiber means carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories: a) edible carbohydrate polymers naturally occurring in the food consumed; b) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities; c) synthetic carbohydrate polymers that have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities”.

The first method used for the analysis and characterization of DF was that of Weende crude fiber, which gives a measure for cell wall contents somewhere between the NDF and ADF (see Figure 1). The Van Soest method (Van Soest et al., 1991) is used worldwide and measures the fiber fraction that is insoluble in neutral or acid detergent solutions, allowing a more precise differentiation of hemicelluloses, celluloses, and lignin. The main limitation of this method is that the fraction measured may contain some crude protein and starch linked to the fibrous fraction of the ingredient or feed. Moreover, none of these two methods includes and analyzes pectin content. In this respect, the total DF method seems the most appropriate, because includes and differentiates between soluble (pectins, β -glucans, fructans, and other soluble sugars) and insoluble (hemicellulose, cellulose) fibers.

Figure 1. Carbohydrate fraction of the diet and methods for dietary fiber analysis (adapted from Degen, 2010)

2.1.2. Dietary fibre: chemical structure and associated functions

The structure determines at a great extent the physiological effects and metabolic impact of DF. Plant cell walls are the main source of DF. Depending on the polysaccharides present in the plants, two types of cell walls can be described: type I (mainly in flowering plants during cell division and growth) and type II (mainly in grasses after growth, conferring rigidity to the plant) (Carpita and McCann, 2000; Mertens, 2003). The first type are composed mostly of polysaccharides (constituents of soluble fiber), glycoproteins, esters, and minerals, whereas the second type (constituents of insoluble fiber) are composed of celluloses, hemicelluloses, and lignin.

The main components of the DF are the non-starch polysaccharides (NSP) and lignin. In this respect, cellulose, hemicellulose, pectins, and lignin are the main fractions:

- *Cellulose*, a long chain of β (1-4) linked D-glucose present in all plant cell walls. It is the main component of insoluble fiber. Due to the lack of enzymes able to hydrolyze β linkages, cellulose is not digested in the GIT of the animals (Van Soest, 1994).
- *Hemicellulose*, a heteropolysaccharide that includes xylan, xyloglucan, arabinoxylan, glucuronoxylan, and glucomannan. Hemicellulose digestibility depends on the degree of lignification of the plant (Van Soest, 1994) because it is often linked to lignin.
- *Pectins*, composed by a wide range of polysaccharide, rich in galacturonic acid and rhamnose (Willats et al., 2001). This heteropolysaccharide is involved and affect wall porosity, cell adhesion, and pathogen recognition (Guillon et al., 2007). Pectins are the most soluble components of the plant cell walls.

- *Lignin*, a polymer characterized by its heterogeneity and lack of a defined primary structure. It fills cell wall spaces between cellulose, hemicellulose, and pectins, conferring mechanical strength to the plant (Chabannes et al., 2001). Because of its hydrophobic behavior, lignin also allows water conduction along the plant.

The composition of the plant tissues, the source of fiber, the processing method of the plant material, and the form in which they are ingested, define the physico-chemical properties of DF (Guillon and Champ, 2000; Guillon et al., 2000). Particle size, ion exchange capacity, hydration properties, or viscosity are some of the known associated functions to DF (Bach Knudsen, 2001). Particle size depends on the nature of the cell walls and it is reduced in the bird gizzard. Particle size will define at a high extent the rate of digesta passage through the GIT. It is thought that some polysaccharides, such as pectins, because of its ion exchange capacity, have the capability of binding metal ions impairing mineral absorption. The most important hydration properties of DF are swelling capacity (SWC), water holding capacity (WHC), and solubility. The SWC evaluates the ability of DF to increase size when retaining water. The WHC evaluates the amount of water retained by the fiber when water is in excess and therefore, measures the ability to immobilize free water. Solubility measures the capacity of fiber diffusion in water. All these properties will change particle size and therefore, gut transit. Viscosity is determined by measuring the amount of soluble polysaccharides which interact each other, leading to a resistance to flow.

2.1.3. Fiber effects on the gastrointestinal tract of poultry

Having a look at the different segments of the digestive tract, it can be assumed that fiber plays an important role on the anatomy and physiology of the GIT (Figure 2). In first place, in the proximal part we find the crop, an esophageal diverticulum used by poultry for storing the feed. When fibrous ingredients are fed, the particles are retained for longer in this organ (Vergara et al., 1989) leading to a sensation of satiety which inhibits feed intake (Richardson, 1970). When the feed particles are released from the crop they pass to the next organ, the proventriculus or glandular stomach where pepsinogen and HCl are secreted. Next, the digesta is ground and mixed with the proventriculus secretions in the muscular stomach, commonly known as gizzard. There are numerous published researches demonstrating the beneficial effects of the fiber on gizzard function, mostly because of mechanical stimulation (Hetland et al., 2005; Hetland and Svihus, 2007). Once feed particle size is reduced because of gizzard function, the digesta passes to the small intestine where there are intensive gastroduodenal refluxes of the digesta to compensate for its small dimensions and fast rate of transit (Sklan et al., 1978; Duke, 1986a). The inclusion of fiber in the diet is thought to improve the number and intensity of these refluxes (Hetland et al., 2003). Along the small intestine, the nutrients are

Figure 2. Alimentary canal of a chicken (McFetridge, 1920)

digested and then absorbed. However, the GIT of poultry does not produce the necessary enzymes to digest the fiber. But, some of the water soluble particles including different fiber fractions enter into the pair of ceca by antiperistaltic movements. In the ceca, one of the large intestine segments, the microorganisms that are present ferment part of the fiber obtaining short chain fatty acids that reduce ceca pH and can be used as a preferent source of energy by the colonocytes and the bird. Therefore, fiber has an important role on the digestive system of the birds. However, because of the different physico-chemical characteristics of the available fiber sources, a more detailed study of each particular fiber source is necessary to fully understand the role and function of the DF in poultry.

2.1.4. Fiber in poultry feeding

Dietary fiber has been widely studied in ruminant nutrition. Ruminant animals have adapted their digestive system to consume feeds with a high content of non-lignified fiber and are able to obtain energy from them by fermentation. This ability places the ruminant animals in a unique position in the economy of the world (Van Soest, 1994) and gives them an advantage over non-ruminants. However, the role of fiber in non-ruminant feeding, especially in poultry, has been for a long time an area of debate. Traditionally, feeding fiber to poultry has been thought to affect negatively palatability, feed intake (**FI**), and nutrient digestibility (Hamberg et al., 1989; Mateos et al., 2002). However, recent studies conducted with broilers (Amerah et al., 2009; González-Alvarado et al., 2010; Jiménez-Moreno et al., 2016), laying hens (Hetland et al., 2005), and turkeys (Sklan et al., 2003) have shown that certain fiber sources might improve the development of the GIT, nutrient digestibility, and growth performance. Also, it has been reported that DF might improve the structure and consistency of the excreta

(Pottguter, 2008; Amerah et al., 2009), reduce cannibalism and mortality (Van Krimpen et al., 2009; Mateos et al., 2012), and improve animal welfare (Hocking et al., 2004).

Data from different authors indicate that when low fiber diets are fed to broilers the development of the GIT, including the gizzard, is penalized, leading to a decrease in nutrient digestibility and feed efficiency (Mateos et al., 2012; Verdal et al., 2013). Under these circumstances, the inclusion of moderate amounts of certain fibers may benefit GIT development (González-Alvarado et al., 2007; Sacranie et al., 2012). Moreover, the inclusion of insoluble fiber increases the secretion of HCl, bile acids, and endogenous enzymes (Rogel et al., 1987; Svihus et al., 2011) which in turn might affect and help in the control of digestive processes. Also, DF affects microbial profile of the bird (Amerah et al., 2009; Rochell et al., 2012). However, the response of poultry to the inclusion of fiber in the diet depends on numerous factors, some of them interact each other (Montagne et al., 2003). Some of the factors to be considered are: type and level of fiber, species and age of the bird, and health status of the chickens.

a) Type of fiber. The analytical method for determination of the DF divides the total NSP into soluble and insoluble fractions (Bach Knudsen, 1997). This classification into soluble and insoluble provides important nutritional information because their effects on the digestion process and intestinal health are different (Bach Knudsen, 2001; Mertens, 2003). For example, soluble fiber sources, such as sugar beet pulp (SBP), have a high content in pectins and therefore, a high WHC that might lead to an increase in the bulk and viscosity of the digesta (Mosenthin et al., 2001; Noblet and Le Goff, 2001), slowing transit time in the GIT because of the suppression of the intestinal contractions (Cherbut et al., 1990) which in turn reduces the mixing of dietary components with digestive enzymes, and therefore, nutrient digestibility and utilization (Pettersson and Åman, 1989; Choct and Annison, 1990; McNab and Smithard, 1992; Johnston et al., 2003).

However, soluble fiber sources can be fermented in the distal part of the GIT, producing volatile fatty acids that will reduce the pH of the digestive system and will be used as a source of energy. Unlike soluble fibers, insoluble fiber sources, such as cereal straw and sunflower hulls (SFH), have usually a higher content in lignin, and therefore, shorten the transit time of the digesta through the distal part of the GIT. This reduction in transit time might decrease the time available for digestive enzymes to degradate the feed, penalizing the effectiveness of the digestion process (Morel et al., 2006). On the other hand, the high lignin content of most insoluble fiber sources leads to a longer retention of the feed in the gizzard, favoring the development of the muscular layers of this organ and improving its function (Hetland and Svihus, 2001; Hetland et al., 2003; Jiménez-Moreno et al., 2010), and reducing the incidence of gizzard erosion and ulceration (Kaldhusdal et al., 2012).

b) Level of fiber. The data available shows that broilers fed low fiber diets benefit from additional fiber but that an excess might have an opposite effect. The recommended level of fiber in poultry diets might depend on the type of fiber used and the age of the birds. An excess of soluble fiber, rich in pectins, may reduce FI and nutrient digestibility at a higher extent than an excess of insoluble fiber (Jiménez-Moreno et al., 2011, 2013a).

c) Species and age. Numerous studies have demonstrated the benefits of moderate amounts of DF on broiler and laying hen productivity. However, few papers are available showing the effects of DF on pullet performance. The information available suggests that pullets might be less susceptible to changes in the content of DF than broilers of the same age (Walugembe et al., 2013). Broilers may respond better to the inclusion of moderate amounts of fiber than pullets and laying hens because diets for

broilers are low in fiber and broilers are more voracious than pullets or adult laying hens (Mateos et al., 2014).

d) Health status. The inclusion of fiber in the diet affects in different ways the mechanisms of defense of the host. Some of these effects might have negative connotations on growth (increased digesta viscosity, effects on mucosa integrity, etc.), but others might protect the mucosa of the GIT against the adherence of pathogens favouring digestive tract motility (reduced pH, improved reverse peristalsis, changes in rate of passage, etc.). Moreover, the response to DF of birds susceptible to microbial infection will depend on the health status of the animals, with more noticeable effects under poor management practices (Mateos et al., 2013; Berrocoso et al., 2015).

2.2. Feed form and particle size of the diet

Pelleting is a common practice used to maximize growth performance and reduce feed wastage in broilers (Abdollahi et al., 2011; Serrano et al., 2013). The pelleting process modifies the structure of the protein and might increase AA digestibility because of the heat, moisture, and pressure applied (Lemme et al., 2006; Abdollahi et al., 2011). Moreover, pelleting requires fine grinding of the ingredients to improve the cohesion of feed particles, and fine particles might affect GIT function. For example, the rate of passage through the GIT is faster with pellets than with mash. Consequently, birds will satiate faster and will reduce feed intake when fed coarse mash diets as compared with pellet diets. As a result, BWG will increase when the feed is pelleted (Amerah et al., 2007; Serrano et al., 2012; Jiménez-Moreno et al., 2016). In contrast, gizzard function and GIT development will improve with coarse grinding as compared with the fine grinding required for pelleting diets and consequently, nutrient digestibility might be reduced with pelleting (Svihus and Hetland, 2001; Abdollahi et al., 2010; Svihus, 2011; Serrano et al., 2013). Moreover, the incidence of wet litter,

Escherichia coli counts, and *Salmonella spp* contamination of carcasses will increase when the diets are fed as pellets (Engberg et al., 2002; Huang et al., 2006). However, the information available on the influence of feed form of the diet on productive performance and GIT development in pullets is limited (Frikha et al, 2009b; Saldaña et al., 2015 a,b). Probably, the effects of pelleting might be less noticeable in pullets than in broilers because of the reduced growth rate and lower ingesta capacity of these birds.

2.3. Energy content of the diet

Energy concentration of the diet affects FI and growth performance of broilers (Brickett et al., 2007), pullets (Frikha et al., 2009a; Saldaña et al., 2015b), and laying hens (Grobas et al., 1999a; Pérez-Bonilla et al. 2012b). Birds eat to satisfy their energy requirements and therefore, FI will decrease as the energy content of the diet increases (Leeson et al., 1996; Veldkamp et al., 2005). Usually, high energy diets contain more fat and are more palatable than low energy diets which might favor energy intake (**EnI**) of the birds (Grobas et al., 1999b; Frikha et al., 2009a). Moreover, supplemental fat may reduce the digesta transit time and improve the utilization of other components of the diet as has been demonstrated by Mateos and Sell (1980, 1981). In contrast, if the diet is diluted excessively, birds might not be able to consume enough feed to maintain EnI constant, leading to a reduction in productive performance (Nielsen, 2004; Pérez-Bonilla et al., 2012b). On the other hand, when the energy concentration of the diet is reduced the fiber content is usually increased, which benefits the development and function of the GIT (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009a; Sacranie et al., 2012).

**CHAPTER 3: PRODUCTIVE PERFORMANCE OF BROWN-EGG LAYING
PULLETS FROM HATCHING TO 5 WEEKS OF AGE AS AFFECTED BY
FIBER INCLUSION, FEED FORM, AND ENERGY CONCENTRATION OF
THE DIET**

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3.1. Introduction

The inclusion of fibrous ingredients in poultry diets reduces feed intake (**FI**) (Kondra et al., 1974; Sklan et al., 2003) and nutrient digestibility (Rougière and Carré, 2010) and might affect the incidence of enteric disorders in poultry (Montagne et al., 2003; Shakouri et al., 2006; Mateos et al., 2012). Recent research conducted with young broilers, however, have shown that the inclusion of moderate amounts of insoluble fiber sources, such as oat hulls (**OH**) and sunflower hulls (**SFH**), in the diet stimulates the development and physiology of the gastrointestinal tract (**GIT**) (González-Alvarado et al., 2008; Svihus, 2011; Sacranie et al., 2012) and might improve broiler performance (Jiménez-Moreno et al., 2009a; González-Alvarado et al., 2010). However, the information available on the effects of including fiber sources differing in physico-chemical characteristics on growth performance of pullets is very limited.

Pelleting improves ADG and feed conversion ratio (**FCR**) in broilers (Amerah et al., 2007; Serrano et al., 2012; Abdollahi et al., 2013a) with most of the benefits associated to higher FI and reduced feed wastage (Serrano et al., 2013). However, the information available on the influence of feed form of the diet on productive performance in pullets is scarce (Frikha et al, 2009b). Probably pelleting of the feed might be of less benefit in pullets than in broilers because of the lower growth rate and reduced capacity for voluntary FI of pullets.

Energy concentration of the diet affects FI and growth performance in broilers (Brickett et al., 2007), pullets (Frikha et al., 2009a), and laying hens (Pérez-Bonilla et al. 2012b). Birds eat to satisfy their energy requirements and therefore, voluntary FI decreases as the energy content of the diet increases (Leeson et al., 1996; Veldkamp et al., 2005). High energy diets contain usually more fat and are more palatable than low energy diets which might favor energy intake (**EnI**) of the birds (Frikha et al., 2009a). Moreover, supplemental fat reduces rate of feed passage and might improve the utilization of other components of the diet (Mateos and Sell, 1980, 1981). In contrast, if the diet is diluted excessively, birds might not consume enough feed to maintain EnI constant, which may reduce productive performance (Nielsen, 2004; Pérez-Bonilla et al., 2012b).

The hypothesis tested in this research was that the inclusion of moderate amounts of fiber in the diet could improve growth performance of young pullets. Also, it was hypothesized that feeding crumbles and increasing the energy content of the diet could improve ADG and FCR of the birds. The purpose of this research was to investigate in 2 experiments the effect of including 3 fiber sources with different physico-chemical characteristics, and of feed form and energy concentration of the diet on growth performance of brown egg-laying pullets from hatching to 5 wk of age.

3.2. Materials and Methods

The experimental procedures described in this research were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish guidelines for the care and use of animals in research (Boletín Oficial Del Estado, 2007).

3.2.1. Experiment 1

3.2.1.1. Husbandry. In total, 3,500 one-day-old Lohmann Brown pullets (37.3 ± 1.27 g) were housed in an environmentally controlled barn. Pullets were weighed individually and randomly allotted in groups of 50 to 70 cages (40 cm x 80 cm x 68 cm; Facco, Padova, Italy) provided with an open trough feeder and 2 low pressure nipple drinkers. The average BW of the pullets was similar for all cages. Pullets were beak-trimmed at the hatchery and kept on a 23 h/d light program for the first week of life. Then, the light was decreased 2 h per wk until reaching 15 h/d at the end of the trial. Barn temperature was maintained at $32 \pm 1.5^{\circ}\text{C}$ for the first 3 d of life, and was reduced gradually until reaching 21°C at the end of the trial. Because of the dimensions of the cage only 22 pullets chosen at random, remained in these cages after 21 d of age. During the rearing period, the pullets were managed and vaccinated against main diseases (Infectious Bronchitis Disease, Marek Disease, Infectious Bursal Disease, Newcastle Disease, and *Salmonella* spp.) according to accepted commercial practices.

3.2.1.2. Ingredients, Diets, and Experimental Design. The cereal straw and the sugar beet pulp (**SBP**) were received as 9-mm pellets whereas the SFH was received in meal form. The straw used was a 80:20 mixture of wheat and barley straw and was treated before pelleting with a 2% sodium hydroxide solution. Before being included in their respective experimental diets, all fiber sources were ground using a hammer mill provided with a 4-mm screen. The calculated and determined chemical analysis, geometric mean diameter (**GMD**), water-holding capacity (**WHC**, mL/g DM), swelling capacity (**SWC**, mL/g DM), and buffer properties of the fiber sources are shown in Table 1.

Table 1. Calculated and determined analysis (% as fed basis, unless otherwise indicated), and physico-chemical characteristics of the fiber sources. Experiment 1

Fiber source	Straw	Sunflower hulls	Sugarbeet pulp
Calculated analysis ¹			
AME _n (kcal/kg)	200	340	900
Ether extract	1.6	3.0	0.8
Crude fiber	36.0	48.7	18.2
Neutral detergent fiber	72.0	72.2	42.8
Crude protein	3.7	5.7	9.2
Arg	-	0.46	0.42
Ile	-	-	0.35
Lys	-	0.17	0.54
Met	-	0.10	0.16
Met + Cys	-	0.17	0.28
Thr	-	0.18	0.44
Trp	-	0.05	0.09
Val	-	0.27	0.55
Determined analyses			
DM	91.3	90.1	92.0
Gross energy (kcal/kg)	3,854	4,201	3,729
CP	2.8	5.9	9.2
Total ash	6.7	4.3	5.6
Neutral detergent fiber	69.9	58.2	31.7
Acid detergent fiber	41.5	40.5	15.6
Acid detergent lignin	5.6	13.1	1.7
Total dietary fiber	78.5	84.0	64.7
Soluble dietary fiber	3.8	3.0	10.6
Insoluble dietary fiber	74.7	81.0	54.1
Physical properties			
Particle size ²			
2,500	0.2	0.8	1.2
1,250	12.3	8.7	33.5
630	40.4	55.7	35.2
315	28.4	24.6	18.4
160	15.3	9.9	9.0
80	2.9	0.3	2.3
GMD ³ ± GSD ⁴	602 ± 2.01	701 ± 1.75	835 ± 2.12
WHC ⁵ ± SD	7.5 ± 0.14	6.6 ± 0.06	8.7 ± 0.27
SWC ⁶ ± SD	3.6 ± 0.23	3.3 ± 0.25	6.6 ± 0.56
Buffer properties			
Initial pH ⁷	8.39 ± 0.052	5.64 ± 0.023	5.62 ± 0.033
Base-buffering capacity ⁸	-	80.7 ± 6.4	50.9 ± 5.6
Acid-buffering capacity ⁹	134.2 ± 10.2	124.1 ± 16.7	96.3 ± 5.2

¹According to Fundación Española para el Desarrollo de la Nutrición Animal (2010).

²Measured after grinding with a hammer mill provided with a 4-mm screen. The percentage of particles bigger than 2,500 µm or smaller than 80 µm were negligible for all diets.

³Geometric mean diameter (µm).

⁴GSD = Log normal SD.

⁵Water holding capacity (mL/g DM).

⁶Swelling capacity (mL/g DM).

⁷pH of 10 g DM of the fiber source suspended in 200 mL double distilled water.

⁸ μ Eq of NaOH required to increase the pH of 10 g DM of the fiber source suspended in 200 mL double distilled water from the initial pH to pH 7 divided by pH change. The base-buffereing capacity of the straw could not be measured because the initial pH was above 7.00.

⁹ μ Eq of HCl required to decrease the pH of 10 g DM of the fiber source suspended in 200 mL double distilled water from pH 7 to pH 4 divided by pH change.

The control diet was based on corn, wheat, and soybean meal. The other 6 experimental diets resulted from the inclusion of 2 or 4% straw, SBP, or SFH at the expense (wt/wt) of the whole control diet. All diets met or exceeded the nutrient requirements of pullets as recommended by the Fundación Española Desarrollo Nutrición Animal (2008) but feeds that included a fiber source had more DF and slightly less AME_n and CP than the control feed. All diets contained a commercial enzyme complex with xylanase and β -glucanase activities (Roxazyme, DSM S.A., Madrid, Spain). In the formulation of the diets it was accepted that the inclusion of the enzyme complex increased the energy content of the wheat by 2% (from 3,150 to 3,213 kcal AME_n/kg) but had no effects on the energy content of the corn (Fundación Española Desarrollo Nutrición Animal, 2010). Diets were offered for ad libitum consumption in mash form. New feed was added into the feeders twice a day (7.00 am and 4.00 pm) to reduce feed spillage. The ingredient composition and the calculated and determined chemical analysis of the diets are shown in Table 2 and their particle size distribution and GMD are shown in Table 3.

The experimental design was completely randomized with 7 treatments that consisted in a control diet without any additional fiber and 6 additional diets that formed a 3x2 factorial with 3 fibers sources (straw, SBP, and SFH) and 2 levels of inclusion (2 and 4%). Each treatment was replicated 10 times and the experimental unit was the cage for all measurements.

Table 2. Ingredient composition and nutrient content (% as fed basis, unless otherwise indicated) of the experimental diets. Experiment 1

Ingredient	Control	Straw		Sunflower hulls		Sugarbeet pulp	
		2%	4%	2%	4%	2%	4%
Corn	38.0	37.2	36.5	37.2	36.5	37.2	36.5
Wheat, 11.2% CP	20.0	19.6	19.2	19.6	19.2	19.6	19.2
Soybean meal, 47% CP	35.0	34.3	33.6	34.3	33.6	34.3	33.6
Straw	-	2.0	4.0	-	-	-	-
Sunflower hulls	-	-	-	2.0	4.0	-	-
Sugarbeet pulp	-	-	-	-	-	2.0	4.0
Poultry fat	2.7	2.6	2.5	2.6	2.5	2.6	2.5
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Calcium carbonate	1.26	1.27	1.18	1.27	1.18	1.27	1.18
Sodium chloride	0.36	0.35	0.35	0.35	0.35	0.35	0.35
DL-Met, 99%	0.18	0.18	0.17	0.18	0.17	0.18	0.17
Vitamin and mineral premix ²	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis ¹							
AME _n (Kcal/kg)	2,956	2,900	2,843	2,904	2,852	2,915	2,874
Ether extract	5.0	5.0	4.9	5.0	5.0	5.0	4.9
Crude fiber	2.8	3.5	4.2	3.7	4.7	3.2	3.5
Neutral detergent fiber	8.3	9.6	10.8	9.6	10.9	9.0	9.8
Crude protein	21.5	21.1	20.7	21.1	20.8	21.2	21.0
Digestible amino acids							
Arg	1.30	1.28	1.25	1.28	1.25	1.28	1.25
Lys	1.01	0.99	0.97	0.99	0.97	0.99	0.97
Met	0.47	0.46	0.45	0.46	0.45	0.46	0.45
Met + Cys	0.77	0.76	0.74	0.76	0.74	0.76	0.74
Thr	0.69	0.68	0.66	0.68	0.66	0.68	0.66
Trp	0.23	0.23	0.22	0.23	0.22	0.23	0.22
Calcium	0.97	0.96	0.95	0.96	0.95	0.97	0.97
Total phosphorus	0.84	0.83	0.81	0.83	0.82	0.83	0.81
Digestible phosphorus	0.53	0.52	0.51	0.52	0.51	0.52	0.51
Determined analyses							
DM	90.4	90.9	91.4	91.8	90.9	91.1	91.8
Gross energy (kcal/kg)	4,017	4,085	4,048	4,082	4,056	4,094	4,109
Crude fiber	2.9	3.5	4.2	3.3	4.7	2.9	3.1
Neutral detergent fiber	8.9	10.3	11.4	9.3	11.2	9.3	10.0
Acid detergent fiber	3.3	4.0	4.7	3.5	4.7	3.4	3.5
Acid detergent lignin	0.3	0.4	0.5	0.6	0.8	0.3	0.3
CP	20.8	20.2	21.1	21.5	20.1	21.1	20.9
Arg	1.44	1.37	1.36	1.37	1.35	1.36	1.40
Lys	1.12	1.13	1.10	1.10	1.08	1.11	1.08
Met	0.52	0.49	0.46	0.48	0.46	0.47	0.47
Met + Cys	0.85	0.85	0.81	0.82	0.82	0.83	0.81
Thr	0.81	0.81	0.78	0.77	0.78	0.76	0.76
Trp	0.27	0.25	0.26	0.26	0.24	0.27	0.24
Ash	1.03	7.1	7.5	7.2	6.8	7.3	6.8

¹According to Fundación Española Desarrollo Nutrición Animal (2010).²Provided the following (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (all-*rac*-tocopherol-acetate), 30 mg;

vitamin B₁, 2 mg; vitamin B₂, 8 mg; vitamin B₆, 4 mg; vitamin B₁₂ (cyanocobalamin), 0.025 mg; vitamin K₃ (bisulphatemenadione complex), 3mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; betaine anhydrous, 80 mg; D-biotin, 0.15 mg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg iron (FeCO₃), 60 mg; copper (CuSO₄ · 5H₂O), 8 mg; iodine (KI), 2 mg; selenium (Na₂SeO₃), 0.2 mg; Roxazyme, 200 mg [1,600 U of endo-1,4-β-glucanase (EC 3.2.1.4), 3,600 U of endo-1,3 (4)-β-glucanase (EC 3.2.1.6), and 5,200 U of endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM S.A., Madrid, Spain; and Natuphos 5000, 60 mg (300 FTU/kg) supplied by Basf Espanola S.A., Tarragona, Spain.

Table 3. Particle size distribution and geometric mean diameter (GMD, μm) of the experimental diets. Experiment 1

	Control ¹	Straw		Sunflower hulls		Sugarbeet pulp	
		2%	4%	2%	4%	2%	4%
Particle size distribution ²							
> 2,500	7.6	4.4	3.8	2.4	3.8	3.3	2.8
1,250	40.6	42.4	38.2	31.6	38.2	37.2	31.4
630	31.5	33.3	35.8	39.7	35.8	36.7	39.5
315	12.4	12.4	14.6	17.4	14.6	14.9	17.0
160	7.6	7.4	7.4	8.6	7.4	6.8	9.1
GMD $\pm$ GSD ³	1,080 $\pm$ 2.03	1,050 $\pm$ 1.97	995 $\pm$ 1.96	902 $\pm$ 1.94	996 $\pm$ 1.95	991 $\pm$ 1.93	899 $\pm$ 1.96

¹The water holding capacity ($\pm$ SD) and the swelling capacity ($\pm$ SD) of the control diet, measured in triplicate, were 2.1 ± 0.17 and 2.8 ± 0.30 , respectively.

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

³GSD = Log normal SD.

3.2.1.3. Measurements. Feed disappearance and BW were determined by cage at week intervals and mortality was recorded and weighed as produced. Feed wastage was not measured. From these data, ADG, ADFI, and FCR were determined by week and for the entire experimental period. In addition, EnI expressed as kcal of AME_n consumed per day and energy conversion ratio (**ECR**), expressed as kcal of calculated AME_n per g of ADG, were also estimated for the entire experimental period. At the end of the experiment (5 wk of age) the pullets were weighed individually and BW uniformity was determined by replicate as indicated by Peak et al. (2000). Briefly, the CV of the individual BW of the pullets of each cage was generated and this variable was used as an indirect measurement of BW uniformity of the birds.

3.2.1.4. Statistical Analysis. Data on growth performance and BW uniformity were analyzed as a completely randomized design with 7 treatments using the GLM procedure of SAS (SAS Institute, 1990). Pre-planned polynomial contrasts were performed as indicated by García et al. (2008), taking into account that 6 of the diets formed a 3 x 2 factorial. The model studied the effects of 1) control diet vs. the average of the 6 diets that contained a fiber source, 2) main effect of the fiber source, 3) main effect of level of fiber source, and 4) the interaction between source and level of inclusion of the fiber source. Differences among treatment means were considered significant at $P < 0.05$. When a significant difference was detected, means were separated using the Tukey test. Results in tables are presented as means.

3.2.2. Experiment 2

3.2.2.1. Husbandry. In total, 3,000 brown-egg laying pullets (36.7 ± 2.91 g) were used in this experiment. The experimental procedures, including type and age of the

birds, management, growth performance controls, and duration of the trial, were similar to those indicated for experiment 1.

3.2.2.2. Diets and Experimental Design. Five diets differing in AME_n content (2,850, 2,900, 2,950, 3,000, and 3,050 kcal AME_n/kg) were used. The 2 extreme diets (2,850 and 3,050 kcal/kg) were formulated to have similar indispensable amino acids (AA) and nutrient content per unit of energy (Fundación Española Desarrollo Nutrición Animal, 2008). The intermediate feeds (2,900, 2,950, and 3,000 kcal AME_n/kg) were obtained by judicious mixing in adequate proportions of the 2 summit diets. The batches of each of the 5 experimental diets were divided into 2 portions; the first portion was used as such and the second portion was steam-conditioned at 72°C for 60 s, passed through a pellet press (Model PVR 180 2T, Mabrik, Barcelona, Spain) provided with a 60-mm-thick and a 3-mm screen die and crumbled. The ingredient composition and the calculated and the determined chemical analysis of the diets are shown in Table 4. The particle size distribution and GMD of the diets are shown in Table 5. The experiment was completely randomized with 10 treatments in a factorial arrangement with 5 energy levels (2,850, 2,900, 2,950, 3,000, 3,050 kcal AME_n/kg) and 2 feed forms (crumble vs. mash). Each treatment was replicated 6 times and the experimental unit was the cage for all measurements.

Table 4. Ingredient composition and nutrient content (% as fed basis, unless otherwise indicated) of the experimental diets. Experiment 2

AME _n , kcal/kg	2,850	2,900	2,950	3,000	3,050
Ingredient					
Corn	35.0	35.0	35.0	35.0	35.0
Wheat, 11.2% CP	19.1	18.2	17.4	16.5	15.6
Soybean meal, 45.5% CP	33.1	34.8	36.5	38.2	39.9
Sunflower meal, 28% CP	6.0	4.5	3.0	1.5	-
Soy oil	2.7	3.4	4.1	4.7	5.4
Dicalcium phosphate	2.07	2.07	2.07	2.06	2.06
Calcium carbonate	1.05	1.05	1.05	1.04	1.04
Sodium chloride	0.35	0.35	0.35	0.35	0.35
DL-methionine, 99%	0.13	0.14	0.14	0.15	0.15
Vitamin and mineral premix ²	0.50	0.50	0.50	0.50	0.50
Calculated analyses¹					
AME _n (kcal/kg)	2,850	2,900	2,950	3,000	3,050
Ether extract	5.1	5.8	6.4	7.1	7.7
Crude fiber	4.5	4.2	3.9	3.6	3.3
Neutral detergent fiber	11.1	10.6	10.0	9.5	8.9
CP	21.7	22.0	22.2	22.5	22.7
Digestible amino acids					
Arg	1.32	1.34	1.35	1.37	1.38
Lys	0.97	1.00	1.02	1.05	1.08
Met	0.43	0.44	0.44	0.45	0.46
Met + Cys	0.72	0.73	0.74	0.75	0.76
Thr	0.68	0.70	0.71	0.72	0.73
Trp	0.23	0.23	0.23	0.24	0.24
Calcium	1.10	1.10	1.10	1.10	1.10
Total phosphorus	0.82	0.82	0.81	0.81	0.80
Digestible phosphorus	0.44	0.44	0.44	0.43	0.43
Determined analysis³					
DM	93.3	91.3	92.2	91.8	92.6
Gross energy (kcal/kg)	4,134	4,145	4,192	4,212	4,279
Ether extract	5.0	6.2	6.5	7.0	7.3
Crude fiber	4.4	4.2	3.9	3.8	3.5
CP	21.1	22.2	21.9	22.2	23.5
Arg	1.42	1.50	1.48	1.50	1.52
Lys	1.11	1.15	1.16	1.22	1.25
Met	0.49	0.50	0.51	0.49	0.48
Met + Cys	0.84	0.84	0.86	0.85	0.87
Thr	0.80	0.84	0.84	0.85	0.85
Ash	6.0	7.5	7.6	6.1	6.1

¹According to Fundación Española Desarrollo Nutrición Animal (2010).

²Provided the following (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (all-*rac*-tocopherol-acetate), 30 mg; vitamin B₁, 2 mg; vitamin B₂, 8 mg; vitamin B₆, 4 mg; vitamin B₁₂ (cyanocobalamin), 0.025 mg; vitamin K₃ (bisulphatemenadione complex), 3mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; betaine anhydrous, 80 mg; D-biotin, 0.15 mg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg iron (FeCO₃), 60 mg; copper (CuSO₄

▪ 5H₂O), 8 mg; iodine (KI), 2 mg; selenium (Na₂SeO₃), 0.2 mg; Roxazyme, 200 mg [1,600 U of endo-1,4-β-glucanase (EC 3.2.1.4), 3,600 U of endo-1,3 (4)-β-glucanase (EC 3.2.1.6), and 5,200 U of endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM S.A., Madrid, Spain; and Natuphos 5000, 60 mg (300 FTU/kg) supplied by Basf Espanola S.A., Tarragona, Spain.

³Data presented correspond to the average of the mash and crumble feeds, except for amino acids for which only the mash diets were analyzed.

Table 5. Particle size distribution and geometric mean diameter (GMD, μm) of the experimental diets. Experiment 2

AME _n , kcal/kg	2,850		2,900		2,950		3,000		3,050	
	Mash	Crumbles	Mash	Crumbles	Mash	Crumbles	Mash	Crumbles	Mash	Crumbles
Particle size ¹										
> 2500	12.1	8.8	10.2	10.1	12.2	7.1	11.8	4.6	9.8	4.7
1,250	27.2	66.1	29.6	56.6	28.1	54.2	27.2	50.6	26.8	52.3
630	32.1	18.6	32.8	21.7	33.4	25.0	34.3	28.7	35.0	27.7
315	18.0	4.6	17.1	8.3	17.0	9.6	17.5	11.2	18.3	10.4
160	10.5	18.0	10	3.1	9.2	3.9	9.2	4.5	10.1	4.6
GMD $\pm$ GSD ²	968 $\pm$ 2.22	1,491 $\pm$ 1.69	986 $\pm$ 2.18	1,384 $\pm$ 1.86	1,000 $\pm$ 2.18	1,272 $\pm$ 1.89	968 $\pm$ 2.17	1,158 $\pm$ 1.90	940 $\pm$ 2.15	1,144 $\pm$ 1.90

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

²GSD = Log normal SD.

3.2.2.3. Measurements. The same variables studied in experiment 1 were measured in this experiment. The only difference was that in the present experiment, pullet uniformity was determined at weekly intervals whereas in experiment 1, uniformity was determined only at 5 wk of age.

3.2.2.4. Statistical Analysis. Data on growth performance and BW uniformity were analyzed as a completely randomized design with 10 treatments arranged as a 2x5 factorial using the GLM procedure of SAS (SAS Institute, 1990). The model included feed form and energy concentration of the diets as main effects and their interactions. Treatment sums of squares for the effects of AME_n content of the diets on the different variables studied were partitioned into linear (L) and quadratic (Q) effects. Differences among treatment means were considered significant at $P < 0.05$. Significant differences between treatment means were separated using the Tukey test. Results in tables are presented as means.

3.2.3. Laboratory Analysis

Representative samples of the fiber sources used in experiment 1 and of the diets of both experiments were ground in a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) equipped with a 1-mm screen and analyzed for moisture by the oven-drying (method 930.15), total ash by a muffle furnace method (942.05), and nitrogen by Dumas (method 968.06) using a Leco analyzer (Model FP-528, Leco Corp., St. Joseph, MI) as indicated by AOAC International (2005). The AA content of the diets was determined by ion-exchange chromatography (Hewlett-Packard 1100, Waldbronn, Germany) after acid hydrolysis, as indicated by De Coca-Sinova et al. (2008). Crude fiber (CF) content of the diets used in both experiments and ether extract of the diets used in experiment 2 were determined as specified by Pérez-Bonilla et al. (2011). Gross energy (GE) of the fiber sources and diets was determined using an adiabatic bomb calorimeter (model

1356, Parr Instrument Company, Moline, IL, USA). Neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and acid detergent lignin (**ADL**) of the fiber sources and diets from experiment 1 were determined as described by Van Soest et al. (1991) and expressed on an ash-free basis. Also, DF (method 985.29) and the insoluble fraction of DF (method 991.43) of the fiber sources were analyzed as proposed by AOAC International (2005). The soluble fraction of DF was calculated by difference between total and insoluble DF. The WHC, SWC, and buffer properties of the fiber sources were determined as indicated by Jiménez-Moreno et al. (2009b). The mean particle size of the fiber sources and diets, expressed as GMD, was determined in 100 g samples using a Retsch shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 μm , as described by the ASAE (1995). All the analyses were conducted in duplicate except for buffer properties and GMD determination that were conducted in triplicate.

3.3. Results

3.3.1. Experiment 1

The straw, SFH, and SBP contained by analyses 2.8, 5.9, and 9.2 % CP and 69.9, 58.2, and 31.7 % NDF, respectively (Table 1). The GMD was 602, 701, and 835 μm , for straw, SFH, and SBP, respectively. The determined nutrient content of the experimental diets, including the AA composition, was close to expected values, confirming that the ingredients were mixed correctly (Table 3). As the level of fiber increased, the GMD of the diet decreased (1,080, 981, and 963 μm for the control diet and for the average of the diets that contained 2 or 4% of the fiber sources, respectively).

Mortality was 2.9% and was not related to treatment (data not shown). Most of the mortality (2.6%) occurred during the first week of life. For the entire experimental period (from hatching to 5 wk of age), the inclusion of fiber in substitution of the whole diet increased ($P < 0.05$) ADFI and ADG (Table 6) and improved ($P < 0.05$) ECR (Table 7). Most of the benefits of fiber inclusion were observed after the first week of life. In fact, pullets fed the fiber containing diets showed, as an average, higher ADG than pullets fed the control diet for the second (6.3 vs. 5.8 g/d; $P < 0.001$), third (8.9 vs. 8.5 g/d; $P < 0.01$), and fourth week (10.8 vs. 10.1 g/d; $P < 0.01$) of age.

From hatching to 5 wk of age, pullets fed the SFH containing diets tended to have higher ($P = 0.072$) ADG than pullets fed the SBP containing diets, with pullets fed the straw containing diets being intermediate. However, ADFI, FCR, and ECR were not affected by type of fiber. Similarly, an increase in the level of inclusion of fiber from 2 to 4% did not affect ADFI, ADG, or EnE, but hindered ($P < 0.05$) FCR. Body weight uniformity at 5 wk of age was not affected by the inclusion of fiber in the diet (Table 8).

Table 6. Influence of fiber source and level of fiber inclusion in the diet on growth performance of pullets from hatching to 5 wk of age. Experiment 1

	0 to 1 week			1 to 2 week			2 to 3 week			3 to 4 week			4 to 5 week			0 to 5 week		
	ADFI (g)	ADG (g)	FCR ¹	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR
Fiber inclusion																		
Control	8.17	4.57	1.79	12.9 ^b	5.83 ^b	2.21	19.6 ^b	8.51 ^b	2.30	24.8	10.1 ^b	2.46	30.7	11.6	2.66	19.2 ^b	8.11 ^b	2.37
Fiber	8.81	4.71	1.87	13.9 ^a	6.30 ^a	2.20	20.4 ^a	8.91 ^a	2.29	25.6	10.8 ^a	2.37	30.9	11.4	2.70	19.9 ^a	8.44 ^a	2.36
Fiber source																		
Straw	8.77	4.75	1.85	13.8	6.26 ^b	2.21	20.4	8.97	2.27	25.5	10.8	2.37	30.9	11.5	2.69	19.9	8.42	2.36
SFH ²	8.92	4.77	1.87	14.1	6.49 ^a	2.17	20.4	8.95	2.28	25.8	11.0	2.36	30.9	11.6	2.66	20.0	8.56	2.34
SBP ³	8.74	4.62	1.89	13.8	6.24 ^b	2.20	20.4	8.80	2.31	25.6	10.7	2.39	30.8	11.2	2.74	19.9	8.32	2.39
Inclusion level (%)																		
2	8.84	4.74	1.87	13.9	6.37	2.18	20.2	8.93	2.26 ^b	25.6	10.9	2.34	30.6	11.5	2.66	19.8	8.49	2.34 ^b
4	8.78	4.69	1.87	13.9	6.28	2.21	20.6	8.88	2.31 ^a	25.7	10.7	2.41	31.1	11.4	2.73	20.0	8.38	2.39 ^a
Sd ⁴	1.030	0.490	0.229	0.58	0.660	0.105	0.72	0.408	0.089	1.85	0.64	0.175	1.47	0.90	0.216	0.75	0.320	0.092
	Probability																	
Control vs. fiber diets ⁵	0.074	0.426	0.327	<0.001	<0.001	0.646	0.002	0.006	0.606	0.185	0.001	0.062	0.803	0.662	0.594	0.010	0.004	0.632
Fiber source	0.859	0.625	0.806	0.164	0.023	0.121	0.977	0.371	0.347	0.829	0.473	0.844	0.952	0.330	0.417	0.713	0.072	0.284
Inclusion level	0.813	0.603	0.749	0.723	0.726	0.990	0.089	0.639	0.035	0.697	0.142	0.121	0.262	0.571	0.199	0.392	0.218	0.047
Fiber source x inclusion level	0.812	0.738	0.986	0.982	0.648	0.549	0.879	0.643	0.339	0.838	0.611	0.975	0.922	0.878	0.945	0.884	0.528	0.675

^{a-b} Within a column, means without a common superscript differ ($P < 0.05$).

¹Feed conversion ratio.

²Sunflower hulls.

³Sugar beet pulp.

⁴Standard deviation: n=20 for fiber source and n=30 for level of inclusion of fiber, respectively.

⁵Control without additional fiber vs. average of the 6 diets containing a fiber source.

Table 7. Influence of fiber source and level of fiber inclusion in the diet on energy intake¹ (EnI, kcal AME_n/d) and energy conversion ratio (ECR, kcal AME_n/g ADG) of the pullets from hatching to 5 wk of age. Experiment 1

	0 to 5 week	
	EnI	ECR
Fiber inclusion		
Control	56.9	7.03 ^a
Fiber	57.4	6.81 ^b
Fiber source		
Straw	57.0	6.78
Sunflower hulls	57.6	6.73
Sugar beet pulp	57.5	6.91
Inclusion level (%)		
2	57.6	6.80
4	57.1	6.82
Sd ²	2.17	0.267
Probability		
Control vs. fiber diets ³	0.480	0.020
Fiber source	0.661	0.107
Inclusion level	0.357	0.743
Fiber source x inclusion level	0.850	0.548

^{a-b} Within a column, means without a common superscript differ ($P < 0.05$).

¹Calculated energy intake (kcal AME_n/d). The calculated AME_n values are presented in Table 2.

²Standard deviation: n = 20 replicates for fiber source and n = 30 replicates for level of inclusion of fiber, respectively.

³Control without additional fiber vs. average of the 6 diets containing fiber source.

Table 8. Influence of fiber source and level of fiber inclusion in the diet on BW uniformity¹ of pullets at 5 wk of age. Experiment 1

	CV, %
Fiber inclusion	
Control	8.12
Fiber	8.75
Fiber source	
Straw	9.21
Sunflower hulls	8.43
Sugar beet pulp	8.63
Inclusion level (%)	
2	8.61
4	8.90
Sd ²	1.467
Probability	
Control vs. fiber diets ³	0.209
Fiber source	0.225
Inclusion level	0.456
Fiber source x inclusion level	0.815

¹Evaluated as the CV (%) of BW (Peak et al., 2000).

²Standard deviation: n=20 for fiber source and n=30 for level of inclusion of fiber, respectively.

³Control without any additional fiber vs. the average of the 6 diets containing a fiber source.

3.3.2. Experiment 2

The determined nutrient content of the experimental diets were close to expected values and similar for the mash as for their corresponding crumble diets, confirming that the ingredients were mixed correctly (Table 4). The GMD of the 2,850, 2,900, 2,950, 3,000, and 3,050 kcal AME_n/kg diets was 968, 986, 1,000, 968, and 940 μ m for the mash diets and 1,491, 1,384, 1,272, 1,158, and 1,144 μ m for the crumble diets, respectively. Mortality was 2.7% and was not related to treatment (data not shown). Most of the mortality (2.6%) occurred during the first week of life.

3.3.2.1. Feed Form. From hatching to 5 wk of age, pullets fed crumbles had lower ADFI (21.1 vs. 21.5 g/d; $P < 0.01$), greater ADG (9.8 vs. 9.2 g/d; $P < 0.001$), and better FCR (2.14 vs. 2.33; $P < 0.001$) than pullets fed mash (Table 9). Consequently, ECR (6.32 vs. 6.87 kcal/AME_n/g ADG; $P < 0.001$) was improved when the diets were crumbled (Table 10). The beneficial effects of crumbling on ADG and FCR were observed at all ages. Average daily FI, however, was higher ($P < 0.05$) in pullets fed crumbles than in pullets fed mash for the first week of life, but an opposite effect was observed after this age.

Crumbling tended to improve BW uniformity of the pullets at all ages, with differences being significant at 3 wk (8.8 vs. 10.2%; $P < 0.05$) and 4 wk (8.0 vs. 8.5%; $P < 0.05$) of age (Table 11).

Table 9. Influence of feed form and energy content (AME_n, kcal/kg) of the diet on growth performance of the pullets from hatching to 5 wk of age. Experiment 2

Feed form	AME _n (kcal/kg)	0 to 1 week			1 to 2 week			2 to 3 week			3 to 4 week			4 to 5 week			0 to 5 week		
		ADFI (g)	ADG (g)	FCR ¹	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR	ADFI (g)	ADG (g)	FCR
Mash	2,850	8.8 ^{ab}	5.1	1.73	13.9	6.6	2.11	21.0	9.8	2.16	30.0 ^{abc}	12.0	2.51	32.3 ^{ab}	12.1	2.68	21.2 ^{ab}	9.1	2.33 ^{ab}
	2,900	8.8 ^{ab}	5.3	1.67	14.1	6.8	2.07	21.2	10.1	2.10	30.6 ^{abc}	12.5	2.45	33.2 ^{ab}	11.7	2.84	21.5 ^{ab}	9.3	2.32 ^{ab}
	2,950	8.9 ^{ab}	5.1	1.76	14.0	6.6	2.12	20.9	9.6	2.18	31.6 ^a	12.6	2.50	33.3 ^{ab}	11.7	2.85	21.7 ^a	9.1	2.38 ^a
	3,000	9.0 ^{ab}	5.2	1.75	13.6	6.5	2.09	21.0	10.3	2.04	30.5 ^{abc}	12.6	2.43	33.7 ^{ab}	12.1	2.78	21.6 ^{ab}	9.3	2.31 ^{ab}
	3,050	8.4 ^{ab}	5.1	1.64	13.3	6.6	2.02	22.2	10.4	2.14	30.9 ^{ab}	12.5	2.49	32.9 ^{ab}	12.3	2.69	21.5 ^{ab}	9.4	2.30 ^{abc}
Crumbles	2,850	9.4 ^a	5.6	1.68	13.9	6.9	2.01	20.9	10.1	2.06	30.0 ^{abc}	13.2	2.27	34.4 ^a	13.0	2.67	21.7 ^a	9.8	2.22 ^{bcd}
	2,900	9.4 ^a	5.7	1.64	13.2	6.7	1.97	22.0	10.6	2.07	28.9 ^{abc}	13.1	2.21	33.3 ^{ab}	13.2	2.53	21.4 ^{ab}	9.9	2.16 ^{cde}
	2,950	9.4 ^a	5.7	1.65	13.1	6.8	1.95	21.3	10.2	2.08	29.1 ^{abc}	13.1	2.23	32.7 ^{ab}	12.8	2.56	21.1 ^{ab}	9.7	2.17 ^{cde}
	3,000	9.1 ^{ab}	5.5	1.65	13.2	7.0	1.87	21.1	10.4	2.03	28.2 ^{bc}	13.3	2.12	32.3 ^{ab}	13.1	2.46	20.8 ^{ab}	9.9	2.10 ^{de}
	3,050	8.2 ^b	5.5	1.48	13.1	6.9	1.90	20.9	11.1	1.88	27.9 ^c	12.8	2.18	32.2 ^b	13.3	2.43	20.4 ^b	9.9	2.06 ^e
Sd ²		0.57	0.32	0.107	0.98	0.36	0.142	1.69	0.92	0.230	1.46	0.75	0.120	1.11	0.86	0.168	0.59	0.32	0.071
Probability																			
Feed form		0.042	<.001	0.001	0.045	0.015	<.001	0.945	0.049	0.114	<.001	<.001	<.001	0.889	<.001	<.001	0.007	<.001	<.001
AME _n																			
Linear		0.002	0.356	0.022	0.063	0.781	0.067	0.690	0.062	0.265	0.244	0.717	0.138	0.078	0.400	0.063	0.026	0.137	<.001
Quadratic		0.015	0.722	0.037	0.988	0.749	0.808	0.927	0.408	0.665	0.493	0.201	0.400	0.729	0.249	0.176	0.337	0.829	0.230
Feed form * AME _n		0.032	0.633	0.114	0.926	0.4338	0.588	0.339	0.897	0.549	0.017	0.258	0.357	0.001	0.800	0.120	0.001	0.591	0.023

^{a-b-c} Within a column, means without a common superscript differ ($P < 0.05$).

¹Feed conversion ratio.

²Standard deviation: n = 30 replicates for feed form and n = 12 replicates for AME_n content of the diet.

Table 10. Influence of feed form and energy content (AME_n, kcal/kg) of the diet on energy intake¹ (EnI, kcal AME_n/d) and energy conversion ratio (ECR, kcal AME_n/g) of the pullets from hatching to 5 wk of age. Experiment 2

Feed form	AME _n (kcal/kg)	0 to 5 week	
		EnI	ECR
Mash	2,850	60.5 ^c	6.65 ^{bc}
	2,900	62.5 ^{abc}	6.74 ^{ab}
	2,950	64.1 ^{ab}	7.03 ^a
	3,000	64.7 ^{ab}	6.94 ^a
	3,050	65.7 ^a	7.02 ^a
Crumbles	2,850	61.9 ^{bc}	6.32 ^c
	2,900	62.0 ^{bc}	6.28 ^c
	2,950	62.3 ^{abc}	6.41 ^{bc}
	3,000	62.3 ^{abc}	6.30 ^c
	3,050	62.4 ^{abc}	6.29 ^c
Sd ²		1.71	0.208
Probability			
Feed form		0.005	<.001
AME _n			
Linear		<.001	<.001
Quadratic		0.337	0.201
Feed form *AME _n		0.001	0.013

^{a-b-c} Within a column, means without a common superscript differ ($P < 0.05$).

¹Calculated energy intake (kcal AME_n/d). The calculated AME_n values of the diets are presented in Table 4.

²Standard deviation: n = 30 replicates for feed form and n = 12 replicates for AME_n content of the diet.

Table 11. Influence of feed form and energy content (AME_n, kcal/kg) of the diet on BW uniformity¹ of the pullets. Experiment 2

	Hatching	1 week	2 week	3 week	4 week	5 week
Feed form						
Mash	7.67	9.96	9.77	10.19 ^a	8.76 ^a	8.50
Crumbles	7.79	9.24	9.35	8.81 ^b	8.01 ^b	7.97
AME _n (kcal/kg)						
2,850	7.58	8.86	9.47	10.61	8.45	8.55
2,900	7.67	9.47	9.09	8.71	9.00	7.99
2,950	7.81	9.82	9.61	9.40	7.77	8.18
3,000	7.63	10.01	9.45	9.08	8.00	8.03
3,050	7.96	9.84	10.18	9.72	8.69	8.42
Sd ²	0.841	1.597	1.184	2.286	1.386	1.155
Probability						
Feed form	0.572	0.089	0.175	0.023	0.042	0.077
AME _n						
Linear	0.360	0.094	0.101	0.515	0.694	0.827
Quadratic	0.877	0.324	0.236	0.103	0.266	0.215
Feed form * AME _n	0.738	0.856	0.105	0.092	0.628	0.989

¹Evaluated as the CV (%) of BW (Peak et al., 2000).

²Standard deviation: n = 30 replicates for feed form and n = 12 replicates for AME_n content of the diet.

3.3.2.2. Energy Concentration. Pullet performance was affected in different ways by energy concentration of the diet. From hatching to 5 wk of age, an increase in the energy concentration of the diet decreased ADFI and improved FCR when the feeds were fed in crumble form but no differences were detected when fed in mash form ($P < 0.01$ and $P < 0.05$ for the interaction, respectively) (Figure 1). Consequently, EnI was similar for all pullets fed crumbles, irrespective of the energy concentration of the diet, but increased in pullets fed mash as the AME_n increased ($P < 0.01$ for the interaction). The interactions between feed form and energy concentration of the diet were more evident during the last part of the experiment. BW uniformity was not affected by the energy content of the diet at any age (Table 11).

Figure 1. Interaction between feed form and energy content (AMEn, kcal/kg) of the diet on ADFI (A), FCR (B), energy intake (EnI) (C), and energy conversion ratio (ECR) (D) from hatching to 5 wk of age (n = 6). Experiment 2

3.4. Discussion

3.4.1. Experiment 1

The inclusion of additional fiber in the diet increased FI by 3.6% as an average and improved ADG by 4.1% from hatching to 5 wk of age and consequently, FCR was not affected. The authors have not found any research on the effects of DF on growth performance of pullets to compare with the results reported herein. In broilers, Jiménez-Moreno et al. (2009a) observed that the inclusion of 3% OH in the diet from 1 to 21 d of age improved FCR by 5.5% and ADG by 6.1% as compared with broilers fed a control diet. Similarly, González-Alvarado et al. (2007) reported that the inclusion of 3% OH or 3% soy hulls in the diet of broilers from 1 to 21 d of age improved ADG by 5.4% for both fiber sources and FCR by 2.9% and 2.2%, respectively. However, FI was not affected by fiber inclusion in any of these researches conducted with broilers whereas a significant increase of 3.6% was detected in the current research conducted with pullets.

The inclusion of fiber in the diet improved ECR within the range of 0.9% (4% SBP containing diet) and 4.4% (2% SFH containing diet) as compared with the control diet. González-Alvarado et al. (2010) reported a 5.8% increase in ECR with 3% OH inclusion in broilers from 1 to 42 d of age and Jiménez-Moreno et al. (2013a) showed a 4.3% improvement in ECR in broilers fed diets with 5.0% SBP from 1 to 18 d of age as compared with broilers fed the control diet. The data also suggest that during the initial stages of the rearing period, pullets might benefit from the inclusion of moderate amounts of fiber in the diet but that the benefits on ADG and ECR might be less pronounced in pullets than in broilers. Broilers had higher capacity for FI and grew faster than pullets. Also, fiber content is lower in commercial diets for broilers than in commercial diets for pullets. Consequently, under practical conditions, additional DF might be more beneficial in young broilers than in young pullets.

From hatching to 5 wk of age pullets fed SFH grew as an average 2.8% faster than pullets fed SBP with pullets fed straw being intermediate. Jiménez-Moreno et al. (2010) also observed that broilers fed 3% OH, an insoluble fiber source, grew 6.3% faster from 1 to 21 d of age than broilers fed 3% SBP, a soluble fiber source. Similarly, González-Alvarado et al. (2010) reported 7.1% higher ADG in broilers fed 3% OH than in broilers fed 3% SBP from 1 to 42 d of age. The differences in ADG observed between birds fed SFH and SBP might be related to differences in the physico-chemical characteristics of the 2 fiber sources. For example, pectins content is higher in SBP than in SFH and pectins increases water retention and volume and viscosity of the digesta in the GIT which may lead to faster satiety and less growth of the birds.

Pullets fed diets with 4% added fiber were less efficient than pullets fed diets with 2% added fiber. Jiménez-Moreno et al. (2013a) reported also a reduction in feed efficiency in broilers from 1 to 18 d of age as the level of OH or SBP in the diet increased from 2.5 to 7.5%. Similarly, Pettersson and Razdan (1993) showed a reduction in feed efficiency in broilers from 1 to 21 d of age when the SBP of the diet was increased from 2.3% to 4.6% or 9.2%. The data suggest that similar to what have been observed in broilers, young pullets might benefit when additional fiber sources are included in low fiber diets but that an excess of fiber might not be of benefit. Possibly, pullets might require a minimum of 3.2 to 3.5% crude fiber (equivalent to 9.2 to 9.5% NDF, approximately) to maximize growth performance from hatching to 5 wk of age. In this respect, Fundación Española Desarrollo Nutrición Animal (2008) recommended at least 3.0% CF in this period. However, neither the National Research Council (1994) nor the main companies involved in poultry genetics (e.g., Hy-Line International, 2013; Lohmann, 2013) have made any suggestion on the most convenient level of fiber in diets for young pullets.

Body weight uniformity was not affected by the inclusion of fiber in the diet. The authors have not found any published report on the influence of fiber inclusion in the diet on this trait. The data indicate that when moderate amounts of fiber are included in the diet, BW uniformity will not be affected.

3.4.2. Experiment 2

3.4.2.1. Feed Form. The GMD of the mash diets was not affected by energy concentration of the feed but that of the crumble diets decreased as the AME_n increased. The high energy diets contained more fat than the low energy diets, and an increase in fat content might reduce crumble quality, increasing the percentage of fines (Briggs et al., 1999). In contrast, dust formation is reduced in mash diets when the level of fat is increased and therefore, no reduction in GMD should be expected.

Numerous studies have reported that crumbling or pelleting of the diets improves performance in broilers (Amerah et al., 2007; Brickett et al., 2007; Serrano et al., 2012) and pullets (Frikha et al., 2009b). The heat and pressure applied during the pelleting process reduces further the particle size of some of the ingredients of the feed, fracturing the endosperm and disrupting the outer coat of the seeds (Svihus et al., 2004; Amerah et al., 2007). Consequently, pelleting facilitates the release of the intracellular oil contained in the ingredients, as well as the access of digestive enzymes to nutrients. Also, the heat applied during the pelleting process might modify at some extent the structure of the protein fraction, reducing the activity of some antinutritional factors present in plant ingredients and increasing CP digestibility (Herkelman et al., 1991). In addition, pellets might reduce the energy spent by the bird in feed ingestion, which may decrease further energy requirements (Abdollahi et al., 2012). From hatching to 5 wk of age pullets fed crumbles had 6.5% higher ADG and 8.2% better FCR than pullets fed mash. These results agree with data of Frikha et al. (2009b) who reported in 45-d old

pullets a 5.9% improvement in ADG when fed pellets, although in this experiment FCR was not affected.

For the first week of age, FI was greater in pullets fed crumbles than in pullets fed mash but opposite results were observed after this age. In contrast, ADG and FCR were better for pullets fed crumbles at all ages. The reason for the lower FI with mash feeding during the first week of life is not known. Mash feeding increases the time and energy spent by the chick in the apprehension of the feed (Jensen et al., 1962; Savory, 1974) and therefore, very young chicks, that have a limited GIT capacity, might not be able of consuming enough feed to optimize performance. In this respect, pullets were beak-trimmed immediately after hatching which might have reduced FI early in life in birds fed mash. Also, the size of the crumbles used might have been bigger than required for very young pullets and might not have fit well the beak of the bird, resulting in an increase in feed wastage (Workman and Rogers, 1990). After the first week of life, however, GIT development and capacity might be sufficient to meet energy needs of the pullets. Moreover, crumbling might reduce feed wastage as has been reported in broilers by Mateos et al. (2002) and Serrano et al. (2013). GIT capacity and feed wastage, however, were not measured in this experiment and therefore, this hypothesis needs further testing.

At 5 wk of age, pullets fed crumbles tended to have better BW uniformity than pullets fed mash, consistent with data of Brickett et al. (2007) in 5 wk-old broilers. In contrast, Frikha et al. (2009b) did not find any difference in BW uniformity of 45-d old pullets fed mash or 2-mm pellet diets. The discrepancies reported on BW uniformity of the birds among researches suggest that other factors, such as type and age of the birds, and method used for beak-trimming, as well as the ingredient composition of the diet, might affect the response to feed form.

3.4.2.2. Energy Concentration. Poultry eats to satisfy their energy requirements and therefore, ADFI should decrease and FCR should improve with increases in energy density of the diet. In the current experiment, however, pullets fed mash tended to overconsume energy as the AME_n of the diet increased whereas pullets fed crumbles were able of adjusting their EnI. Consequently, ECR was not affected by energy concentration of the diet in pullets fed crumbles but was hindered in pullets fed mash. The authors have not found any published research in pullets on the potential interaction between energy content of the diet and feed form to compare with the results reported herein. The data suggest that pullets fed mash were either unable to regulate feed consumption to fit energy requirements or wasted more feed than pullets fed crumbles. Consequently, an increase in the energy content of the diet resulted in an EnI that was higher than the real consumption. The greater feed wastage that possibly occurred when pullets were fed mash as compared with pullets fed crumbles, might explain the absence of effects of an increase in the energy concentration of the diet on reducing ADFI and improving FCR in these birds.

The distinct effect of energy concentration of the diet on FI in pullets fed mash or crumble diets are of considerable practical interest. The data reported herein suggest that young pullets have greater ability to regulate ADFI when fed a pellet diet than when fed a mash diet that possibly is less palatable, as has been shown in broilers by Scott (2002) and Brickett et al. (2007). In fact, Brickett et al. (2007) observed that broilers regulate better voluntary FI of diets varying in energy concentration when fed pellets than when fed mash.

Body weight uniformity was not affected by energy concentration of the diet at any age, in agreement with data of Frikha et al. (2009a) in 45 d-old brown-egg pullets fed diets varying in AME_n content from 2,735 to 3,025 Kcal/kg. Also, Brickett et al. (2007)

reported in 35 d-old broilers that BW uniformity was not affected by the energy concentration of the diet. However, Keshavarz et al. (1998) observed a significant improvement in BW uniformity of 18 wk-old pullets when the AME_n of the mash diet was increased from 2,815 to 3,035 Kcal/kg. The reason for the discrepancies reported among authors is unknown although factors such as type and age of the birds, flock management, including rearing density, and the characteristics of the experimental diets, might affect the outcome of the researches.

In summary, the inclusion of 2% of a fiber source in a corn-wheat-soybean meal diet improved pullet performance from hatching to 5 wk of age, an effect that tended to be more pronounced with SFH than with straw or SBP inclusion. An increase in the level of fiber from 2 to 4% reduced growth performance, especially when SBP was used. Feeding crumbles to pullets improved ADG and FCR and tended to improve BW uniformity as compared with feeding mash. An increase in the energy content of the diet decreased ADFI in pullets fed crumbles but no effects were detected in pullets fed mash. Neither ADG nor BW uniformity were affected by energy content of the diet. The results indicate that from hatching to 5 wk of age, pullets might require a minimal amount of DF to maximize growth performance. Moreover, at this early stage of the rearing period crumbled feeds might be preferred to mash feeds. The beneficial effect of increasing the energy content of the diet on pullet performance might depend on feed form and on the relative cost of available ingredients.

**CHAPTER 4: INCLUSION OF FIBER IN DIETS FOR BROWN-EGG LAYING
PULLETS: EFFECTS ON GROWTH PERFORMANCE AND DIGESTIVE
TRACT TRAITS FROM HATCHING TO 17 WEEKS OF AGE**

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4.1. Introduction

Traditionally, the inclusion of fiber in poultry diets was limited because of the poor adaptation of the gastrointestinal tract (**GIT**) of young birds to fibrous ingredients (Sell 1996; Uni et al., 1999) which affects feed intake, nutrient digestibility, and growth (Janssen and Carré, 1985; Sklan et al., 2003). Recent research, however, has shown that the inclusion in the diet of moderate amounts of certain insoluble fiber sources might improve gastrointestinal function and maximize growth performance in broilers (Jiménez-Moreno et al., 2009a; Svihus, 2011; Incharoen, 2013) and pullets (Guzmán et al., 2015a) during the first stage of life. Numerous ingredients, including oat hulls (**OH**), sunflower hulls (**SFH**), pea hulls, rice hulls, soy hulls, and sugar beet pulp (**SBP**), have been tested as a source of additional fiber in poultry diets (González-Alvarado, 2010; Kalmendal et al., 2011; Jiménez-Moreno et al., 2011, 2013a). Straw is an insoluble fiber source of uniform quality but the information available on its use as a dietary component in poultry diets is scarce. In fact, no research is available on the effects of straw on growth performance and GIT development of pullets from hatching to 17 wk of age. On the other hand, SBP, a soluble fiber source, has been largely studied as a source of DF in broilers (Pettersson and Razdan, 1993; Jiménez-Moreno et al., 2009b) but little information is available in pullets. Most papers that compared the effects of fiber in broilers, reported greater benefits with the inclusion of insoluble

sources than with the inclusion of soluble sources (González-Alvarado et al., 2010; Jiménez-Moreno et al., 2013a). Insoluble fiber stimulates more the development of the upper part of the GIT than soluble fiber (González-Alvarado et al., 2008) which in turn, improves the development and facilitates the adaptation of the digestive tract of the birds to consume more feed. However, no information is available comparing insoluble and soluble fiber sources in diets for pullets from hatch to 17 wk of age.

The hypothesis of this research was that the inclusion in the diet of adequate amounts of an insoluble fiber source, but not of a soluble fiber source, could improve GIT function and pullet performance. The aim of this research was to evaluate the inclusion in the diet of 2 fiber sources varying in physicochemical characteristics at 2 levels, on performance and GIT development of brown-egg laying pullets from hatching to 17 wk of age.

4.2. Materials and Methods

4.2.1. Husbandry

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, 2007).

In total, 2,500 one day-old (37.1 ± 1.31 g) Lohmann Brown Classic pullets were housed in an environmentally controlled barn and randomly allotted in groups of 50 to 50 cages (40 cm x 80 cm x 68 cm; Facco, Padova, Italy) provided with an open trough feeder and 2 low pressure nipple drinkers. The average BW of the pullets was similar for all cages. Because of the equipment and dimensions of the cages, only 22 pullets chosen at random formed the experimental unit after 3 wk of age. Pullets were beak-

trimmed at the hatchery and kept on a 22 h/d light program for the first week of life. The light was decreased gradually until reaching 10 h/d at 10 wk, kept constant from 10 to 15 wk of age and then, increased gradually to 13 h/d to the end of the experiment. Barn temperature was maintained at $32 \pm 1.5^{\circ}\text{C}$ for the first 3 d of life and reduced gradually until reaching 20°C at 6 wk. From 6 to 17 wk, the temperature of the barn varied between 22 and 25°C depending on weather conditions. Pullets were vaccinated against main diseases (Infectious Bronchitis Disease, Marek Disease, Infectious Bursal Disease, Newcastle Disease, and *Salmonella* spp.) and managed according to accepted commercial practices (Lohmann, 2013).

4.2.2. Fiber Sources, Diets, and Experimental Design

Batches of straw and SBP were received as 9-mm pellets from a trader. The straw used was a mixture of 80% wheat and 20% barley straw, and was processed with 2% sodium hydroxide solution before pelleting. Both fiber sources were ground using a hammer mill fitted with a 4-mm screen before being included in the corresponding experimental diets. The chemical analyses and the physical characteristics of the 2 fiber sources are shown in Table 1.

Table 1. Chemical analyses (% as fed basis, unless otherwise indicated) and physicochemical characteristics of the fiber sources

Fiber source	Straw	Sugar beet pulp
Calculated chemical analysis ¹		
AME _n (kcal/kg)	200	900
Ether extract	1.6	0.8
Crude fiber	36.0	18.2
Determined chemical analysis		
DM	91.3	92.0
Gross energy (kcal/kg)	3,854	3,729
CP	2.8	9.2
Total ash	6.7	5.6
Neutral detergent fiber	69.9	31.7
Acid detergent fiber	41.5	15.6
Acid detergent lignin	5.6	1.7
Total dietary fiber	78.5	64.7
Soluble dietary fiber	3.8	10.6
Insoluble dietary fiber	74.7	54.1
Physical properties		
Particle size ² (μm)		
2,500	0.2	1.2
1,250	12.3	33.5
630	40.4	35.2
315	28.4	18.4
160	15.3	9.0
80	2.9	2.3
GMD ³ ± GSD ⁴	602 ± 2.01	835 ± 2.12
WHC ⁵ ± SD	7.5 ± 0.14	8.7 ± 0.27
SWC ⁶ ± SD	3.6 ± 0.23	6.6 ± 0.56
Buffer properties		
Initial pH ⁷	8.39 ± 0.052	5.62 ± 0.033
Base-buffering capacity ⁸	-	50.9 ± 5.6
Acid-buffering capacity ⁹	134.2 ± 10.2	96.3 ± 5.2

¹According to Fundación Española para el Desarrollo de la Nutrición Animal (2010).

²The percentage of particles bigger than 2,500 μm or smaller than 80 μm were negligible for all diets.

³Geometric mean diameter (μm).

⁴GSD = Log normal SD.

⁵Water holding capacity (mL/g DM).

⁶Swelling capacity (mL/g DM).

⁷pH of 10 g DM of the fiber source suspended in 200 mL double distilled water.

⁸μEq of NaOH required to increase the pH of 10 g DM of the fiber source suspended in 200 mL double distilled water from the initial pH to pH 7 divided by pH change. The base-buffering capacity of the straw could not be measured because the initial pH was above 7.00.

⁹μEq of HCl required to decrease the pH of 10 g DM of the fiber source suspended in 200 mL double distilled water from pH 7 to pH 4 divided by pH change.

The feeding program consisted of 3 phases: starter (0 to 5 wk), grower (5 to 10 wk), and developer (10 to 17 wk), and the control diets used were based on corn and soybean meal. For the manufacturing of these diets, the ingredients were ground with a hammer mill fitted with a 2-mm (starter period) or a 3-mm (grower and developer periods) screen. The remaining experimental diets resulted from the inclusion of 2 or 4% straw or SBP at the expense (wt:wt) of the whole control diets. Diets were mixed using a horizontal ribbon mixer (Mecafa S.A., Ciudad Real, Spain). All diets met or exceeded the nutritional requirements of pullets as recommended by the Fundación Española Desarrollo Nutrición Animal (2008), but the diets that included a fiber source, had more crude fiber and slightly less AME_n and CP than the control diets. All diets included a commercial enzyme complex with xylanase and β -glucanase activity (Roxazyme, DSM S.A., Madrid, Spain). In the formulation of the diets, it was accepted that the inclusion of the enzyme complex increased the AME_n content of the wheat and barley grains by 2% (from 3,150 to 3,213 kcal/kg for wheat and from 2,800 to 2,856 kcal/kg for barley) but did not affect the energy content of the other ingredients (Fundación Española Desarrollo Nutrición Animal, 2010). Diets were offered for ad libitum consumption in mash form. The ingredient composition of the starter, grower, and developer diets is shown in Table 2 and the calculated and determined chemical analysis, as well as their particle size distribution, are shown in Tables 3, 4, and 5, respectively.

The experimental design was completely randomized with 5 treatments that consisted in a control diet without any additional fiber source and 4 extra diets arranged as a 2x2 factorial with 2 fiber sources (straw and SBP) at 2 levels of inclusion (2 and 4%). Each treatment was replicated 10 times and the experimental unit was the cage for all measurements.

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

	0 to 5 week			5 to 10 week			10 to 17 week		
	Control diet	Fiber diets		Control diet	Fiber diets		Control diet	Fiber diets	
		2%	4%		2%	4%		2%	4%
Barley	-	-	-	5.0	4.9	4.8	26.0	25.5	25.0
Corn	40.0	39.3	38.5	40.0	39.2	38.4	40.0	39.2	38.4
Wheat	18.0	17.6	17.3	15.0	14.7	14.4	1.10	1.08	1.06
Soybean meal, 47% CP	35.0	34.3	33.6	25.2	24.7	24.2	18.8	18.4	18.0
Wheat bran	-	-	-	10.1	9.9	9.7	10.2	10.0	9.8
Fiber source ¹	-	2.0	4.0	-	2.0	4.0	-	2.0	4.0
Poultry fat	2.65	2.54	2.44	1.04	1.02	0.98	0.50	0.49	0.48
Dicalcium phosphate	2.05	2.01	1.95	1.35	1.32	1.30	0.96	0.94	0.92
Calcium carbonate	1.26	1.22	1.20	1.28	1.26	1.23	1.50	1.47	1.45
Sodium chloride	0.36	0.35	0.34	0.33	0.32	0.31	0.35	0.34	0.34
DL-Met, 99%	0.18	0.18	0.17	0.16	0.15	0.15	0.08	0.08	0.07
L-Lys HCl, 78%	-	-	-	0.04	0.03	0.03	-	-	-
L-Thr, 98%	-	-	-	-	-	-	0.01	0.01	0.01
Vitamin and mineral premix ²	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

¹The fiber sources used were sugar beet pulp and straw, depending on the experimental treatment.

²Provided the following (per kilogram of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (all-*rac*-tocopherol-acetate), 30 mg; vitamin B₁, 2 mg; vitamin B₂, 8 mg; vitamin B₆, 4 mg; vitamin B₁₂ (cyanocobalamin), 0.025 mg; vitamin K₃ (bisulphatemenadione complex), 3mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; betaine anhydrous, 80 mg; D-biotin, 0.15 mg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg iron (FeCO₃), 60 mg; copper (CuSO₄ · 5H₂O), 8 mg; iodine (KI), 2 mg; selenium (Na₂SeO₃), 0.2 mg; Roxazyme, 200 mg [1,600 U of endo-1,4-β-glucanase (EC 3.2.1.4), 3,600 U of endo-1,3 (4)-β-glucanase (EC 3.2.1.6), and 5,200 U of endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM S.A., Madrid, Spain; and Natuphos 5000, 60 mg (300 phytase units/kg) supplied by Basf Espanola S.A., Tarragona, Spain.

Table 3. Chemical analyses (% as fed basis, unless otherwise indicated) and particle size distribution of the starter diets (0 to 5 wk of age)

	Control diet ¹	Straw (2%)	Straw (4%)	SBP ² (2%)	SBP (4%)
Calculated chemical analysis ³					
AME _n (kcal/kg)	2,960	2,898	2,839	2,914	2,870
Digestible amino acids					
Lys	1.01	0.99	0.97	0.99	0.97
Met	0.47	0.47	0.45	0.47	0.45
Met+cys	0.77	0.76	0.74	0.76	0.74
Thr	0.69	0.68	0.66	0.68	0.67
Trp	0.23	0.22	0.22	0.22	0.22
Ether extract	5.1	5.0	4.8	4.9	4.8
Crude fiber	2.9	3.5	4.1	3.2	3.5
Calcium	1.10	1.08	1.06	1.09	1.09
Phosphorus	0.74	0.73	0.71	0.73	0.71
Digestible phosphorus	0.43	0.42	0.41	0.43	0.42
Determined chemical analysis					
DM	90.4	90.9	91.4	91.1	91.8
Gross energy (kcal/kg)	4,017	4,085	4,048	4,094	4,109
CP	20.8	20.4	20.3	20.7	20.5
Neutral detergent fiber	9.4	10.3	11.4	9.7	10.0
Acid detergent fiber	3.8	4.6	5.3	4.0	4.1
Acid detergent lignin	0.6	0.7	0.8	0.6	0.6
Total ash	7.1	7.1	7.5	7.3	6.8
Particle size ⁴ (μm)					
2,500	3.8	4.1	5.6	3.9	3.7
1,250	28.4	26.5	31.9	27.6	28.9
630	36.9	37.0	34.8	36.7	36.1
315	21.7	21.7	17.9	21.8	21.8
160	9.0	10.2	9.1	9.5	9.3
80	0.3	0.3	0.5	0.4	0.3
GMD ⁵ ± GSD ⁶	862±2.01	840±2.04	924±2.08	851±2.03	859±2.02

¹The water holding capacity (± SD) and the swelling capacity (± SD) of the control diet, measured in triplicate, were 2.10 ± 0.172 mL/g DM and 2.80 ± 0.298 mL/g DM, respectively.

²Sugar beet pulp.

³According to Fundación Española Desarrollo Nutrición Animal (2010).

⁴The percentage of particles bigger than 2,500 μm and smaller than 80 μm were negligible for all diets.

⁵Geometric mean diameter.

⁶GSD = Log normal SD.

Table 4. Chemical analyses (% as fed basis, unless otherwise indicated) and particle size distribution of the grower diets (5 to 10 wk of age)

	Control diet ¹	Straw (2%)	Straw (4%)	SBP ² (2%)	SBP (4%)
Calculated chemical analysis ³					
AME _n (kcal/kg)	2,820	2,767	2,712	2,783	2,743
Digestible amino acids					
Lys	0.84	0.81	0.80	0.82	0.81
Met	0.41	0.40	0.39	0.40	0.40
Met+cys	0.68	0.66	0.65	0.66	0.65
Thr	0.58	0.56	0.55	0.57	0.56
Trp	0.19	0.19	0.19	0.19	0.19
Ether extract	3.7	3.6	3.6	3.6	3.5
Crude fiber	3.6	4.2	4.8	3.9	4.2
Calcium	0.93	0.92	0.90	0.93	0.93
Phosphorus	0.66	0.65	0.64	0.65	0.64
Digestible phosphorus	0.34	0.33	0.33	0.33	0.33
Determined chemical analysis					
DM	90.8	90.2	91.4	88.9	90.1
Gross energy (kcal/kg)	4,026	3,956	4,070	4,004	4,048
CP	18.4	17.2	18.2	17.9	17.9
Neutral detergent fiber	10.7	11.7	13.6	11.1	11.4
Acid detergent fiber	4.1	4.5	5.6	4.1	4.3
Acid detergent lignin	0.9	1.0	1.3	1.0	0.9
Total ash	5.2	5.2	5.5	5.1	5.1
Particle size ⁴ (μm)					
2,500	7.6	4.4	3.8	3.3	2.7
1,250	40.6	42.4	38.2	37.2	31.3
630	31.5	33.3	35.8	36.7	39.5
315	12.4	12.4	14.6	14.9	17.0
160	7.6	7.3	7.4	6.7	9.1
GMD ⁵ ± GSD ⁶	1,080±2.03	1,050±1.97	995±1.96	991±1.93	899±1.96

¹The water holding capacity (± SD) and the swelling capacity (± SD) of the control diet, measured in triplicate, were 2.03 ± 0.102 mL/g DM and 2.93 ± 0.111 mL/g DM, respectively.

²Sugar beet pulp.

³According to Fundación Española Desarrollo Nutrición Animal (2010).

⁴The percentage of particles bigger than 2,500 μm and smaller than 160 μm were negligible for all diets.

⁵Geometric mean diameter.

⁶GSD = Log normal SD.

Table 5. Chemical analyses (% as fed basis, unless otherwise indicated) and particle size distribution of the developer diets (10 to 17 wk of age)

	Control diet ¹	Straw (2%)	Straw (4%)	SBP ² (2%)	SBP (4%)
Calculated chemical analysis ³					
AME _n (kcal/kg)	2,790	2,741	2,687	2,754	2,718
Digestible amino acids					
Lys	0.67	0.66	0.64	0.66	0.65
Met	0.30	0.30	0.29	0.30	0.29
Met+cys	0.55	0.54	0.52	0.54	0.52
Thr	0.51	0.50	0.49	0.50	0.49
Trp	0.17	0.16	0.16	0.16	0.16
Ether extract	3.2	3.2	3.1	3.1	3.1
Crude fiber	3.9	4.5	5.1	4.2	4.5
Calcium	0.91	0.89	0.88	0.91	0.91
Phosphorus	0.59	0.58	0.57	0.58	0.57
Digestible phosphorus	0.28	0.27	0.27	0.27	0.27
Determined chemical analysis					
DM	93.1	92.8	91.7	91.4	91.9
Gross energy (kcal/kg)	3,964	3,961	3,957	3,899	3,971
CP	17.6	16.8	16.7	17.2	17.1
Neutral detergent fiber	14.3	14.9	17.3	14.5	15.2
Acid detergent fiber	4.6	5.0	5.9	4.7	4.9
Acid detergent lignin	1.1	1.0	1.0	0.9	1.0
Total ash	6.7	7.0	6.7	7.5	6.6
Particle size ⁴ (µm)					
2,500	10.0	11.1	10.8	10.2	9.8
1,250	36.6	29.3	29.6	30.1	29.9
630	29.5	30.5	30.9	31.3	31.7
315	14.9	17.3	16.8	17.5	17.3
160	8.6	11.0	11.0	10.4	10.6
80	0.3	0.7	0.9	0.4	0.6
GMD ⁵ ± GSD ⁶	1,046±2.15	953±2.26	951±2.26	960±2.21	949±2.21

¹The water holding capacity (± SD) and the swelling capacity (± SD) of the control diet, measured in triplicate, were 2.10 ± 0.204 mL/g DM and 3.16 ± 0.149 mL/g DM, respectively.

²Sugar beet pulp.

³According to Fundación Española Desarrollo Nutrición Animal (2010).

⁴The percentage of particles bigger than 2,500 µm and smaller than 80 µm were negligible for all diets.

⁵Geometric mean diameter.

⁶GSD = Log normal SD.

4.2.3. Growth Performance

Feed disappearance was recorded by cage and pullets were individually weighed at 5, 10, and 17 wk of age. Feed wastage was not measured. Any mortality was recorded and weighed as produced. From these data, ADG, ADFI, and feed conversion ratio (**FCR**) were determined by feeding phase and cumulatively. In addition, energy intake (**EI**), expressed as kilocalories of AME_n ingested per day, and energy conversion ratio (**ECR**), expressed as kilocalories of AME_n required per gram of gain, were measured at same ages. Body weight uniformity was determined by replicate as indicated by Peak et al. (2000). Briefly, the CV of the individual BW of the pullets of each cage replicate was generated and this variable was used as an indirect measurement of BW uniformity.

4.2.4. Gastrointestinal Tract Traits and Body Measurements

At 5, 10, and 17 wk of age, after the corresponding productive performance controls, 2 birds per replicate were randomly selected, weighed, and euthanized by CO₂ inhalation. The GIT, from the beginning of the proventriculus to the cloaca, including digesta content, liver, pancreas, and spleen, but not the crop and the esophagus, was removed aseptically and weighed. Then, the liver and the full proventriculus and gizzard, were excised and weighed and expressed relative (%) to live BW. The pH of the gizzard was measured in situ in duplicate at 10 wk of age using a digital pH meter fitted with a fine tip glass electrode (model 507, Crison Instruments S.A., Barcelona, Spain) as indicated by Jiménez-Moreno et al. (2009b). The mean value of the two measurements was used for further statistical evaluation. The proventriculus and gizzard were emptied from any digesta, cleaned, dried with desiccant paper, and weighed again, and the fresh digesta content was calculated as the difference between the full and empty organ weights and expressed relative (%) to full organ weight. The length of the

duodenum (from the gizzard to the pancreo-biliary ducts), jejunum (from the pancreo-biliary ducts to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileo-cecal junction), and 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. The length of the small intestine (**SI**) was determined by adding the length of the duodenum, jejunum, and ileum. Body length of the pullets, measured from the tip of the beak to the end of the longest phalanx, was also determined on extended birds. Finally, the length and diameter (in the middle point of the bone) of the tarsus were measured using a digital caliper. All length measurements were expressed in absolute (cm) and relative (cm/kg BW) terms.

4.2.5. Laboratory Analysis

Representative samples of the fiber sources and diets were ground in a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) equipped with a 1-mm screen and analysed for moisture by oven-drying (method 930.15), total ash by muffle furnace method (942.05), and nitrogen by Dumas (method 968.06) using a Leco analyzer (Model FP-528, Leco Corp., St. Joseph, MI) as indicated by AOAC International (2005). Gross energy was determined using an adiabatic bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL, USA). Neutral detergent fiber (**NDF**), acid detergent fiber, and acid detergent lignin were determined as described by Van Soest et al. (1991) and expressed on an ash-free basis. Also, the total DF and the insoluble fraction of DF of the fiber sources were analyzed (methods 985.29 and 991.43) as proposed by AOAC International (2005). The soluble fraction of DF was calculated by difference between total and insoluble DF. The water-holding capacity (**WHC**, mL/g DM), swelling capacity (**SWC**, mL/g DM), and buffer properties of the fiber sources were determined as indicated by Jiménez-Moreno et al. (2009b). The particle size distribution and mean

particle size of the fiber sources and diets, expressed as geometric mean diameter (**GMD**), were determined in 100 g samples using a shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 μm , as described by the ASAE (1995). All the analyses were conducted in duplicate, except for buffer properties and GMD determinations that were conducted in triplicate.

4.2.6. Statistical Analysis

Data were analysed as a completely randomized design using the GLM procedure of SAS (SAS Institute, 1990) as reported by Beccaccia et al. (2015). Briefly, main effects (fiber source and level of inclusion of fiber) and the interactions were studied for the 4 treatments arranged as a 2 x 2 factorial. In addition, the Dunnett test was used to compare data for all traits between pullets fed the control diet and pullets fed each of the other treatments. Differences were considered significant at $P < 0.05$ and tendencies at $0.10 < P < 0.05$. Results in tables are presented as means.

4.3. Results

The straw and the SBP contained by analyses 2.8 and 9.2% CP and 69.9 and 31.7% NDF, and had a GMD of 602 and 835 μm , respectively (Table 1). The NDF of the diets increased as the level of fiber increased (Tables 3 to 5). The GMD was similar for all the diets that included a fiber source and slightly lower than for that of the control diets in the starter, grower, and developer phases (average of 984, 869, and 954 for the fiber containing diets vs. 1,080, 870, and 1,046 for the control diets, respectively).

4.3.1. Growth Performance

Mortality was 2.5% and not related to treatment (data not shown). Most of the mortality (96%) occurred during the first week of the experiment. No interactions between main effects (fiber source and level of fiber) were detected at any age. From

hatch to 17 wk of age, the inclusion of 4% SBP at the expense of the whole diet, affected negatively FCR ($P < 0.001$) and ADG and EI ($P < 0.05$) (Table 6). Also, the inclusion of 4% straw, but not of 2% straw, worsened FCR ($P < 0.01$) and EI ($P < 0.05$). Straw inclusion, however, improved ADG ($P < 0.05$) and ECR ($P < 0.01$) as compared with SBP inclusion. An increase in the fiber content of the diet from 2 to 4% tended to reduce EI ($P = 0.059$) and worsened FCR ($P < 0.05$). Most of the negative effects of 4% SBP inclusion were observed during the last part of the rearing period. In fact, from 0 to 5 wk of age, none of the variables studied was affected by the inclusion of fiber in the diet. Moreover, ECR tended to improve with the inclusion of straw ($P = 0.052$) as compared with the inclusion of SBP. From 5 to 10 wk of age, ADFI was lower ($P < 0.01$) in pullets fed the control or the SBP diets than in pullets fed the 4% straw diets, with pullets fed 2% straw being intermediate. In this period, pullet growth responded with higher ADFI and ADG ($P < 0.001$) and EI ($P < 0.01$), and better ECR ($P < 0.05$) to the inclusion of straw as compared to the inclusion of SBP. In fact, pullets fed the 4% SBP diets showed worse ADG ($P < 0.01$), FCR ($P < 0.01$), and EI ($P < 0.05$) than pullets fed the control diet. Body weight uniformity was not affected by diet at any age (Table 7).

Table 6. Influence of source (SOU) and level (LEV) of fiber in the diet on growth performance, energy intake¹ (EI, kcal AME_n/d), and energy conversion ratio (ECR, kcal AME_n/g ADG) of the pullets from hatching to 17 wk of age

	Control	Straw		SBP ²		Fiber source		Inclusion level		Sd ³	Probability ⁴	
		2%	4%	2%	4%	Straw	SBP	2%	4%		SOU	LEV
0 to 5 week												
ADFI	19.6	20.1	20.3	20.1	20.2	20.2	20.2	20.1	20.2	0.72	0.827	0.580
ADG	8.55	8.78	8.79	8.75	8.57	8.79	8.66	8.77	8.68	0.350	0.138	0.305
FCR ⁵	2.29	2.29	2.31	2.30	2.36	2.30	2.33	2.30	2.33	0.083	0.266	0.171
EI	58.1	58.4	57.6	58.7	58.2	58.0	58.5	58.6	57.9	2.09	0.447	0.285
ECR ⁶	6.80	6.65	6.55	6.71	6.80	6.60	6.76	6.68	6.68	0.242	0.052	0.916
5 to 10 week												
ADFI ⁷	49.7	50.7	51.2	49.7	49.5	50.9	49.6	50.2	50.4	1.01	<.001	0.645
ADG ⁸	15.1	15.2	15.2	14.8	14.5	15.2	14.6	15.0	14.8	0.38	<.001	0.190
FCR ⁸	3.30	3.34	3.37	3.36	3.42	3.36	3.39	3.35	3.40	0.080	0.143	0.075
EI ⁹	140.1	140.4	138.8	138.3	136.1	139.6	137.2	139.3	137.5	2.80	0.007	0.031
ECR	9.31	9.25	9.15	9.35	9.41	9.20	9.38	9.30	9.28	0.249	0.021	0.752
10 to 17 week												
ADFI	68.0	67.7	68.0	67.7	68.0	67.9	67.9	67.7	68.0	2.21	0.995	0.644
ADG ¹⁰	12.1	11.6	11.5	11.6	11.5	11.5	11.5	11.6	11.5	0.61	0.984	0.644
FCR ¹¹	5.61	5.86	5.92	5.85	5.93	5.89	5.89	5.85	5.93	0.211	0.484	0.938
EI ¹²	186.9	182.1	179.6	183.5	181.7	180.9	182.6	182.8	180.7	5.95	0.397	0.286
ECR	15.4	15.8	15.6	15.9	15.8	15.7	15.9	15.8	15.7	0.57	0.511	0.535
0 to 17 week												
ADFI	48.9	49.3	49.6	49.0	49.1	49.4	49.0	49.1	49.3	0.95	0.209	0.490
ADG ¹³	12.0	11.8	11.8	11.7	11.5	11.8	11.6	11.8	11.7	0.31	0.042	0.231
FCR ¹⁴	4.09	4.16	4.20	4.18	4.27	4.18	4.22	4.17	4.23	0.072	0.128	0.016
EI ¹⁵	136.8	134.9	133.1	134.9	133.4	134.0	134.2	134.9	133.3	2.57	0.860	0.059
ECR	11.4	11.4	11.3	11.5	11.6	11.3	11.6	11.5	11.4	0.20	0.004	0.550

¹Energy intake calculated from the AME_n of the diets presented in Tables 3 to 5.²Sugar beet pulp.³Standard deviation (n = 10 for each treatment and n = 20 for main effects).

⁴The interaction between source and level of fiber inclusion was not significant ($P > 0.10$).

⁵Feed conversion ratio.

⁶Contrast of control vs. 4% straw ($P = 0.089$).

⁷Contrast of control vs. 2% straw ($P = 0.095$) and control vs. 4% straw ($P = 0.005$).

⁸Contrast of control vs. 4% SBP ($P = 0.005$).

⁹Contrast of control vs. 4% SBP ($P = 0.010$).

¹⁰Contrast of control vs. 4% straw ($P = 0.087$) and control vs. 4% SBP ($P = 0.081$).

¹¹Contrast of control vs. 2% straw ($P = 0.035$), control vs. 4% straw ($P = 0.006$), control vs. 2% SBP ($P = 0.036$), and control vs. 4% SBP ($P = 0.012$).

¹²Contrast of control vs. 4% straw ($P = 0.030$).

¹³Contrast of control vs. 4% SBP ($P = 0.008$).

¹⁴Contrast of control vs. 4% straw ($P = 0.004$), control vs. 2% SBP ($P = 0.025$), and control vs. 4% SBP ($P < 0.001$).

¹⁵Contrast of control vs. 4% straw ($P = 0.010$) and control vs. 4% SBP ($P = 0.019$).

Table 7. Influence of source (SOU) and level (LEV) of fiber in the diet on BW uniformity¹ of the pullets

	Control	Straw		SBP ²		Fiber source		Inclusion level		Sd ³	Probability ⁴	
		2%	4%	2%	4%	Straw	SBP	2%	4%		SOU	LEV
5 week	8.02	9.33	8.88	8.64	9.07	9.11	8.85	8.99	8.97	1.569	0.618	0.982
10 week	8.37	8.99	7.99	9.08	8.83	8.49	8.96	9.04	8.41	1.543	0.373	0.233
17 week	7.26	8.36	7.83	8.68	8.68	8.09	8.68	8.52	8.25	1.731	0.301	0.638

¹Evaluated as the CV (%) of BW (Peak et al., 2000).

²Sugar beet pulp.

³Standard deviation (n = 10 for each treatment and n = 20 for main effects).

⁴The interaction between source and level of fiber inclusion was not significant ($P > 0.10$).

4.3.2. Gastrointestinal Tract Traits and Body Measurements

No interactions between main effects were detected for any of the traits studied and therefore, only main effects are discussed. At 5 wk of age, the inclusion of fiber did not affect the relative weight of any of the organs of the GIT (Table 8). However, in absolute terms the gizzard tended to be heavier in pullets fed straw than in pullets fed SBP ($P = 0.056$) (Table 9). Moreover, an increase in fiber from 2% to 4% tended to increase the relative weight (5.22 vs. 5.56%; $P = 0.056$) of the gizzard. Also at this age, the relative length of the SI was higher in pullets fed SBP than in pullets fed straw (296 vs. 279 cm/kg BW; $P = 0.051$) but cecum length was not affected (Table 10). At 10 wk of age, pullets fed the control diet had lighter gizzards in relative and absolute terms ($P < 0.01$) than pullets fed the 4% straw diets. Pullets fed SBP had greater relative weight of the GIT ($P < 0.01$) than pullets fed straw. Also at this age, gizzard relative weight increased as the level of fiber increased ($P < 0.01$). Moreover, the relative length of the SI was higher ($P < 0.05$) in pullets fed SBP than in pullet fed straw but no differences were detected for the cecum.

At 5 and 10 wk of age, the relative length of the pullets ($P = 0.058$ and $P < 0.05$, respectively) and of the tarsus ($P = 0.079$ and $P < 0.05$, respectively) were higher with SBP than with straw. Dietary treatment, however, had little effect on GIT development at 17 wk of age. Opposite to data reported in relative terms, the absolute length was higher in pullets fed straw than in pullets fed SBP, with differences tending to be significant at 5 wk of age ($P = 0.054$) (data not shown).

Table 8. Influence of source (SOU) and level (LEV) of fiber in the diet on the relative weight (% BW) of selected digestive organs of the pullets

	Control	Straw		SBP ¹		Fiber source		Inclusion level		Sd ²	Probability ³	
		2%	4%	2%	4%	Straw	SBP	2%	4%		SOU	LEV
5 week												
BW	343	361	357	340	338	359	339	350	348			
GIT ⁴	19.7	20.0	20.0	20.4	20.7	20.0	20.6	20.2	20.4	1.22	0.169	0.625
Liver	4.06	4.12	3.90	4.16	3.91	4.01	4.03	4.14	3.90	0.387	0.849	0.045
Proventriculus ^{5,6}	0.79	0.85	0.90	0.85	0.89	0.87	0.87	0.85	0.89	0.092	0.896	0.206
Gizzard ⁵	5.08	5.27	5.52	5.17	5.60	5.39	5.38	5.22	5.56	0.531	0.957	0.056
10 week												
BW	874	901	899	868	837	900	852	884	868			
GIT	13.6	13.8	14.1	14.8	14.7	14.0	14.8	14.3	14.4	1.29	0.007	0.819
Liver ⁷	2.67	2.50	2.41	2.63	2.53	2.46	2.58	2.56	2.47	0.250	0.142	0.288
Proventriculus	0.65	0.60	0.62	0.63	0.63	0.61	0.63	0.61	0.63	0.062	0.257	0.428
Gizzard ⁸	4.21	4.33	4.70	4.31	4.60	4.51	4.45	4.32	4.65	0.316	0.584	0.004
17 week												
BW	1,512	1,500	1,502	1,507	1,452	1,501	1,479	1,503	1,477			
GIT ⁹	11.5	11.9	11.9	11.5	12.5	11.9	12.0	11.7	12.2	0.93	0.769	0.092
Liver	2.06	2.04	2.02	2.00	2.11	2.03	2.06	2.02	2.07	0.291	0.766	0.627
Proventriculus	0.55	0.51	0.55	0.52	0.54	0.53	0.53	0.51	0.54	0.058	0.936	0.084
Gizzard	3.60	3.80	3.98	3.74	3.91	3.89	3.82	3.77	3.94	0.400	0.643	0.192

¹Sugar beet pulp.²Standard deviation (n = 10 for each treatment and n = 20 for main effects).³The interaction between source and level of fiber inclusion was not significant ($P > 0.10$).⁴Full gastrointestinal tract including liver, pancreas, and spleen but not the crop and the esophagus.⁵With contents.⁶Contrast of control vs. 4% straw ($P = 0.039$), and control vs. 4% SBP ($P = 0.077$).⁷Contrast of control vs. 4% straw ($P = 0.091$).⁸Contrast of control vs. 4% straw ($P = 0.005$) and control vs. 4% SBP ($P = 0.029$).⁹Contrast of control vs. 4% SBP ($P = 0.083$).

Table 9. Influence of source (SOU) and level (LEV) of fiber in the diet on the absolute full organ weight (g) and digesta content (expressed as % to full organ weight) of the proventriculus and the gizzard and pH of the gizzard

		Control	Straw		SBP ¹		Fiber source		Inclusion level		Sd ²	Probability ³	
			2%	4%	2%	4%	Straw	SBP	2%	4%		SOU	LEV
5 week													
Proventriculus	Weight	2.95	2.94	2.99	3.13	3.08	2.97	3.10	3.04	3.03	0.363	0.256	0.975
	Content	6.21	4.84	5.06	6.12	5.75	4.95	5.94	5.48	5.40	2.926	0.270	0.932
Gizzard	Weight ⁴	17.3	19.0	19.6	17.5	18.9	19.3	18.2	18.2	19.2	1.76	0.056	0.082
	Content	36.8	33.0	35.6	33.8	35.1	34.3	34.4	33.4	35.4	4.47	0.946	0.152
10 week													
Proventriculus	Weight	5.64	5.39	5.58	5.43	5.29	5.49	5.36	5.41	5.44	0.528	0.391	0.851
	Content	9.04	5.50	7.97	9.74	7.53	6.73	8.64	7.62	7.75	4.124	0.133	0.916
Gizzard	Weight ⁵	36.6	38.9	42.1	37.4	38.3	40.5	37.8	38.2	40.2	3.21	0.016	0.062
	Content	29.2	29.8	30.5	29.9	28.7	30.1	29.3	29.8	29.6	4.43	0.553	0.873
	pH ⁶	2.94	2.60	2.43	2.84	2.60	2.51	2.72	2.72	2.51	0.481	0.188	0.199
17 week													
Proventriculus	Weight	8.28	7.63	8.22	7.88	7.80	7.92	7.84	7.76	8.01	1.107	0.805	0.440
	Content	8.09	6.02	7.31	8.77	7.18	6.67	7.97	7.39	7.25	3.537	0.282	0.902
Gizzard	Weight	54.1	58.3	59.7	56.4	56.8	59.0	56.6	57.4	58.3	6.65	0.291	0.694
	Content ⁷	27.5	30.8	28.7	28.8	29.5	29.6	29.1	29.8	29.1	3.52	0.524	0.510

¹Sugar beet pulp.²Standard deviation (n = 10 for each treatment and n = 20 for main effects).³The interaction between source and level of fiber inclusion was not significant ($P > 0.05$).⁴Contrast of control vs. 4% straw ($P = 0.025$).⁵Contrast of control vs. 4% straw ($P = 0.002$).⁶Contrast of control vs. 4% straw ($P = 0.066$).⁷Contrast of control vs. 2% straw ($P = 0.040$).

Table 10. Influence of source (SOU) and level (LEV) of fiber source in the diet on the relative length (cm/kg BW¹) of the organs of the gastrointestinal tract, pullets², and the length and diameter³ of the tarsus

	Control	Straw		SBP ⁴		Fiber source		Inclusion level		Sd ⁵	Probability ⁶	
		2%	4%	2%	4%	Straw	SBP	2%	4%		SOU	LEV
5 week												
Duodenum	51.7	48.3	49.3	52.3	53.0	48.8	52.6	50.3	51.1	5.07	0.032	0.627
Jejunum	131	124	127	129	136	125	132	126	131	12.4	0.088	0.233
Ileum	109	104	105	109	113	105	111	106	109	10.4	0.064	0.350
Small intestine	292	276	281	290	302	279	296	283	292	26.2	0.051	0.306
Cecum	54.3	53.8	51.9	55.4	56.4	52.8	55.9	54.6	54.1	6.28	0.150	0.821
Pullet	115	111	112	117	114	111	116	114	113	7.4	0.058	0.636
Tarsus	18.1	17.5	17.9	18.7	18.1	17.7	18.4	18.1	18.0	1.22	0.079	0.774
Tarsus diameter	1.87	1.79	1.82	1.88	1.85	1.81	1.86	1.83	1.84	0.135	0.172	0.916
10 week												
Duodenum ⁷	23.3	22.2	21.1	23.5	24.0	21.7	23.7	22.9	22.5	2.22	0.008	0.660
Jejunum	65.2	61.1	61.6	65.7	64.6	61.3	65.2	63.4	63.1	4.73	0.023	0.829
Ileum	52.2	50.3	50.2	53.4	51.5	50.2	52.5	51.9	50.8	5.45	0.216	0.563
Small intestine	141	134	133	143	140	133	141	138	136	10.1	0.021	0.615
Cecum	28.0	27.2	25.8	28.1	27.1	26.5	27.6	27.7	26.5	2.74	0.222	0.191
Pullet	67.0	64.6	65.8	67.7	68.8	65.2	68.2	66.2	67.3	2.82	0.002	0.243
Tarsus	8.43	8.07	8.18	8.45	8.58	8.13	8.52	8.26	8.38	0.519	0.028	0.485
Tarsus diameter	0.94	0.94	0.94	0.97	0.98	0.94	0.97	0.95	0.96	0.061	0.112	0.910
17 week												
Duodenum	14.4	14.4	14.7	14.4	15.2	14.6	14.8	14.4	14.9	1.32	0.602	0.240
Jejunum	39.3	39.7	39.5	39.4	40.7	39.6	40.0	39.6	40.1	3.62	0.698	0.662
Ileum	33.0	33.9	34.1	33.5	34.6	34.0	34.1	33.7	34.3	2.95	0.954	0.524
Small intestine	86.7	88.1	88.2	87.3	90.4	88.2	88.9	87.7	89.3	7.31	0.759	0.491
Cecum	11.1	11.2	11.2	11.1	11.5	11.2	11.3	11.1	11.3	0.82	0.720	0.456
Pullet	43.4	43.3	43.0	43.4	44.7	43.2	44.0	43.4	43.8	3.26	0.371	0.635
Tarsus	5.05	5.08	5.20	5.19	5.33	5.14	5.26	5.14	5.26	0.531	0.528	0.500
Tarsus diameter	0.61	0.59	0.63	0.62	0.63	0.61	0.62	0.61	0.63	0.059	0.591	0.301

¹BW are shown in table 8.

²Measured from the tip of the beak to the end of the longest phalanx.

³Measured with a caliper above the spur.

⁴Sugar beet pulp

⁵Standard deviation (n = 10 for each treatment and n = 20 for main effects).

⁶The interaction between source and level of fiber inclusion was not significant ($P > 0.05$)

4.4. Discussion

The CP and NDF contents of the straw were slightly lower than values reported by García et al. (1996) for barley straw and Gidenne (2003) for wheat straw. For the SBP, the analyzed values of CP and NDF were lower than those reported by González-Alvarado et al. (2010) and Jiménez-Moreno et al. (2013b), although the differences were of little practical interest. The determined chemical analyses of the diets were similar to expected values, indicating that the diets were mixed correctly.

4.4.1. Growth Performance

Body weight at 17 wk of age is one of the most important factors affecting egg production and egg size at the initiation of the egg cycle (Summers et al., 1987). Moreover, FCR from 0 to 17 wk of age is significantly correlated with pullet cost. For the entire experimental period, pullets fed the control diets showed 1.7 and 3.3% higher ADG and 2.7 and 3.7% better FCR than pullets fed the 4% straw or 4% SBP containing diets. From hatching to 5 wk of age, however, the inclusion of 2 or 4% of the fiber sources at the expense (wt:wt) of the whole diet did not affect ADG or FCR. Guzmán et al (2015a) reported that pullets fed diets containing 2% straw or SBP from hatching to 5 wk of age, had 3.7% greater ADG in both cases than pullets fed the control diet, consistent with the results reported herein. González-Alvarado et al. (2010) reported that FCR improved with the inclusion of 3% SBP in the diet in broilers from 1 to 10 d of age but not from 10 to 42 d of age. The maximal growth of all organs and segments of the GIT in broilers, is reached before d 9 post-hatching (González-Alvarado et al., 2008). Consequently, during the first stages of life, DF might stimulate the development of the upper part of the GIT, benefiting bird performance. Sugar beet pulp, however, is rich in pectins, a fiber fraction with a high WHC and SWC, that increases the bulk and

the viscosity of the digesta (Bach Knudsen, 2001), reduces the rate of digesta passage along the distal part of the GIT, and decreases feed intake in older broilers (Jiménez-Moreno et al., 2009a; González-Alvarado et al., 2010). The authors, however, have not found any published research on the effects of the inclusion of additional fiber in the diet on performance of pullets for the entire rearing phase.

Poultry eat to satisfy their energy requirements and therefore, EI should be similar for all diets, irrespective of their fiber content. In this respect, Leeson et al. (1996) reported that broilers from 0 to 49 d of age adapted feed intake to maintain EI, when the diet was diluted with up to 3.75% OH. The influence of additional fiber on EI, ECR, and FCR, however, is a subject of debate. In the current research, ADFI from 0 to 17 wk of age increased as the level of fiber in the diet increased although the differences were not significant. However, pullets fed the control diet had 2.5 and 2.7% higher EI than pullets fed the 4% SBP or straw containing diet, respectively. The results indicate that from 0 to 17 wk of age, pullets were not able to consume enough feed to regulate precisely EI to meet their needs, and that ADG and FCR were worsened when fed 4% straw or SBP. Jiménez-Moreno et al. (2016) reported also 5.0 and 4.4% higher EI in broilers when fed a control diet with only 3.6% NDF than when fed diets with 5.0% OH or SFH that contained 7.1 and 7.2% NDF, respectively. Additional fiber increases the bulk of the feed and satiates faster bird appetite, which in turn might result in pullets unable to regulate EI. Consequently, unless nutrient digestibility is improved, FCR might be worsened when extra amounts of fiber are included in the diet. In this respect, González-Alvarado et al. (2010) reported 6.3 and 3.8% better FCR in broilers from 1 to 42 d of age when fed 3% OH or SBP than when fed the control diet. In the current research, the level of NDF of the control diet was higher than that used in most experiments conducted with broilers. For example, the basal broiler diet used by

González-Alvarado et al. (2010) contained 5.3% NDF in the starter period (1 to 25 d of age) and 5.7% NDF in the finisher period (26 to 42 d of age) whereas in the current research, the control pullet diet had 9.4% NDF in the starter period and 14.3% NDF in the finisher period. We hypothesize that the beneficial effects of including additional fiber in the diet could be more evident in broilers than in pullets because of the lower NDF level of their diets and their higher appetite and rate of growth. Consequently, less effect of additional fiber on pullet growth should be expected in the current research because the need for fiber of the birds was already satisfied when fed the control diet (Mateos et al., 2012).

From hatching to 17 wk of age, ADG was better for pullets fed straw than for pullets fed SBP. Guzmán et al (2015a) observed that from 0 to 5 wk of age, pullets fed 2 and 4% SFH, an insoluble fiber source, had as an average 2.8% greater ADG than pullets fed 2 and 4% SBP, a soluble fiber source, in agreement with the results reported herein. In broilers from 1 to 42 d of age, González-Alvarado et al. (2010) reported also 6.7% greater ADG when fed a diet with 3% OH than when fed a diet with 3% SBP. The differences in ADG and also in ECR observed at 17 wk of age between birds fed straw and birds fed SBP might be related to the distinct physicochemical properties of these 2 fiber sources. Acid detergent fiber and lignin contents are higher for the straw than for the SBP and therefore, straw particles will be more resistant to grinding and will be retained for longer in the gizzard than SBP particles. As a result, the gizzard will be heavier and the pH of the digesta lower with straw than with SBP (Bach Knudsen, 2001; Jiménez-Moreno et al., 2013b). On the other hand, WHC and SWC are higher in SBP than in straw because of differences in pectins content (Bach Knudsen, 2014). Also, the soluble fraction of SBP might encapsulate part of the phospholipids and bile acids present in the digesta (Story and Kritchersky, 1982) reducing fat digestibility

(Forman and Schneeman, 1980). Moreover, the fiber fraction of the diet modifies the environmental conditions within the GIT, which in turn might interfere with the digestion and absorption processes and affect the growth and profile of the existing microbiota (Kalmendal et al., 2011; Mateos et al., 2012; Bach Knudsen, 2014). Consequently, type and level of fiber might affect in different ways GIT environment, nutrient digestibility, and pullet growth.

The available information suggests that the inclusion of moderate amounts of fiber in commercial diets for pullets had little effect on growth during the first period of life but that negative effects might occur as the bird ages, especially when a high level of a soluble fiber source, such as SBP, is used. In this respect, no recommendations on the level and type of fiber of diets for pullets are available from the National Research Council (1994) or from the main companies involved in genetic development of laying hens (e.g., Lohmann, 2013; Hy-Line International, 2015). However, Fundación Española Desarrollo Nutrición Animal (2008) recommends a minimum of CF of 3% in starter (0 to 5 wk) and 4% in finisher (10 to 17 wk) diets for pullets, values that are consistent with the results of the current research.

4.4.2. Gastrointestinal Tract Traits and Body Measurements

The relative weight of the full GIT, proventriculus, and gizzard, and of the liver, and the relative length of the different segments of the SI, decreased with age, results that agree with data of Ravindran et al. (2006), Gracia et al. (2009), and Jiménez-Moreno et al. (2010) and are consistent with the allometric growth rate of these organs and tissues in the chicken (Zuidhof, 2005).

The gizzard was heavier at all ages in pullets fed the 4% fiber containing diets than in pullets fed the control diet. In fact, the differences were significant at 10 wk, an age at which the gizzard was 10.4 and 8.5% heavier in pullets fed 4% straw or 4% SBP than in

pullets fed the control diet. Moreover, gizzard weight increased when the fiber level was increased from 2 to 4%, with differences being significant at 5 and 10 wk of age. González-Alvarado et al. (2007) suggested that the fiber fraction of the diet is retained for longer in the gizzard. An increase in retention time leads to an increase in size of the muscular walls and of this organ (Jiménez-Moreno et al., 2009a). In this respect, González-Alvarado et al. (2010) reported 45 and 31% heavier gizzards in 42 d-old broilers fed 3% OH or 3% SBP than in broilers fed the control diet. Similarly, Hetland et al. (2003) observed that the gizzard was 21% heavier in 33 d-old broilers fed 10% OH than in broilers fed the control diet. All this information suggests that insoluble fiber impacts more GIT development in broilers than in pullets, a phenomenon that might be related to the lower fiber content of the broiler diets but also to differences in feed intake and rate of feed passage between the 2 chicken lines.

In the current research, the relative weight of the GIT was greater in pullets fed SBP than in pullets fed straw, although the differences were significant only at 10 wk of age. These differences in relative weight of the GIT might be related to the distinct physicochemical characteristics of the fiber sources used. Jiménez-Moreno et al. (2013b) reported also heavier GIT in broilers fed SBP than in broilers fed OH. Similarly, Jørgensen et al. (1996) observed heavier GIT in broilers fed pea hulls, a high pectin fiber source, than in broilers fed oat bran, a more insoluble fiber source.

The relative weight of the gizzard was similar for pullets fed straw than for pullets fed SBP. In contrast, Jiménez-Moreno et al. (2013b) observed heavier gizzards in broilers fed OH than in broilers fed SBP. The lower BW of pullets fed SBP than of pullets fed straw might have distorted the results when comparing data based on relative rather than on absolute weights. In this respect, in absolute terms, the gizzard was heavier in pullets fed straw than in pullets fed SBP at all ages, with differences being

significant at 5 and 10 wk of age. Consequently, the data on the effects of fiber source on GIT development might be taken with caution. The digesta content of the gizzard was not affected by fiber inclusion at any age and therefore, the higher gizzard weight in pullets fed straw than in pullets fed SBP might be due to a greater development of the muscular layer of this organ, a consequence of the different physicochemical properties of the two fiber sources.

At 10 wk of age, the inclusion of 4% straw reduced gizzard pH by 17.3% as compared with that of pullets fed the control diet. Jiménez-Moreno et al. (2010) reported also a 19.7% reduction in gizzard pH in 21 d-old broilers with the inclusion of 3% OH in the diet. Insoluble fiber sources, such as straw or OH, are retained for longer in the gizzard, leading to a higher HCl production (Duke, 1986b; Hetland et al., 2005; Jiménez-Moreno et al., 2013b). A low pH in the gizzard increases pepsin activity, mineral salts solubility, and nutrient digestibility (Rogel et al., 1987; Gabriel et al., 2003; Jiménez-Moreno et al. 2009c), effects that might favor bird performance.

The relative length of the SI was higher in pullets fed SBP than in pullets fed straw, with differences being significant at 10 wk of age, in agreement with data of Jiménez-Moreno et al. (2013a) who reported longer relative length of the SI in broilers fed SBP than in broilers fed OH. However, when the length values were compared in absolute terms, no differences were detected.

The relative length of the body and tarsus of the pullets were higher in pullets fed SBP than in pullets fed straw with differences being significant at 5 and 10 wk of age. The authors have not found in the literature any research on the effects of type of fiber on these traits to compare with the results reported herein. An increase in body and tarsus length suggests a better bone development (Cleasby et al., 2011) of the pullet which might be of benefit for future egg production (Senar and Pascual, 1997; Mendes

et al., 2008). Opposite to these findings, the absolute length of the body and tarsus were higher for pullets fed straw than for pullets fed SBP. Consequently, data on the effects of fiber inclusion in the diet on the relative length of the SI, body, and tarsus must be taken with caution.

In summary, the inclusion (wt:wt) of fiber in the diet did not affect pullet development from 0 to 5 wk of age. The inclusion of 2% straw, at the expense of the whole diet, might benefit the development of the GIT of the pullets without any adverse effect on growth performance. Sugar beet pulp inclusion, however, reduced pullet performance after 5 wk of age. Therefore, up to 10% NDF (equivalent to 3.5% CF in the current research) from 0 to 5 wk and up to 15% NDF (4.5% CF) from 10 to 17 wk of age can be included in pullet diets, provided that insoluble fiber sources are used.

**CHAPTER 5: EFFECT OF LEVEL OF FIBER OF THE REARING PHASE
DIETS ON EGG PRODUCTION, DIGESTIVE TRACT TRAITS, AND BODY
MEASUREMENTS OF BROWN EGG-LAYING HENS FED DIETS
DIFFERING IN ENERGY CONCENTRATION**

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5.1. Introduction

Hen productivity is affected by numerous factors, including the physico-chemical characteristics of the diets fed during the rearing and the laying phases (Leeson and Summers, 2005; Pérez-Bonilla et al., 2012a). Pullets with a low BW at the onset of egg production produce fewer and smaller eggs than heavy pullets during the whole egg laying cycle (Leeson et al., 1997; Pérez-Bonilla et al., 2012a,b). Consequently, the implementation of strategies that favor feed intake during the rearing phase should result in an increase in BW gain (**BWG**) and in an improvement in hen productivity.

Dietary fiber has been considered traditionally as a diluent of poultry diets with negative effects on palatability and feed intake (Janssen and Carré, 1985; Mateos et al., 2002). However, recent studies have shown that the inclusion of moderate amounts of fiber in the diet might benefit the development of the gastrointestinal tract (**GIT**) and the production of HCl, bile acids, and endogenous enzymes (Hetland et al., 2005; González-Alvarado et al., 2008, 2010). In fact, DF often results in improved nutrient digestibility and growth in broilers (Sklan et al., 2003; Jiménez-Moreno et al., 2011, 2016) and pullets (Guzmán et al., 2015a,b). Mateos et al. (2012) suggested that the effects of additional fiber on poultry performance might depend on the type of fiber used, with more pronounced effects with the inclusion of insoluble fiber sources.

Before the start of the laying period, pullets need to adapt their GIT to the consumption of increased amounts of feed. A common commercial practice used by the industry to increase the digestive capacity of the bird consists in increasing the fiber content of the diets fed from 10 to 17 wk of age. However, the scientific information on the benefits of this practice on hen productivity is very limited.

The use of low energy laying diets might result in hens not being able to consume enough energy to satisfy their requirements for egg production, especially early in the egg production cycle, when the GIT is not yet well developed (Pérez-Bonilla et al., 2012b). Consequently, the use of high energy diets in the laying phase might be more beneficial in hens that were fed low fiber diets during the rearing phase than in those that were fed high fiber diets because of differences in the development and physical capacity of the GIT.

Body measurements [body length, body mass index (**BMI**), tarsus length, and tarsus width] are useful criteria to predict the growth and future size of the birds (Senar and Pascual, 1997; Mendes et al., 2008; Cleasby et al., 2011) and the production potential of avian species (Ortiz et al., 2011; Saldaña et al., 2015a,b). However, the information available on the influence of nutritional practices during the rearing and production phases on these variables, and its relationship with BW and egg production of the laying hens is very limited.

The hypothesis tested in this research was that the inclusion of additional insoluble fiber, but not of soluble fiber, in the rearing phase diets could improve GIT development and subsequent production of the hens, especially at the onset of the egg cycle. Also, it was hypothesized that the use of high energy diets during the laying phase could be more beneficial in pullets that were fed low fiber diets during the rearing phase, because of the lower GIT capacity of these birds at 17 wk of age. This research

evaluated the effects of the inclusion in the rearing phase diets of 2 fiber sources differing in their physico-chemical characteristics, and the energy concentration of the laying phase diets on production, egg quality, GIT traits, and body measurements of brown egg-laying hens from 17 to 46 wk of age.

5.2. Materials and Methods

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 del Estado, 2007).

5.2.1. Husbandry, Diets, and Experimental Design

Details on diet composition and bird management from hatching to 17 wk of age have been reported elsewhere (Guzmán et al., 2015b). Briefly, 2,500 recently hatched Lohmann Brown Classic pullets were placed in an environmentally controlled barn, in groups of 50, in 50 cages with similar initial average BW. During the rearing phase, the diets of the 3 feeding periods (1 to 5 wk, 5 to 10 wk, and 10 to 17 wk of age) were arranged as a 1 + (2 x 2) factorial with a control diet based on corn and soybean meal and 4 additional diets which resulted from the combination of including 2 fiber sources differing in physico-chemical properties (straw and sugar beet pulp; **SBP**) at 2 levels (2 and 4%). The fiber sources were included in their respective diets at the expense (wt/wt) of the whole control diet.

At 17 wk of age (start of the laying phase), pullets were moved to an environmentally controlled barn and placed in enriched cages (40 cm × 80 cm × 68 cm; Facco S.p.A., Padova, Italy) equipped with an open trough feeder and 2 low pressure nipple drinkers. Because of the number and dimensions of the layer cages and the lack of space in the experimental barn, only 8 out of the 10 replicates of each of the previous

treatments and 10 out of 16 pullets of each rearing cage, chosen at random, were used. All the remaining birds were discarded. The pullets were placed in the layer cages according to previous treatment, without any commingling. Consequently, all the hens in each of the laying cages belonged to the same cage used during the rearing phase. Half of the cage replicates ($n = 4$) of each of the previous rearing treatments received a low energy layer diet (2,650 kcal AME_n/kg) and the other half received a higher energy layer diet (2,750 kcal AME_n/kg). Both diets met or exceeded the nutrient requirements for brown egg-laying hens (FEDNA, 2008) and had the same AME_n to indispensable amino acids (AA) ratio (Table 1). Feed in mash form and water were provided for ad libitum consumption. The experiment lasted for 7 periods of 4 wk, with the exception of the first period that lasted 5 wk (17 to 22 wk). The longer length of the first period was planned to equalize egg production of the young hens fed different diets during the rearing period. Hens were vaccinated against main diseases and managed according to commercial practices (Lohmann, 2013). The light program consisted of 13 h for the first week, then increased 1 h per week until reaching 16 h at 20 wk of age, and kept constant to the end of the experiment. The temperature inside the barn was recorded daily and varied, as an average, from $26 \pm 3^{\circ}\text{C}$ in June (first period of the experiment) to $21 \pm 3^{\circ}\text{C}$ in January (last period of the experiment).

The experimental design was completely randomized with 10 diets arranged as a 5 x 2 factorial with 5 rearing phase diets and 2 laying phase diets. The 5 rearing phase diets consisting in a control diet and the combination of 2 fiber sources (straw and SBP) at 2 levels of inclusion (2 and 4%). The 2 laying phase diets differed in energy content (2,650 vs. 2,750 kcal AME_n /kg).

Table 1. Ingredient composition and calculated analysis (% as fed bases) of the experimental diets

Ingredient	AME _n (kcal/kg)	
	2,650	2,750
Wheat	40.0	40.0
Yellow corn	20.0	17.0
Soybean meal (47% CP)	17.1	18.0
Sunflower meal (34% CP)	10.3	11.0
Sunflower oil soapstock	1.45	3.39
Dicalcium phosphate	1.08	1.18
Calcium carbonate	8.24	8.50
Sodium chloride	0.28	0.30
<i>DL</i> -methionine (99%)	0.11	0.13
Sepiolite ¹	0.94	-
Vitamin and mineral premix ²	0.50	0.50
Calculated analysis ³		
DM	89.4	89.8
AME _n (kcal/kg)	2,650	2,750
CP	17.6	18.2
Digestible amino acids		
Lys	0.68	0.71
Met	0.37	0.40
Met+cys	0.63	0.66
Thr	0.53	0.55
Trp	0.18	0.19
Crude fiber	4.4	4.6
Neutral detergent fiber	11.1	11.4
Ash	12.4	12.9
Calcium	3.75	3.85
Sodium	0.14	0.14
Total phosphorus	0.61	0.64
Digestible phosphorus	0.35	0.37

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

²Provided the following (per kilogram of diet): vitamin A (*trans*-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,750 IU; vitamin E (dl- α -tocopheryl acetate), 10 mg; vitamin B₁, 1.3 mg; vitamin B₂, 5 mg; vitamin B₆, 1.8 mg; vitamin B₁₂ (cyanocobalamin), 13 mg; niacin, 25 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 1.3 mg; biotin, 1.3 mg; choline (choline chloride), 250 mg; manganese (MnO), 88 mg; zinc (ZnO), 63 mg; iron (FeSO₄ H₂O), 38 mg; copper (CuSO₄ 5H₂O), 8 mg; iodine [Ca(IO₃)₂], 0.6 mg; selenium (Na₂SeO₃), 0.4 mg; 6-phytase (EC 3.1.3.26) 375 FTU/kg; endo-1,3- β -xylanase (EC 3.2.1.8), 190 UI/g, endo-1,4(4)- β -glucanase (EC 3.2.1.6), 610 U/g; etoquin, 200 mg.

³According to Fundación Española Desarrollo Nutrición Animal (2010).

5.2.2. Hen productivity and Egg Quality

Egg production was measured daily by replicate. All the eggs laid the last 2 d of each week were weighed by replicate and the average value of the 4 wk (or 5 wk for the first period) was used to estimate the average weight for each of the 7 experimental periods. Feed disappearance was recorded per replicate by period and for the entire experiment. In addition, the hens were weighed by replicate at the start of the experiment and at the end of each of the 7 experimental periods. Any mortality was recorded and weighed as it occurred. From these data, egg production, egg weight, egg mass, ADFI, feed conversion ratio per kilogram and per dozen of eggs, and BWG were calculated by period and cumulatively. In addition, the number of dirty, broken, and shell-less eggs was recorded daily by replicate in all eggs produced. An egg was considered as dirty when a spot of any kind or size was detected on the shell (Lázaro et al., 2003). Other egg quality traits, including Haugh units, yolk and shell color, shell strength, and shell thickness, were measured in 12 fresh eggs collected randomly from each replicate for the last 2 d of the 7 experimental periods. Haugh units and yolk color (Roche color fan) were measured in fresh eggs using a multitester equipment (QCM System, Technical Services and Supplies, Dunnington, York, UK) as indicated by Safaa et al. (2009). Shell color was measured using a Minolta colorimeter (Chroma Meter Model CR-200, Minolta Corp., Ramsey, NJ) and the Hunter values, L* (lightness), a* (green to red), and b* (blue to yellow) were recorded. Egg shell strength (kg/cm^2) was evaluated applying increased pressure to the broad pole of the egg using a press meter (Egg Force Reader, SANOVO Technology A/S, Odense, Denmark), as indicated by Safaa et al. (2008a). Shell thickness was measured at the two pole ends and at the middle section of the egg, using a digital micrometer (model IT-014UT, Mitotuyo,

Kawasaki, Japan). The average of the 3 measurements per egg was used for further analyses.

5.2.3. Digestive Traits and Body Measurements

At 46 wk of age, after the corresponding control on hen productivity, 2 hens per replicate were randomly selected, weighed individually, and euthanized by CO₂ inhalation. The full GIT, from the beginning of the proventriculus to the cloaca, including the spleen, liver, and pancreas, but not the crop and the esophagus, was removed aseptically and weighed. Liver, proventriculus, and gizzard were excised and weighed, and the results expressed relative to BW (**RW**, %). Then, the gizzard was emptied from any digesta, cleaned, dried with desiccant paper, and weighed again. The fresh digesta content was measured as the difference between the full and empty gizzard weight and expressed relative (%) to the weight of the full organ. The pH of the gizzard was measured in duplicate in situ using a digital pH meter fitted with a fine tip glass electrode (model 507, Crison Instruments S.A., Barcelona, Spain) as indicated by Jiménez-Moreno et al. (2009b) and the mean value was used for further evaluation. The length of the duodenum (from the end of the gizzard to pancreo-biliary ducts), jejunum (from pancreo-biliary ducts to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileo-cecal junction), and the two ceca (from the ostium to the tip of the right and left ceca) were measured on a glass surface using a flexible tape with a precision of 1 mm and expressed relative to live BW (**RL**, cm/kg BW). The length of the small intestine (**SI**) was determined by adding the length of the duodenum, jejunum, and ileum. Body length of the hens was measured from the tip of the beak to the end of the longest phalanx with the same flexible tape used for SI measurements. Tarsus length and tarsus width, measured in the middle point of the bone, were determined using a digital caliper. In addition, the BMI was estimated by dividing the BW (g) of the hen by

the body length² (cm), as indicated by Mendes et al. (2007). The average value of the 2 hens per replicate was used for further statistical analysis of all the traits studied.

5.2.4. Laboratory Analyses

The determined chemical analyses of the rearing phase diets have been reported elsewhere (Guzmán et al., 2015b). Representative samples of the laying hen diets were ground in a laboratory mill (Model Z-I, Retsch Stuttgart, Germany) equipped with a 1-mm screen and analyzed for moisture by oven-drying (method 930.15), total ash using a muffle furnace (method 942.05), and nitrogen by Dumas (method 968.06) using a Leco analyzer (Model FP-528, Leco Corp., St. Joseph, MI) as described by AOAC International (2005). Ether extract was determined by Soxhlet analysis after 3N HCl acid hydrolysis (Boletín Oficial del Estado, 1995) and gross energy using an adiabatic bomb calorimeter (Model 1356, Parr Instrument Company, Moline, IL, USA). The AA content of the diets was determined by ion-exchange chromatography (Hewlett-Packard 1100, Waldbronn, Germany) as described by De Coca-Sinova et al. (2008) and the linoleic acid as indicated by Grobas et al. (1999a). The particle size distribution and the geometric mean diameter (**GMD**) of the diets were determined in 100 g samples using a shaker (Retsch, Stuttgart, Germany) equipped with 8 sieves ranging in mesh from 5,000 to 40 µm as outlined by the ASAE (1995). All the analysis were conducted in duplicate, except for particle size distribution that was conducted in triplicate. The determined physico-chemical analyses of the diets are shown in Table 2.

Table 2. Determined chemical analysis¹ and particle size distribution² of the laying phase diets (% as fed basis, unless otherwise indicated)

	AME _n (kcal/kg)	
	2,650	2,750
Chemical analyses		
Gross energy (kcal/kg)	3,848	3,872
DM	90.0	89.5
CP	18.2	18.5
Indispensable amino acids		
Lys	0.82	0.84
Met	0.41	0.43
Met+cys	0.74	0.75
Thr	0.66	0.67
Trp	0.21	0.22
Ether extract	5.4	6.8
Linoleic acid	1.9	2.7
Total ash	13.0	13.3
Particle size ³ (μm)		
2,500	11.70	12.86
1,250	37.41	35.91
630	28.21	29.95
315	17.90	15.01
160	4.75	6.16
80	0.03	0.11
GMD ⁴ ± GSD ⁵	1,119± 2.06	1,124± 2.10

¹Analyzed in duplicate.²Sieve diameter (μm).³The percentage of particles bigger than 2,500 μm or smaller than 80 μm was negligible.⁴Geometric mean diameter.⁵GSD = Log normal SD.

5.2.5. Statistical Analysis

Data were analyzed as a completely randomized design by two-way ANOVA using the GLM procedure of SAS (SAS Institute, 2004). Main effects (rearing and laying phase diets) and the interactions between main effects were analyzed. Because 4 of the rearing phase diets were arranged as a 2 x 2 factorial, the main effects (fiber source and fiber level) and the interactions between these 2 main effects were also studied. The experimental unit was the cage for all measurements. Differences were considered significant at $P < 0.05$. Results in tables are presented as means. In addition, the Pearson correlation analyses (SAS Institute, 2004) was conducted to study the relation between BW and BMI, body length, tarsus length, and tarsus width of the hens at 46 wk of age.

5.3. Results

5.3.1. Hen Productivity and Egg Quality Traits

Mortality during the laying period was 1.8% and was not related to treatment (data not shown). No interactions between the rearing and the laying phase diets were detected for any of the traits studied and therefore, only main effects are presented. Source and level of fiber of the rearing phase diets did not affect any of the production variables studied, except egg production that was lower in hens fed SBP during the rearing phase than in hens fed straw (91.6 vs. 94.1%: $P < 0.05$; Table 3). The negative effects of the inclusion of SBP in the pullet diets on hen performance were more noticeable in the last periods of the laying phase (Figure 1). Egg production was not affected by the level of fiber of the pullet diets in any of the periods considered (Figure 2).

For the entire laying period, a 3.6% decrease in the energy content of the diet (2,750 vs. 2,650 kcal AME_n/kg) increased ADFI by 3.0% (111.3 vs. 114.6 g; $P <$

0.001), worsened feed conversion ratio per kilogram of eggs by 2.6% (1.94 vs. 1.99; $P < 0.01$), and decreased BWG of the hens by 10.3% (379 vs. 340 g; $P < 0.05$). However, egg production, egg weight, and egg mass were not affected. Data on the effects of energy content of the laying diet on egg production by period are shown in Figure 3.

Egg quality traits were not affected by the type of diet fed during the pullet or the laying phase in any of the periods studied (Table 4).

Table 3. Influence of type of pullet diet and energy content of the laying diet (kcal AME_n/kg) on productive performance of the hens from 17 to 46 wk of age

	Egg production (%)	ADFI (g/hen)	Egg weight (g)	Egg mass (g/d)	Feed conversion ratio		BW (g)		BW gain (g)
					(kg:kg)	(kg:dozen)	17 wk	46 wk	
Laying diet									
2,650 kcal/kg	93.4	114.6 ^a	61.9	57.7	1.99 ^a	1.48 ^a	1,453	1,792	340 ^b
2,750 kcal/kg	92.7	111.3 ^b	62.0	57.5	1.94 ^b	1.44 ^b	1,442	1,821	379 ^a
Pullet diet									
Control	93.9	113.8	62.2	58.4	1.95	1.46	1,467	1,844	376
Straw, 2%	94.0	113.1	61.9	58.1	1.95	1.44	1,451	1,801	350
Straw, 4%	94.0	113.7	61.5	57.8	1.97	1.45	1,455	1,815	359
Sugar beet pulp, 2%	91.9	111.9	62.5	57.5	1.95	1.47	1,447	1,783	336
Sugar beet pulp, 4%	91.5	112.4	61.6	56.4	2.00	1.47	1,417	1,791	375
Main effects, pullet diet									
Fiber source									
Straw	94.1 ^a	113.4	61.6	57.9	1.96	1.45	1,453	1,808	355
SBP	91.6 ^b	112.1	62.1	56.9	1.97	1.47	1,432	1,788	356
Fiber level									
2%	92.9	112.5	62.2	57.8	1.95	1.45	1,449	1,792	343
4%	92.7	113.1	61.6	57.1	1.98	1.46	1,436	1,803	367
SD ¹	2.60	2.52	0.28	1.76	0.052	0.042	45.9	56.6	49.2
					Probability ²				
Laying diet	0.401	0.001	0.690	0.676	0.008	0.016	0.481	0.117	0.017
Pullet diet	0.107	0.433	0.463	0.210	0.367	0.582	0.272	0.259	0.448
Contrast									
Fiber source in pullet diets	0.012	0.147	0.414	0.114	0.427	0.138	0.198	0.315	0.957
Fiber level in pullet diets	0.809	0.494	0.153	0.261	0.228	0.489	0.425	0.593	0.179

^{a,b}Means with different superscripts are significantly different ($P < 0.05$).¹Standard deviation (20 replicates for each laying diet, 8 replicates for each pullet diet, and 16 replicates for fiber source and fiber level).²The interactions between pullet and laying diets and between source and level of fiber of the pullet diets were not significant ($P > 0.05$).

Table 4. Influence of source and level of fiber of the rearing phase diets (hatching to 17 wk) and of the AME_n (kcal/kg) of the laying phase diet on egg quality from 17 to 46 wk of age

	Dirty eggs (%)	Haugh Units	RCF ¹	Undergrades ² (%)	Shell quality				
					Strength (kg/cm ²)	Thickness (mm)	Color ³		
							L	A	B
Laying diet									
2,650 kcal/kg	2.51	90.9	10.4	0.781	5.09	0.381	61.4	15.5	32.6
2,750 kcal/kg	2.67	90.7	10.4	0.838	5.15	0.383	61.7	15.3	32.7
Pullet diet									
Control	2.74	90.8	10.5	0.919	5.07	0.378	62.1	15.1	32.6
Straw, 2%	2.50	91.0	10.4	0.962	5.07	0.384	61.1	15.7	32.8
Straw, 4%	2.75	90.8	10.4	0.736	5.21	0.381	61.6	15.3	32.5
Sugar beet pulp, 2%	2.64	91.0	10.5	0.665	5.03	0.382	61.3	15.5	32.8
Sugar beet pulp, 4%	2.33	90.5	10.5	0.765	5.22	0.383	61.2	15.6	32.4
Main effects, pullet diet									
Fiber source									
Straw	2.62	90.9	10.3	0.849	5.14	0.383	61.4	15.5	32.6
SBP	2.48	90.8	10.4	0.715	5.12	0.383	61.2	15.5	32.6
Fiber level									
2%	2.57	91.0	10.4	0.814	5.05	0.383	61.2	15.6	32.8
4%	2.54	90.7	10.3	0.750	5.22	0.382	61.4	15.4	32.5
SD ⁴	0.812	1.38	0.35	0.2918	0.380	0.0088	1.08	0.72	0.70
					Probability ⁵				
Laying diet	0.532	0.794	0.928	0.537	0.654	0.831	0.362	0.261	0.721
Pullet diet	0.820	0.942	0.951	0.229	0.760	0.693	0.436	0.564	0.805
Contrast									
Fiber source in pullet diets	0.625	0.751	0.532	0.209	0.916	0.984	0.757	0.714	0.822
Fiber level in pullet diets	0.919	0.456	0.677	0.548	0.208	0.858	0.637	0.543	0.227

¹Roche color fan.²Obtained as the addition of broken and shell-less eggs.³A high L value means lighter color; a high A value means redder color, and a high B value means a more yellow color.⁴Standard deviation (20 replicates for each laying diet, 8 replicates for each pullet diet, and 16 replicates for fiber source and fiber level).⁵The interactions between pullet and laying diets and between source and level of fiber of the pullet diets were not significant ($P > 0.05$).

Figure 1. Effect of type of fiber (straw vs. sugar beet pulp; SBP) used in the rearing phase diets on ADFI (A), egg production (B), egg mass (C), feed conversion ratio per kilogram of eggs (D), and BW gain (E) of the hens by period from 17 to 46 wk of age ($n = 16$). NS = $P \geq 0.05$, * $P \leq 0.05$, and ** $P \leq 0.01$.

Figure 2. Effect of level of fiber inclusion (2 vs. 4%) in the rearing phase diets on ADFI (A), egg production (B), egg mass (C), feed conversion ratio per kilogram of eggs (D), and BW gain (E) of the hens by period from 17 to 46 wk of age (n = 16). NS = $P \geq 0.05$ and $*P \leq 0.05$.

Figure 3. Effect of energy content of the laying phase diets (2,650 vs. 2,750 kcal AMEn/kg) on ADFI (A), egg production (B), egg mass (C), feed conversion ratio per kilogram of eggs (D), and BW gain (E) of the hens by period from 17 to 46 wk of age ($n = 20$). NS = $P \geq 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$.

5.3.2. Digestive Tract Traits and Body Measurements

At 46 wk of age, the relative weight of the organs of the GIT and of the fresh digesta content and pH of the gizzard, were not affected by the type of pullet or layer diet used (Table 5). Neither the relative length of the SI and cecum nor the body and tarsus length were affected by dietary treatment (Table 6). Significant correlations between BW of the hens and BMI ($r = 0.762$; $P < 0.001$), body length ($r = 0.500$; $P < 0.01$), and tarsus length ($r = 0.758$; $P < 0.001$) were detected at this age (Table 7).

Table 5. Influence of source and level of fiber of the rearing phase diets (hatching to 17 wk) and energy content (kcal AME_n/kg) of the laying phase diets on BW, the relative digestive organ weight (% BW), and gizzard traits of laying hens at 46 wk of age

	BW (g)	GIT (%)	Liver (%)	Proventriculus ¹ (%)	Gizzard traits			
					Weight ¹ (g)	Weight ¹ (%)	Content (%)	pH
Laying diet								
2,650 kcal/kg	1,861	12.5	2.61	0.551	51.6	2.78	33.3	4.04
2,750 kcal/kg	1,827	12.5	2.70	0.560	49.5	2.71	32.2	4.01
Pullet diet								
Control	1,872	12.2	2.65	0.512	49.9	2.66	34.3	4.08
Straw, 2%	1,810	12.5	2.73	0.555	50.8	2.81	32.6	4.01
Straw, 4%	1,878	12.6	2.57	0.565	53.3	2.86	31.5	4.00
Sugar beet pulp, 2%	1,841	12.8	2.66	0.554	49.7	2.70	30.9	4.17
Sugar beet pulp, 4%	1,811	12.8	2.65	0.590	50.4	2.79	32.5	4.06
Main effects, pullet diet								
Fiber source								
Straw	1,843	12.5	2.65	0.560	52.1	2.83	32.0	4.00
SBP	1,826	12.7	2.66	0.572	50.0	2.74	31.7	4.11
Fiber level								
2%	1,825	12.6	2.69	0.555	50.2	2.75	31.7	4.09
4%	1,844	12.6	2.61	0.577	51.8	2.82	32.0	4.02
S								
SD ²	137.2	0.69	0.248	0.0990	6.12	0.257	3.89	0.281
				Probability ³				
Laying diet	0.680	0.676	0.118	0.829	0.454	0.446	0.438	0.653
Pullet diet	0.774	0.453	0.595	0.659	0.763	0.569	0.500	0.726
Contrast								
Fiber source in pullet diets	0.717	0.436	0.912	0.770	0.351	0.343	0.806	0.260
Fiber level in pullet diets	0.691	0.922	0.230	0.571	0.471	0.485	0.866	0.538

¹With contents.²Standard deviation (20 replicates for each laying diet, 8 replicates for each pullet diet, and 16 replicates for fiber source and fiber level).³The interactions between pullet and laying diets and between source and level of fiber of the pullet diets were not significant ($P > 0.05$).

Table 6. Influence of source and level of fiber of the rearing phase diets (from hatching to 17 wk) and of the AME_n (kcal/kg) of the laying phase diet on body mass index (BMI, g BW/length cm²), relative lengths (cm/kg BW¹) of the hen, tarsus, small intestine (SI) and cecum, and relative width (cm/kg BW) of the tarsus at 46 wk of age

	BMI	Relative length							Tarsus relative width
		Tarsus	Body	Duodenum	Jejunum	Ileum	SI ²	Cecum	
Laying diet									
2,650 kcal/kg	0.432	5.47	35.5	13.9	42.0	38.4	94.3	12.4	0.702
2,750 kcal/kg	0.431	5.50	35.9	13.9	42.2	37.9	94.0	12.3	0.701
Pullet diet									
Control	0.444	5.40	34.8	13.9	41.2	37.5	92.5	12.0	0.688
Straw, 2%	0.419	5.55	36.4	13.6	42.0	37.9	93.4	12.9	0.709
Straw, 4%	0.429	5.45	35.4	14.6	41.7	38.7	94.9	12.5	0.694
Sugar beet pulp, 2%	0.431	5.47	35.8	13.6	42.8	38.1	94.4	12.1	0.697
Sugar beet pulp, 4%	0.435	5.55	35.9	13.9	43.5	39.3	96.7	12.6	0.718
Main effects, pullet diet									
Fiber source									
Straw	0.424	5.50	35.9	14.1	41.8	38.2	94.1	12.7	0.702
SBP	0.433	5.51	35.8	13.7	43.1	38.7	95.5	12.3	0.708
Fiber level									
2%	0.425	5.51	36.1	13.6	42.4	37.9	93.9	12.4	0.703
4%	0.432	5.50	35.6	14.2	42.6	39.0	95.8	12.5	0.706
SD ³	0.0296	0.339	2.48	1.65	3.52	3.45	5.15	3.68	0.0487
		Probability ⁴							
Laying diet	0.836	0.821	0.881	0.932	0.757	0.545	0.885	0.856	0.878
Pullet diet	0.602	0.899	0.763	0.729	0.589	0.854	0.818	0.851	0.746
Contrast									
Fiber source in pullet diets	0.455	0.706	0.963	0.455	0.237	0.735	0.589	0.612	0.706
Fiber level in pullet diets	0.527	0.926	0.616	0.267	0.859	0.415	0.467	0.895	0.918

¹The BW is shown in Table 5.²Small intestine was calculated based on the data of duodenum, jejunum and ileum.³Standard deviation (20 replicates for each laying diet, 8 replicates for each pullet diet, and 16 replicates for fiber source and fiber level).⁴The interactions between pullet and laying diets and between source and level of fiber of the pullet diets were not significant ($P > 0.05$).

Table 7. Correlations between BW¹ and body length, tarsus length, tarsus width, and body mass index (BMI) of the hens at 46 wk of age

	Body length (cm)	Tarsus length (cm)	Tarsus width (cm)	BMI (g/cm ²)
<i>r</i>	0.500	0.758	0.295	0.762
<i>Probability</i>	0.001	<.001	0.064	<.001

¹Average of 2 hens per replicate. The average BW of the hens used is shown in Table 5.

5.4. Discussion

5.4.1. *Hen Productivity and Egg Quality*

From 17 to 46 wk of age, hen performance was not affected by the inclusion of fiber in the pullet diets, except for egg production that was significantly lower in hens fed SBP than in hens fed straw. The authors have not found any published research on the effects of the inclusion of SBP in the rearing diets on egg production to compare with the results reported herein, and we do not have any clear explanation for these results. Pullets fed SBP during the rearing phase were 1.5% lighter at 17 wk of age and had 1.2% lower ADFI during the laying phase than hens fed straw, although the differences were not significant. Probably, the combined effect of lighter BW at the onset of egg production and reduced ADFI early in the egg cycle resulted in lower egg production in these pullets (Leeson et al., 1997; Pérez-Bonilla et al., 2012a). In this respect, Almirall et al. (1997) reported a reduction in egg production in hens fed a cereal-soybean meal diet diluted with 7.5 or 15.0% SBP, although in this research all hens received a common feeding program during the rearing phase. Similar reduction in BWG and feed efficiency has been reported in broilers by González-Alvarado et al. (2010) and Sadeghi et al. (2015) with the inclusion of 3% SBP in the diet. Sadeghi et al. (2015) reported also an increase in intestinal viscosity and a reduction in villus height of the duodenum and ileum mucosa when 3% SBP was included in the diet. The fiber fraction of SBP is more soluble than that of straw (Brøkner et al., 2012; Bach Knudsen, 2014). Story and Kritchersky (1982) reported that the soluble part of the fibrous fraction might interfere with the metabolism of the lipid (Forman and Schneeman, 1980; Anderson et al., 1994) and other dietary fractions (Pettersson and Razdan, 1993; Razdan and Pettersson, 1994), causing a reduction in egg production.

From 17 to 46 wk of age, egg production was high in all hens, irrespective of the energy concentration of the diet, in agreement with data of Grobas et al. (1999a,b) comparing diets with 2,680 and 2,810 kcal AME_n/kg and Saldaña et al. (2016) comparing diets with 2,650 and 2,750 kcal/kg in brown egg-laying hens. Similar data have been reported by Jalal et al. (2006) and Murugesan and Persia (2013) in Single Comb White Leghorn hens fed diets containing 2,800 to 2,900 kcal AME_n/kg. In contrast, Pérez-Bonilla et al. (2012b) observed an increase in egg production from 88.8% to 91.2% as the AME_n concentration of the diet increased from 2,650 to 2,750 kcal/kg. In the research of Pérez-Bonilla et al. (2012b), the hens ingested 3.0% more energy when the high energy diet was used, which might justify the increase in egg production observed. However, in the current research, hens compensated for the lower energy content of the diet by increasing feed intake proportionally and consequently, no differences in egg production were expected.

Egg weight was not affected by energy concentration of the diet, in agreement with data of Çiftci et al. (2003), Jalal et al. (2006), and Murugesan and Persia (2013). In contrast, Harms et al. (2000), Wu et al. (2007), and Bouvarel et al. (2010) reported that egg weight increased as the AME_n content of the diet increased. Under practical conditions, egg size depends primarily of the methionine, fat, and linoleic acid content of the diet (Safaa et al., 2008b). As the energy concentration of the diet increases, the level of supplemental fat and linoleic acid are often increased, which may result in heavier eggs (Grobas et al., 1999c; Pérez-Bonilla et al., 2011). The methionine, linoleic acid, and fat content of the diets used in the current research, however, were above requirements (NRC, 1994; FEDNA, 2008) and consequently, no differences in egg weight between treatments should be expected.

An increase of 100 kcal of AME_n/kg diet resulted in an increase in BW of the hens of 0.18 g/d, a value that was within the range of 0.08 to 0.45 g/d reported by others (Grobas et al., 1999a; Harms et al., 2000; Pérez-Bonilla et al., 2012b). Body weight gain and egg mass production of the hens depends on their genetic background. When birds are fed low energy diets, they might not be able to consume enough feed to keep their energy intake constant, resulting in a reduction in BW (Nielsen, 2004; Pérez-Bonilla et al., 2012b). On the other hand, when hens are fed diets with a high energy content, they over consume energy which might be stored as body fat rather than used for extra increases in egg mass, leading to moderate increases in BWG (Pérez-Bonilla et al., 2012b). The higher BWG of the hens fed the high energy diet might be also a consequence of the higher level of supplemental fat (1.4 vs. 3.4%) of this diet.

Egg quality traits were not affected by the energy content of the layer diet, in agreement with data of Grobas et al. (1999c), Junqueira et al. (2006), and Saldaña et al. (2016). In contrast, Wu et al. (2005) and Pérez-Bonilla et al. (2012b) reported a linear decrease in albumen quality as the energy concentration of the diet increased. In the research of Wu et al. (2005) diets were not iso-aminoacidic per unit of energy, and the intake in indispensable AA was lower when fed the high energy diet than when fed the low energy diet, which might have affected albumen quality. Also, the high energy diet used by Pérez-Bonilla et al. (2012b) had more fat and wheat and less barley than the low energy diet. In the current research, however, AA intake and the ingredient composition of the diets were similar for both groups of hens. Consequently, no differences in egg quality traits should be expected.

5.4.2. Digestive Tract Traits and Body Measurements

Fiber inclusion affects GIT development in both broilers (Jiménez-Moreno et al., 2010; Sadeghi et al., 2015) and pullets (Guzmán et al., 2015b). Dietary fiber increases

the retention time of the digesta in the proximal part of the GIT of young birds, improving gizzard weight and function (Frikha et al., 2009a; Jiménez-Moreno et al., 2013b) and increasing the relative length of the SI (Van der Klis and Van Vorst, 1993; Guzmán et al., 2015b). These changes in GIT development might result in an increase in the capacity for feed ingestion during the start of the laying period. At 46 wk of age, however, none of the GIT traits studied was affected by the characteristics of the pullet diets. The authors have not found any published report on the effects of fiber content of the rearing phase diets on GIT traits at the end of the laying period. The data presented herein indicate that the potential beneficial effects of DF on the size and development of the GIT are not long lasting and disappear once the birds are fed a common commercial layer diet (Mateos et al., 2012). In this respect, Saldaña et al. (2016) reported similar size and development of the different organs of the GIT in 46 wk-old hens that were fed mash or crumble diets during the rearing phase, consistent with the results reported herein. Also, Saldaña et al (2015a) reported that the effects of feed form on the GIT traits of pullets from 0 to 17 wk of age, reverted quickly when the birds were changed from receiving crumbles to receiving mash diets.

Body length, BMI, and tarsus measurements are useful criteria to evaluate body size and composition of avian species, such as sparrows (Cleasby et al., 2011), broilers (Mendes et al., 2007; Van Roover-Reijrink; 2013), pullets (Lamazares et al. 2006; Ortiz et al. 2011), and hens (Ojedapo et al., 2012). In the current research, an increase in the fiber content of the rearing diets or in the energy of the laying hen diets had no effects on these traits at 46 wk of age. Similarly, Saldaña et al. (2016) did not find any difference in body length or tarsus measurements in 46 wk-old hens that were fed mash or crumble diets differing in 50 kcal AME_n/kg during the rearing phase or diets differing in 100 kcal AME_n/kg from 17 to 46 wk of age.

In summary, the inclusion of straw in the rearing phase diets did not affect any of the egg production traits studied. However, SBP reduced egg production without affecting any other trait. The inclusion of straw or SBP in the rearing phase diets did not affect GIT development at 46 wk of age. An increase in the energy content of the laying phase diet of 100 kcal AME_n/kg reduced ADFI, improved feed efficiency, and increased BWG of the hens but did not affect any of the other production or GIT traits studied. Consequently, the ingredient composition and nutritive value of diets for pullets and laying hens can be adjusted, within the range of values studied, according to the cost of available ingredients.

CHAPTER 6: GENERAL DISCUSSION

Adequate management and nutrition of the pullets during the rearing phase are two important points to improve egg production and egg quality of laying hens. High peak production and persistency of egg production, together with a high proportion of large eggs are often correlated with adequate BW and uniformity of the pullets at the end of the rearing period (Summers and Leeson, 1994). However, the information available on the nutrient requirements and the influence of feed form, energy concentration, and fiber content of the diet on growth performance and GIT development of the pullets is very limited.

Effect of feed form, energy concentration, and fiber content of the diet on BW uniformity of pullets

Gous and Cherry (2004) reported that not only average BW at the onset of egg production is an important factor defining subsequent laying performance but also the shape of the growth curve, a good indicative of pullet uniformity. Consequently, one of the main objectives for egg producers is to obtain flocks with an adequate BW and uniformity (ISA Brown, 2007; Hy-Line Brown, 2008).

Two strategies that can be used to improve BW and uniformity of the pullets are the feeding of pellets instead of mash and the increase of the AME_n concentration of the diet. In this respect, in experiment 1 pullets fed crumbles from hatching to 5 wk of age showed better BW uniformity than pullets fed mash, consistent with data of Saldaña et al. (2015a,b) in 5, 10, and 17 wk-old pullets and Brickett et al. (2007) in 5 wk-old broilers. In contrast, Frikha et al. (2009b) did not find any difference in BW uniformity in 6-wk old pullets fed mash or pellet diets. In the current study, BW uniformity was not affected by the energy concentration of the diet in experiment 1, or by fiber content in

experiments 1 and 2. Also, Frikha et al. (2009a) in 6 wk-old brown-egg pullets and Brickett et al. (2007) in 35 d-old broilers reported that BW uniformity was not affected by the energy concentration of the diet. However, Keshavarz et al. (1998) observed an improvement in BW uniformity of pullets at 18 wk of age when the AMEn of the diet was increased from 2,815 to 3,035 Kcal/kg. The reason for the discrepancies reported among authors is unknown. Factors such as type and age of the birds, flock management, including rearing density, and the characteristics of the experimental diets, might affect the outcome of the researches.

No information has been found on the influence of fiber inclusion in the diet on BW uniformity. In laying hens and broiler breeders, the inclusion of insoluble fiber in the diet reduced nervousness of the birds, feather pecking, and cannibalism which in turn improves flock uniformity (Mateos et al., 2012). The results of the current research indicate that when the main objective is to improve flock uniformity, pullets respond better to changes in feed form than to increases in energy content of the diet, and that the inclusion of moderate amount of fiber in the diet does not affect BW uniformity.

Effect of feed form of the diet on productive performance of pullets during the early stage of the rearing period

In experiment 1, pullets fed crumbles ate more feed than pullets fed mash during the first week of life, but opposite results were observed after this age. For the entire experimental period, BWG and FCR were better in pullets fed crumbles than in pullets fed mash. There is not any clear explanation for the higher FI in pullets fed crumbles during the early stage of life. Probably, very young birds prefer crumbles to mash feed because of the better adaptation of the beak to consume processed feeds. Also, it was probable that FI was not higher in pullets fed the crumble diets but that the feed wastage

was higher because the crumble might not have fitted the beak of the pullets (Workman and Rogers, 1990). In any case, after the first week of age, the increase in beak size facilitated feed consumption and crumble was not a potential problem anymore, leading to a reduction of feed wastage with crumbles compared with mash (Mateos et al., 2002; Serrano et al., 2013). In experiment 1, however, feed wastage could not be measured, and therefore, further testing of this suggestion is warranted. The benefits of feeding crumbles on growth performance is well documented in broilers (Amerah et al., 2007; Brickett et al., 2007; Serrano et al., 2012; Abdollahi et al., 2013b; Jiménez-Moreno et al., 2016) but not in pullets. Saldaña et al. (2015a,b) reported lower ADFI and better BWG and FCR in pullets fed crumbles than in pullets fed mash from 0 to 5 wk of age. Similarly, Gous and Morris (2001) observed a reduction in FI and an increase in BW in pullets fed crumbles from 1 to 4 wk of age when compared to pullets fed mash. Frikha et al. (2009b) also reported an improvement in BWG in 6-wk-old pullets fed pellets compared to mash, although in this experiment FCR was not affected by feed form. During the process of pelleting, the particle size of the feed is reduced and the endosperm is fractured, releasing intracellular oil of some of the ingredients, facilitating the contact between exogenous and endogenous enzymes and nutrients (Svihus et al., 2004; Amerah et al., 2007), thereby increasing energy utilization (Huang, 1992). Consequently, FCR may improve with crumbled or pelleted diets compared with mash diets. In addition, the poorer FCR reported in pullets fed mash compared with pullets fed crumbles might be due, at least in part, to greater feed wastage, as has been reported in broilers (Corchero et al., 2008; Serrano et al., 2013) and piglets (Medel et al., 2004).

Effect of energy concentration of the diet on productive performance of pullets and laying hens

Poultry eat to satisfy their energy requirements, and therefore, they regulate FI according to the energy concentration of the diet. Consequently, ADFI should decrease and FCR should improve as the AME_n content of the diet increases. In experiment 1, pullets responded differently to changes in AME_n depending on feed form. Apparently, pullets fed crumbles adjusted their EnI to requirements whereas pullets fed mash tended to overconsume energy as the AME_n of the diet increased. Consequently, no differences were detected in ECR when pullets were fed crumbles, but in pullets fed mash ECR was hindered. Similar results were reported by Saldaña et al. (2015b) in rearing pullets and Scott (2002) and Brickett et al. (2007) in broilers. Probably, young birds are not able to increase voluntary FI when fed low-energy, bulkier, diets in mash form. However, the possibility that the differences observed in FI between feed forms with increases in the level of energy of the diet are a consequence of greater wastage with mash diets can not be ruled out.

Latshaw (2008) reported that energy content of the diet is not the only variable affecting FI; diet composition should also be considered. In the research of this author, EnI of broilers increased with the inclusion of 5% fat in the diet compared to a control diet with no added fat. Moreover, broilers fed a diet containing 4% fiber showed a reduction in EnI compared with broilers fed a diet without additional fiber. These results are consistent with those reported in experiment 2, in which the pullets fed the 4% fiber containing diets from hatching to 17 wk of age had lower EnI than pullets fed the the control diet with no added fiber. Additional fiber increases the bulk of the feed and satiate the appetite of the bird wich in turn might result in birds unable to consume enough feed to regulate EnI according to their requirements.

The available information suggests that young pullets can maintain growth performance when fed diets varying widely in energy concentration, provided that the

feed is crumbled. Moreover, although EnI in poultry depends primarily on the energy content of the diet, other factors, such as feed form, ingredient composition, and nutrient content will affect also this variable.

In experiment 3, an increase in the energy content of the laying hen diet from 2,650 to 2,750 kcal AME_n/kg decreased ADFI, but increased BWG resulting in improved FCR (kg:kg) from 17 to 46 wk of age. However, egg production, egg weight, and egg mass were not affected by energy content of the diet. Similar results have been reported by Saldaña et al. (2016) in hens fed diets with 2,650 and 2,750 kcal/kg and Grobas et al. (1999a,b) in hens fed diets with 2,680 and 2,810 kcal AME_n/kg. Also, Jalal et al. (2006) and Murugesan and Persia (2013) reported similar results in Single Comb White Leghorn hens fed diets with 2,800 and 2,900 kcal AME_n/kg. In contrast, Peguri et al. (1991) in hens fed diets varying from 2,700 to 2,910 kcal AME_n/kg, reported an increase in egg production with increases in energy concentration. Also, Valkonen et al. (2008) and Pérez-Bonilla et al. (2012b) reported greater egg production when the AME_n content of the diet increased from 2,340 to 2,630 kcal/kg and from 2,650 to 2,750 kcal/kg, respectively. The data suggest that an increase in the energy content of the diet may be more beneficial when low energy diets are used. In the research of Pérez-Bonilla et al. (2012b), hens ate more energy when the high energy diet was used, which might explain the increase in egg production and BW gain observed. However, in experiment 3 of the current research, hens compensated for the lower energy content of the diet by increasing FI proportionally and consequently, no differences in egg production were expected. The extra energy content of the high energy diet consumed by the hens was probably stored as body fat rather than used for increases in egg mass and size, leading to an increase in BW, in agreement with other authors (Grobas et al., 1999a; Harms et al., 2000; Pérez-Bonilla et al., 2012b; Saldaña et al., 2016). The

increase in energy concentration of the diets was obtained mainly by increasing the level of supplemental fat, which has been shown to reduce rate of feed passage, facilitating the contact between digesta and enzymes and improving digestibility and utilization of other nutrients such as lipids and carbohydrates (Mateos and Sell, 1980b, 1981). A decrease in the energy concentration is usually attained by increasing the fiber content of the feed, leading to bulkier diets. Probably, hens might not be able to consume enough feed to keep their energy intake constant when the diets are very low in energy, resulting in a reduction in BW (Nielsen, 2004; Pérez-Bonilla et al., 2012b).

In experiment 3, neither egg weight nor egg quality was affected by the energy content of the diet. Egg size depends mainly on the methionine, fat, and linoleic acid content of the diet (Safaa et al., 2008b). In this experiment all nutrients were supplied above requirements (NRC, 1994; FEDNA, 2008) in both diets and consequently, no differences in egg weight were expected. These results are in agreement with those reported by Çiftci et al. (2003), Jalal et al. (2006), Murugesan and Persia (2013), and Saldaña et al. (2016). Egg quality traits might not depend on the AME_n content of the diets, if they are conveniently balanced (Grobbs et al., 1999c; Junqueira et al., 2006; Saldaña et al., 2016). The results reported herein suggest that an excess in energy content of the hen diet, results in a slightly increase in EnI that is derived mainly to increased BW rather than to increases in egg mass production.

Effect of fiber on growth performance of rearing pullets and subsequent hen productivity

In experiment 1 (from hatching to 5 wk of age), pullets fed the fiber containing diets (straw, SFH, and SBP) had as an average higher ADFI and better ADG than pullets fed the control diet. However, no effects were detected for FCR. The authors have not found any recent information on the effects of DF on growth performance of

brown pullets, but similar positive effects with DF have been reported in broilers. In this respect, González-Alvarado et al. (2007) and Jiménez-Moreno et al. (2009a) showed that the inclusion of 3% fiber in the broiler diet from 0 to 21 d of age improved ADG and FCR, without any effect on ADFI. Moreover, in experiment 1, an improvement in ECR was reported in pullets fed the fiber containing diet as compared with pullets fed the control diet. Similar improvement in ECR has been reported by González-Alvarado et al. (2010) and Jiménez-Moreno et al. (2013a) in broilers fed diets with supplemental insoluble fiber compared to broilers fed a control diet.

The beneficial effects of DF on pullet performance might be less pronounced after the first weeks of age, as reported in experiment 2. In this research, pullets fed the control diet had as an average higher ADG and better FCR for the entire rearing period (0 to 17 wk of age) than pullets fed the fiber containing diets (straw and SBP), especially at the highest level of inclusion (4%). Similarly, González-Alvarado et al. (2010) reported that FCR improved with the inclusion of 3% SBP in the diet in broilers from 1 to 10 d of age but not from 10 to 42 d of age. The maximal growth of all organs and segments of the GIT in broilers is reached before d 9 post-hatching (González-Alvarado et al., 2008). Consequently, during the first stages of life, moderate amounts of insoluble DF might stimulate the development of the upper part of the GIT, benefiting bird performance. In experiment 2, unlike the results of experiment 1, no differences were detected from hatching to 5 wk of age between pullets fed the control diet and pullets fed the fiber diets. These contrasting results may be a consequence of the different fiber sources used in both experiments. In experiment 1 straw, SFH, and SBP were used, but in experiment 2 only straw and SBP were used. In this respect, the SFH inclusion showed the best results in experiment 1. Moreover, different authors have shown that soluble fiber sources, such as SBP, might reduce voluntary FI of the

birds because of its high WHC. The data suggest that during the initial stages of the rearing period, pullets might benefit from the inclusion of moderate amounts of insoluble fiber in the diet but that the benefits might be less pronounced than in broilers. Broilers have higher capacity for FI and grow faster than pullets. Consequently, under practical conditions, additional DF might be more beneficial in young broilers than in young pullets.

No information was found on the effects of additional DF during the rearing period on subsequent egg production, once the hens are fed a common commercial laying diet. The results of the current research (experiment 3) indicate that the effects of feeding high fiber diets to rearing pullets to increase GIT capacity are not long lasting and that hens adapt quickly to changes in diet during the laying cycle. In fact, no differences in hen productivity or egg quality traits were detected between hens fed the control diet during the rearing phase and hens fed the fiber diets during this period.

In general, pullets fed an insoluble fiber source, such as SFH and straw, had better performance than pullets fed a soluble fiber source, such as SBP, as shown in experiments 1 and 2. In experiment 1 (hatching to 5 wk of age), pullets fed SFH had higher ADG than pullets fed SBP, with pullets fed straw being intermediate. These results are consistent with data reported in experiment 2 for the entire rearing period, in which pullets fed straw grew more than pullets fed SBP. Similar results have been reported in broilers from 1 to 21 d of age by Jiménez-Moreno et al. (2010) and from 1 to 42 d of age by González-Alvarado et al. (2010). These authors reported higher growth when fed 3% OH, an insoluble fiber source, than when fed 3% SBP, a soluble fiber source. The differences in growth reported might be a consequence of the distinct physicochemical properties of the fiber sources used. For example, as a consequence of the higher pectin content SBP has higher WHC and SWC than SFH or straw (Bach

Knudsen, 2014). Consequently, SBP feeding increases the bulk of the digesta and satiates faster bird appetite, reducing growth rate. On the other hand, the content in ADF and lignin is higher in insoluble fibers than in soluble fiber sources. Thus, SFH and straw will be more resistant to grinding and will be retained for longer in the gizzard than SBP, increasing gizzard size and resulting in a better acidification of its content (Bach Knudsen, 2001; Jiménez-Moreno et al., 2013b). Changes in pH within the GIT might interfere with the digestion and absorption processes affecting the growth and profile of the existing microbiota (Kalmendal et al., 2011; Mateos et al., 2012; Bach Knudsen, 2014).

Not only the nature of the fiber source affects pullet performance but also the level of fiber included in the diet. In this respect, in experiment 1, pullets fed the 4% fiber diets from hatching to 5 wk of age were less efficient than pullets fed the 2% fiber diets. These results were maintained in experiment 2 for the entire rearing period (hatching to 17 wk of age). Pettersson and Razdan (1993) showed also a reduction in feed efficiency when the level of SBP in the broiler diet (1 to 21 d of age) increased from 2.3% to 4.6%. Similarly, Jiménez-Moreno et al. (2013a) reported poor feed efficiency in broilers from 1 to 18 d of age as the level of OH or SBP in the diet increased from 2.5 to 7.5%. The available information suggests that, similar to what has been reported in broilers, the inclusion of moderate amounts of fiber in commercial diets for pullets might be beneficial for growth but that an excess might have detrimental effects on pullet performance, especially when soluble fiber sources, such as SPB, are used. In this respect, no recommendations on the type and level of fiber are given by NRC (1994) or the main guidelines of the genetic companies involved in egg production (e.g., Lohmann, 2013; Hy-Line International, 2015). FEDNA (2008), however, recommends

a minimum of CF of 3% in starter (0 to 5 wk) and 4% in finisher (10 to 17 wk) pullet diets, values that are consistent with data shown in experiments 1 and 2.

As shown in experiment 3, type of fiber fed from hatching to 17 wk of age affected egg production during the laying cycle. From 17 to 46 wk of age, egg production was reduced in hens fed SBP during the rearing period compared with hens fed straw. At 17 wk of age, pullets fed SBP were lighter and had lower ADFI than pullets fed straw which might have resulted in lower egg production (Leeson et al., 1997; Pérez-Bonilla et al., 2012a) although differences were not significant. Almirall et al. (1997) reported a reduction in egg production in hens fed diets containing 7.5 SBP, although in this research all hens were fed a common feed during the rearing phase. Sadeghi et al. (2015) reported an increase in intestinal viscosity and a reduction in villus height of the duodenum and ileum mucosa of broilers when 3% SBP was included in the diet. The SBP could have reduced the absorption of nutrients, leading to lower ADG. Story and Kritchersky (1982) reported that the soluble part of the fibrous fraction might interfere with the metabolism of the lipids (Forman and Schneeman, 1980; Anderson et al., 1994) and other dietary fractions (Pettersson and Razdan, 1993; Razdan and Pettersson, 1994), reducing its digestibility and egg production.

Effect of fiber on gastrointestinal tract traits and body measurements of rearing pullets and further effect on laying hens

The effects of fiber inclusion in diets for rearing pullets on GIT traits and body measurements were studied in experiment 2. In general, the size and weight of the GIT increased with increases in DF intake (Jørgensen et al., 1996; González-Alvarado et al., 2008). In fact, in this experiment fiber inclusion, especially SBP at the higher level of inclusion (4%), increased the relative weight of the full GIT and the gizzard compared with the control diet, which might be indicative of a hypertrophy of the digestive tissues

(Hansen et al., 1992, Jørgensen et al., 1996). These results are consistent with data reported by Hetland et al. (2003) in broilers fed 10% OH and González-Alvarado et al. (2010) in broilers fed 3% SBP or 3% OH. The fiber fraction of the diet is retained for longer in the gizzard (González-Alvarado et al., 2007), improving gizzard weight and function (Jiménez-Moreno et al., 2009a; Frikha et al., 2009a) and increasing the relative length of the SI (Van der Klis and Van Vorst, 1993). The longer retention time of fiber in the gizzard might be due to differences in the physicochemical characteristics of the fiber sources used. Insoluble fiber sources, such as straw, are more resistant to gizzard grinding due to the higher lignin content than soluble fiber sources such as SBP. Consequently, insoluble fibers might lead to a greater development of the muscular layers of the gizzard (González-Alvarado et al., 2008). On the other hand, soluble fiber sources have higher pectin content and thus, higher WHC and SWC (Michel et al., 1988; Ramanzin et al., 1994). Consequently, soluble fibers increase the bulk of the digesta and produce physical dilation of the gizzard. As a consequence of the longer retention time in the gizzard of the fiber containing diets, HCl production increases and pH decreases (Duke, 1986b; Hetland et al., 2005; Jiménez-Moreno et al., 2013b). These data are consistent with the results reported at 10 wk of age in experiment 2, although in this trial the differences were significant only in pullets fed 4% straw diet. A low gizzard pH increases pepsin activity, mineral salts solubility, and nutrient digestibility (Rogel et al., 1987; Gabriel et al., 2003; Jiménez-Moreno et al. 2009c), effects that might favor bird performance.

The relative weight of the GIT of the pullets was greater with SBP than with straw inclusion. Similar results were reported in broilers by Jørgensen et al. (1996) and Jiménez-Moreno et al. (2013b). The differences observed between fibers might be related to the distinct physicochemical properties of the two sources. No differences in

the relative weight of the gizzard were detected in chicks fed these two fiber sources, although the value was numerically higher with the use of straw, results that are consistent with data on the absolute weight of the gizzard supporting the findings of Hetland and Svihus (2001), González-Alvarado et al. (2010), and Jiménez-Moreno et al. (2013b). No differences among diets were observed for digesta content of the gizzard, suggesting that the heavier gizzards reported with the use of straw were probably a consequence of the increase in the development of the muscular layers.

The effects of fiber inclusion on the relative length of the SI, body, and tarsus depended also on the fiber source. All these parameters were longer in pullets fed SBP than in pullets fed straw. An increase in body and tarsus length suggests a better skeletal development (Cleasby et al., 2011) which might be of benefit for future egg production (Senar and Pascual, 1997; Mendes et al., 2008). However, in absolute terms, no differences in length were detected for SI and in fact, opposite results were obtained for the body and tarsus. The reduced BW of the pullets fed SBP compared to pullets fed straw, might have distorted the results when data used was based on the relative rather than on absolute weights. Consequently, data on the effects of fiber inclusion in the diet on the relative length of the SI, body, and tarsus must be taken cautiously.

No differences on any of the GIT traits and body measurements studied were detected at 46 wk of age in hens fed diets differing in 100 kcal AME_n/kg from 17 to 46 wk of age. No information is available on the effects of fiber content of the rearing diets on digestive traits at the end of the laying cycle. Saldaña et al. (2016) reported similar size and development of the different organs of the GIT and body measurements in 46 wk-old hens that were fed mash or crumble diets during the rearing phase. The data presented in the current research indicate that the potential benefits of DF of the pullet

diets on the size and development of the GIT disappeared once the birds were fed a common commercial layer diet consistent with the suggestion of Mateos et al. (2012).

CONCLUSIONS AND IMPLICATIONS

- Changes in feed form were more effective than changes in diet composition for increasing body weight gain and body weight uniformity.
- At early ages, pullets responded better to crumble diets than to mash diets.
- Feed intake could be regulated by modifying the energy content of the feed, provided that the diets were fed as crumbles.
- Pullets needed a minimum amount of fiber to maximize growth performance and gastrointestinal tract development.
- Type and level of fiber affected in different ways pullet growth. In general, insoluble fiber sources were more beneficial than soluble fiber sources.
- Feeding moderate amounts of fiber improved pullet growth during the first period of life but the effects disappeared and were even negative as the birds aged.
- An increase in the level of fiber from 2 to 4% reduced growth performance, especially when sugar beet pulp, a soluble fiber source, was used.
- The effects of fiber inclusion in the diet on the length of the SI, body, and tarsus depended on the method used (absolute vs. relative). Consequently, fiber source effects on these traits must be taken cautiously.
- The beneficial effects of fiber inclusion in rearing diets on gastrointestinal tract traits were not long lasting and disappeared once the hens were fed a common commercial laying diet.
- Hen productivity was not affected by the straw fed to the birds during the rearing period. However, sugar beet pulp feeding reduced egg production without showing any effect on the other traits.
- Consequently, it is highly recommendable to include at least 2% of an insoluble fiber source in the diet for pullets in order to improve gastrointestinal tract development

and maximize growth performance. Moreover, the recommended energy content of the pullet diets will depend on feed form and the relative cost of available ingredients.

CONCLUSIONES E IMPLICACIONES

- Cambios en la presentación del pienso fueron más efectivos que cambios en la composición para aumentar el peso vivo y mejorar la uniformidad de las aves.
- A edades tempranas las pollitas respondieron con mejor crecimiento a piensos en miga que a piensos en harina.
- El consumo energético de las aves pudo regularse modificando la concentración energética del pienso cuando se suministró en forma de migas.
- Para maximizar su crecimiento y el desarrollo del tracto gastrointestinal las pollitas necesitaron durante el periodo de recría un mínimo de fibra en la dieta.
- Las pollitas respondieron de manera diferente a la fibra del pienso en función del tipo y nivel de inclusión de la misma. En general, fuentes de fibra insoluble fueron más beneficiosas que fuentes de fibra solubles.
- La inclusión de fibra en el pienso mejoró el crecimiento de las pollitas durante los primeros estadios de vida. Sin embargo, ese efecto fue menos pronunciado e incluso negativo a medida que las aves se desarrollaron.
- Un aumento del nivel de fibra del 2 al 4% redujo el rendimiento productivo de las pollitas, especialmente con el uso de pulpa de remolacha, una fibra de tipo soluble.
- El efecto de la fibra sobre la longitud corporal, tarsos e intestino dependió del método utilizado (absoluto vs. relativo). Por lo tanto, el efecto de la fibra sobre estos parámetros se debe considerar con precaución.
- El efecto de la fibra dietética en piensos de recría sobre el desarrollo del tracto gastrointestinal disminuyó y llegó a desaparecer durante la fase de puesta, una vez que las gallinas fueron alimentadas con un pienso comercial común.
- El uso de paja de cereales en los piensos de recría no afectó a ninguno de los parámetros productivos o de calidad del huevo estudiados. Sin embargo, el uso de

pulpa de remolacha redujo el índice de puesta sin afectar ningún otro de los parámetros productivos.

- Por lo tanto, es recomendable incluir al menos un 2% de fibra insoluble en los piensos de pollitas para mejorar el desarrollo del tracto gastrointestinal y maximizar el crecimiento. Además, el nivel energético del pienso para pollitas dependerá de su presentación y del coste de las materias primas disponibles.

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