UNIVERSIDAD POLITÉCNICA DE MADRID

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

**COMPARATIVE STUDY OF THE INCLUSION OF SUNFLOWER
HULLS IN THE DIET ON GROWTH PERFORMANCE AND
DIGESTIVE TRACT TRAITS OF BROILERS AND PULLETS
FROM 0 TO 21 DAYS OF AGE**

TESIS DOCTORAL

MOHAMMAD VAHID KIMIAEITALAB

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DEDICATION

This dissertation is dedicated to God for every things that I have. My family that was always supportive of what I did for no other reason than their love for me. To my parents, for teaching me, through examples, to love and ask everything from God, for dedicating exhausting years of work for me, for giving me wonderful memories, for their humbleness, kindness, and unconditional love, my deepest appreciation goes to them. They are always the biggest supporters in my life, and give me the strength to face the challenges in life and taught me to become an independent person, which give me all those wonderful opportunities to experience different cultures, different environments, and encouraged to explore the world of the unknown. I could not have done any of this without support of them.

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ABBREVIATION LIST

ADFI, average daily feed intake

ADG, average daily gain

AME_n, apparent metabolizable energy nitrogen corrected

BW, body weight

CD, crypt depth

CF, crude fiber

cm, centimeter

CP, crude protein

CTTAR, coefficient of total tract apparent retention

DF, dietary fiber

DM, dry matter

ECR, energy conversion ratio

EI, energy intake

Exp., experiment

FCR, feed conversion ratio

GE, gross energy

GIT, gastrointestinal tract

GMD, geometric mean diameter

GSD, geometric standard deviation

IU, international units

N, nitrogen

N, normality

OM, organic matter

P, probability

RSD, residual standard deviation

SCFA, short chain fatty acid

SEM, standard error of means

SFH, sunflower hulls

TTAR, total tract apparent retention

VH, villus height

RESUMEN

Se realizaron dos experimentos (Exp.) para evaluar los efectos de la inclusión de un 3% de cascarilla de girasol (CG) en piensos para pollos de engorde y pollitas de recría de puesta sobre el crecimiento, la retención fecal aparente de los nutrientes (RFA), el desarrollo del tracto gastrointestinal (TGI), y la concentración de ácidos grasos de cadena corta (AGCC) a nivel del ciego en aves de 0 a 21 d de edad. En el Exp. 1 se utilizaron cuatro tratamientos organizados como un factorial 2×2 , con 2 tipos de aves (broilers hembra y pollitas de puesta comercial) y 2 niveles de inclusión de CG (0 vs 3%). Las aves recibieron un pienso típico de iniciación de broilers con 2,980 EMA_n kcal, 22.2% proteína bruta, 1.25% lisina digestible y 8.7% FND. La dieta experimental incluía un 3% de CG a expensas (peso:peso) de la dieta control. Las aves se alojaron en grupos de 10, dentro de cada línea, en 28 jaulas en una nave de ambiente controlado. El pienso, en forma de harina y el agua fueron ofrecidos ad libitum durante todo el experimento. Las variables estudiadas fueron el crecimiento, el desarrollo del TGI y la RFA a los 9 y 21 días de edad, y la producción de AGCC en el ciego y la morfología ileal a los 21 días de edad. Se observó un mayor crecimiento ($P<0.001$) y mejores RFA de los nutrientes y de la EMA_n de la dieta ($P=0.097$ a $P<0.001$) en broilers que en pollitas. La humedad de las excretas fue mayor ($P<0.004$) en las pollitas que en los broilers a los 9 d pero se observó un efecto opuesto ($P<0.05$) a los 21 días de edad. En valores absolutos, todos los órganos del TGI pesaron más ($P<0.001$) y el intestino delgado y el ciego fueron más largos ($P<0.001$) en broilers que en pollitas. A los 21 d de edad, el peso relativo (% peso vivo) de todos los órganos del TGI ($P<0.001$) y la longitud relativa (cm/kg peso vivo) del intestino delgado y del ciego ($P<0.01$) fueron mayores en pollitas. El pH de la molleja ($P<0.001$), la concentración total de AGCC en el ciego ($P=0.098$) y la altura de las vellosidades ($P<0.001$) y la profundidad de las criptas ($P<0.001$) de la mucosa del íleon fueron mayores en broilers que en pollitas. La inclusión de CG en el pienso aumentó el peso de

la molleja y redujo el pH de la misma ($P<0.001$) a ambas edades. Se detectó una interacción entre tipo de ave e inclusión CG en el pienso; la inclusión de CG aumentó el peso absoluto de la molleja vacía ($P<0.05$) y del contenido de la molleja ($P<0.05$) a ambas edades en broilers, pero no en pollitas. La inclusión de 3% CG (peso:peso) no afectó ni al rendimiento de las aves, ni al contenido de humedad de las excretas, ni a la concentración o el perfil de AGCC en el ciego. En conclusión, los broilers crecieron más y tuvieron una mayor retención de nutrientes y capacidad de absorción a nivel del íleon que las pollitas. La inclusión de 3% CG en el pienso no tuvo efecto negativo alguno sobre los rendimientos productivos. De hecho, aumentó el peso y redujo el pH de la molleja y mejoró el contenido en EMA_n de la dieta.

En el Exp. 2, las variables estudiadas, el diseño experimental y las condiciones de manejo y los controles fueron similares a los del Exp. 1. La principal diferencia entre ambos ensayos fue el tipo de pienso control utilizado, que en este experimento fue un pienso de iniciación de pollos que contenía 2,850 kcal EMA_n , 19.1% de proteína bruta, 0.98% de lisina digestible y 10.1% FND. El pienso experimental, al igual que en el Exp. 1, incluía un 3% de CG a expensas (peso:peso) de la dieta control. Los crecimientos fueron superiores ($P<0.001$) en broilers que en pollitas a ambas edades. El contenido en humedad de las excretas a 21 d fue mayor ($P<0.001$) en broilers que en pollitas, pero se observó un efecto contrario a los 9 d de edad. Se detectó una interacción entre el tipo de ave y la inclusión de CG a los 21 d de edad; la inclusión de CG redujo el contenido de humedad ($P<0.05$) de las excretas en broilers, pero no en pollitas. La retención de nutrientes ($P<0.001$) y la EMA_n de la dieta ($P<0.01$) fueron superiores en broilers que en pollitas a los 9 d de edad. Sin embargo, a los 21 d de edad, la única diferencia detectada fue para la retención de N ($P<0.001$) que fue superior en broilers. En valores absolutos (g o cm), los órganos digestivos fueron más pesados ($P<0.001$) y el intestino delgado y el

ciego fueron más largos en broilers que en pollitas a ambas edades. No obstante, en valores relativos, el TGI ($P=0.062$), el proventrículo ($P<0.001$), la molleja ($P<0.001$), y el páncreas ($P<0.01$) fueron más pesados en pollitas que en los broilers a 21 d de edad. Además, la longitud relativa del intestino delgado y del ciego fueron superiores ($P<0.001$) en pollitas que en broilers a ambas edades. La inclusión de CG no afectó ni al crecimiento, ni al contenido de humedad de las excretas, ni al peso de los órganos a ninguna edad, pero aumentó la EMA_n del pienso a 21 d ($P<0.05$). Además, la inclusión de CG aumentó la longitud absoluta del intestino delgado a 21 d y del ciego en ambas edades ($P<0.05$). El pH de los diferentes órganos del TGI y la concentración de AGCC en el ciego no se vieron afectados por el tipo de ave o por la inclusión de CG en el pienso. La altura de las vellosidades y la profundidad de las criptas fueron superior ($P<0.001$) en broilers que en pollitas, pero no se vieron afectadas por la inclusión de CG en el pienso. En resumen, los broilers presentaron un mayor rendimiento productivo a ambas edades y una mejor retención de los nutrientes a los 9 d, así como una mejor capacidad de absorción ileal a los 21 d de edad, que las pollitas. La dilución de la dieta con 3% CG no afectó al rendimiento de las aves pero mejoró la EMA_n de la dieta en ambos tipos de ave a 21 d de edad.

En conclusión:

1. Los broilers crecen más rápido y son más eficientes que las pollitas. Alimentar una dieta de broilers, aumente la ganancia de peso corporal y mejorar el rendimiento de crecimiento en pollitas.
2. La densidad de nutrientes, el cereal principal (maíz versus trigo y cebada) y el contenido de fibra de la dieta pueden afectar la respuesta fisiológica de las líneas de aves a la inclusión de una cantidad moderada de fibra insoluble en la dieta.

3. La retención de nutrientes y EMA_n de las dietas fue mayor para broilers que para pollitas. Se observó una mayor digestibilidad de N en broilers que en pollitas de todas las edades.
4. En términos absolutos, todos los órganos TGI eran más pesados y intestino delgado y ciego eran más largos en broilers que en pollitas. Sin embargo, a menudo se observaron resultados adversos en términos relativos. La molleja estaba más desarrollada y más pesada en broilers que en pollitas. El pH de la molleja, afectado por el contenido de fibra de la dieta.
5. La concentración de ácidos grasos de cadena corta en el ciego fue 11% mayor en broilers que en pollitas. La composición de la dieta puede modificar la proporción de ácidos grasos en el ciego.
6. Altura de las vellosidades y profundidad de la cripta del íleon fueron mayores en broilers que en pollitas. Sin embargo, la inclusión de CG no afectó altura de las vellosidades, profundidad de la cripta y su relación de las aves.
7. La inclusión de CG (peso: peso) mejoró el EMA_n de la dieta sin ningún efecto negativo en el rendimiento de crecimiento de las aves. Estos efectos fueron más pronunciados cuando el CG fue incluido en la dieta de broilers que cuando se incluyó en la dieta de pollitas.
8. La falta de interacciones en muchos de los rasgos estudiados en los principales rasgos estudiados, como el rendimiento y el tamaño de los órganos, indicó que los datos existentes disponibles para broilers se generalizarían en cierta medida a pollitas.

SUMMARY

Two experiments (Exp.) were conducted to evaluate the effects of inclusion of 3% sunflower hulls (SFH) in the diet of broilers and pullets, in substitution of the whole diet, on growth performance, total tract apparent retention (TTAR), gastrointestinal tract (GIT) traits, and cecal short chain fatty acid (SCFA) concentration from 0 to 21 d of age. The main objectives of the experiments were to ascertain if broilers and pullets responded in similar way to the inclusion of additional fiber in the diet. Experiment 1 had four treatments organized as a 2×2 factorial with 2 chicken lines (female broilers vs. brown pullets laying hens) and 2 levels of SFH inclusion (0 vs. 3%). In this experiment, both chicken lines received a commercial broiler diet that contained 2,980 kcal AME_n/kg, 22.2% crude protein, 1.25% digestible Lys, and 8.7% NDF. Birds were housed in groups of 10 in 28 batteries according to chicken line, and placed in an environmentally controlled room. Feed in mash form and water were offered for ad libitum consumption throughout the experiment. The variable studied were growth performance, TTAR, and GIT traits at 9 and 21 d of age, and SCFA production in the cecum and ileal morphology at 21 d of age. Growth performance was greater ($P<0.001$) and TTAR of nutrients and AME_n of the diet were better ($P=0.097$ to $P<0.001$) in broilers than in pullets. Excreta moisture was higher ($P<0.01$) in pullets than in broilers at 9 d of age but an opposite effect ($P<0.05$) was observed at 21 d of age. In absolute terms, all the organs of the GIT were heavier ($P<0.001$) and the small intestine (SI) and cecum were longer ($P<0.001$) in broilers than in pullets. At 21 d of age, the relative weight (% BW) of all organs of the GIT ($P<0.001$) and relative length (cm/kg BW) of SI and cecum ($P<0.01$) were greater in pullets. Gizzard pH ($P<0.001$), total SCFA concentration in the cecum ($P=0.098$), and villus height ($P<0.001$), crypt depth ($P<0.001$), and villus height to crypt depth ratio ($P<0.001$) of the ileal mucosa were greater in broilers. Dietary SFH increased gizzard weight and reduced gizzard pH ($P<0.001$) at both ages. An interactions between chicken

line and SFH inclusion on absolute weight of the empty gizzard and on the fresh digesta content of the gizzard were detected; at both ages inclusion of SFH increased ($P<0.05$) both weights in broilers but not in pullets. Inclusion (wt:wt) of 3% SFH did not affect bird performance, excreta moisture, or the concentration and profile of SCFA in the cecum and in fact, increased the AME_n content of the diet ($P<0.05$). In conclusion, broilers showed better growth performance, nutrient retention, and ileal absorptive capacity than pullets. The inclusion of 3% SFH increased gizzard weight, reduced gizzard pH, and improved the energy content of the diet.

In Exp. 2, the variables studied, the design, and the experimental controls, were similar to those indicated for Exp. 1 with the main difference being the type of control diet used. In this experiment, both chicken lines received a commercial pullet diet that contained 2,850 kcal AME_n/kg, 19.1% crude protein, 0.98% digestible Lys, and 10.1% NDF. Growth performance was greater ($P<0.001$) in broilers than in pullets at all ages. Excreta moisture was higher ($P<0.001$) in broilers at 21 d but an opposite effect was observed at 9 d of age. An interaction between chicken line and SFH inclusion on excreta moisture was detected at 21 d of age; the inclusion of SFH reduced ($P<0.05$) excreta moisture in broilers but not in pullets. Nutrient retention ($P<0.001$) and AME_n of the diet ($P<0.01$) were higher in broilers than in pullets at 9 d of age but at 21 d of age the only difference detected was for N retention ($P<0.001$). In absolute terms (g or cm) broilers had heavier ($P<0.001$) digestive organs and longer SI and cecum than pullets. In relative terms, however, the GIT ($P=0.062$), proventriculus ($P<0.001$), gizzard ($P<0.001$), and pancreas ($P<0.01$) were heavier at 21 d, and the SI and cecum were longer ($P<0.001$) at both ages in pullets. The inclusion of SFH did not affect growth performance, excreta moisture, or organ weight at any age but increased the AME_n of the diet at 21 d of age ($P<0.05$). Also, SFH inclusion increased the absolute length of the SI at 21 d of age and

that of cecum at both ages ($P<0.05$). The pH of the different organs of the GIT and the concentration of SCFA in the cecum were not affected by chicken line or SFH inclusion. Villus height and crypt depth were higher ($P<0.001$) in broilers than in pullets but were not affected by SFH inclusion. In summary, broilers showed greater growth performance and nutrient retention at 9 d of age and ileal absorptive capacity at 21 d than pullets. The dilution of the diet with 3% SFH did not affect chick performance, but improved the AME_n of the diet in both chicken lines at 21 d of age.

In conclusion:

1. Broilers growth faster and were more efficient than pullets. Feeding a broiler diet, increased BW gain and improve growth performance in pullets.
2. Nutrient density, main cereal (corn vs. wheat and barley), and fiber content of the diet can affect the physiological response of chicken lines to inclusion of a moderate amount of insoluble fiber in the diet.
3. Nutrients retention and AME_n of the diets were higher for broilers than for pullets. Higher digestibility of N was noticeable in broilers than in pullets at all ages.
4. In absolute terms, all GIT organs were heavier and SI and ceca were longer in broilers than in pullets. However, often adverse results were observed in relative terms. The gizzard was more developed and heavier in broilers than in pullets. The pH of the gizzard, affected by fiber content of the diet.
5. Short chain fatty acid concentration in the cecum was 11% higher in broilers than in pullets. Diet composition can modify the fatty acid proportion in the cecum.
6. Ileal VH and CD of the ileum were greater in broilers than in pullets. However, the inclusion of SFH did not affect VH, CD, or VH:CD ratio of birds.

7. The inclusion of SFH (wt:wt basis), improved the AME_n of the diet without any negative effects on growth performance of the birds. These effects were more pronounced when SFH was included in the broiler diet than when included in the pullet diet.

8. Lack of interactions in many of traits studied in main traits studied such as performance and organ size indicated that existing data available for broilers would be generalized to a certain degree to pullets.

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CHAPTER 1

LITERATURE REVIEW

AND

OBJECTIVES

1. Livestock and poultry meat production in the world. Future trends

Livestock and poultry are the world largest users of land, either directly through grazing or indirectly through consumption of forages, legumes, and feed grains. Global meat production has increased steadily in the last decade, with poultry and pig meat leading the growth (Organization for Economic Cooperation and Development OECD, 2015). Livestock production accounts for almost 40% of the gross value of agricultural production worldwide. In developing countries, which accounts for one-third of all livestock meat consumption, meat intake is increasing quickly a result of the fast growth in population, increased income, and changes in lifestyle. For the next 15 years the annual meat consumptions growth rate is expected to be 0.33% for bovine, 0.95% for ovine, -0.09% in pig, and 1.6% in poultry. Table 1 gives a summary of worldwide meat consumption, according to the Food and Agriculture Organization (FAO, 2015).

Table 1. World meat consumption by type and region (kg/capita/year)¹

	1997-99	2015	2030	2015-30 ²
World	36.4	41.3	45.3	0.65
Bovine ³	9.8	10.1	10.6	0.33
Ovine ⁴	1.8	2.1	2.4	0.95
Pig	14.6	15.3	15.1	-0.09
Poultry	10.2	13.8	17.2	1.6
Region of the world				
Sub-Saharan Africa	9.4	10.9	13.4	1.5
Near East-North Africa	21.2	28.6	35.0	1.5
Latin America and Caribbean	53.8	65.3	76.6	1.2
South Asia	5.3	7.6	11.7	3.6
East Asia	37.7	50.0	58.5	1.1
Developed countries	88.2	95.7	100.1	0.31
Developing countries	25.5	31.6	36.7	1.1
Under-developing countries	46.2	53.8	60.7	0.86

¹FAO (2015). ²Annual growth rate (%). ³Includes buffalo meat. ⁴Includes sheep and goat meat.

Red meat (ruminants) accounted for almost 31% of total meat production in the developing countries in 1999, but declined to 28.8% in 2015, a reduction that is expected to continue in the future. Growth in poultry production has been highest in the developing

countries. Table 2 summarizes data for livestock and poultry meat production by region from 1997-99 to 2015, and the expected annual growth rate for the next 15 years.

Table 2. Livestock and poultry meat production by region¹ (MT)

	1997-99	2015	2030	2015-30 ²
World	217.8	300.1	376.3	1.7
Bovine ³	58.7	74.0	88.4	1.3
Ovine ⁴	10.8	15.3	20.1	2.1
Pig	86.5	110.2	124.5	0.87
Poultry	61.8	100.6	143.3	2.8
Sub-Saharan Africa	0.9	1.9	4.1	7.7
Near East-North Africa	3.2	7.1	11.6	4.2
Latin America and Caribbean	10.5	18.2	27.3	4.2
South Asia	1.1	3.9	10.6	11.5
East Asia	15.5	27.9	39.9	2.9
Developed countries	27.7	37.5	44.1	1.2
Developing countries	31.3	59.1	93.5	3.9
Under-developing countries	2.9	4.1	5.7	2.6

¹FAO (2015). ²Annual growth rate (%). ³Includes buffalo meat. ⁴Includes sheep and goat meat.

1.1. Chicken meat production

The poultry sector has undergone major structural changes for the past two decades mostly because of the introduction of intensive production systems, improved genetic, and implementation of preventive measures of control of diseases, including vaccination and biosecurity programs. The rapid growth in poultry production that occurred in the last four decades was accompanied by changes on the ways in which meat and eggs are produced, processed, and marketed. All these changes have led to improved productivity in controlled costs, encompassing the needs of poultry products for an increasing population in a society with greater incomes and more urbanized lifestyle. There are, however, major regional differences in poultry meat and egg production. FAO (2015) data show that the poultry meat production has increased steadily for the last 15 years. In developing countries, growth of the poultry industry has been driven by the demand with the main factors being: 1) higher income of the population, 2) lower relative

cost of chicken meat, 3) preference for poultry, 4) relative ease of industrialization, 5) more rapid urbanization of rural population, and 6) lack of religious connotations.

Table 3. Global poultry meat production by region (MT)¹

Region	2000	2005	2010	2016	2000-2016 ²
World	68.4	82.4	100.6	114.4	4.2
Africa	2.1	2.5	3.3	3.5	4.2
America	29.9	37.3	43.2	47.5	3.7
Asia	21.3	25.3	32.8	38.1	4.9
Europe	11.8	13.4	16.6	19.9	4.3
Oceania	0.76	0.96	1.1	1.4	5.3

¹OECD-FAO (2015). ²Annual growth rate (%).

The move from extensive production to confined poultry operations has resulted in an increase in the number of birds that a single farmer can manage. Advances in breeding technology and management, together with better disease control and new feedings practices, improved growth rates and feed efficiency of the chicks with the extra benefit of rearing more flocks per year. Table 3 gives data on poultry meat production by region. In 2016, America with 47.5 MT of poultry meat, leads the table in poultry production followed by Asia, Europe, Africa, and Oceania. Also, the top poultry meat producing countries are shown in table 4.

Table 4. Top poultry meat producer countries in the world¹ (MT)

	2000	2005	2010	2016 ²	2000-16 ³
World	68.5	82.7	100.9	115.2	4.2
USA	16.4	18.6	19.3	20.9	1.7
China	11.9	13.4	16.6	18.6	3.5
Brazil	6.1	9.7	12.6	13.5	7.6
Russian Federation	0.77	1.4	2.8	4.2	27.8
Mexico	1.8	2.5	2.7	3.1	4.5
Iran	0.83	1.3	1.9	2.2	10.3
Spain ³	0.96	1.1	1.2	1.2	1.6

¹Data for Iran and Spain is also included. ²OECD-FAO (2015). ³Annual growth rate (%). ⁴MAGRAMA (2015).

OECD-FAO (2015) data indicates that the USA produced 20.9 MT of poultry meat in 2016, followed by China, Brazil, Russian Federation, and Mexico. Russian Federation had the greatest annual growth rate, 27.8% followed by Brazil, Mexico, and China. In the case of Iran and Spain, two countries of special consideration in this thesis, poultry meat

output showed an annual growth rate of 10.3 and 1.6%, from 0.83 and 0.96 MT in 2000 to almost 2.2 and 1.2 MT in 2016, respectively (MAGRAMA, 2015; OECD-FAO, 2015).

1.2. Egg production

Egg production has increased steadily for the last two decades, with growth rates that depended on the regions considered. According to FAO (2015), global shell egg production saw a rise from 51.7 MT in the 1997-99 to 70.4 MT in 2015 (a 36.2% increase), and it is expected to rise by 1.85% annually, reaching 89.9 MT in 2030. The contribution expected is of 17.2% for the developed, 76.7% for the developing, and 6.1% for under-developing countries.

Table 5. Egg production by region¹ (MT)

	1997-99	2015	2030	2015-30 ²
World	51.7	70.4	89.9	1.8
Africa				
Sub-Saharan Africa	0.9	1.8	3.4	5.9
Near East/North Africa	2.2	3.6	5.3	3.1
Latin America and Caribbean	4.6	7.3	10.4	2.8
Asia				
South Asia	2.2	5.7	9.9	4.9
East Asia	23.8	32.1	40.0	1.6
Developed countries	13.7	14.8	15.5	0.31
Developing countries	33.7	50.7	69.0	2.4
Under-developing countries	4.3	5.0	5.5	0.67

¹FAO (2015). ²Annual growth rate (%).

In 1997-1999, the industrialized countries contributed to 26.5% of the global egg, a contribution that was reduced to 21.0% in 2015 (Table 5). In 1997-1999, developing countries contributed over 65.2% of the global egg production, a proportion that increased to 72.0% in 2015. Most of the rises observed resulted from fast growth in Asia, especially in China. In developed countries a slight decline in egg consumption is expected because of consumer concerns on the high content in cholesterol of the eggs. The expected decrease in consumption of table eggs will be offset by the increased use of egg in further processed products. In 2013, Iran and Spain, with 0.66 MT and 0.74 MT egg production, respectively, ranked 19 and 14 among egg producers (FAO, 2013) (Table 6).

Table 6. Top egg producers in the world, Iran and Spain¹

	World	China	USA	India	Japan	Mexico	Spain	Iran
2000	51.0	18.9	5.0	2.0	2.5	1.8	0.66	0.58
2013	68.3	24.4	5.6	3.8	2.5	2.5	0.74	0.67

¹FAO, FAOSTAT database (2013).

2. Chicken line

2.1. Genetic selection in poultry according to chicken line. Objectives

Poultry breeding and genetic improvement depend primarily on the work of a small number of scientists and professionals from key genetic companies, universities, and research centers. In the last 20 years, broilers have been selected for fast growth and also for improved breast meat. Modern broilers can reach a BW of 3.6 kg at 50 d of age, a weight that is almost double than that reported 35 years ago. In addition, the breast is 80% heavier than that in 60 years ago (Zuidhof et al., 2014). The impact of the strong selection pressure applied improved FCR but resulted often in an increase in the incidence of metabolic problems such as ascites, sudden death syndrome, foot and leg lesions, and wooden breast. These problems affect meat quality and poultry welfare and therefore require to focus on the genetic potential of the birds compatible with animal welfare and meat quality issues.

During the last few decades, the genetic improvement for egg production has resulted hens with reduced body size and feed intake together with longer egg laying cycle and egg persistency, which results in an improvement in egg production of considerable economic interest. It is clear that this increase results from the combination of improved genetics, management, nutrition, and disease prevention. The number of eggs produced is the main trait studied but other characteristics such as egg size and quality are also important. Moreover, new issues related to animal welfare, sustainability, environment and food safety are becoming key in a wide increasing segment of the population.

Correct rearing of the pullets is key to maximize future egg production. Pullets are not frequently the center of the discussions among nutritionists. Yet, a successful rearing

program is the key factor for better life and improved performance. An early-maturing pullet might start producing eggs sooner, but egg will be smaller and probably, fewer eggs will be produced during the entire laying cycle. In general, BW of the hens and egg size will be probably optimized in 2016, and therefore, no big changes in FI are expected. In the future, genetic improvement in feed conversion will take place through additional egg mass output and not through decreasing further FI. Currently, the main objective of the selection process in hens is to increase laying persistency to get production of 300 eggs per hen in 100 weeks of life with good shell quality and without the need of molting the birds. Consequently, the selection pressure is focused on shell strength, shell surface properties, shell color, and internal egg quality. The main objectives of the genetic programs for egg production are shown in table 7.

Table 7. Breeding traits in laying hens¹

Objective	Trait
Egg production	Age at first egg, hen-day egg production, persistency of production, pauses, egg size.
Egg quality	Egg deformation, shell thickness, shell color, texture, porosity and shape, albumen quality, blood spots.
Feed efficiency	Mature body weight, feed consumption, egg mass.
Functional traits	Disease resistance, leg strength survival, feeding behavior, cannibalism
Others	Feathering color and coverage.

¹Groen (2003).

2.2. Development of the gastrointestinal tract in broilers and pullets

Gastrointestinal tract is a complex ecosystem, in which commensal bacteria act in symbiosis within the host. The GIT of poultry has been developing fast from the first days before hatching until 7 d of life (Geyra et al., 2001; Sklan, 2001; Uni et al., 2003). At hatch, concentration, profile, and diversity of the microbiota of the GIT are quite limited but change quickly with time. Physico-chemical changes within the GIT produced by the feed and the environment, affect the ecosystem, which in turn will modify the profile of

the coexisting microflora. The GIT exposure to contact with foreign bacteria from the environment and exogenous feed contributes to the establishment early in the life of a stable diverse intestinal bacterial community. The development of the GIT differs between genetic lines and is affected by the type of diet used including CP content and type and source of fiber.

The main difference within poultry species is the age at which the maximal growth rate is reached and consequently, the development of the GIT will vary between species (Lilja, 1983; Katanbaf et al., 1988). Several studies compared growth performance, nutrient retention, and GIT development between meat and egg type birds as well as among meat lines varying in lean potential (Uni et al., 1995b; Hetland et al., 2003; Walugembe et al., 2014, 2015). Dror et al. (1977) compared the relative weight of the organs of the GIT of light (New Hampshire $\times$ White Leghorn) and a heavy (White Rock) breeds. These authors reported that heavy breeds had 17% and 60% higher BW than light breed at hatching and at 21 d of age, respectively, and that light breeds had heavier relative weight of the pancreas. At 21 d of age, the relative weight of the whole GIT was similar for both breeds but the duodenum and the jejunum were heavier in the light breed, whereas an opposite effect was observed for the ileum and cecum. Also, Yamauchi and Isshiki (1991) reported a faster development of SI in broilers than pullets. Dunnington and Siegel (1995) compared the GIT development in meat type chicks selected for high or low BW from hatch to 42 d of age. These authors reported that the high BW chicks had heavier relative GIT weights for the first 10 d after hatch but that an opposite effect was observed after this age. Mitchell and Smith (1991) compared highly selected (a common commercial line), relaxed selected (a second commercial line), and unselected (a SCWL line) pullets at 42 d of age. The highly selected birds were the heaviest, had the highest absolute weights of all the segments of the SI and also the greatest villus surface area, followed by the relaxed and then the unselected lines. However, on relative weight

basis the unselected birds had the heaviest weights for all segments of SI. Yamauchi et al. (1990) reported that at hatch, pullets and broilers had similar uniform finger-like villus structure, but that at 10 d of age broilers had a more developed villus and goblet cells than pullets. This research demonstrated that within the first 10 d of age all the segments of the SI developed faster in broilers than in pullets. Moreover, Yamauchi et al. (1990) suggested that rapid development of GIT could be the main contributing factor for the increased rate of BW gain observed for broilers. Uni et al. (1995ab) reported larger and more organized villus in all segments of the SI of broilers than those of pullets at 7 d of age. Furthermore, in these researches pullets had lower amylase, trypsin, and lipase activity and rate of food passage through the SI than broilers. Feed passage rate is affected by numerous factors including genetic, age, and certain characteristics of the diet, such as level of fat inclusion (Mateos and Sell, 1981, 1982; Shires et al., 1987) and level and type of fiber (Almirall and Esteve-Garcia, 1994; Lázaro et al., 2003; Hetland et al., 2004; Rochell et al., 2012). Broilers and pullets had similar overall mean retention time of the digesta through the GIT but the time that the feed stays in the different parts of the GIT varied (Table 8). Probably, the higher retention time of the digesta in the proventriculus and gizzard of the pullets (36 vs. 71 min) reduced the pH in these organs in pullets compared to broilers. Consequently, pullets should have fewer problems for pepsin activity and phytate solubility than broilers, at least at young age. Diet composition depends on the availability and cost of the ingredients. Changes in ingredient composition will affect the development and the pH of the GIT (Hetland et al., 2004; Rochell et al., 2012). Non-starch polysaccharides (NSP) are major components of cereal grains with anti-nutritional factors which will affect GIT development and bird performance (Smiths and Annison, 1996). The presence of NSP has been reported to increase CD and VH in the ileum while reduced enterocyte numbers and maltase and alkaline phosphatase activity in the jejunum of young chicks (Iji et al., 2001). Moderate amounts of insoluble

fiber seemed to have beneficial effects on the function and development of the gizzard of the birds (Hetland et al., 2005; Mateos et al., 2012; Jiménez-Moreno et al., 2016).

Table 8. Mean retention time (MRT) and digesta pH in various segments of the gastrointestinal tract (GIT)

GIT segment	MRT (min)			pH ³
	Broiler ¹	Broiler ²	Leghorn ¹	
Crop	31	41	48	4-6
Proventriculus + gizzard	39	33	71	2-5
Duodenum	10	5	7	6-6.5
Jejunum	84	71	85	6-6.7
Ileum	97	90	84	6-7.2
Cecum	119	--	112	5-7
Rectum	56	26	51	6-7

¹Shires et al. (1987). ²van Der Klis et al. (1990). ³Denbow (1986)

3. Dietary fiber

3.1. Definition of dietary fiber

The term "fiber" has been used broadly to describe the NSP and lignin content of the feeds, the latter being a polyphenolic compound. There are many definitions of the term "fiber" which are difficult to differentiate and create confusion. The Proximate Analysis System defined at the Weende Research Station in Germany (Henneberg and Stohmann 1859, 1860) classified feed carbohydrates into a more digestible component, called nitrogen free extract (NFE) and a less digestible fibrous component, called crude fiber (CF). The advantage of the Weende system was that the separation of feed components (NFE, CF, moisture, ash, ether extract, crude fat, and crude protein) was based on detailed analytical procedures. Crude fiber refers to the organic part of the feedstuffs that was insoluble in hot, dilute sulphuric acid, and sodium hydroxide. It includes variable portions of the insoluble NSP, including cellulose and some hemicellulose. Consequently, the CF value obtained using this methodology depends on the ingredient evaluated and the physicochemical nature of the NSP present. For instance, soluble NSP, such as pectic polysaccharides, mixed linked β -glucans, and arabinoxylans are not accounted in CF measurement. In addition, some of the amorphous components,

less crystalline, less lignified cellulose might not be qualified and will be missed when measuring CF as it relies on extraction with hot acid and alkali over an extended period of time. Nevertheless, the poultry industry still uses CF in feed formulation.

In a series of excellent studies, van Soest (1963) found that the "true fiber" content of many feedstuffs. In the system he proposed for fiber evaluation, there are two types of fiber: Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). From a chemical point of view, the NDF and ADF values do not represent any specific entities. In a proximate term, the two fiber values overlap because NDF refers to the insoluble portion of the NSP plus lignin, whereas ADF refers to a portion of insoluble NSP consisting largely, but not exclusively, of cellulose and lignin. The differences between the Weende and the van Soest methods are presented in Figure 1. Dietary fiber (DF) is a term widely used in human nutrition and was used first by Hipsley (1953) and later defined by Trowell et al. (1976) as the residue of plant food resistant to hydrolysis by human alimentary enzymes. The definition of DF created a great deal of controversy because of the numerous, at times confusing, variable definitions over the years, including concepts based on the physiological effects of DF on the animal and concepts based on the methods used for their determination. In figure 2 we presented data reported by NRC (2012) on the different fractions of the plant carbohydrate. Dietary fiber consists of diverse polymers with variable physiochemical properties, when included in the diet, result in differences in ion exchange capacity, viscosity, bulking, and fermentation in the GIT. It is not easy to measure DF, but differentiating components into soluble and insoluble fractions helps to explain the physiological effects of fiber in human and animal nutrition (Newman et al., 1992; Mertens, 2003).

Weende system	Chemical composition	van Soest
Crude protein	<ul style="list-style-type: none"> ✓ Protein ✓ Free nitrogen 	Soluble in neutral detergent
Ether extract	<ul style="list-style-type: none"> ✓ Lipids ✓ Pigments 	
Nitrogen-free extract	<ul style="list-style-type: none"> ✓ Sugar ✓ Organic acids ✓ Pectin 	
	<ul style="list-style-type: none"> ✓ Hemicellulose ✓ Soluble lignin in alkaline 	ADL
Crude fiber	<ul style="list-style-type: none"> ✓ Insoluble lignin in alkaline ✓ Nitrogen bound to fiber ✓ Cellulose 	
		ADF
Ash	<ul style="list-style-type: none"> ✓ Mineral insoluble in detergent ✓ Mineral soluble in detergent 	
		NDF

Figure 1. Dietary fiber measurements as per Weende (Henneberg and Stohmann, 1859, 1860) and van Soest (1963) methodologies.

Figure 2. Classification of dietary carbohydrates based on the analytical procedures used (NRC, 2012).

Based on new developments and on more sophisticated methods of fiber determination, it is clear that different fiber fractions have different properties and should consequently be evaluated differently. Solubility is an important feature of fibers as it largely determines their effect on production performance (Choct, 2002). More specifically, the new definition of DF allows differentiation of its effects on the anatomy and physiology of the digestive tract (Table 9).

Table 9. Effects of soluble and insoluble NSP on GIT physiology. Signs represent increases (+) or decreases (-).¹

	Soluble NSP	Insoluble NSP
WHC ² and viscosity	++	+
GIT motility	-	+
MRT	+	-
Nutrient digestibility	-	+
Bacterial growth substrate	++	+

¹Kalmendal (2012). ²Water holding capacity.

3.2. The role of “fiber” in animal feeding

The role of fiber in poultry diet is a subject of debate. Traditionally, DF was associated with negative attributes such as energy dilution of the diet, reduced FI, and usually, it was not recommended to be included at high levels in poultry diets (Mateos et al., 2012). In fact, most nutritionists found out that the requirements of broiler for CF are low and recommended to be reduced its content in diets for broilers to less than 3.0-4.0% (FEDNA, 2008; Swennen et al., 2010). Consequently, “fiber” has been used commonly as a negative coefficient in prediction equations of the nutritive value of feeds. However, recent studies suggested that moderate amounts of different fiber sources in the diet improved digestive organ development and increased HCl, bile acids, and enzyme secretion (González-Alvarado et al., 2008; Jiménez-Moreno et al., 2016) in the chicks. These new ideas might result in an increase in the level of fiber inclusion in the diet to improve nutrient digestibility, growth performance, GIT health, and eventually, animal

welfare (Mateos et al., 2012). Poultry requires a certain amount of DF for proper development, function, and normal physiology of the GIT. In addition, depending on the amount and type of DF, and on the composition of the basal diet, the profile of the microbiota in the distal part of the GIT might change (Amerah et al., 2009). The interest in changes in diet composition to promote or maintain GIT health in poultry has grown in the last decades. Two new areas of interest in the inclusion of fiber in poultry diets are a) the availability of non-traditional ingredients with large concentrations of fiber for commercial diets and b) public concern regarding the use of in-feed antimicrobials for livestock production, because of the potential transmission of antibiotic resistance from animals to humans (McDermott et al., 2002; Witte et al., 2002). Also, feeding diets with moderate amounts of DF is a useful strategy to control FI in pullet and broiler breeders and to improve animal welfare.

The main areas of research on the effects of DF on poultry relate to its influence on 1) feed passage rate and organ size development, 2) secretion of HCl, bile salts, digestive enzymes, and nutrient digestibility, 3) feed intake and growth performance, and 4) intestinal health and mucosa integrity, bird behavior, and microbial growth (Mateos et al., 2012). Differences in the structure and properties of the existing fiber sources affect the rate of passage in different ways as well as SCFA production and digesta pH in the different segments of the GIT (Montagne et al., 2003; Raninen et al., 2011). Consequently, the effects of DF on voluntary FI, organ size, GIT motility, enzyme production, microbial profile, and bird behavior differ, depending on the source and level of fiber (Jørgensen et al., 1996; Jiménez-Moreno et al., 2011), nature and form of the basal diet (Amerah et al., 2009; Jiménez-Moreno et al., 2009b), physical characteristics of the fiber source (Kritchevsky and Story 1974; Jiménez-Moreno et al., 2010), and the type and age of the birds (González-Alvarado et al., 2010). In practice, as indicated by

Mateos et al. (2012), response to these variables depends on nutritional and management practices, including rearing conditions and birds access to litter materials (battery cages vs. floor pens), feed form (mash vs. pellets), composition of the basal diet (i.e., the main cereal used, type and level of supplemental fat, and DF content), and health status of the birds (i.e., hygiene conditions and incidence of diseases and digestive disturbances).

3.3. Effects of inclusion of insoluble fiber in poultry diets

3.3.1. Growth performance and nutrient digestibility

The influence of fiber content of the diet on voluntary FI and TTAR of nutrients in poultry is a highly debated issue (Moran, 2006). Traditionally, DF has been considered a diluent in poultry diets, with negative effects on voluntary FI and nutrient digestibility (Mateos et al., 2002). For example, Janssen and Carré (1985) found that the fibrous components of feedstuffs negatively affect chick growth and, consequently, they recommended diets low in DF for young chicks. However, insoluble fibers have beneficial effects on fat and starch digestibility and on the energy content of the diet in broilers (Jiménez-Moreno et al., 2009a). Svihus and Hetland (2001) reported that the dilution of the diet with 10% cellulose increased the ileal digestibility of the starch without any negative affect on broiler performance. Similarly, Hetland and Svihus (2001) reported that the inclusion of 10% OH improved ileal starch digestibility but did not affect average daily gain (ADG) or average daily feed intake (ADFI). González-Alvarado et al. (2007) reported an improvement in ADG and FCR in broilers with the inclusion of 3% OH in the diet. González-Alvarado et al. (2010) reported improvement in DM, organic matter (OM), soluble ash, nitrogen (N), and ether extract (EE) retention, and AME_n of the diet with the inclusion of 3% OH in 42 d-old broilers. Similar improvement in DM, OM, N, and AME_n of the diet, has been reported by Jiménez-Moreno et al. (2011) with the inclusion of up to 5% pea hulls in 18 d-old broilers. Sacranie et al. (2012) reported that

the inclusion of 15% OH or barley hulls in the diet increased starch digestibility but reduced feed efficiency in 32 d-old broiler. The beneficial effect of insoluble fiber on nutrient utilization might be a result of an increase in gizzard size and function and higher enzyme activity (Jiménez-Moreno et al., 2009b; Mateos et al., 2012). The inclusion of insoluble fiber in the diet affects gizzard development and the mean retention time of the digesta (Hetland and Svihus, 2007; Steenfeldt et al., 2007; van Krimpen et al., 2009). In this respect, an increase in mean retention time of the digesta in the gizzard accumulates fiber in this organ (Hetland et al., 2005; Hetland and Svihus, 2007; van Krimpen et al., 2009). Higher proportion of fiber in the gizzard digesta might be explained because fiber is harder to grind than other components and requires a longer time for grinding (Hetland et al., 2005). Moreover, the stimulatory effects of fiber on the muscular contractions lead to an enlargement of the gizzard (Steenfeldt et al., 2007; van Krimpen et al., 2009) and increase its grinding capacity (Hetland and Svihus, 2007).

Information available on the effects of additional fiber sources in the diet of other poultry species is limited. In laying hens, DF reduces the aggressiveness and cannibalism of the flock (van Krimpen et al., 2009; Qaisrani et al., 2013) with improvement in welfare and livability. An increase in weight and digesta content of the gizzard has been reported in laying hens (Hetland et al., 2003, 2005; Hetland and Svihus, 2007) with the inclusion of insoluble fiber sources. Hetland et al. (2003) reported that the inclusion of OH and wood shaving increased starch digestibility in laying hens. Information available suggests that the beneficial effects of dietary fiber in poultry diet might depend on the birds age with more noticeable effects in young chicks than in pullets and in both type of birds than in laying hens. Research conducted by Guzmán et al. (2015a) in which 2% of cereal straw, sugar beet pulp, or SFH were included in pullet diets, showed improved growth performance and energy efficiency with fiber inclusion. However, a further increase to

4% had negative effects on FCR in pullets at 5 wk of age. In a second paper of same author (Guzmán et al., 2015b), inclusion of straw improved ADG compared to SBP from hatch to 17 wk but a further increase to 4% reduced energy intake and feed efficiency.

Few research has compared the effects of DF on growth performance and GIT development in different chicken lines. Moreover, most of the available information was obtained in studies conducted more than 20 years ago, this fact limits its practical interest. Recently, Walugembe et al. (2014) compared the response of broilers and pullets to the inclusion of 16% mixture of distillers dried grains with solubles (DDGS) and wheat bran in the diet on growth performance, and AME_n of the diet from 1 to 21 d of age. They reported that fiber inclusion reduced ADG in broilers, but had no effects in pullets. In these research, the AME_n was similar in control and high fiber diets for both chicken lines. Also, pullets utilized 21% more efficient NDF of the diet than broilers.

3.3.2. Gastrointestinal tract development

A good development of the GIT benefits nutrient digestibility and chick growth (Noy and Sklan, 1998). Insoluble fiber has positive effects on GIT development (Hetland and Svihus, 2001; Gonzalez-Alvarado et al., 2007; Jiménez-Moreno et al., 2009b) and stimulates secretion of HCL, bile acids, and endogenous enzymes (Hetland et al., 2003; Svihus, 2011). In poultry, GIT size touched a high growth rate around hatching and continued for 7 d after hatch (Sklan 2001; Geyra et al., 2001; Uni et al., 2003). Dietary fiber affects GIT development in different ways. Hetland and Svihus (2001) reported that the inclusion in the diet of 10% coarse OH increased the relative weight of the GIT and of the empty gizzard and reduced feed passage rate in broilers. However, González-Alvarado et al. (2008) did not find any effect of the inclusion of 3% SH or OH on the relative weight of the GIT but reported an increase in the relative weight of the gizzard. Jiménez-Moreno et al. (2009a,b) reported that inclusion of 3% OH or SH decreased

gizzard pH and increased the relative weight of the GIT in broilers. In this respect, González-Alvarado et al. (2007) reported a reduction in gizzard pH with the inclusion of fiber in the diet, an effect that was more pronounced in diets based on rice than in diets based on corn. The authors suggested that additional fiber had more beneficial effects when added to low fiber diet, as happened with the rice diet. Hetland et al. (2003) reported that the inclusion of 10% OH in 33 d-old broilers and 10% wood shavings in 29 wk-old laying hens increased the relative weight of the full gizzard as well as the relative weight of the empty gizzard and the NDF content of the gizzard digesta. Also, Guzmán et al. (2015b) reported that the inclusion of 2 and 4% straw increased the relative weight of the gizzard in 35 d-old pullets. A well-developed GIT is crucial for utilization and adequate nutrient digestion of exogenous nutrients in modern chicken lines. The insoluble fraction of the DF improves gizzard function, gut physiology, and bird productivity. The amount of DF required for adequate GIT function depends on the physico-chemical characteristics of the fiber source, especially of its water holding capacity (WHC), lignin content, feed form, and particle size (Mateos et al., 2012).

3.3.3. Cecal SCFA production and intestinal morphology

In chicken, total concentration of SCFA is the highest in the ceca, followed by the crop, ileum, and gizzard (Annison et al., 1968; Montagne et al., 2003). The SCFA is produced in the GIT by organisms such as *Lactobacillus spp.* and *Bifidobacterium spp.* during fermentation process of the indigestible carbohydrates (Ricke, 2003; Miller, 2004). Annison et al. (1968) reported that the highest concentration of SCFA in poultry was observed in the ceca which included primarily acetate, propionate, and butyrate acids. Fibers are the main constituents of plant cell walls and consist primarily of NSP. Upon ingestion, DF can change feed passage rate, metabolite production, gut microbial activities, and digestive efficacy in birds (Bach Knudsen, 2001). Differences in structure

and properties of the fiber sources, modify SCFA production and digesta pH in the different segments of the GIT (Mateos et al., 2012). Several studies (Jørgensen et al., 1996; Jamroz et al., 2002; Guo et al., 2003; Saengkerdsut et al., 2006) reported that DF fractions are fermented by cecal microorganism ending in SCFA, ammonia, CO₂, and methane as major end product. Guo et al. (2003) suggested that certain fractions of DF, including the oligosaccharides are potential prebiotics and might modify the profile of the microbiota present in the large intestine. In vitro studies using inoculum of layers (Dunkley et al., 2007), and broilers (Lan et al., 2005), indicated that acetate production was highest, followed by propionate and butyrate whereas isobutyrate and isovalerate production were only present in trace amounts. Moreover, Rehman et al. (2009) reported a wide variation in SCFA concentration in the ceca of broilers among researches and that most of the variation could be explained by the ingredient composition of the diet. The most important factors of impact on the fermentability of DF include source, solubility, degree of lignification, processing method, level of inclusion of DF in the diet, intestinal transit time, age and weight of the animal, and microbial profile (Bach Knudsen and Hansen, 1991; Macfarlane and Cummings, 1991; Williams et al., 2001). The concentration of SCFA in the ceca increases from undetectable levels in one day-old broilers to a culmination at 15 d of age (van der Wielen et al., 2000). Tsukahara and Ushida (2000) reported that plant protein diets generated higher concentrations of SCFA than animal protein diet in chicks. These authors concluded that the differences were due to the higher fiber content in the plant based diets. Type of cereal can affect also the pattern of fermentation in the ceca. Feeding wheat to broilers increased butyrate proportion compared with feeding corn (Kiarie et al., 2014; Cámara et al., 2015). Singh et al. (2012) reported that low energy diets increased total SCFA production in the cecum of 42 d-old broilers. The amount of energy obtained from fermentation processes does

not add significantly to meet requirements of modern strains. Morton (1978) concluded that the amount of energy obtained from SCFA in the chickens is around 3 to 4% of total energy needs. However, SCFA have antibacterial activity, which probably plays an important role in maintaining in balance the ceca ecology (Cherrington et al., 1990, 1991; Davidson, 1997).

Figure 3. Concentration of SCFA ($\mu\text{mol/g}$ fresh digesta) in the cecum of broilers and pullets at 21 d of age fed a low or a high fiber containing diet (Walugembe et al., 2015).

Broilers and pullets differ in GIT capacity (Uni et al., 1995b; Walugembe et al., 2014) and in the digesta passage rate through the GIT (Shires et al., 1996). Consequently, the pattern of fermentation and the production of SCFA in the different GIT segments will vary between chicken lines (Annison et al., 1968). However, little information is available which compare SCFA production in broilers and pullets fed commercial poultry diets with or without insoluble fiber supplementation. Walugembe et al. (2015) observed that the inclusion of 16% of a 50:50 mixture of DDGS and wheat bran did not affect the total concentration of SCFA or the concentration of acetate and propionate in the cecum but decreased that of butyrate (Figure 3).

The VH to CD ratio is a useful criterion for estimating the absorptive capacity of the small intestine of the chicken (Montagne et al., 2003). A high VH to CD ratio is

indicative of adequate function and maturity of the intestinal mucosa. The effects of fiber inclusion on the epithelial morphology and cell turnover depended on its physicochemical characteristics, source and level, bird's age, and the site in the intestinal tract studied (Iji et al., 2001). Sarikhan et al. (2010) and Rezaei et al. (2011) observed that the inclusion of 0.25 to 0.75% of Vitacel (a micronized insoluble fiber consisting mostly of cellulose), increased VH to CD ratio in the ileum of 42 d-old broilers. Jiménez-Moreno et al. (2011) reported that the inclusion of 2.5% pea hulls increased VH to CD ratio. However a further raising to 5% had no extra benefits. Similarly, Jiménez-Moreno et al. (2013b) reported that dilution of a control diet with up to 7.5% OH, reduced VH and CD in 12 d-old broilers and a appositive effect was observed with the inclusion of 2.5% OH. An excess of DF could increase the abrasion of the mucosal surface of the small intestine, increasing mucus production and affecting villus structure. Consequently, the inclusion of an excessive amount of insoluble fiber could reduce absorptive villus surface and hinder nutrient retention (Jiménez-Moreno et al., 2011, 2013b).

4. Objectives

The hypothesis tested in this thesis was that the inclusion of SFH in the diet, could affect GIT development and improve growth performance in both chicken lines, but that the effects could possibly be more pronounced in broilers. It was hypothesized that because of their lower rate of feed passage, lower digestive capacity, and ADFI, the effects could be less evident in pullets. The main objective of this study was to evaluate the response of broilers and pullets to the inclusion of SFH in the diets differ in nutrient density and fiber content on growth performance and GIT traits from 0 to 21 d of age.

CHAPTER 2

Effects of the inclusion of sunflower hulls in the diet on growth performance and digestive tract traits of broilers and pullets fed a broiler diet from 0 to 21 days of age. A comparative study

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2.1. Introduction

Dietary fiber (DF) affects feed intake and nutrient digestibility in poultry, with effects that depend at a high extent on the type and level of fiber inclusion (Mateos et al., 2002, 2012; Hetland and Svihus 2007). In this respect, the inclusion of moderate amounts of insoluble fiber in the diet increases the retention time of the digesta in the upper part of the gastrointestinal tract (GIT) and improves the development and function of the gizzard and other digestive organs in broilers (Hetland and Svihus, 2001; González-Alvarado et al., 2007, 2008). In addition, DF stimulates HCl and bile acid production and improves amylase activity, which results in increased nutrient utilization (Hetland et al., 2003).

Broilers are selected for faster growth and improved feed to gain ratio (F:G) whereas pullets are selected for optimal future egg mass production. The divergent selection targets for both chicken lines result in birds differing in the anatomical and physiological characteristics of the GIT (Masic et al., 1974; Nir et al., 1993; Hetland et al., 2003; Rideau et al., 2014). Consequently, the response of the GIT of broilers and pullets to fiber inclusion and its effects on nutrient digestibility might differ between chicken lines (Slinger et al., 1964; Walugembe et al., 2014). Most of the research on the effects of the inclusion of a fiber source in the diet on GIT development and growth has been conducted with broilers (Hetland and Svihus, 2001; Jiménez-Moreno et al., 2009a; González-Alvarado et al., 2010; Mateos et al., 2012) but little information is available in pullets. Research comparing the effects of insoluble fiber on growth performance and GIT traits in modern strains of these 2 chicken lines could help to adapt the existing information in broilers to pullets.

The hypothesis tested in this research was that sunflower hulls (SFH) could affect GIT development and growth performance in both chicken lines. In addition, it was

hypothesized that because of their lower rate of feed passage, limited digestive capacity, and reduced potential for voluntary feed intake, the effects could be less evident in pullets. The objective of this research was to compare the effects of including 3% SFH in the diet on growth performance, nutrient digestibility, and GIT traits of broilers and pullets fed a broiler diet from 0 to 21 d of age.

2.2. Materials and methods

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

2.2.1. Diets and fiber source

The control diet was based on corn and soybean meal and contained 2,980 kcal AME_n/kg, 22.2% CP, 1.25% digestible Lys, and 8.7% neutral detergent fiber (NDF). Celite, an acid-washed diatomaceous earth (Celite Hispánica S.A., Alicante, Spain), was included at 2% in the diet to increase the acid insoluble ash content of feeds and excreta. The experimental diet had similar ingredient composition to that of the control diet but was diluted (wt:wt) with 3% SFH. Before incorporation to the diet, the SFH was ground using a hammer mill (Model DFZC-635, Bühler AG, Uzwil, Switzerland) fitted with a 2.5 mm screen. Both diets met or exceeded the nutritional requirements of pullets as recommended by FEDNA (2008). The chemical analyses and physical characteristics of the SFH are shown in Table 10, and the ingredient composition and physico-chemical characteristics of the experimental diets are shown in Table 11.

2.2.2. Husbandry and experimental design

In total, 280 one-d-old chicks, 140 Ross 308 female broilers with an initial BW of 46.7 ± 3.80 g and 140 Lohmann Brown pullets with an initial BW of 37.5 ± 2.84 g, were used. Birds were housed in groups of 10, according to chicken line, in 28 battery cages

(0.36 m × 1.00 m; Alternative Design, Siloam Springs, AR) equipped with 2 nipple drinkers and an open trough feeder (65 cm length). Within each chicken line, all cages had similar average BW. The batteries were placed in an environmentally controlled room with an ambient temperature that was reduced gradually from 31 ± 1°C at the arrival of the chicks to 24 ± 2°C at 21 d of age.

Table 10. Analysed chemical composition and physical characteristics (% as-fed basis) of the sunflower hulls

Item	%
Chemical analyses	
Dry matter	93.0
Gross energy (kcal/kg)	4,423
AME _n ¹ (kcal/kg)	340
Crude protein	4.7
Ether extract	3.6
Total ash	2.8
Crude fiber	50.7
Neutral detergent fiber	70.0
Acid detergent fiber	51.3
Acid detergent lignin	13.1
Total dietary fiber	86.8
Soluble fiber	2.0
Insoluble fiber	84.8
Physical characteristics	
Particle size distribution, sieve screen ² (µm)	
> 2,500	0.4
1,250	4.6
630	60.1
315	24.4
160	10.3
< 80	0.2
GMD ³ ± GSD ⁴ (µm)	675 ± 1.68
WHC ⁵ ± SD (mL/g DM)	6.88 ± 0.35
SWC ⁶ ± SD (mL/g DM)	3.85 ± 0.19

¹Calculated value (FEDNA, 2010).

²The percentage of particles bigger than 5,000 or smaller than 40 µm was negligible.

³Geometric mean diameter.

⁴Log geometric standard deviation.

⁵Water holding capacity.

⁶Swelling water capacity.

Table 11. Ingredient composition and calculated and analysed chemical composition of the experimental diets (% , as-fed basis, unless otherwise indicated)

Ingredient	Sunflower hulls	
	0%	3%
Corn	34.0	33.0
Wheat	20.0	19.4
Sunflower hulls	0.0	3.0
Soy oil	1.31	1.27
Soybean meal (47% CP)	21.0	20.4
Full fat soybeans	14.8	14.3
Fish meal (60% CP)	3.00	2.92
Calcium carbonate	1.35	1.32
Monocalcium phosphate	1.20	1.16
Sodium chloride	0.31	0.30
L-Lysine (78%)	0.27	0.26
DL-Methionine (99%)	0.32	0.31
L-Threonine (99%)	0.11	0.10
L-Valine (99%)	0.03	0.03
Celite	2.00	1.94
Vitamin and mineral premix ¹	0.30	0.29
Analysed chemical composition		
Dry matter	88.4	88.7
Crude protein	22.2	21.7
Ether extract	5.8	6.1
Ash	6.3	6.2
Crude fiber	3.0	4.4
Neutral detergent fiber	8.7	10.3
Acid detergent fiber	3.9	5.7
Acid detergent lignin	1.5	1.8
GMD ² ± GSD ³ (µm)	975 ± 2.07	884 ± 2.07
Initial pH	5.82	5.88
Calculated chemical composition ⁴		
AME _n (kcal/kg)	2,980	2,900
Digestible amino acid		
Arg	1.33	1.29
Lys	1.25	1.22
Met	0.63	0.61
Met + Cys	0.92	0.89
Thr	0.81	0.78
Trp	0.23	0.22
Calcium	1.02	0.99
Available phosphorus	0.45	0.44

¹Provided the following (per kilogram of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3, (cholecalciferol), 3,000 IU; vitamin E (all-rac-tocopherol acetate), 30 IU; vitamin K (bisulphate menadione complex), 3 mg; riboflavin, 5 mg; pantothenic acid (D-Ca pantothenate), 10 mg; nicotinic acid, 30 mg; pyridoxine (pyridoxine, HCl), 3 mg; thiamin (thiamin-mononitrate), 1 mg; vitamin B₁₂

(cyanocobalamin), 12 µg; D-biotin, 0.15 mg; choline (choline chloride), 300 mg; folic acid, 0.5 mg; Fe (FeSO₄·7H₂O), 30 mg; Zn (ZnO), 100 mg; Mn (MnSO₄·H₂O), 100 mg; Se (Na₂SeO₃), 0.2 mg; I (KI), 2.0 mg; Cu (CuSO₄·5H₂O), 10 mg; phytase (EC 3.1.3.26), 0.06% Natuphos (BASF Española, S.A., Tarragona, Spain); [(Endo-1,3 (4)-β-glucanase (EC 3.2.1.6), 150 IU; Endo-1,4-β-xylanase (EC 3.2.1.8) 150 IU; (Endofeed, GNC Bioferm, Saskatchewan, SK, Canada)]; and ethoxyquin, 110 mg.

²Geometric mean diameter.

³Log geometric standard deviation.

⁴According to FEDNA (2010).

The light program consisted of 23 h of light during the first 7 d of life followed by 16 h to the end of the experiment. Feed in mash form and water were offered for ad libitum consumption. The experiment was conducted as a completely randomized design with 4 treatments arranged as a 2 × 2 factorial with 2 chicken lines (female broilers vs. pullets) and 2 levels of SFH (0 vs. 3%). Each treatment was replicated 7 times and the experimental unit was a cage with 10 birds.

2.2.3. Growth performance and TTAR of nutrients

Body weight of the chicks and feed disappearance were determined by cage at 9 and 21 d of age and the data used to estimate ADFI, ADG, and F:G by period (0 to 9 d and 10 to 21 d) and cumulatively (0 to 21 d). In addition, energy intake (EI; kcal AME_n/day) and energy conversion ratio (ECR; kcal AME_n ingested/g BW gain) were estimated at the same ages. Total tract apparent retention (TTAR) of DM, organic matter, N, and GE was determined by the indigestible marker method and the AME_n of the diets as indicated by De Coca-Sinova et al. (2011). Briefly, clean plastic pans were placed beneath the cages at 7 and 19 d of age, and representative samples of the excreta were collected daily for 2 d. The samples were, dried in oven at 60°C for 72 h, ground with a laboratory mill (model Z-I, Retsch, Stuttgart, Germany) fitted with a 1-mm screen, mixed by replicate, and stored at 4°C until further analyses. At 9 and 21 d of age, after the collection of the excreta for TTAR determination, the trays placed beneath the cages were cleaned and the

excreta produced for the next 3 h was collected and immediately oven-dried (60°C for 72 h) for determination of the DM content.

2.2.4. *Gastrointestinal tract traits*

After each BW determination, 2 birds per replicate were randomly selected, weighed individually, and euthanized by CO₂ asphyxiation. The GIT were excised and divided into 7 portions: crop, proventriculus, gizzard, duodenum (from the gizzard junction to the entry of the bile and pancreatic ducts), jejunum (from end of the duodenum to Meckel's diverticulum), ileum (from Meckel's diverticulum to the ileocecal junction), and cecum. The segments of the GIT were clamped to avoid bolus contamination and the pH of all organs was measured in situ using a digital pH meter (Model 507, Crison Instruments S.A., Barcelona, Spain) as indicated by Jiménez-Moreno et al. (2009b). The mean value of 2 measurements taken in each of the 2 birds was used for further evaluation.

In addition, 2 and 4 extra birds per replicate were slaughtered at 9 d and at 21 d of age, respectively, and the full GIT, from the post-crop to the cloaca, without the annex organs (liver, pancreas, and spleen), was excised, dried with desiccant paper, and weighed. The weight of the full proventriculus and gizzard, and that of the liver and pancreas were also measured in these birds and expressed in absolute (g) and in relative (% BW) terms. The gizzard of all these birds was opened and the contents were weighed. The weight of the empty gizzard was calculated by difference between the weight of the full organ and its content. The DM of the digesta of the gizzard was also determined by drying the contents at 60°C for 72 h. In addition, the absolute (cm) and relative (cm/kg BW) length of the small intestine (SI; duodenum, jejunum, and ileum) and of the 2 cecum were measured in all these birds using a flexible ruler with a precision of 1 mm.

At 21 d of age, the concentration (μmol/g fresh digesta) of acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate and total SCFA was determined in the cecum

of the 2 birds used for pH determination, and the proportion of each individual fatty acid (% total SCFA) was calculated. In addition, ileum tissue samples of approximately 3 cm in length, were taken from the midpoint of the ileum in these 2 birds and fixed in a buffer solution with 10% formalin. Villus height (VH), from the tip of the villi to the villus crypt junction, and the associated crypt depth (CD), defined as the depth of the invagination between adjacent villi of the samples, were measured as described by Jiménez-Moreno et al. (2011). Two representative cross sections from each bird were viewed at 40× magnification using an Olympus BX-40 light microscope. Images were digitally captured (Soft Imaging System, version 3.2 C4040Z, Olympus, GmbH, Hamburg, Germany) and the average of at least 30 measurements taken from cross-sectional samples were used for further analysis.

2.2.5. Laboratory analysis

Sunflower hulls, diets, and excreta were analyzed for moisture by oven-drying (method 930.15), total ash by muffle furnace (method 942.05), and nitrogen (N) by Dumas (method 968.06), using a LECO analyzer (Model FP-528, Leco Corporation, St. Joseph, MI) as described by AOAC International (2000). Gross energy was measured using an isoperibol bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL) and ether extract was determined, after 3 N HCl acid hydrolysis, using an Ankom XT10 extraction system (Ankom Technology Corp. Macedon, NY) by method Am 5–04 of the AOCS (2004). Neutral detergent fiber, acid detergent fiber, and acid detergent lignin of the fiber source and diets were determined sequentially using a filter bag system (Ankom Technology Corp. Macedon, NY) and expressed on an ash-free basis (van Soest et al., 1991). Also, total DF (method 985.29) and the insoluble fraction of the DF (method 991.43) were analyzed as proposed by the AOAC International (2005). The soluble fraction was calculated by difference between total and insoluble DF. The pH of the

drinking water and diets was measured as described by Giger-Reverdin et al. (2002) using a portable pH meter (Model 507, Crison Instruments S.A., Barcelona, Spain). Particle size distribution and mean particle size of the SFH and diets, expressed as geometric mean diameter $\pm$ log geometric standard deviation (GMD $\pm$ GSD), were determined in 100 g samples using a Retsch shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 40 to 5,000 μ m, as described by the ASAE (1995). The water-holding (WHC, mL/g DM) and swelling (SWC, mL/g DM) capacity of the SFH were determined as indicated by Jiménez-Moreno et al. (2009b). Acid-insoluble ash was analyzed as proposed by De Coca-Sinova et al. (2011). The concentration of total and of the individual short chain fatty acids (SCFA) was determined in cecum samples by gas chromatography, using a Perkin Elmer Autosystem XL (*Perkin Elmer Inc.*, Waltham, MA) equipped with a flame-ionization detector and a TRB-FFAP capillary column (30 m $\times$ 0.53 mm $\times$ 1 μ m; Supelco, Barcelona, Spain), as described by Carro et al. (1992). Briefly, 1.5 g sample of fresh cecal contents was mixed with 1.5 mL of 0.5 M HCl, and frozen at -20°C . Before analyses, samples were thawed at 4°C , homogenized, and centrifuged ($13,000 \times g$) for 15 min at 4°C . One mL of the supernatant was mixed with 0.5 mL of a deproteinized solution consisting of a mixture of 20 g metaphosphoric acid and 0.6 g of crotonic acid/L and left overnight at 4°C .

2.2.6. Statistical analysis

All data were analyzed as a completely randomized design with 4 treatments arranged as a 2×2 factorial using the GLM program of SAS (1990). The model included the chicken line (female broilers vs. pullets) and the level of SFH inclusion (0 and 3%) as main effects as well as the interaction. The experimental unit was the cage with 10 (0 to 9 d) or 6 (10 to 21 d) birds for growth performance and TTAR data, 2 (9 d) and 4 (21 d) birds for GIT measurements, and 2 birds for pH and ileum mucosa morphology. The

Tukey test was used to detect difference among treatment means and the differences were considered significant at $P < 0.05$.

2.3. Results

The analytical values of the SFH batch were within the range reported by other authors (Cancalon, 1971; FEDNA, 2010; Guzmán et al., 2015a). The NDF content was 70.0% and the GMD $\pm$ GSD was $675 \pm 1.68 \mu\text{m}$. The chemical composition of the experimental diets was close to expected values, confirming that the ingredients were mixed correctly.

2.3.1. Growth performance and TTAR of nutrients

Mortality was low (1.0%) and not related to any of the treatments (data not shown). No interactions between main effects were detected for any of the traits studied and therefore, only main effects are presented.

Effects of chicken line

Feed intake and ADG were greater ($P < 0.001$) and F:G and ECR were better ($P < 0.001$) for broilers than for pullets at all ages (Tables 12). The moisture content of the excreta was higher ($P < 0.01$) in broilers than in pullets at 21 d of age but an opposite effect ($P < 0.05$) was observed at 9 d of age (Table 13).

The TTAR of all nutrients and the AME_n content of the diets were higher ($P = 0.097$ to $P < 0.001$) for broilers than for pullets at both ages with the most pronounced differences observed for N retention (Table 14).

Effects of sunflower hulls inclusion

The inclusion of SFH in the diet did not affect growth performance of the birds, but increased ECR ($P < 0.01$) (Table 12). Sunflower hulls did not affect moisture content of the excreta (Table 13) or TTAR of the nutrients at any age, but improved the AME_n of the diets at 9 ($P < 0.05$) and 21 ($P < 0.01$) d of age (Table 14).

Table 12. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on growth performance of the chicks from 0 to 21 d of age

	Line		SFH		SEM ¹	P-value ⁷	
	Broiler	Pullet	0%	3%		Line	SFH
0 to 9 d							
ADG ²	21.3	6.3	13.9	13.7	0.23	0.001	0.679
ADFI ³	26.0	11.5	18.8	18.8	0.19	0.001	0.898
F:G ⁴	1.223	1.814	1.511	1.526	0.0131	0.001	0.425
EI ⁵	77.1	34.2	55.1	56.2	0.58	0.001	0.217
ECR ⁶	3.63	5.28	4.41	4.50	0.039	0.001	0.085
10 to 21 d							
ADG	40.9	9.8	25.4	25.3	0.37	0.001	0.749
ADFI	58.5	18.4	38.2	38.6	0.44	0.001	0.576
F:G	1.430	1.876	1.644	1.662	0.0093	0.001	0.183
EI	179.6	55.7	116.3	119.0	1.34	0.001	0.166
ECR	4.39	5.72	5.00	5.12	0.029	0.001	0.006
0 to 21 d							
ADG	34.5	8.7	21.6	21.6	0.27	0.001	0.956
ADFI	47.6	16.1	31.7	32.0	0.31	0.001	0.598
F:G	1.387	1.860	1.615	1.632	0.0088	0.001	0.192
EI	135.7	46.4	90.1	92.1	0.86	0.001	0.113
ECR	4.18	5.58	4.82	4.94	0.027	0.001	0.005

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

²Average daily gain (g).

³Average daily feed intake (g).

⁴Feed to gain ratio.

⁵Energy intake (kcal AME_n per bird/d).

⁶Energy conversion ratio (kcal AME_n ingested/g BW gain).

⁷The interactions between chicken line and SFH inclusion were not significant (P>0.05).

Table 13. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on moisture content of the excreta

	Line		SFH		SEM ¹	P-value ²	
	Broiler	Pullet	0%	3%		Line	SFH
9 d	79.1	80.4	79.8	79.7	0.29	0.004	0.865
21 d	75.1	73.7	74.3	74.5	0.48	0.043	0.876

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

²The interactions between chicken line and SFH inclusion were not significant (P>0.05).

Table 14. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the total tract apparent retention (TTAR, %) of the nutrients and AME_n content of the diets

	Line		SFH		SEM ¹	P-value ²	
	Broiler	Pullet	0%	3%		Line	SFH
At 9 d of age							
Dry matter	67.5	63.9	65.5	65.8	0.34	0.001	0.559
Organic matter	71.2	67.1	69.4	69.0	0.38	0.001	0.367
Nitrogen	62.5	50.5	55.8	57.1	0.69	0.001	0.185
Gross energy	72.6	71.3	71.8	72.1	0.34	0.009	0.461
AMEn (kcal/kg)	2,965	2,912	2,918	2,959	13.7	0.013	0.049
At 21 d of age							
Dry matter	68.9	67.3	67.8	68.4	0.28	0.001	0.152
Organic matter	73.0	71.6	72.3	72.4	0.27	0.001	0.669
Nitrogen	64.1	55.0	58.7	60.5	0.73	0.001	0.103
Gross energy	75.3	74.7	74.8	75.2	0.21	0.072	0.239
AMEn (kcal/kg)	3,072	3,052	3,041	3,083	8.4	0.097	0.002

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

²The interactions between chicken line and SFH inclusion were not significant (P>0.05).

2.3.2. Gastrointestinal tract traits

Effects of chicken line

In absolute terms, all the organs of the GIT were heavier (P<0.001) in broilers than in pullets (Table 15). Significant interactions between chicken line and SFH inclusion were observed for the absolute weight (g) of the proventriculus at 21 d of age (P<0.05) and of the gizzard at 9 d (P<0.05) and 21 d (P<0.001) of age (Figure 4) and for the empty weight and fresh digesta content of the gizzard at both ages (Figure 5). In relative terms (g/kg BW), however, no interactions between main effects were detected for any organ. Moreover, at 21 d of age, all the digestive organs were heavier (P<0.001) in pullets than

in broilers. Fresh digesta content (g) of the gizzard was greater in broilers than in pullets ($P<0.001$) at both ages but the DM was not affected (Table 16).

The SI and the cecum were longer ($P<0.001$) in absolute terms (cm) but shorter ($P<0.001$) in relative terms (cm/kg BW) in broilers than in pullets at both ages (Table 17). The pH of the gizzard was higher in broilers ($P<0.001$) at both ages (Table 18).

Effects of sunflower hulls inclusion

The inclusion of SFH in the diet had little effect on the absolute weight of the different organs of the GIT, except for the full proventriculus at 21 d of age that was reduced ($P<0.05$) and the full gizzard at both ages ($P<0.001$) that was increased (Table 15). In relative terms, the only differences observed were for the weight of the GIT at 21 d of age ($P<0.05$) and of the gizzard at both ages ($P<0.001$) that increased with SFH inclusion.

The empty weight ($P<0.001$) and the fresh digesta content (g) of the gizzard at 9 ($P<0.01$) and 21 ($P<0.001$) d of age increased with SFH inclusion (Table 16). Sunflower hulls did not affect the absolute or relative length of the SI and cecum (Table 17) but decreased the pH of the gizzard ($P<0.001$) and cecum ($P<0.05$ to $P<0.01$) at both ages (Table 18).

Table 15. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the absolute (g) and relative (% BW) weight of the organs of the gastrointestinal tract (GIT) of the chicks

	Line		SFH		SEM ⁵	P-value ⁶	
	Broiler	Pullet	0%	3%		Line	SFH
At 9 d of age							
BW ¹ (g)	197	82	141	139	4.5	0.001	0.735
Absolute weight (g) ²							
GIT	39.3	15.6	27.2	27.6	0.92	0.001	0.637
Proventriculus ³	2.16	1.01	1.59	1.58	0.059	0.001	0.764
Gizzard ⁴	11.3	5.5	7.69	9.08	0.355	0.001	0.001
Pancreas	0.87	0.35	0.64	0.58	0.043	0.001	0.160
Liver	8.74	3.56	6.18	6.11	0.203	0.001	0.726
Relative weight (% BW)							
GIT	20.0	19.0	19.2	19.9	0.64	0.131	0.326
Proventriculus	1.10	1.24	1.17	1.17	0.043	0.002	1.000
Gizzard	5.74	6.72	5.74	6.72	0.259	0.001	0.001
Pancreas	0.44	0.43	0.46	0.41	0.027	0.481	0.088
Liver	4.44	4.35	4.41	4.38	0.156	0.584	0.887
At 21 d of age							
BW (g)	786	224	510	500	16.3	0.001	0.353
Absolute weight (g) ²							
GIT	92.1	31.0	61.3	61.9	2.11	0.001	0.716
Proventriculus	4.50	1.87	3.31	3.07	0.168	0.001	0.045
Gizzard	24.2	10.1	14.6	19.7	1.07	0.001	0.001
Pancreas	2.74	0.96	1.83	1.86	0.117	0.001	0.733
Liver	27.6	9.11	18.8	17.9	0.79	0.001	0.129
Relative weight (% BW)							
GIT	11.8	13.9	12.6	13.1	0.31	0.001	0.027
Proventriculus	0.57	0.84	0.72	0.70	0.027	0.001	0.300
Gizzard	3.09	4.51	3.26	4.35	0.158	0.001	0.001
Pancreas	0.35	0.43	0.38	0.40	0.021	0.001	0.208
Liver	3.52	4.07	3.84	3.75	0.107	0.001	0.276

¹Average of 2 and 4 birds at 9 and 21 d of age, respectively.

²Full gastrointestinal tract without the annex organs (pancreas, spleen, and the liver).

³Full proventriculus.

⁴Full gizzard.

⁵Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 and 4 birds per replicate at 9 and 21 d of age, respectively.

⁶The interactions between chicken line and SFH inclusion were not significant for the relative weight of all the organs (P>0.05).

Table 16. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the absolute (g) and relative (% BW) empty gizzard weight and gizzard digesta characteristics of the chicks¹

	Line		SFH		SEM ²	P-value ³		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
At 9 d of age								
Absolute weight	6.63	3.50	4.74	5.39	0.155	0.001	0.001	0.031
Relative weight	3.39	4.28	3.58	4.09	0.134	0.001	0.001	0.821
Fresh digesta content (g)	4.61	2.00	2.96	3.65	0.259	0.001	0.009	0.053
Absolute empty:full gizzard weight	59.7	64.1	62.8	60.9	1.54	0.007	0.236	0.501
Digesta DM (%)	36.2	36.8	36.4	36.5	0.52	0.288	0.913	0.805
At 21 d of age								
Absolute weight	14.4	6.83	9.39	11.9	0.44	0.001	0.001	0.001
Relative weight ²	1.85	3.06	2.17	2.73	0.077	0.001	0.001	0.238
Fresh digesta content (g)	9.81	3.26	5.23	7.83	0.745	0.001	0.001	0.004
Absolute empty:full gizzard weight	61.4	68.3	67.3	62.4	1.85	0.001	0.001	0.176
Digesta DM (%)	36.5	37.1	36.7	36.9	0.46	0.363	0.824	0.877

¹The average BW of the birds used is shown in table 15.

²Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 and 4 birds per replicate at 9 and 21 d of age, respectively.

³The interactions between chicken line and SFH inclusion were significant (P<0.05) for the absolute weight of the gizzard at 9 d (P<0.05) and 21 d (P<0.001) and for the fresh digesta content at 9 d (P=0.053). See Figures 1 and 2, respectively.

Figure 4. Interaction between chicken line (Line) and sunflower hulls (SFH) inclusion on the absolute weight of the full proventriculus at 21 d (A) and full gizzard at 9 d (B) and 21 d (C) of age.

Figure 5. Interaction between chicken line (Line) and sunflower hull (SFH) inclusion in the diet on the absolute weight of the empty gizzard at 9 d (A) and 21 d (B) of age, and on the fresh digesta content of the gizzard at 9 d (C) and 21 d (D) of age.

Table 17. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the absolute (cm) and relative length (cm/kg BW) of the small intestine (SI) and the cecum of the chicks¹

	Line		SFH		SEM ⁴	P-value ⁵	
	Broiler	Pullet	0%	3%		Line	SFH
At 9 d of age							
Absolute length							
SI ²	114	74	93.2	94.3	2.00	0.001	0.607
Cecum ³	8.93	6.39	7.50	7.82	0.26	0.001	0.221
Relative length							
SI	581	907	739	749	25.4	0.001	0.698
Cecum	44.6	75.5	58.7	61.3	2.80	0.001	0.356
At 21 d of age							
Absolute length							
SI	145	94.0	119	120	2.20	0.001	0.647
Cecum	14.1	9.43	11.7	11.8	0.36	0.001	0.889
Relative length							
SI	186	419	303	307	7.86	0.001	0.166
Cecum	18.0	41.6	29.6	30.0	1.31	0.001	0.715

¹The average BW of the birds used is shown in table 15.

²Small intestine (duodenum, jejunum, and ileum).

³Length of the 2 cecum.

⁴Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 and 4 birds per replicate at 9 and 21 d of age, respectively.

⁵The interactions between chicken line and SFH inclusion were not significant (P>0.05).

Table 18. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the pH of the organs of the gastrointestinal tract of the chicks¹

	Line		SFH		SEM ²	P-value ³	
	Broiler	Pullet	0%	3%		Line	SFH
At 9 d of age							
Crop	4.32	4.35	4.32	4.35	0.023	0.267	0.465
Proventriculus	2.43	2.41	2.43	2.42	0.021	0.454	0.645
Gizzard	2.70	2.40	2.58	2.52	0.012	0.001	0.001
Duodenum	5.76	5.79	5.77	5.79	0.016	0.128	0.271
Jejunum	5.68	5.72	5.68	5.71	0.018	0.161	0.276
Ileum	6.37	6.38	6.37	6.39	0.020	0.980	0.352
Cecum	5.93	5.88	5.94	5.87	0.021	0.133	0.018
At 21 d of age							
Crop	4.52	4.49	4.49	4.52	0.014	0.108	0.141
Proventriculus	2.46	2.47	2.47	2.46	0.013	0.418	0.340
Gizzard	2.57	2.37	2.55	2.37	0.020	0.001	0.001
Duodenum	6.07	6.06	6.05	6.09	0.017	0.635	0.198
Jejunum	5.90	5.95	5.91	5.94	0.018	0.111	0.365
Ileum	6.65	6.69	6.67	6.68	0.019	0.161	0.711
Cecum	6.01	5.96	6.05	5.93	0.024	0.148	0.002

¹The pH of the drinking water was 6.88.

²Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 birds per replicate at 9 and 21 d of age.

³The interactions between chicken line and SFH inclusion were not significant (P>0.05).

2.3.3. Short chain fatty acid concentration and ileum morphology

No interactions between chicken line and SFH inclusion were detected for any of the traits studied and therefore, only main effects are presented.

Effects of chicken line

Broilers tended to have higher concentration of total SCFA (P=0.098) and higher proportion of acetate (P=0.077) but lower of valerate (P=0.052) than pullets (Table 19). Chicken line affected (P<0.001) the mucosa structure of the ileum, with broilers showing higher VH and CD than pullets (Table 20).

Effects of sunflower hulls inclusion

The inclusion of SFH in the diet did not affect the concentration of total SCFA or the FA profile in the cecum (Table 19). Similarly, ileum mucosa structure was not affected by SFH inclusion (Table 20).

Table 19. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet short chain fatty acids (SCFA) concentration ($\mu\text{mol/g}$ fresh digesta) and proportion fatty acids proportion (% SCFA) in the cecum at 21 d of age

	Line		SFH		SEM ¹	P-value ²	
	Broiler	Pullet	0%	3%		Line	SFH
SCFA ($\mu\text{mol/g}$)	83.03	74.32	78.17	79.18	3.60	0.098	0.845
Fatty acid (% of total SCFA)							
Acetate	78.2	75.9	76.2	77.9	0.89	0.077	0.215
Propionate	4.95	5.66	4.89	5.72	0.49	0.322	0.254
Butyrate	14.81	15.46	16.07	14.20	0.91	0.619	0.158
Isobutyrate	0.397	0.391	0.368	0.420	0.095	0.964	0.704
Isovalerate	0.717	1.438	1.388	0.767	0.379	0.175	0.347
Valerate	0.926	1.151	1.084	0.993	0.078	0.052	0.413

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 birds per replicate.

²The interactions between chicken line and SFH inclusion were not significant (P>0.05).

Table 20. Influence of chicken line (Line) and inclusion of 3% sunflower hulls (SFH) in the diet on ileal mucosa morphology at 21 d of age

	Line		SFH		SEM ¹	P-value ³	
	Broiler	Pullet	0%	3%		Line	SFH
Villus height (μm)	1,075	931	1,001	999	21.9	0.001	0.775
Crypt depth (μm)	132	122	127	127	2.41	0.012	0.791
VH:CD ²	8.14	7.64	7.86	7.90	0.056	0.030	0.942

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 birds per replicate.

²Villus height (VH) to crypt depth (CD) ratio ($\mu\text{m}/\mu\text{m}$).

³The interactions between chicken line and SFH inclusion were not significant (P>0.05).

2.4. Discussion

Significant interactions between main effects were observed for the absolute weight of the full proventriculus at 21 d and gizzard at 9 and 21 d of age and for the empty weight and fresh digesta content of the gizzard at both ages. The interactions detected showed a similar trend, with SFH showing more pronounced effects in broilers than in pullets (Figures 1 and 2). Consequently, the discussion will focus mostly on main effects.

2.4.1. Growth performance and *TTAR* of nutrients

Effects of chicken line

Growth performance was greater for broilers than for pullets at all ages. From 0 to 21 d of age, ADFI and ADG were 3 and 4 times greater and F:G ratio was 25% better for broilers, consistent with the objectives of the current programs of selection for poultry based on fast growth rate and feed efficiency for broilers and harmonic growth of body tissues during the rearing phase for pullets. In the current research, female broilers grew 15.4% less than expected based on the Ross (2014) guidelines whereas pullets grew 16.0% faster than indicated in the Lohmann (2014) guidelines. The lower than expected ADG observed in broilers was caused primarily by the feeding of mash diets as reported by Serrano et al. (2012) and Jiménez-Moreno et al. (2016). On the other hand, the higher than expected ADG reported for pullets fed a broiler diet suggests that modern layer strains might respond to increases in the nutrient content of the starter diet with improved growth.

Nutrient retention and AME_n of the diet increased with age, irrespective of chicken line, consistent with most published research in broilers (Batal and Parsons, 2002; Gracia et al., 2009; Kim and Corzo, 2012). In the current research, nutrient retention was higher in broilers than in pullets, with the most pronounced effects observed for N. In contrast, Walugembe et al. (2014) reported that the AME_n of 2 isonutritive diets that varied widely

in NDF content, were similar for both chicken lines. Modern broiler strains are more voracious and had shorter retention time of the digesta in the proximal part of the GIT than pullets, which may have a negative effect on nutrient digestibility. Kaminiska (1979) reported that at the same age, pullets utilized feed energy more efficiently than broilers, suggesting that the longer SI and reduced rate of digesta passage through the GIT was the main reason for the improvement observed. Uni et al. (1995b), however, observed that at 4 d of age broilers secreted more amylase, trypsin, and lipase per gram of feed and had better villus development and higher nutrient digestibility than pullets. The greater enzyme production and absorption capacity of broilers may counteract the negative effects of a faster rate of passage and the final outcome might depend on factors such as the age of the experimental birds and the physico-chemical characteristics (e.g. mash vs. pellets) of the experimental diets. In this respect, little effect of rate of feed passage on nutrient digestibility was expected in the current research, because the diets were fed as mash, and mash diets have a slower rate of passage through the upper part of the GIT than pelleted diets (Serrano et al., 2013; Svihus, 2014). The lower N retention observed in pullets was expected because pullets require less protein and have lower capacity for N retention than broilers. In addition, the diet used (pre-starter broiler feed) contained more CP than a regular commercial pullet diet, increasing further N excretion. The data suggest that the greater enzyme production and better development of the ileum villus surface of modern broiler strains overcome the problems related to their faster rate of passage, resulting in a net increase in nutrient retention.

Excreta moisture was higher in pullets than in broilers at 9 d of age but an opposite effect was observed at 21 d, although, the differences were of small magnitude. An excess of dietary N increases uric production and eventually, water intake. Consequently, moisture content of the excreta will increase, with effects being more pronounced in

pullets than in broilers, consistent with the results reported for 9 d-old chicks. Other mechanisms, however, might regulate and affect water metabolism and excretion in older birds. In this respect, van der Hoeven-Hangoor et al. (2014) reported that the moisture content of the excreta increased as feed intake increased and Shires et al. (1987) observed that broilers had faster rate of feed passage and greater feed intake than pullets. Consequently, the moisture content of the excreta should be higher in broilers than in pullets, in agreement with the results reported herein for chicks at 21 d of age.

Effects of sunflower hulls inclusion

The dilution of the diet with 3% SFH did not affect chick performance at any age. The authors have not found any research comparing the effects of dietary SFH on growth performance of these 2 chicken lines. In pullets, Guzmán et al. (2015a) observed a 6.4% increase in ADG from 0 to 21 d of age with the inclusion of 2% SFH in the diet. Similar results were reported by González-Alvarado et al. (2007) and Jiménez-Moreno et al. (2011, 2013a, 2016) in broilers. In contrast, Walugembe et al. (2014) observed that the inclusion of 16% of a combination of dried distiller's grains with solubles (DDGS) and wheat bran, in isocaloric diets based on corn and soybean meal, reduced ADG and voluntary FI in broilers but not in pullets. Probably, the high fiber content (15% NDF) of the diet used by Walugembe et al. (2014) caused the 9.3% reduction in feed intake in broilers. However, no effects of fiber on feed intake in any of the 2 chicken lines were observed in the current research in which the NDF content of the SFH diet was 10.3%.

Sunflower hulls did not affect moisture content of the excreta at any age but visually, the appearance and consistency of the excreta improved when SFH was included in the diet (data not shown). These results agree with data of Jiménez-Moreno et al. (2016) in broilers fed diets that contained 2.5 or 5.0% SFH. Moreover, van der Hoeven-Hangoor

et al. (2014) reported drier excreta when the control diet was supplemented with 2.5% OH.

In general, SFH inclusion improved nutrient retention although the differences were not significant. In fact, the AME_n of the diet, increased significantly with SFH inclusion at both ages. Jiménez-Moreno et al. (2013a) and González-Alvarado et al. (2010) in broilers reported also a 2.1% and 4.4% increase in the energy content of the diet with the inclusion of 2.5 and 3.0% OH, respectively. In this respect, Hetland et al. (2003) showed that OH inclusion increased amylase activity and bile acid concentration in the jejunum of laying hens fed a corn-soybean meal diet. Moreover, Yokhana et al. (2016) showed an increase in proteolytic enzyme activity in the pancreas of 13 wk-old pullets fed a diet supplemented with 1% of a fiber source with 65% crude fiber and 20% lignin (Arbocel; JRS Co. Inc., Rosenberg, Germany). Also, Kalmendal et al. (2011) reported higher ether extract digestibility when 20% sunflower meal was included in the diet, although in this research, DM digestibility and AME_n of the diet decreased. In the current experiment, the control and the experimental diets contained 3.0 and 4.4% crude fiber whereas in the research of Kalmendal et al. (2011) the values were 2.6 and 8.0%, respectively. Jiménez-Moreno et al. (2013a) reported that the inclusion of up to 5% OH in a low fiber diet increased nutrient retention in broiler but that a further increase to 7.5% tended to reduce it.

2.4.2. Gastrointestinal tract traits

Effects of chicken line

In absolute terms, the GIT was heavier and the SI and cecum were longer in broilers than in pullets, in agreement with most published reports (Nir et al., 1993; Mahagna and Nir, 1996; Zavarize et al., 2012). In relative terms, however, an opposite effect was observed in most cases. In fact, at 21 d of age, the relative weight of all the organs of the

GIT, and the relative length of the small intestine and cecum, were significantly greater in pullets. Similar data have been reported by Mahagna and Nir (1996) and Zavarize et al. (2012) comparing broilers and pullets at young ages. The data suggest that when comparing chicken lines with marked differences in rate of growth, the interest of comparing in relative terms the weight and length of the GIT organs, should be taken cautiously (Buzala et al., 2015).

Chicken line did not affect the pH of the different segments of the GIT, except for gizzard pH that was reduced at both ages. Shires et al. (1987) observed that the retention time of the digesta in the upper part (proventriculus and gizzard) of the GIT was longer in 86 d-old pullets than in 44 d-old broilers (86 vs. 54 min). A longer retention time of the digesta in the gizzard increases HCl concentration and reduces pH (Jiménez-Moreno et al., 2009a), consistent with the results reported herein. In addition, the digesta content was proportionally lower in pullets than in broilers, which results in higher proportion of HCl per gram of digesta and consequently, in a reduction in pH.

Effects of sunflower hulls inclusion

Fiber inclusion had little effect on the development of the GIT in both chicken lines, except for the gizzard that was heavier and had higher fresh digesta content and lower pH with additional SFH. In pullets, Guzmán et al. (2015b) reported also a 8.7% increase in gizzard weight at 35 d of age with the inclusion of 4% straw in the diet. Moreover, González-Alvarado et al. (2008) observed that the inclusion of 3% OH increased gizzard weight by 32% in 22 d-old broilers, in agreement with the data reported herein.

Most available data in broilers (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009b; 2013b; Sacranie et al., 2012) and pullets (Guzmán et al., 2015b) observed a reduction in gizzard pH with the inclusion of insoluble fiber sources in the diet. Highly lignified insoluble fiber sources, such as OH and SFH are retained for longer in the

gizzard, resulting in an increase in HCl production and consequently, in a reduction in gizzard pH (Mateos et al. 2012).

2.4.3. Short chain fatty acid in the cecum and ileal morphology

Effects of chicken line

Substrates entering the cecum are a mixture of digesta fractions that escaped from digestion in the upper part of the GIT, with SCFA being the major end products. In the current research, the concentration of total SCFA was higher in broilers than in pullets. Walugembe et al. (2015) suggested that the higher feed intake in broilers as compared with pullets provided more nutrients to the microorganisms of the cecum, resulting in higher SCFA production. In this respect, the cecum was longer in broilers than in pullets which may be indicative of a higher potential for fermentation of the digesta.

The proportion of valerate and isovalerate in the cecum was higher in pullets than in broilers. Most SCFA produced in the gut results from the fermentation of complex carbohydrates, but proteins and peptides are also net contributors. In fact, these SCFA originate primarily from the oxidative deamination and decarboxylation of the branched chain amino acids (Allison, 1978; Andries et al., 1987). The higher proportion of these two FA reported for pullets was consistent with the excess of protein of the diets as compared with their requirements, with more N compounds available for fermentation in the cecum.

The VH and CD of the ileum mucosa were higher in broilers than in pullets, in agreement with data of Uni et al. (1995b) and consistent with the improved nutrient digestibility observed for broilers. Zavarize et al. (2012) reported also higher VH and CD in broilers than in pullets at 7 d of age, although in this research the difference in CD was not significant.

Effects of sunflower hulls inclusion

The inclusion of SFH in the diet did not affect the concentration of total SCFA in the cecum, in agreement with the results of Walugembe et al. (2015). Sunflower hulls did not affect the structure of the ileal mucosa. In contrast, Kalmendal et al. (2011) reported a linear decrease in VH as the level of sunflower meal increased from 0 to 30%. In the research of Kalmendal et al. (2011), the crude fiber content of the diet increased from 2.3 to 11.0% with sunflower meal inclusion, whereas in the current research, the increase was from 3.0 to 4.4%. In this respect, Jiménez-Moreno et al. (2011) reported that the inclusion of 2.5% pea hulls in the diet tended to increase VH in the jejunum mucosa but that a further increase to 7.5% had an opposite effect.

In summary, broilers grew faster and had greater nutrient digestibility and ileum absorption capacity than pullets. The inclusion of 3% SFH at the expense of the whole diet (wt:wt) reduced the pH of the gizzard and improved the AME_n content of the diet, without showing any negative effect on chick performance. Broilers and pullets responded in a similar way to an increase in the insoluble fiber content of the diet. The lack of interactions between chicken lines and SFH inclusion for most of the variables studied, suggests that the information available on the inclusion of insoluble fiber sources in broiler diets might be used, at some extent, in research conducted with pullets.

CHAPTER 3

A comparative study on the effects of dietary sunflower hulls on growth performance and digestive tract traits of broilers and pullets fed a pullet diet from 0 to 21 days of age

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3.1. Introduction

Current genetic selection programs for poultry aim to improve growth rate, feed to gain ratio (F:G) and meat yield in broilers and to maximize future egg production in pullets. Consequently, the two chicken lines differ not only in voluntary feed intake and body weight (BW) gain but also in basal metabolism (Kuenzel and Kuenzel, 1977), protein turnover rate (Jones et al., 1986; Saunderson and Leslie, 1988) and organ development (Lilburn and Loeffler, 2015; Kimiaetalab et al., 2016)

Under commercial conditions, the inclusion of fibrous ingredients is limited in diets for young birds because of its negative impact on feed intake and nutrient utilization (Sklan et al., 2003; Rougiere and Carré, 2010). Recent researches, however, have shown that moderate amounts of certain insoluble fiber sources, such as oat hulls (OH) and sunflower hulls (SFH), stimulates the development and health of the gastrointestinal tract (GIT) in broilers (González-Alvarado et al., 2008; Svihus, 2011; Sacranie et al., 2012). However, few researches are available on the effects of fiber on growth performance, GIT traits and nutrient digestibility in pullets. Kimiaetalab et al. (2016) reported that SFH inclusion had similar effects on growth, nutrient retention and villus development in both chicken lines. Walugembe et al. (2014), however, observed that the inclusion of 160 g/kg of a mixture of a wheat distiller's dried grains with solubles (DDGS) and wheat bran, reduced feed intake and BW gain in broilers but had no effects in pullets. The reasons for these discrepancies, however, are not well understood.

In commercial practice, pullet diets contain less energy and crude protein (CP) and more neutral detergent fiber (NDF) than broiler diets. Moreover, because of their lower energy requirements, pullets are fed often diets based on wheat and barley with limited use of maize. As a consequence differences, in chick growth and in the characteristics and ingredient composition of the diets, the two chicken lines might respond in different

ways to additional fiber. The aim of this experiment was to compare the effects of the inclusion of moderate amount of an insoluble fiber source in the diet, on growth performance, nutrient retention and GIT development of broilers and pullets fed a pullet diet in mash form from 0 to 21 d of age. A second objective was to compare the data of the current research, using a pullet diet based on wheat and barley, with data of a previous research (Kimiæitalab et al., 2016) in which both chicken lines were fed a broiler diet based on maize.

3.2. Materials and methods

3.2.1. Sunflower hulls and diets

The physico-chemical characteristics of the batch of the SFH were reported by Kimiæitalab et al. (2016). Briefly, SFH contained 4.7% CP and 70% NDF and had a geometric mean diameter $\pm$ geometric standard deviation of $675 \pm 1.68 \mu\text{m}$.

The control diet used in this experiment contained 2,850 kcal AME_n, 19.1% crude protein, 0.98 % digestible Lys and 10.1% NDF, and included 2% of celite, an acid-washed diatomaceous earth (Celite Hispanica S.A., Alicante, Spain), to increase the acid insoluble ash content of feeds and excreta. The fiber containing diet was similar to the control diet but was diluted (wt:wt) with 3% SFH. Consequently, the experimental diet had less AME_n and CP and more NDF than the control diet (Table 21). Both diets, however, met or exceeded the nutritional requirements of pullets (FEDNA, 2008).

3.2.2. Husbandry and experimental design

The husbandry and care of the chicks were similar to those reported by Kimiæitalab et al. (2016). Briefly, 280 one day-old chicks, 140 Ross 308 female broilers and 140 Lohmann Brown Classic pullets were used. The birds were housed at random in groups of 10 according to chicken line, in 28 battery cages. The experiment was conducted as a completely randomized design with 4 treatments arranged as a 2×2 factorial with two

chicken lines (female broilers vs. pullets) and two levels of dietary SFH (0 vs. 3%). Each treatment was replicated seven times and the experimental unit was a cage with 10 birds.

Table 21. Ingredient composition and calculated and determined chemical analysis of the experimental diets (% as-fed basis).

Ingredient	SFH	
	0%	3%
Wheat	44.0	42.7
Barley	20.1	19.5
Soybean meal, 47% CP	27.6	26.8
Lard	2.83	2.75
Sunflower hulls	0.0	3.00
Calcium carbonate	0.98	0.95
Dicalcium phosphate	1.52	1.47
Sodium chloride	0.34	0.33
<i>L</i> -Lysine HCL, 78%	0.10	0.10
Methionine-OH, 88%	0.23	0.22
Celite ¹	2.0	1.94
Vitamin and mineral premix ²	0.30	0.29
Calculated analyses ³		
AMEn (kcal/kg)	2,850	2,777
Crude fiber	3.2	4.6
Calcium	1.0	0.97
Available phosphorous	0.44	0.43
Digestible amino acids		
Arginine	1.21	1.17
Lysine	0.98	0.95
Methionine	0.49	0.48
Methionine + Cysteine	0.80	0.78
Threonine	0.63	0.61
Tryptophan	0.22	0.21
Determined analyses		
Dry matter	91.9	92.0
Total ash	5.7	5.9
Crude protein	19.1	18.7
Ether extract	3.9	4.1
Neutral detergent fiber	10.1	12.1
Acid detergent fiber	5.1	6.9
Acid detergent lignin	1.4	1.6
Particle size distribution (µm)		
>2,500	4.1	3.3
1,250	37.6	29.0
630	34.8	39.3
315	14.8	17.3
160	8.6	11.1
<80	0.1	0.2
GMD ± GSD ⁴ (µm)	962 ± 2.01	867 ± 2.01

¹Acid washed diatomaceous earth (Celite Hispánica, S.A., Alicante, Spain).

²Provided the following per kilogram of diet: vitamin A (trans-retinyl acetate), 11,500 IU; vitamin D3 (cholecalciferol), 2,800 IU; vitamin E (all-rac-tocopherol-acetate), 40 IU; vitamin B₁, 2 mg; vitamin B₂, 8 mg; vitamin B₆, 4 mg; vitamin B₁₂

(cyanocobalamin), 0.025 mg; vitamin K₃ (bisulphate menadione complex), 3 mg; choline (choline chloride), 300 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; D-biotin, 150 µg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg; iron (FeCO₃), 60 mg; copper (CuSO₄, 5 H₂O), 8 mg; iodine (KI), 2 mg; selenium (Na₂SeO₃), 0.2 mg; Roxazyme, 200 mg [1,600 U endo-1,4-β-glucanase (EC 3.2.1.4), 3,600 U endo-1,3 (4)-β-glucanase (EC 3.2.1.6) and 5,200 U endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM, S.A., Madrid, Spain; and Natuphos 5000, 60 mg [300 Phytase units (FTU)/kg] supplied by BASF Española S.A., Tarragona, Spain.

³According to FEDNA (2010).

⁴Geometric mean diameter ± log geometric standard deviation.

3.2.3. Growth performance and TTAR of nutrients

Average daily feed intake (ADFI), BW gain and feed to gain ratio (F:G) were determined by period (0 to 9 d and 10 to 21 d) and for the entire experiment (0 to 21 d). The total tract apparent retention (TTAR) of dry matter (DM), organic matter (OM), nitrogen (N) and gross energy (GE) of the diets were determined at the end of the two experimental periods. Energy intake (EI; kcal AME_n/day) and energy conversion ratio (ECR; kcal AME_n ingested/g BW gain) were estimated, using the average AME_n content of the diets determined at 9 and 21 d of age.

3.2.4. Gastrointestinal tract traits

Details on the procedures used for measuring the GIT traits were presented by Kimiaieitalab et al. (2016). Briefly, two birds per replicate were euthanized by CO₂ asphyxiation after each growth performance control. The GIT was excised and divided into seven portions (crop, proventriculus, gizzard, duodenum, jejunum, ileum, and cecum) and the pH of all these segments was measured in situ as indicated by Jiménez-Moreno et al. (2009). In addition, two extra birds at 9 d and four extra birds at 21 d of age were slaughtered, and the full GIT, from the post-crop to the cloaca, was excised and weighed. The weight of the annex organs (liver, spleen and pancreas) were not included in the determination of the weight of the full GIT. The weights of the full proventriculus, full gizzard, liver and pancreas were measured also in these birds. In addition, the weight

of the empty gizzard and its contents, and the DM of the gizzard digesta were determined. Because of the extreme differences in BW and ADFI between broilers and pullets, data on organ weight were expressed in absolute (g) and relative (% BW) terms. The length of the small intestine (SI; duodenum, jejunum and ileum) and of the two cecum was measured also in all these birds and the results expressed in absolute (cm) and relative (cm/kg BW) terms. The concentration SCFA in cecum samples ($\mu\text{mol/g}$ fresh digesta) and the villus height (VH) and crypt depth (CD) in ileum tissue samples were measured at 21 d of age in two of the birds used for pH determination, as indicated by Kimiaeitalab et al. (2016).

3.2.5. Statistical analysis

Data were analyzed as a completely randomized design with four treatments arranged as a 2×2 factorial using the GLM program of SAS (1990). The model included the chicken line (female broilers vs. pullets) and SFH inclusion (0 vs. 3%) as main effects as well as their interactions. The experimental unit was the cage for all traits. Differences between treatment means were considered significant at $P < 0.05$ and tendencies at $0.05 \leq P \leq 0.10$.

3.3. Results

The determined analyses of the experimental diets were close to expected values, confirming that the feeds were manufactured correctly. The mean particle size of the control and the SFH containing diet, expressed as the geometric mean diameter $\pm$ geometric standard deviation was 962 ± 2.01 and $867 \pm 2.01 \mu\text{m}$, respectively.

3.3.1. Growth performance and coefficient of TTAR of nutrients

Mortality was low (1.0%) and not related to any of the treatments (data not shown). The only interaction detected between chicken line and inclusion of SFH in the diet was for moisture content of the excreta at 21 d of age (Figure 6).

Effects of chicken line

Feed intake and BW gain were greater ($P<0.001$) and F:G was better ($P<0.001$) in broilers than in pullets at all ages (Table 22). From 0 to 21 d of age, ECR was better ($P<0.001$) for broilers than for pullets (Table 23).

Moisture content of the excreta was higher ($P<0.001$) in broilers than in pullets at 9 d of age but no differences were detected at 21 d of age (Table 24). The TTAR of all nutrients increased with age and were higher ($P<0.01$ to $P<0.001$) for broilers than for pullets at 9 d of age. At 21 d of age, however, the only difference detected was for N that was higher ($P<0.001$) in broilers (Table 25).

Effects of sunflower hulls inclusion

The dilution of the diet with 3% SFH did not affect chick performance or TTAR of nutrients at any age. The AME_n of the diet increased ($P<0.05$) with SFH inclusion at 21 d of age but no differences were detected at 9 d (Tables 25). Sunflower hulls did not affect moisture content of the excreta at 9 d of age. At 21 d of age, however, SFH inclusion reduced the moisture content of the excreta in broilers but had no effects in pullets ($P<0.05$ for the interaction) (Figure 6).

Table 22. Influence of the chicken line and the inclusion of sunflower hulls (SFH) in the diet on growth performance from 0 to 21 days of age.

Age	Line		SFH		SEM ¹	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
0 to 9 d								
BWG ²	15.7	4.5	10.1	10.0	0.08	0.001	0.479	0.409
ADFI ³	21.5	10.2	15.8	15.9	0.07	0.001	0.270	0.941
F:G ⁴	1.371	2.287	1.819	1.839	0.0146	0.001	0.360	0.816
10 to 21 d								
BWG	30.7	7.7	19.4	19.1	0.33	0.001	0.422	0.272
ADFI	52.3	17.9	35.2	35.0	0.49	0.001	0.675	0.316
F:G	1.704	2.307	2.000	2.012	0.0133	0.001	0.487	0.881
0 to 21 d								
BWG	24.3	6.3	15.4	15.2	0.20	0.001	0.366	0.231
ADFI	39.1	14.5	26.9	26.8	0.28	0.001	0.788	0.346
F:G	1.611	2.301	1.949	1.963	0.0116	0.001	0.379	0.863

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

²BW gain (g).

³Average daily feed intake (g).

⁴Feed to gain ratio.

Table 23. Influence of the chicken line and the inclusion of sunflower hulls (SFH) in the diet on calculated and determined energy intake (EI, kcal AME_n/d) and energy conversion ratio (ECR, kcal AME_n ingested/g BW gain) from 0 to 21 days of age.

P-value								
	Line		SFH		SEM ¹	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
Calculated								
EI	110.2	40.9	76.7	74.3	0.79	0.001	0.044	0.079
ECR	4.53	6.49	4.98	4.89	0.033	0.001	0.031	0.495
Determined ²								
EI	109.2	39.4	74.3	74.3	3.3	0.001	0.848	0.821
ECR	4.48	6.21	5.31	5.38	0.031	0.001	0.130	0.370

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

²The AME_n used for estimation was the average of values determined at 9 and 21 d of age from 10 to 21 d of age (See Table 25).

Table 24. Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on moisture content (%) of the excreta.

Age	Line		SFH		SEM ¹	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
9 d	79.8	82.2	81.1	80.9	0.43	0.001	0.687	0.845
21 d	74.6	73.0	74.1	73.5	0.25	0.001	0.104	0.034

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

Table 25. Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the of total tract apparent retention (TTAR) of the nutrients and AME_n content of the diets.

TTAR	Line		SFH		SEM ¹	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
At 9 d of age								
Dry matter	69.5	64.5	67.3	66.6	0.71	0.001	0.484	0.663
Organic matter	71.7	66.5	69.4	68.7	0.67	0.001	0.465	0.899
Nitrogen	56.9	47.2	51.0	53.1	1.09	0.001	0.196	0.537
Gross energy	71.6	68.0	70.0	69.5	0.72	0.002	0.612	0.862
AMEn (kcal/kg)	2,734	2,610	2,667	2,677	26.8	0.003	0.800	0.926
At 21 d of age								
Dry matter	72.3	72.1	72.1	72.4	0.39	0.734	0.658	0.639
Organic matter	74.8	74.6	74.8	74.7	0.44	0.761	0.866	0.480
Nitrogen	61.5	54.5	57.6	58.5	0.94	0.001	0.509	0.629
Gross energy	75.5	75.6	75.6	75.6	0.34	0.883	0.906	0.334
AMEn (kcal/kg)	2,885	2,899	2,873	2,911	12.4	0.424	0.044	0.263

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

Figure 6. Interaction between chicken line and SFH inclusion in the diet on moisture content of the excreta at 21 d of age.

3.3.2. Gastrointestinal tract traits

Effects of chicken line

In absolute terms, all the organs of the GIT were heavier ($P < 0.001$) in broilers than in pullets (Table 26). In relative terms, however, an opposite effect was observed in some of the organs. In this respect, at 9 d of age the relative weights of the full proventriculus ($P = 0.06$) and full and empty gizzard ($P < 0.001$) were lower in broilers than in pullets, but an opposite effect was observed for the full GIT ($P < 0.001$), pancreas ($P < 0.05$) and liver ($P < 0.05$). At 21 d of age, pullets had heavier (% BW) full GIT ($P = 0.062$), full proventriculus ($P < 0.001$), full and empty gizzard ($P < 0.001$) and pancreas ($P < 0.01$) than broilers (Tables 26 and 27). In absolute terms, the fresh digesta content of the gizzard was greater ($P < 0.001$) in broilers at both ages but the DM content of the digesta was not affected by chicken line (Table 27).

In absolute terms, the SI and cecum were longer ($P < 0.001$) in broilers than in pullets but in relative terms an opposite effect ($P < 0.001$) was observed (Table 28). The pH of the different organs of the GIT were not affected by chicken line (Table 29).

Effects of sunflower hulls inclusion

Sunflower hulls inclusion did not affect the characteristics of the gizzard or the weight (g or % BW) and the pH of the organs of the GIT at any age (Tables 26, 27 and 29). Sunflower hulls increased the absolute length of the GIT, with differences that were significant for the SI ($P<0.05$) at 21 d and for the cecum ($P\leq 0.01$) at both ages. In relative terms, an opposite effect was observed for the cecum ($P<0.05$) at 9 d of age (Table 28).

Table 26. Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the absolute (g) and relative (% BW) weight of the organs of the gastrointestinal tract (GIT) of the chicks.

	Line		SFH		SEM ¹	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
At 9 d								
BW ² (kg)	0.158	0.71	0.113	0.116	0.018	0.001	0.263	0.639
Absolute weight (g)								
GIT ³	37.5	15.1	25.9	26.7	0.59	0.001	0.330	0.418
Proventriculus ⁴	1.69	0.84	1.29	1.24	0.033	0.001	0.254	0.249
Gizzard ⁵	11.1	5.71	8.17	8.63	0.195	0.001	0.111	0.208
Pancreas	0.87	0.35	0.63	0.59	0.029	0.001	0.319	0.319
Liver	7.28	3.10	5.15	5.23	0.120	0.001	0.654	0.275
Relative weight (% BW)								
GIT	23.9	21.2	22.6	22.6	0.42	0.001	0.941	0.665
Proventriculus	1.09	1.18	1.16	1.10	0.032	0.060	0.209	0.472
Gizzard	7.05	8.0	7.46	7.59	0.168	0.001	0.579	0.528
Pancreas	0.560	0.484	0.542	0.502	0.024	0.037	0.262	0.402
Liver	4.60	4.35	4.52	4.43	0.065	0.014	0.304	0.125
At 21 d								
BW ⁶ (kg)	0.615	0.181	0.388	0.408	0.102	0.001	0.180	0.384
Absolute weight (g)								
GIT	103	32.0	67.3	68.1	1.13	0.001	0.606	0.416
Proventriculus	3.99	1.51	2.69	2.82	0.080	0.001	0.260	0.573
Gizzard	23.2	9.71	16.3	16.7	0.49	0.001	0.567	0.595
Pancreas	2.57	0.86	1.66	1.78	0.062	0.001	0.180	0.479
Liver	23.3	6.85	15.0	15.2	0.57	0.001	0.813	0.826
Relative weight (% BW)								
GIT	17.1	17.9	17.6	17.4	0.27	0.062	0.610	0.117
Proventriculus	0.659	0.845	0.753	0.751	0.018	0.001	0.956	0.657
Gizzard	3.86	5.47	4.75	4.58	0.100	0.001	0.215	0.767
Pancreas	0.423	0.479	0.447	0.455	0.013	0.007	0.657	0.934
Liver	3.82	3.81	3.89	3.75	0.081	0.951	0.251	0.951

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 or 4 birds per replicate at 9 and 21 d of age, respectively.

²Average of 2 birds chosen at random per replicate.

³Full gastrointestinal tract without the annex organs (pancreas, spleen, and the liver).

⁴Full proventriculus.

⁵Full gizzard.

⁶Average of 4 birds chosen at random per replicate.

Table 27. Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the absolute (g) and relative (% BW) empty gizzard weight and gizzard digesta characteristics.¹

	Line		SFH		SEM ²	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
At 9 d								
Absolute weight	7.03	3.82	5.33	5.53	0.13	0.001	0.278	0.617
Relative weight	4.46	5.37	4.90	4.93	0.108	0.001	0.839	0.989
Fresh digesta content (g)	4.04	1.87	2.87	3.04	0.075	0.001	0.119	0.115
Absolute empty:full gizzard	63	67	66	65	0.6	0.001	0.278	0.121
Digesta dry matter (%)	36.3	36.2	36.0	36.6	0.56	0.925	0.484	0.417
At 21 d								
Absolute weight	14.7	6.80	10.6	11.0	0.31	0.001	0.374	0.475
Relative weight	24.6	38.4	31.9	31.0	0.07	0.001	0.369	0.774
Fresh digesta content (g)	8.51	2.91	5.70	5.72	0.215	0.001	0.944	0.818
Absolute empty:full gizzard	64	70	67	63	0.6	0.001	0.453	0.933
Digesta dry matter (%)	37.3	36.9	37.0	37.3	0.41	0.558	0.709	0.655

¹The average BW of the chicks are shown in table 26.

²Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 or 4 birds per replicate at 9 and 21 d of age, respectively.

3.3.3. Short chain fatty acid in the cecum and morphology of the ileum

Effects of chicken line

The concentration of SCFA in the cecum was 6.7 percent units higher in broilers than in pullets but the differences were not significant (Table 30). The proportions of the individual SCFA were not affected by chicken line, except for butyrate ($P=0.054$) and isobutyrate ($P=0.074$) that tended to be higher for broilers. Villus height and CD were greater ($P<0.001$) in broilers than in pullets (Table 31).

Effects of sunflower hulls inclusion

SFH inclusion did not affect total SCFA concentration or the proportion of the individual SCFA in the cecum. Similarly, villus morphology of the ileum was not affected by fiber inclusion.

Table 28. Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the absolute (cm) and relative length (cm/kg BW) of the small intestine (SI) and the cecum.¹

Age	Line		SFH		SEM ²	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
At 9 d								
Absolute length								
SI ³	112	70.1	89.2	93.1	1.70	0.001	0.115	0.564
Cecum ⁴	9.08	6.35	7.40	8.03	0.16	0.001	0.010	0.751
Relative length								
SI	727	991	854	863	18.3	0.001	0.737	0.844
Cecum	58.5	89.2	71.6	76.2	1.46	0.001	0.035	0.260
At 21 d								
Absolute length								
SI	150	99	118	132	3.9	0.001	0.022	0.941
Cecum	15.3	10.1	11.9	13.6	0.51	0.001	0.028	0.769
Relative length								
SI	252	561	390	423	17.6	0.001	0.209	0.463
Cecum	25.6	57.2	39.5	43.3	1.80	0.001	0.158	0.439

¹The average of the birds used is shown in table 26.

²Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 or 4 birds per replicate at 9 and 21 d of age, respectively.

³Small intestine (duodenum, jejunum, and ileum).

⁴Average length of the 2 cecum.

Table 29. Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the pH of the organs of the gastrointestinal tract.¹

	Line		SFH		SEM ²	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
At 9 d								
Crop	4.50	4.39	4.42	4.47	0.092	0.400	0.736	0.803
Proventriculus	2.82	2.98	2.91	2.89	0.182	0.556	0.920	0.958
Gizzard	2.62	2.64	2.67	2.59	0.079	0.895	0.441	0.855
Duodenum	5.77	5.83	5.78	5.82	0.064	0.513	0.719	0.925
Jejunum	5.56	5.67	5.60	5.63	0.049	0.111	0.628	0.887
Ileum	6.54	6.74	6.57	6.71	0.113	0.225	0.383	0.849
Cecum	5.84	5.75	5.77	5.81	0.098	0.524	0.763	0.955
At 21 d								
Crop	4.74	4.86	4.77	4.83	0.082	0.343	0.588	0.937
Proventriculus	2.80	2.94	2.90	2.84	0.115	0.398	0.690	0.862
Gizzard	2.28	2.21	2.26	2.23	0.042	0.235	0.540	0.943
Duodenum	5.90	5.89	5.87	5.91	0.029	0.769	0.381	0.931
Jejunum	5.66	5.71	5.67	5.69	0.032	0.230	0.685	0.851
Ileum	6.71	6.83	6.72	6.82	0.107	0.415	0.498	0.944
Cecum	5.84	5.81	5.79	5.86	0.079	0.753	0.563	0.909

¹The pH of the drinking water was 6.88.²Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 birds at 9 and 21 d of age.

Table 30. Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on short chain fatty acid (SCFA) concentration (μmol/g fresh digesta) and profile (% total SCFA) in the cecum at 21 d of age.

	Line		SFH		SEM ¹	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
SCFA (μmol/g)	71.8	65.1	69.3	67.5	4.30	0.291	0.780	0.910
Percentage of each individual SCFA								
Acetate	73.6	76.0	75.2	74.5	1.04	0.127	0.616	0.465
Propionate	1.85	2.60	2.29	2.16	0.40	0.203	0.826	0.855
Butyrate	23.4	20.6	21.4	22.6	0.103	0.065	0.430	0.431
Isobutyrate	0.109	0.021	0.077	0.052	0.035	0.087	0.615	0.185
Isovalerate	0.621	0.480	0.690	0.410	0.177	0.599	0.274	0.464
Valerate	0.364	0.305	0.323	0.345	0.083	0.621	0.854	0.407

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 birds per replicate.

Table 31. Influence of chicken line and inclusion of 3% sunflower hulls (SFH) in the diet on ileal mucosa morphology at 21 d of age.

	Line		SFH		SEM ¹	P-value		
	Broiler	Pullet	0%	3%		Line	SFH	Line × SFH
Villus height (µm)	1,149	847	968	1,028	40.2	0.001	0.307	0.225
Crypt depth (µm)	138	109	122	124	4.3	0.001	0.665	0.438
VH:CD ²	8.38	7.88	8.00	8.26	0.339	0.310	0.596	0.774

¹Standard error of means (n=7 for each treatment and n=14 for main effects). The experimental unit was formed by 2 birds per replicate.

²Villus height (VH) to crypt depth (CD) ratio.

3.4. Discussion

3.4.1. Growth performance and coefficient of TTAR of nutrients

Effects of chicken line

Broilers grew 4 times faster, ate 3 times more feed and had 30% better F:G than pullets, consistent with the main objectives of the genetic selection programs of the two chicken lines. Broilers are more voracious than pullets, and at young ages, broilers secrete more pancreatic enzymes per gram of feed and have better development of the ileal mucosa than pullets, consistent with data of Uni et al. (1995ab) and Kimiaeitalab et al. (2016).

At 9 d of age, nutrient retention and AME_n of the diets were greater in broilers than in pullets, in agreement with data of Kimiaeitalab et al. (2016) comparing both chicken lines. At 21 d of age, however, the only difference detected was for N. Probably, pullets of this age had the ability to obtain more energy by fermentation of the NDF than broilers, reducing the differences in nutrient retention between the two chicken lines, as reported by Walugembe et al. (2015).

Moisture content of the excreta was higher in pullets than in broilers at 9 d of age but not at 21 d. At 9 d of age, the reduced N retention of the pullets increased the need of extra water to eliminate the excess of uric acid produced through the urine. Consequently, moisture content of the excreta was higher in pullets. At 21 d of age, the difference in N retention between the two chicken lines was reduced and in fact, no differences in CTTAR

were observed for any of the other nutrients. Consequently, less effect of diet on moisture content of the excreta was expected. Moreover, an interaction between chicken line and SFH inclusion was detected at this age; SFH reduced moisture content of the excreta in broilers but not in pullets. The data suggest that the response to fiber inclusion, in respect to litter quality, was more beneficial in broilers than in pullets.

Effects of sunflower hulls inclusion

The dilution of the diet with 30 g SFH/kg did not reduce chick performance at any age, consistent with the results of Kimiaetalab et al. (2016). Jiménez-Moreno et al. (2016) in broilers and Guzman et al. (2015a) in pullets, reported also that the inclusion of 2.0 or 4.0% SFH diet did not affect BW of the chicks from 1 to 21 d of age. In contrast, Walugembe et al. (2014) observed that the inclusion of 16% of a mixture of DDGS and wheat bran diet reduced by 10.9% BW gain in broilers but had no effects in pullets. In the research of Walugembe et al. (2014), fiber inclusion increased the NDF content of the diet from 10.0 to 15.0% and reduced ADFI in broilers by 5.9%. In the current research, however, the inclusion of 3% SFH diet increased the NDF of the diet from 10.1 to 12.1%, an increase that did not affect ADFI or BW gain in any of the two chicken lines. Similar lack of negative effects of fiber inclusion on ADFI and growth of the birds has been reported by other authors (Sacranie et al., 2012; Jiménez-Moreno et al., 2013a; Guzmán et al., 2015). Probably, as suggested by Mateos et al. (2012), the response of young birds to additional insoluble dietary fiber depends on the ingredient composition of the control diet, with effects being more pronounced when this diet is low in fiber.

Moisture content of the excreta was reduced with SFH inclusion in broilers but no effects were observed in pullets. Jiménez-Moreno et al. (2016) reported also a reduction in moisture content the excreta in broilers at same ages. Rezaei et al. (2011) reported also that the inclusion of 0.5% micronized cellulose diet reduced litter moisture content in

broilers. All these data indicate that the inclusion of moderate levels of insoluble fiber in the diet had positive or no effects on excreta quality.

The inclusion of SFH increased the AME_n of the diet at 21 d of age but did not affect the retention of any of the other nutrients studied except N. Kimiaeitalab et al. (2016) reported also that the inclusion of 3% SFH in the diet increased the AME_n of the diet without any significant effect on the retention of the other nutrients. The improvement in AME_n of the diet with SFH inclusion was more pronounced in the research of Kimiaeitalab et al. (2016) than in the current research. The NDF of the control diet used by Kimiaeitalab et al. (2016) was 8.7% whereas in the current research the control diet contained 10.1%. Consequently, a greater benefit of the extra fiber on the AME_n of the diet was expected in the research of Kimiaeitalab et al. (2016) than in the current research (Mateos et al., 2012). In this respect, Jiménez-Moreno et al. (2013a) reported improvements in the CTTAR of the nutrients and AME_n of the diet with the inclusion of up to 5% OH but the benefits decreased with a further increase to 7.5%, consistent with the results reported herein. Insoluble fiber sources are retained for longer in the upper part of the GIT, increasing gizzard function, the intensity of the antiperistaltic movements and the production of HCl, amylase and bile acids (Duke, 1986; Hetland et al., 2003; Jiménez-Moreno et al., 2009). To notice that in the research of Kimiaeitalab et al. (2016) and in the research reported herein, SFH was included at the expense of the basal diet and consequently, the calculated AME_n content of the SFH containing diet was lower than that of the control diet. Consequently, the inclusion of SFH had a considerable beneficial effect on the utilization of the energy of the non-fibrous constituents of the diet in both researches.

3.4.2. Gastrointestinal tract traits

Effects of chicken line

In absolute terms, all the organs of the GIT were heavier and the SI and cecum were longer in broilers than in pullets, consistent with the greater GIT capacity and ADFI of broilers. Uni et al (1995a) and Kimiaeitalab et al. (2016) reported also that the SI of young birds was 30 and 35% longer in broilers than in pullets, respectively. In relative terms, however, the differences in organ weight were less evident and in fact, the gizzard was heavier and the SI and cecum were longer in pullets, in agreement with data of Kimiaeitalab et al. (2016).

The pH of the different segments of the GIT were not affected by chicken line, consistent with data of Kimiaeitalab et al. (2016) for all organs except the gizzard that was lower in pullets. The reason for the inconsistency in gizzard pH between these two experiments, conducted under similar management conditions, are not apparent. In this respect, in the research of Kimiaeitalab et al. (2016) the control diet was based on maize and contained 8.7% whereas in the current research the control diet was based on wheat and barley and contained 10.1% NDF. In broilers, Gonzalez-Alvarado et al. (2007) reported that the pH of the gizzard depended on the main cereal of the diet, with greater response when fed maize than when fed rice. In pullets, Frikha et al. (2009) and Saldaña et al. (2015), however, reported similar gizzard pH with maize or wheat diets, although in these two researches the pH was measured in older birds (7 and 17 wk of age, respectively).

Effects of sunflower hulls inclusion

The inclusion of SFH in the diet did not affect the weight or the pH of the different organs of the GIT but increased the absolute length of the SI at 21 d and of the cecum at both ages. These results are consistent in general, with data reported by Kimiaeitalab et

al. (2016). Wang et al. (2015) reported also an increase in the length of the SI of broilers with the inclusion of 6% DDGS in the diet.

3.4.3. Short chain fatty acid and ileum morphology

Effects of chicken line

The concentration of total SCFA in the cecum was 6.7 percent units higher in broilers than in pullets but the difference was not significant. Walugembe et al. (2015) and Kimiaetalab et al. (2016) reported also a 10.8 and 8.7 percent unit's greater production of SCFA in broilers than in pullets, differences that were significant in both researches. Józefiak et al. (2004) and Rehman et al. (2009) indicated that SCFA concentration in the cecum is highly variable with a range reported in the literature from 53 to 156 $\mu\text{mol/g}$ fresh digesta. Probably, the high variability in SCFA concentration observed in the current research, precluded the detection of any significant difference between the two chicken lines. The SCFA profile in the cecum was similar for the two chicken lines, except for butyrate and isobutyrate that were higher in broilers. Butyrate stimulates the proliferation of epithelial cell and the development and growth of the intestines (Roediger, 1982; Sakata, 1987), consistent with the better development of the ileum mucosa in broilers compared with pullets reported herein.

Villus height of the ileum was greater in broilers than in pullets, in agreement with data of Kimiaetalab et al. (2016). An increase in VH suggests better capacity for absorption of nutrients of the small intestine (Montagne et al., 2003; Chiang et al., 2010), consistent with the better F:G reported in broilers in the current research.

Effects of sunflower hulls inclusion

The concentration of total SCFA in the cecum and the ileal villus morphology were not affected by the inclusion of fiber in the diet, consistent with data of Walugembe et al. (2015) and Kimiaetalab et al. (2016). Similarly, Jiménez-Moreno et al. (2013b) reported

that the inclusion of 2.5% OH did not affect jejunal morphology in 18 d-old broilers. In contrast, Rezaei et al. (2011) observed that the inclusion of 0.5% micronized cellulose diet increased VH in the ileum of 42 d-old broilers. The existing data indicate that the effects of dietary fiber on villus morphology depend primarily on the level of inclusion and the physico-chemical characteristics of the fiber source. For example, Jiménez-Moreno et al. (2011) reported that the inclusion of 2.5% pea hulls/kg but not of 7.5%, increased VH in 15 d-old broilers.

3.5. Conclusions

Broilers and pullets differ in growth performance, nutrient retention, GIT organ development and ileal villus height. Broilers grew faster and were more efficient than pullets at all ages and had better CTTAR of all nutrients at 9 d of age. In absolute terms, the gizzard was heavier and the SI and cecum were longer in broilers than in pullets but an opposite effect was observed in relative terms. The dilution of the control diet with 3% SFH did not reduce chick performance and in fact, improved the AME_n of the diet at 21 d of age. The inclusion of SFH at the expense (wt:wt) of the whole diet did not affect the weight or the pH of the organs of the GIT but increased the absolute length of the SI and the cecum.

No interactions between chicken line and SFH inclusion were detected for any of the variables studied except for moisture content of the excreta at 21 d of age that decreased in broilers with SFH inclusion. The data suggest that most of the information available on the effects of inclusion of insoluble fiber sources in broilers might be used, at some extent, in studies conducted with pullets.

CHAPTER 4

GENERAL DISCUSSION

AND

CONCLUSIONS

Broilers and pullets show differences in ADFI, BWG, and feed utilization, and consequently, fiber inclusion should have different effects in broilers than in pullets. Broiler diets are characterized by their high nutrient density and thus, lower fiber content. Uni et al. (1995b) reported that broilers had greater enzyme secretion, more developed intestinal morphology, and heavier GIT than pullets. Fibrous ingredients dilute the nutrient content of the diet but improve GIT motility and function. The beneficial effects of insoluble fiber sources on growth performance and nutrient digestibility often reported, might be related to an improvement of gizzard function and a decrease in gizzard pH (Jiménez-Moreno et al., 2009a; Mateos et al., 2012). Information available on the effects of inclusion of insoluble fiber on these variables is abundant in broilers but scarce in pullets. Walugembe et al. (2014, 2015) compared response of these two chicken lines to the inclusion of 16% of a 50:50 mixture of wheat DDGS and wheat bran from 1 to 21 d of age, and reported greater growth performance and higher ceca SCFA production in broilers than in pullets. However, the AME_n of the diet, was similar for both chicken lines, and was not affected by fiber inclusion.

In the current research, the control diets used were a commercial broiler diet based on corn and SBM in Exp. 1 and a commercial pullet diet based on wheat, barley, and SBM in Exp. 2. Broiler diet contained 2,980 kcal/kg, 22.2% CP, 1.25% digestible lysine, and 8.7% NDF. Whereas the pullet diet contained 2,850 kcal/kg, 19.1% CP, 0.98% digestible lysine, and 10.1% NDF. Consequently, different effects of the consumption of these diets on GIT development and growth were expected. The diet used in Exp. 1 had the higher nutrient density and indispensable amino acids, and less NDF content, than the diet used in Exp. 2. From hatch to 21 d of age, feeding broiler diet to pullets (Exp. 1) increased BW gain and ADFI by 38.1 and 11.0% respectively, and improved FCR by 19.2%, as compared to data in Exp. 2 where pullets were fed a pullet diet. In Exp. 2, in

which broilers were fed a pullet diet, BW gain and ADFI decreased by 29.6 and 17.9% respectively, and FCR impaired by 16.1%. Broiler diet had higher nutrient density and lower NDF content than the pullet diet. Consequently, both chicken lines had higher energy intake (EI) and better energy conversion ratio (ECR) when fed broiler diet (Exp. 1) than when fed pullet diet (Exp. 2), consistent with the findings of Scott (2002), Brickett et al. (2007), Zhao et al. (2009), and Wang et al. (2014). In both experiments, broilers showed higher EI and lower ECR than pullets at 21 d of age, consistent with the greater GIT capacity and better growth performance reported for broilers (Uni et al., 1995b; Walugembe et al., 2014).

As an average, of the both experiments broilers ate 3.9 times more feed, grew 2.8 times faster, and were 28.7% more efficient than pullets, consistent with results reported by Uni et al. (1995b), Lopez and Leeson (2005), and Walugembe et al. (2014). In Exp. 1, inclusion of SFH in the diet did not affect EI or growth performance but increased ECR of the birds at 21 d of age. In Exp. 2, the calculated values for EI and ECR reduced with SFH inclusion. However, determined values of these two variables were not different. Probably, as indicated by Mateos et al. (2012), fiber inclusion, improved nutrient digestibility via an increase in retention time and gizzard function, consistent with the increase in AME_n of the diet reported in herein.

Chicken line affected the excreta moisture in both experiments. Nitrogen retention was higher in broilers than in pullets in both experiments with differences being more noticeable at 9 d than 21 d of age. The CP content of the broiler diet used in Exp. 1 was more than pullet requirements and consequently, more N was excreted as uric acid via urine, and increased excreta moisture in pullets at 9 d of age. Similar increase in excreta moisture content at 9 d of age was observed in pullets in Exp. 2. The increase in excreta moisture observed might be related to a reduced in N retention at a young age as well as

to the higher soluble NSP content of the pullet diet compared to that of broiler diet (Hartini and Choct 2010; Kiarie et al., 2014).

The inclusion of SFH in the diet did not affect excreta moisture in any of the two experiments at any age, consistent with the results reported by Jiménez-Moreno et al. (2016). An interaction between chicken line and SFH inclusion was detected at 21 d of age in Exp. 2; SFH inclusion decreased the excreta moisture in broilers but not in pullets. Probably, higher GIT capacity and greater FI of broilers were responsible for the differences observed (Lopez and Leeson, 2005; Walugembe et al., 2014).

In general, nutrient retention was higher for broilers than for pullets at 9 d of age, with differences being more pronounced for N retention. Similarly, Uni et al. (1995b) reported that slower growth of 4 d-old pullets compared to broilers was related to their lower ADFI and N retention. Also, Lopez and Leeson, (2005) reported that excreted N was 194% higher in broilers from 10 to 15 d and culminated to 292% from 23 to 28 d of age than pullets, largely because of their higher FI, consistent with the higher excreta moisture of broilers than pullets reported in both experiments at 21 d of age.

The inclusion of SFH increased the AME_n of the diet at both ages in Exp. 1, and at 21 d of age in Exp. 2, consistent with results reported by Jiménez-Moreno et al. (2011, 2013a). In Exp. 2, SFH inclusion did not affect the TTAR of the nutrients. The diet in this experiment had higher NDF content than that in Exp. 1 and as indicated by Jiménez-Moreno et al. (2009a,b), González-Alvarado et al. (2010), and Mateos et al. (2012) the beneficial effects of additional insoluble fiber on growth performance and TTAR are expected to be more pronounced when included in low fiber diets.

In absolute terms all GIT organs were heavier in broilers than in pullets, irrespective of the type of diet used, consistent with results of Katanbaf et al. (1988) and Lilja (1983). In relative terms, however, many of the GIT organs were heavier in pullets. For example,

the proventriculus and the gizzard in Exp. 1, and the GIT, pancreas, and liver in Exp. 2 were heavier in pullets. Dunnington and Siegel (1995) reported that for the first 10 d after hatch the relative weight of the GIT was greater in heavy weight lines than in light weight lines but that the differences disappeared at older ages.

In Exp. 1, the inclusion of SFH in the diet increased the absolute and relative weight of the gizzard at both ages, consistent with results of González-Alvarado et al. (2008) and Jiménez-Moreno et al. (2011). Also, the relative weight of the GIT at 21 d of age increased in Exp. 1 with SFH inclusion in the diet, consistent with data of Jiménez-Moreno et al. (2011). In Exp. 2, however, SFH inclusion did not affect the absolute or relative weight of any of the GIT organs at any age. The contradictory results observed between 2 experiments conducted under similar conditions is not known. The control diet in Exp. 1 was a broiler diet low in fiber, whereas that of Exp. 2 was a pullet diet characterized by a higher fiber content. Probably, the effects of fiber inclusion on organ weight are more pronounced when a low fiber diet is supplemented with additional fiber as reported by Mateos et al. (2012).

In both experiments, empty gizzard was heavier in absolute terms and lighter in relative terms in broilers than in pullets. Also, absolute fresh digesta content of the gizzard was greater in broilers. The inclusion of SFH improved all gizzard traits in Exp. 1, except the DM of gizzard digesta at both ages and the empty to full gizzard ratio at 9 d of age. Hetland et al. (2005) and Hetland and Svihus (2007) reported that the inclusion of wood shavings in the diet weight of the empty gizzard in relative terms in laying hens, consistent with the results reported herein. An interaction between chicken line and SFH inclusion for the absolute weight and fresh gizzard digesta at 9 and 21 d of age was detected. Sunflower hulls inclusion increased the absolute empty weight and fresh digesta content of the gizzard in broilers but not in pullets. Broilers have higher GIT capacity and FI than

pullets (Uni et al., 1995a; Lopez and Leeson, 2005; Walugembe et al., 2014). Consequently, response to dietary SFH should be pronounced of broilers than in pullets, consistent with the results reported herein. In Exp. 2, however, no significant differences were detected probably because of different fiber content of the control diets used.

In absolute terms, broilers had longer SI and cecum than pullets at all ages in both experiments, consistent with the results of Uni et al. (1995). However, an adverse effect was observed in relative terms. The inclusion of SFH did not affect SI or cecum length in absolute and relative terms in Exp. 1. In Exp. 2, the inclusion of SFH increased the absolute and relative lengths of the cecum at 9 d and the absolute length of SI and cecum at 21 d of age. Wang et al. (2015) reported an increased in the length of the SI of birds fed 6% DDGS at 21 d of age. Similarly, Barekattain et al. (2013) reported that birds fed 3% DDGS from sorghum had longer SI at 7 and 21 d of age than birds fed a control diet, consistent with the results reported in Exp. 2. Also, the diet used in Exp. 2 had higher soluble NSP content (Bach Knudsen, 2014) than diet used in Exp. 1. The presence of soluble NSP might have increased the number and activity of the microflora of the large intestine and cecum and increased fermentation rate (Montagne et al., 2003), and consequently size of this organ.

The pH of the different segments of the GIT was not affected by chicken line in any of the 2 experiments, except for that of the gizzard in Exp. 1 that was lower in pullets. Shires et al. (1987) reported that the passage rate of the digesta through the proventriculus/gizzard was lower in pullets compared with broilers. The higher retention time of the digesta increased HCl secretion in the proventriculus and reduced the pH of the gizzard, consistent with this observations in Exp. 1.

Sunflower hulls inclusion reduced gizzard pH an effect that was significant in Exp. 1 but not in Exp. 2. The inclusion of fiber increases the retention time of the digesta in

the upper part of the digestive tract, stimulating gizzard function (Rogel et al., 1987; Hetland et al., 2005) and HCl secretion in the proventriculus (Duke, 1986). Effects on pH of chicken line and SFH inclusion were limited in Exp. 2 compared with Exp. 1, which might accounted for by higher NDF content of the control diet used in Exp. 2, as suggested by Mateos et al. (2012).

The concentration of the SCFA in the cecum was 10.5 and 10.3% higher in broilers than in pullets but the difference was not significant. Most of the SCFA produced in the gut is an outcome of the fermentation of complex, non-digestible carbohydrates but undigested proteins and peptides are also net contributors. Pullets had higher valerate and lower acetate proportion than broilers. Also, higher proportion of isovalerate is noticeable in pullets than in broilers in Exp. 1. The higher proportion of these two FA in pullets was consistent with the excess of protein in the diets which exceeded their requirements, with more N compounds available for fermentation in the cecum. Valerate and isovalerate originate primarily from the oxidative deamination and decarboxylation of the branched chain amino acids (Allison, 1978; Andries et al., 1987). In Exp. 2, the proportion of butyrate and isobutyrate tended to be higher in broilers than in pullets which might be related to the type of cereal used. Kiarie et al (2014) reported higher propionate and lower butyrate concentration when corn replaced wheat in the diet. The inclusion of SFH in the diet did not affect SCFA production in any of the 2 experiments, consistent with data reported by Walugembe et al. (2015) comparing in broilers and pullets a control and a diet that contained 16% of a mixture of DDGS and wheat bran.

The VH and CD were higher in broilers than in pullets in both experiments. In both experiments the VH:CD ratio was higher in broilers than in pullets but the differences were significant only in Exp. 1. An increase in VH and VH:CD ratio suggests a better capacity for absorption of nutrients (Uni et al., 1995b; Montagne et al., 2003), consistent

with the better growth performance and nutrient digestibility reported herein for broilers. Sunflower hulls inclusion in the diet did not affect ileal morphology at any age in any of two experiments, consistent with the results reported by Rezaei et al. (2011) using 0.5% micronized insoluble fiber in the diet of 42 d-old broilers.

In conclusion, broilers and pullets benefits from inclusion of a requirement for a minimal amount of insoluble fiber in the diet. Sunflower hulls inclusion in the diet improved gizzard function, AME_n of the diet. The lack of interactions between chicken line and SFH inclusion for majority of the traits studied suggests that the available information on the response of young broilers to dietary insoluble fiber could be generalized at some extent in pullets.

Conclusions and applications

1. Broilers growth faster and were more efficient than pullets. Feeding a broiler diet, increased BW gain and improve growth performance in pullets.
2. Nutrient density, main cereal (corn vs. wheat and barley), and fiber content of the diet can affect the physiological response of chicken lines to inclusion of a moderate amount of insoluble fiber in the diet.
3. Nutrients retention and AME_n of the diets were higher for broilers than for pullets. Higher digestibility of N was noticeable in broilers than in pullets at all ages.
4. In absolute terms, all GIT organs were heavier and SI and ceca were longer in broilers than in pullets. However, often adverse results were observed in relative terms. The gizzard was more developed and heavier in broilers than in pullets. The pH of the gizzard, affected by fiber content of the diet.

5. Short chain fatty acid concentration in the cecum was 11% higher in broilers than in pullets. Diet composition can modify the fatty acid proportion in the cecum.
6. Ileal VH and CD of the ileum were greater in broilers than in pullets. However, the inclusion of SFH did not affect VH, CD, or VH:CD ratio of birds.
7. The inclusion of SFH (wt:wt basis), improved the AME_n of the diet without any negative effects on growth performance of the birds. These effects were more pronounced when SFH was included in the broiler diet than when included in the pullet diet.
8. Lack of interactions in many of traits studied in main traits studied such as performance and organ size indicated that existing data available for broilers would be generalized to a certain degree to pullets.

CHAPTER 5

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