UNIVERSIDAD POLITÉCNICA DE MADRID

ESCUELA TÉCNICA SUPERIOR DE INGENIEROS AGRÓNOMOS

DEPARTAMENTO DE PRODUCCIÓN AGRARIA

**INFLUENCE OF THE INCLUSION OF CO-PRODUCTS
OF THE SOY OIL INDUSTRY IN POULTRY DIETS ON
NUTRIENT DIGESTIBILITY, GROWTH
PERFORMANCE, AND PRODUCTION**

TESIS DOCTORAL

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Ph.D. THESIS

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DEDICATION

*I dedicate this work to my tenderness **mother** and dear **father** for their help and for all the support during my PhD study. Also, to my lovely wife **Rawaa** and my children **Hayman**, **Ahmed** and **Ramy** for their patience, and for all the support, as well as to my **brothers**, and **sisters**.*

Husham A. Mandalawi

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With many thanks

Husham A. Mandalawi

هشام عبد الستار داود المندلاوي

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ABBREVIATIONS

ABBREVIATIONS

AA	Amino acid
ADG	Average daily gain
ADFI	Average daily feed intake
AID	Apparent ileal digestibility
AME	Apparent metabolizable energy
AMEn	Apparent metabolisable energy, corrected for nitrogen
AS	Aceite de soja
ASO	Acidulated soy oil soapstocks
BW	Body weight
BWG	Body weight gain
CP	Crude protein
CTTAR	Coefficient total tract apparent retention
D	Day
DAG	Diacylglycerols
DM	Dry matter
EB	Energía bruta
EE	Ether extract, extracto etéreo
EMA	Energía metabolizable aparente
EMAn	Energía metabolizable aparente, corregida en nitrógeno
EU	European union
Exp	Experiments, experimentos
FA	Fatty acid
FCR	Feed conversion ratio
FFA	Free fatty acid
F:G	Feed to gain ratio

G	Gram
GE	Gross energy
GIT	Gastrointestinal tract
GLYC	Raw glycerin, glicerina bruta
GMD	Geometric mean diameter
GSD	Geometric standard deviation
H	Hour
IC	Indice de conversión
Kg	kilogram
L	Linear
LNA	Linoleic acid
MAG	Monoacylglycerol
MIU	Moisture, impurities and unsaponifiables
MO	Materia orgánica
MS	Materia seca
N	Nitrogen
OM	Organic matter
P	Probability
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PS	Phosphatidylserine
Q	Quadratic
SEM	Standard error of the mean
SO	Soy oil
TAG	Triacylglycerol
TME	True metabolizable energy

TTAR	Total tract apparent retention
RCF	Roche colour fan
RMAG reesterificado	Re-esterified monoacylglycerol oil, monoacilglicerol
RTAG reesterificado	Re-esterified triacylglycerol oil, triacilglicerol

RESUMEN

RESUMEN

El objetivo general de esta Tesis doctoral fue estudiar la influencia de la inclusión de co-productos de la industria bioenergética en piensos para aves sobre la digestibilidad de los nutrientes, los rendimientos productivos y la producción. A este respecto, la influencia de glicerina, lecitina, y los aceites re-esterificados producidos a partir de aceite de soja, se llevaron a cabo tres estudios.

En el primer estudio (capítulo 2) se estudió la influencia de la fuente y el nivel de inclusión de la glicerina bruta (GLYC) en el pienso sobre los parámetros productivos, las características del tracto digestivo, la retención de nutrientes, y la digestibilidad ileal aparente de los nutrientes en pollos de 1 a 21 d de edad. Se formularon un pienso control a base de maíz y harina de soja y 8 piensos adicionales que siguiendo un diseño factorial 2×4 con 2 fuentes de GLYC y 4 niveles de inclusión (2.5, 5.0, 7.5, y 10%). El GLYC utilizado se obtuvo a partir del mismo lote original de aceite de soja que se secó bajo diferentes condiciones de procesado y contenía 87,5 u 81,6% de glicerol, respectivamente. El tipo de procesado de la GLYC no afectó a ninguna de las variables estudiadas, excepto a la retención de materia seca y materia orgánica ($P < 0,05$) que fue mayor para la pienso de glicerol 87,5%. De 1 a 21 d de edad, el índice de conversión (IC) mejoró de forma lineal (L, $P \leq 0,01$) con el aumento de GLYC del pienso, pero la ganancia media diaria no se vio afectada. A los 21 d, el peso relativo del hígado y del tracto digestivo aumentó de manera lineal con el aumento de GLYC en el pienso (L, $P < 0,01$), pero la concentración de lípidos en el hígado no se vio afectada. La retención de la materia seca (MS) y materia orgánica (MO) aumentó de forma cuadrática (Q, $P < 0,05$) con la inclusión de GLYC, mientras que el contenido de la energía metabolizable aparente, corregida en nitrógeno (EMAn) lo hizo de manera lineal (L, $P < 0,01$). Además, la digestibilidad ileal aparente de la MS (L, $P < 0,05$; Q, P

= 0,07) y la energía bruta (EB) (L, $P < 0,01$) aumentaron con el incremento de GLYC del pienso. Se concluye que GLYC obtenido a partir de la industria del biodiesel se puede utilizar de manera eficiente, hasta un nivel de incorporación en la dieta del 10%, como fuente de energía para los pollos de 1-21 días de edad y que el contenido de energía de GLYC depende principalmente de su contenido de glicerol.

En el segundo estudio (capítulo 3) se estudió el efecto de la inclusión de la glicerina bruta (GLYC) y la lecitina de soja en el pienso sobre la producción y calidad del huevo y la retención de nutrientes en gallinas ponedoras rubias de 23 a 51 semanas de edad. El experimento siguió un diseño completamente al azar con 6 piensos organizados en un factorial 2x3 con dos niveles de glicerina (0 vs 70 g/kg) y 3 ratios grasa animal: lecitina (40:0, 20:20 y 0:40 g/kg). Cada tratamiento se repitió 8 veces y la unidad experimental fue la jaula con diez gallinas. La producción fue estudiada por replica cada periodo de 28 días y de forma acumulativa. Durante todo el experimento, la inclusión de GLYC en el pienso empeoró el IC por kilogramo de huevos (2.071 vs 2.039; $P < 0,05$), pero no afectó a ninguno de los otros parámetros de producción o calidad de huevo del estudiado. El reemplazo de la grasa animal por lecitina (40:0, 20:20 y 0:40 g/kg) aumentó el peso del huevo (60,1, 60,7 y 61,8 g, respectivamente; $P < 0,001$), la masa del huevo (56.8, 57.5 y 58.8 g / d, respectivamente; $P < 0,01$) y mejoró el color de la yema medido mediante el abanico de color DSM (9.2, 9.2 y 9.5, respectivamente, $P < 0,001$) y el IC por kilogramo de huevos (2.072, 2.068 y 2.027, respectivamente ; $P < 0,05$). Sin embargo, el consumo de pienso, la producción de huevos y la ganancia de peso, no se vieron afectados. La inclusión de GLYC en el pienso no afectó a la retención de nutrientes, mientras que la inclusión de lecitina mejoró la retención de MS ($P < 0,05$), MO ($P < 0,05$), extracto etéreo (EE) ($P < 0,001$) y EB ($P < 0,001$). En resumen, la inclusión de 70 g de glicerol/kg de pienso empeoró el IC

por kilogramo de huevos, pero no afectó a ningún otro parámetro productivo o a la digestibilidad. El reemplazo de la grasa animal por lecitina mejoró el peso del huevo, el color de la yema de huevo y la digestibilidad de los nutrientes. En consecuencia, la lecitina se puede usar como una fuente de lípidos en piensos de gallinas ponedoras con efectos beneficiosos sobre la producción de huevo.

En el tercer estudio (capítulo 4) la energía metabolizable aparente (EMA) de fuentes de lípidos tradicionales y re-esterificados y los efectos de su inclusión en el pienso sobre la retención de nutrientes y el rendimiento de los pollos fueron estudiados en dos experimentos (Exp) de 7 a 21 días de edad. En el Exp 1 se formularon un pienso control sin ningún tipo de grasa suplementaria y tres piensos adicionales que incluyeron 25 g/kg de aceite de soja (AS), monoacilglicerol reesterificado (RMAG) o triacilglicerol reesterificado (RTAG) a expensas (wt:wt) del pienso control. La EB (MJ/kg) de los aceites fue 39.66, 38.06 y 38.56, para AS, RMAG y RTAG respectivamente. La EMA de las fuentes de lípidos se determinó mediante dos métodos: a) multiplicando la EB por la digestibilidad del EE de sus respectivos piensos y b) por diferencia entre el EMA del pienso control y el pienso experimental correspondiente. La retención de nutrientes no se vio afectada por la administración de suplementos de grasa, a excepción de la digestibilidad del EE que fue mayor para los piensos de aceite suplementados que para el pienso control ($P < 0,001$). La EMA de la AS, RMAG y RTAG fue 35.44, 33.89 y 34.05 MJ/kg, respectivamente, cuando se determinó directamente de los datos de digestibilidad y 37.70, 35.68 y 35.83 MJ/kg cuando se calculó por diferencia entre el pienso control y el pienso que contenía aceite. La suplementación con grasas mejoró el IC ($P < 0,01$) de 7 a 21 días de edad, con efectos más marcados para los pollos que consumieron los piensos que contenían AS que para aquellos que consumieron RMAG o RTAG. En el experimento 2, el diseño fue el

mismo que en el Exp 1, pero los piensos experimentales incluyeron 50 g/kg de AS, oleínas aciduladas, manteca, RMAG, RTAG o mezcla 20:80 de AS y RMAG (AS-RMAG). La EMA de las grasas suplementadas fue mayor para el AS, AS-RMAG y manteca que para la RMAG, con RTAG y oleínas aciduladas mostrando valores intermedios ($P < 0,01$). La inclusión de grasa mejoró la retención de todos los nutrientes. El contenido de EMA de las grasas suplementadas fue mayor cuando se calculó por diferencia entre el EMA del pienso control y del pienso que contenía la grasa que cuando se determinó directamente de los datos de digestibilidad de la EB y del EE y más alta cuando se incluyó 50 g/kg que cuando se incluyó 25 g/kg. La grasa suplementaria mejoró la ganancia media diaria y el IC de los pollos, con independencia de la fuente de lípidos utilizada ($P < 0.001$). En conclusión, los aceite re-esterificados de monoacilglicerol y triacilglicerol fueron bien digeridos por los pollos, aportando valores de EMA similares a los de fuentes de grasa tradicionales.

SUMMARY

SUMMARY

The general objective of this thesis was to study the effect of inclusion of different co-products from the bioenergy industry in poultry diets on nutrient digestibility, growth performance, and production. To this end, the influence of glycerin, lecithins, and re-esterified oil from soy oil industry, were developed in three studies.

In first study (chapter 2), the influence of source and level of inclusion of raw glycerin (GLYC) in the diet on growth performance, digestive traits, total tract apparent retention (TTAR), and apparent ileal digestibility of nutrients was studied in broilers from 1 to 21 d of age. There was a control diet based on corn and soybean meal and 8 additional diets that formed a 2×4 factorial with 2 sources of GLYC and 4 levels of inclusion (2.5, 5.0, 7.5, and 10%). The GLYC used were obtained from the same original batch of soy oil that was dried under different processing conditions and contained 87.5 or 81.6% glycerol, respectively. Type of processing of the GLYC did not affect any of the variables studied except DM and organic matter retention ($P < 0.05$) that was higher for the 87.5% glycerol diet. From d 1 to 21, feed conversion ratio (FCR) improved linearly ($L, P \leq 0.01$) as the GLYC content of the diet increased, but ADG was not affected. On d 21, the relative weight (% BW) of the liver and the digestive tract increased ($L, P < 0.01$) as the level of GLYC in the diet increased, but lipid concentration in the liver was not affected. The TTAR of DM and organic matter increased quadratically ($Q, P < 0.05$) and the AMEn content of the diet increased linearly ($L, P < 0.01$) with increases in dietary GLYC. Also, the apparent ileal digestibility of DM ($L, P < 0.05$; $Q, P = 0.07$) and gross energy ($L, P < 0.01$) increased as the GLYC content of the diet increased. It is concluded that raw GLYC from the biodiesel industry can be used efficiently, up to 10% of the diet, as a source of energy

for broilers from 1 to 21 d of age and that the energy content of well-processed raw GLYC depends primarily on its glycerol content.

In second study (chapter 3), the effects of the inclusion of raw glycerin (GLYC) and lecithin in the diet on egg production, egg quality and total tract apparent retention (TTAR) of dietary components was studied in brown egg-laying hens from 23 to 51 wk of age. The experimental design was completely randomized with six diets combined as a 2×3 factorial with two levels of GLYC (0 vs. 70 g/kg) and three animal fat to lecithin ratios (40:0, 20:20 and 0:40 g/kg). Each treatment was replicated eight times and the experimental unit was a cage with ten hens. Production was recorded by replicate every 28-d period and cumulatively. For the entire experiment, the inclusion of GLYC in the diet hindered feed conversion ratio per kilogram of eggs (2.071 vs. 2.039; $P < 0.05$) but did not affect any of the other production or egg quality traits studied. The replacement of animal fat by lecithin (40:0, 20:20 and 0:40 g/kg) increased egg weight (60.1, 60.7 and 61.8 g, respectively; $P < 0.001$) and egg mass production (56.8, 57.5 and 58.8 g/d, respectively; $P < 0.01$) and improved yolk color as measured by the DSM color fan (9.2, 9.2 and 9.5, respectively; $P < 0.001$) and feed conversion ratio per kilogram of eggs (2.072, 2.068 and 2.027, respectively; $P < 0.05$). Feed intake, egg production and body weight gain, however, were not affected. The inclusion of GLYC in the diet did not affect nutrient retention but lecithin inclusion improved TTAR of dry matter ($P < 0.05$), organic matter ($P < 0.05$), ether extract ($P < 0.001$) and gross energy ($P < 0.001$). In summary, the inclusion of 70 g glycerol/kg diet hindered feed conversion ratio per kilogram of eggs but did not affect any other production or digestibility trait. The replacement of animal fat by lecithin improved egg weight, egg yolk color and nutrient digestibility. Consequently, lecithin can be used as a lipid source in laying hen diets with beneficial effects on egg production.

In third study (chapter 4), the apparent metabolizable energy (AME) of traditional and re-esterified lipid sources and the effects of its inclusion in the diet on nutrient retention and broilers performance were studied in two experiments (Exp) from 7 to 21 d of age. In Exp 1 there was a control diet without any supplemental fat and three extra diets that included 25 g/kg of soy oil (SO), re-esterified monoacylglycerol (RMAG) or re-esterified triacylglycerol (RTAG) at the expense (wt:wt) of the control diet. The gross energy (GE, MJ/kg) of the oils was 39.66, 38.06 and 38.56, respectively. The AME of the lipid sources was determined by two methods: a) multiplying its GE by the digestibility of the ether extract (EE) of their respective diets and b) by difference between the AME of the control and the corresponding experimental diet. Nutrient retention was not affected by fat supplementation, except for EE that was higher for the oil supplemented diets than for the control diet ($P < 0.001$). The AME of the SO, RMAG and RTAG was 35.44, 33.89 and 34.05 MJ/kg when measured directly from digestibility data and 37.70, 35.68 and 35.83 MJ/kg when calculated by difference between the control and the oil containing diet. Fat supplementation improved feed to gain ratio ($P < 0.01$) from 7 to 21 d of age, with effects being more pronounced for SO than for RMAG or RTAG. In Exp 2, the design was the same than in Exp 1 but the experimental diets included 50 g/kg of SO, acidulated soapstocks (ASO), lard, RMAG, RTAG or a 20:80 mixture of SO and RMAG (SO-RMAG). The AME of the supplemental fats was greater for the SO, SO-RMAG and lard than for the RMAG, with RTAG and ASO being intermediate ($P < 0.01$). Fat inclusion improved the retention of all nutrients. The AME content of the supplemental fats was higher when calculated by difference between the AME of the control and the fat containing diet than when measured directly from the GE and EE digestibility data and higher when included at 50 g/kg than when included at 25 g/kg. Supplemental fat improved ADG and F:G of the

broilers, irrespective of the lipid source used ($P < 0.001$). In conclusion, the re-esterified monoacylglycerol and triacylglycerol oils were well digested by broilers, with AME values within the range of those of traditional fat sources.

CHAPTER 1:
LITERATURE REVIEW AND OBJECTIVES

1. Literature review

1.1. Introduction

Renewable energy sources and biofuels, including biodiesel, have gained increasing attention as a replacement for fossil fuels. This has led to a significant increase in global biofuels production in recent years, with world output growing nearly four-fold between 2005 and 2013 (UNCTAD, 2014). The European Union-28 (EU) has a world leading position in both production and consumption of biodiesel. This has been achieved by the implementation of production policy programs (Directive 2003/30/EC and RED 2009/28/EC) and tax incentives. With a current production of 11 billion liters, biodiesel covers the 80% of the total transport biofuel market in the EU (USDA-FAS, 2014). It is expected that the production of biodiesel in the EU will increase to 16 billion liters in 2020 (OECD-FAO 2012). Germany is the main producer with around 3 billion liters while Spain with almost 1 billion liter, is the fourth (USDA-FAS, 2014). Biodiesel can be produced by a variety feedstocks such as vegetable oils (mainly rapeseed, soybean and palm), animal fats and waste cooking oils (Cooper and Weber 2012). Biodiesel production comes primarily from rapeseed oil in Europe, soybean oil in the USA, Canada, and South America, palm and coconut oil in Asia, palm and soybean oil in Australia and waste vegetable oil and animal fat in New Zealand (Barabás and Todorut, 2011; Cooper and Weber 2012; OECD-FAO 2012).

Co-products from the biodiesel industry such as dried distillers grain with solubles, glycerol, and lecithin are gaining prominence in livestock feed as their implantation bring up several benefits in terms of economy and resource management (Wiesman et al. 2012). These co-products can partially replace higher priced raw materials in animal feeds at lower cost. However, their level of use is depends on the target species, with little research available in many instances (Cooper and Weber

2012). Consequently, studies that include variable amounts of these co-products, under commercial conditions, are needed.

Animal fats and oils are commonly used in animal feeds to increase the energy concentration of the diet, supply essential fatty acids, fat soluble vitamins, and antioxidants or improve the technological properties of the feed (Mateos and Sell, 1980b; Jones et al., 1992; Holland et al., 1998; Grobas et al., 1999; Lammers et al., 2008a; Peña et al., 2014). Three lipid co-products resulting from the biodiesel industry namely; glycerin, lecithin and re-esterified oils, have been selected for the purpose of this thesis. The following literature review will focus on aspects related to their characterization, quality and availability and how they can influence the growth performance, egg production, and nutrient retention in poultry.

1.2. Glycerin

Glycerin, glycerol or 1, 2, 3 propanetriol, a trihydroxy sugar alcohol with molecular $C_3H_8O_3$ is a water-soluble, clear, odorless, viscous liquid with a sweet taste with many applications in the pharmaceutical, cosmetics, and food industries (Pagliaro and Rossi, 2010) (Figure 1).

Figure 1. Chemical structure of glycerin (Robergs and Griffin, 1998).

1.2.1. Production of glycerol

Available glycerol can be obtained either by chemical synthesis or from oils, and fats. Synthetic glycerol is chemically synthesized from petrochemical feedstocks and

propylene (Huang et al., 2002). Because of environmental control requirements, the increase of propylene prices, and the entry in the market of glycerol from the biodiesel industry the production by synthesis decreased significantly during the past years (Perosa et al., 2016). Traditionally, glycerol was a by-product of soap and detergents made from oils and fats. Glycerol is found in plants and animals as a structural component of triglycerides and phospholipids. To obtain the glycerol backbone from the triglyceride, oils and fats must be hydrolyzed and separated by saponification (Figure 2) (Yazdani and Gonzalez, 2007). However, in recent years, the larger amount of glycerol in the market comes from the biodiesel industry. In this case, glycerol is obtained after the transesterification of the oil with methanol. The yield of glycerol is close to 10% (wt:wt bases) of the total biodiesel production (Rahmat et al., 2010).

Figure 2. Production of glycerol from fats and oils (Yazdani and Gonzalez, 2007).

After the transesterification process the resulting product is processed to remove as much methanol as technically possible to insure that the glycerol molecule is harmless to the animal (Hippenstiel et al. 2012). Obtaining a high quality crude glycerol is important to fulfill with legal and animal performance issues (Hippenstiel et al. 2012). According to the German standards, crude glycerol must contain a minimum of 80% glycerine, 10-15% water, and a maximum of 10% ash and 0.2% methanol (Hippenstiel et al. 2012). However, a broader range of raw glycerol with variable contents in glycerine (70-93%), water (8-20%), ash (2-10%) and methanol (0.1-0.75%) exist in the EU market (FEDNA 2012). Current global glycerol production relies primarily on biodiesel production with smaller amount of soap, and fatty. Biodiesel industry has triggered the increase of production from 1 to 2 million tons in the past ten years (Ciriminna et al. 2014).

1.2.2. Absorption and metabolism of glycerol

Intestinal absorption of glycerol is high, around 97%, in non-ruminants animals such as pigs and poultry (Kerr et al., 2011). Glycerol is a small molecule (92 g/mol) and water soluble substance and it is passively absorbed by intestinal epithelial cells (Kerr et al., 2011). However, in rats, the absorption of glycerol seems to be mainly through facilitated transport (Kato et al. 2004). After absorption, glycerol is converted to glycerol-3-phosphate by glycerol kinase (Figure 3) and then becomes an intermediate in carbohydrate and lipid metabolism (Robergs and Griffin, 1998). Within the carbohydrate pathway, glycerol-3-phosphate can be oxidized through the tricarboxylic acid cycle to yield energy. When oxidized as an energy substrate, glycerol is converted to carbon dioxide and water, with the concomitant release of 4.300 kcal/kg GE. As an intermediate in lipid biosynthesis glycerol-3-phosphate becomes the carbon backbone of triglycerides after reacting with two fatty acyl-CoA molecules (Robergs and Griffin,

1998). Also, glycerol-3-phosphate can be converted into dihydroxyacetone phosphate by the action of glycerol phosphate dehydrogenase and then to glucose via gluconeogenesis. The enzymes that catalyze the reactions of glycerol metabolism are tissue specific, in domestic birds glycerol kinase activity is high in liver and very low in the breast muscle (Newsholme and Taylor 1969; Bernardino et al. 2014). However, glycerol phosphate dehydrogenase is active in both tissues muscle and liver (Harding et al. 1975). Liver glycerol kinase activity increases significantly in broilers fed glycerol and seems to be not saturated at high dietary glycerol concentrations, up to 7% feed.

Figure 3. Biochemical reactions involved in glycerol synthesis and metabolic conversion to glycerol-3-phosphate, phosphatidate and triacylglycerol.

DHA = dihydroxyacetone; DHA-P = dihydroxyacetone phosphate; FAD⁺ = oxidised form of flavin adenine dinucleotide; FADH = reduced form of flavin adenine dinucleotide; FFA = free fatty acid; GDH = glycerol dehydrogenase; GK = glycerol kinase; GLUT4 = glucose transport protein; GPD = glycerol phosphate dehydrogenase; L = lipase; NAD⁺ = oxidised form of nicotinamide adenine dinucleotide; NADH = reduced form of nicotinamide adenine dinucleotide (Robergs and Griffin, 1998).

Broiler chickens fed a diet devoid of glycerol showed an average plasmatic concentration of 5.67 nmol of free glycerol/ml as a result of dietary lipolysis (Bernardino et al. 2014). In this study, the values raised up to a mean of 14.45 nmol of free glycerol/ml in plasma in broilers fed 7% crude glycerin. Consequently, broiler chickens can readily metabolize glycerol to glycerol-3-phosphate in the liver. Glycerol phosphate dehydrogenase activity is high in the chicken liver but also there is an active isoform in the skeletal muscle (Harding et al. 1975) of the three known metabolic roles for this enzyme, biosynthesis through the gluconeogenic pathway, catabolic to oxidize glycerol-3-phosphate and redox buffering capacity, the gluconeogenic seems to be the predominant one (Harding et al. 1975). However, the metabolic fate of glycerol towards gluconeogenesis, energy supply or lipogenesis will depend on glycerol dietary concentration and the bird metabolic status (Kerr et al. 2011). In vertebrates, glycerol kinase and glycerol dehydrogenase, have also a high activity in the kidney and an excess of blood glycerol in response to diet is excreted through urine (Robergs and Griffin, 1998).

1.2.3. Use of crude glycerin in poultry diets

Crude glycerin can be included in poultry feed as an energy source replacing carbohydrates (Cerrate et al. 2006). As indicated previously, pure glycerin is highly digestible and yields around 4.300 kcal/kg of GE. In poultry crude glycerin containing 85% glycerol has similar or even higher energy value than maize, yielding around 3,580 kcal/kg AMEn in broilers (FEDNA, 2010). The energy value of raw glycerin depends on the glycerin, methanol, ash, and total fatty acid concentrations. Taking this into account Kerr et al. (2011) proposed the following equation to predict the AMEn of crude glycerin in broilers: $GE \text{ (kcal/kg)} \times (91.63\% - (0.61 \times \% \text{ free fatty acids}) - (1.17 \times \% \text{ methanol}) + (0.60 \times \% \text{ water}))$. They also proposed a predictive value for AMEn to

be approximately the 97% of the GE in glycerol containing less than 0.5% free fatty acids. In laying hens, Swiatkiewicz and Koreleski (2009) reported a AMEn of 3,970 kcal/kg in crude glycerin containing 74% glycerol. Németh et al. (2014), however, found that the AMEn of a crude glycerin containing 86.8% glycerol was 3,669 kcal/kg. The inconsistencies of the data justifies the need of new studies, concluded with well defined characterized and analyzed crude glycerin to validate the energy value and obtain reliable equations to predict GE and ME values in poultry.

Several studies (see review by Kerr et al., 2011) have shown that glycerin is an acceptable feed ingredient for poultry, and can be safely added at levels of 5-10% in poultry diets. In this respect, Simon et al. (1996; 1997) reported in broilers from 1 to 31 d of age improved growth performance with 5 or 10% glycerol in the diets compared with higher levels of inclusion (15, 20, and 25%). Mclea et al. (2011), Sehu et al. (2012; 2013), and Topal and Ozdogan (2013) reported that the inclusion of 5 to 10% glycerol in the diet increased BW gain and improved FCR in broilers from 1 to 28 or 1 to 42 d of age. Moreover, Jung and Batal (2011) and Silva et al. (2012) reported that the inclusion of 10% glycerin in broilers diet did not affect productive performance. Also, Cerrate et al. (2006) reported in broilers that the inclusion of 5 or 10% glycerol in the diets did not affect growth performance. These authors indicated that feeds that included 10% glycerol did not flow well through the feeder system and resulted in reduced feed intake. In addition, Silva et al. (2012) and Romano et al. (2014) observed that an increase in feed intake in broilers fed diets that included 7.5% glycerin at the expense primarily of corn.

In laying hens, Swiatkiewicz and Koreleski (2009) showed no negative effects on performance or egg quality in hens fed with 2, 4, and 6% glycerol from 28 to 53 wk of age. Similarly, Yalçın et al. (2010) did not reported negative effects on hen

performance or egg quality when 7.5% glycerol was included in laying diet from 39 to 55 wk of age. Moreover, Lammers et al. (2008b); Németh et al. (2014) did not report any effect on performance or quality of eggs when 10 to 15% glycerin was include in laying diets. In contrast, Németh et al. (2013) reported that inclusion of 10% of glycerin hindered FCR in hens from 28 to 37 wk of age without affecting any other production trait. In general, the data indicate that glycerol can be included in diets for broiler and laying hens at levels of up to 10%, with no negative effects on performance, carcass yield, or egg and meat quality (Kerr et al. 2011).

1.3. Lecithin

Lecithins are mixtures of phospholipids obtained as a by-product after animal fat and vegetable oil processing (van Nieuwenhuyzen and Tomás, 2008). Phospholipids are structural components of cell membranes composed by two fatty acids and a phosphonic acid moiety esterified to glycerol. The hydrophilic phosphate ester groups and hydrophobic fatty acid chains makes phospholipids to be surface active and thus, emulsifying agents in biological systems (McDonald et al., 1981). Lecithins of animal origin are derived primarily from milk, eggs and brain tissues and are composed by phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) (van Nieuwenhuyzen and Tomás, 2008). On the other hand, lecithins of vegetable origin are devoid of PS and are obtained mainly from soybean, sunflower and rapeseed oil (Table 1). The variations in the composition and fatty acid profile of lecithins depend on the source (soybean, sunflower, etc.) and on natural variations between different batches. The largest source of commercial lecithin is soybean oil, which an usual composition of 60% polar lipids (most phospholipids and glycolipids) and 35-40% soybean oil (van Nieuwenhuyzen and Tomás, 2008; Mateos et

al., 2012). Other minor components are carbohydrates, pigments, sterols and sterol glycosides (Scholfield, 1981) (Figure 4).

Table 1. Composition in phospholipid and fatty acids of vegetable lecithins by origin.

Lecithin	Soy	Sunflower	Rapeseed
Phospholipids			
Phosphatidylcholine	15	16	17
Phosphatidylethanolamine	11	8	9
Phosphatidylinositol	10	14	10
Phosphatidic acid	4	3	4
Others	7	6	6
All phospholipids	47	47	46
Fatty acid			
Palmitic acid (16:0)	16	11	7
Stearic acid (18:0)	4	4	1
Oleic acid (18:1)	17	18	56
Linoleic acid (18:2)	55	63	25
Linolenic acid (18:3)	7	0	6
Others	1	4	5

(van Nieuwenhuyzen and Tomás, 2008).

Figure 4. Molecular structures of the main phospholipids (van Nieuwenhuyzen and Tomás, 2008).

1.3.1. Production of commercial lecithin

Commercial lecithins results from the degumming of the crude oil. Degumming, the first step in vegetable oil refining, consists in removing the phospholipids from the oil (Figure 5). In the process, crude oil is mixed with water resulting in phospholipid rich oil-water emulsions “gums” that are then removed by centrifugation and dried (Szuhaj, 1983). Crude soybean oil contains around 2-3% of commercial lecithin. Although the production soybean oil has increased during the past years reaching around 50 million tons in 2015 (USDA-FAS, 2015), only 15-20% of the potential gums are processed to obtain lecithins with quality standards for food and feed uses (van Nieuwenhuyzen, 2014).

Figure 5. Production of crude lecithin (Mateos et al., 2012).

1.3.2. Quality standards for lecithin

Quality standards for commercial lecithin include the following specifications: phospholipid content (measured as the amount of acetone-insoluble matter; 60 to 62%), acid value (< 36 mg/KOH/g), non-lipid impurities (earth, seed rests) usually expressed as hexane insoluble (lower than 0.3%), moisture ($< 1\%$) and peroxide value < 10 meq O_2 /kg (van Nieuwenhuyzen and Tomás, 2008; FEDNA, 2015).

1.3.3. Absorption and metabolism of lecithin

Lipid digestion and absorption in birds consists of four sequential steps emulsification, enzymatic hydrolysis by specific lipases, micelle formation with bile salts, and absorption (Krogdahl, 1985). As previously described, phospholipids act as emulsifying agents because of its structural composition. Consequently, phospholipids together with bile salts play a critical role in the emulsification process and thus in speeding up the absorption of fat and in increasing the digestibility of the fats (Krogdahl, 1985). The enzymatic digestion of phospholipids is carried out by pancreatic phospholipases yielding free fatty acids and lysophospholipids that are combined with bile salts and then incorporated into mixed micelles. Fatty acids are then disaggregated from micelles and taken up by the enterocytes by diffuse and active transport (Iqbal and Hussain 2009). In chickens, the jejunum seems to be the intestinal segment with a higher capacity for lipid absorption (Krogdahl, 1985). Once in the enterocyte, the absorbed free fatty acids, lysophospholipids, diglycerides, monoglycerides, cholesterol, phosphatidic acid, phosphorylcholine, and choline are used to synthesize new triglycerides and lipoproteins. In contrast to mammals, avian species drain the newly synthesized lipoproteins directly into the portal blood to the liver and peripheral tissues (Krogdahl, 1985). In birds, lipids are mainly metabolized in the liver, so improving organ functionality by the inclusion of lecithins, can lead to a more effective poultry

production (Huang et al., 2008). Moreover, lecithin can be used to prevent excess fat accumulation in poultry due to the its content in lipotropic factors, such as choline and inositol (Attia et al. 2009; Mateos et al. 2012).

1.3.4. Use of soy lecithin in poultry diets

Soy lecithin is a co-product of the soy industry with numerous uses in animal diets. An important use of lecithin is to enhance the absorption and utilization of fats (triglycerides). Lecithin has the ability to emulsify fat, thereby facilitating the formation of micelles and improving the digestion and absorption of lipids. In commercial practice, lecithins are used to enhance the utilization of other fats by the animal, as well a source of energy (Holland, 1994).

Many studies reported that lecithin is an acceptable feed ingredient for poultry (Emmert et al., 1996; Huang et al., 2007; Attia et al., 2009). Lecithin has a GE of 7,860 kcal/kg for poultry (Mateos et al., 2012) making it a good energy source. It has been shown that the inclusion of up to 6% of lecithin increases the AME of the diet, by improving dietary fat digestibility (Sibbald et al., 1962, Polin, 1980, Peña et al. 2014). However, Donaldson and Ward (1988) reported that inclusion of 5% lecithin in the diet at the expense of animal fat, did not affect AMEn in 21-d old broilers. Similarly, Huang et al. (2007) reported equal AMEn content of a control diet with 2% soy oil and of an experimental diet that contained the same amount of soy lecithin in 42-d old broilers. All this information indicates that lecithin may be useful in the utilization of other lipid sources in poultry diets.

The information available on the effects of dietary lecithin on poultry productive performance is very limited. In broilers, some studies report improvements on BW gain, feed intake and FCR with up to 3.5 % lecithin inclusion (Emmert, et al., 1996, Huang et al. 2007) but other research failed to detect significant effects (Summers and Lesson,

1981; Cantor et al., 1997; Azman and Ciftci, 2004). On the other hand, the use of inositol a component of the lecithin, or of lysolecithin improved broiler performance (Żyła et al. 2004, Raju et al., 2011, Cowieson et al., 2013).

Similar results are reported for laying hens, with improvements on productive performance and increased egg weight and egg mass in birds fed 3% lecithin, 5% phospholipids from crude safflower, or 0.15% lysolecithin (An et al. 1997, Attia et al. 2009, Han et al. 2010). However, Wolford and Polin (1975) reported that the inclusion of 1.6% lecithin in the diet did not affect egg production from 31 to 34 wk of age. The information available on the effects of lecithin on egg quality traits is scarce. In this respect, Attia et al. (2009) reported that the inclusion of 6% lecithin in the diet increased Haugh units, yolk colour, and proportion of yolk in the eggs in hens from 47 to 70 wk of age. The difference among authors with respect to the effects of dietary lecithin on poultry production are not known but might depend on the type of bird used as well as on the characteristics of the diet, including the level, source and fatty acids profile of the lipid source used.

1.4. Re-esterified oils

The chemical re-esterification is a de-acidification process used in the oil industry based on the esterification of free fatty acid (FFA) with hydroxyl groups that remain free in the crude oil or that are added in the form of glycerol (Bhosle and Subramanian, 2005). The process yields a blend of neutral lipids including FFA, MAG, DAG, and TAG named re-esterified oil (Figure 6).

Figure 6. The esterification reaction between free fatty acids and glycerol with the formation of monoacylglycerol (A), diacylglycerol (B), triacylglycerol (C).

1.4.1. Re-esterified oil process

The chemical esterification is produced when FFA are heated with glycerol in the presence or absence of a catalyst. Therefore, the yield, quality and characteristics of the resulting re-esterified oil relies on the reaction temperature, the amount of glycerol, the source of FFA, and the type and quantity of the catalyst if used (reviewed by Kombe et al., 2013). The amount of FFA of the resulting re-esterified oil depends on the temperature and the time for the reaction. According to the literature soapstocks containing from 37 to 50% of FFA can be reduced to less than 5% after 3 to 6 hours of a reaction at 200°C, showing lower yields at lower temperatures (Kombe et al., 2013). A higher yield of the reaction can be achieved with the use of catalyst reducing the final FFA concentration and the time for the reaction. Also, the use of glycerol in excess of the theoretical amount required can reduce the final FFA concentration and affect the kinetics of the reaction reducing the time needed to achieve the maximum yield (Kombe et al., 2013). Moreover, the excess of glycerol in the reaction can be used to increase the

yield of MAG in the resulting oil (Blanco et al. 2004). Also, the proportions of the different lipid classes in the final re-esterified oil can be targeted by using different concentrations of the FFA source and glycerol. This is the case of present thesis where FFA and glycerol ratios of 1:1 and 3:1 were used to obtain re-esterified oils rich in MAG (RMAG) or in TAG (RTAG) respectively. Under our experimental conditions the FFA was reduced to 3% with a reaction held at 180 ± 20 °C for 10 hours in the presence of 150 mg/kg of butylated hydroxytoluene as an antioxidant (Figure 7).

Figure 7. Scheme of the re-esterification oils process.

1.4.2. Use of re-esterified oil in animal diets

The information available on the inclusion of re-esterified oils in substitution of other fat sources in animal diets is very limited. Recent research on re-esterified soy and palm oils has been performed in different species at the Department of Animal and Food Science of the Universitat Autònoma de Barcelona, Spain. In chicken, Vilarrasa et al., (2014; 2015 a, b) reported that re-esterified SO or palm oil can be included in broilers diets as alternative fat sources, without any negative effects on growth

performance and fat absorption, when comparing with correspond native oil. In pigs, Tres et al., (2015) and Vilarrasa et al., (2015 c) reported that the inclusion of re-esterified oil in the diets did not affects growth performance, digestibility, and total fatty acid compared to the native oil. In dogs, Fragua et al., (2013) study the effect of re-esterified palm oil diets, presenting in different proportions of MAG, DAG, and TAG, on postprandial serum TAG concentrations in dogs and chickens, comparing with the correspond native oil. In fish, Trullás et al., (2015; 2016) study the effect of inclusion of re-esterified palm and rapeseed oils in the diets of rainbow trout fish, on growth performance, fat and FA digestibility, and plasma parameters. The authors indicated that the re-esterified oils can be included in fish diets as source of fats without negative affect of parameters that studied.

2. Objectives of the thesis

This thesis aims to study the use of by-products of the bioenergy industry from soy oil in poultry diets. To this end, the inclusion of glycerin, lecithin, and re-esterified oils in the diet of broiler chickens and laying hens were developed in three studies. The individual objectives of the different studies of the research were as follow:

In first study (chapter 2), we evaluated the influence of the source and level of raw glycerin in the diet on growth performance, liver variables, and nutrient digestibility in broilers from 1 to 21 d of age.

In second study (chapter 3), we evaluated the effect of the inclusion of raw glycerin and lecithin in the diet and their interaction on performance and nutrient retention of brown egg-laying hens from 23 to 51 wk of age.

In third study (chapter 4), we evaluated the effect of the inclusion of traditional and re-esterified lipid sources on growth performance, nutrient retention, and metabolizable energy content in broilers from 7 to 21 d of age.

CHAPTER 2:

***INFLUENCE OF SOURCE AND LEVEL OF
GLYCERIN IN THE DIET ON GROWTH
PERFORMANCE, LIVER CHARACTERISTICS,
AND NUTRIENT DIGESTIBILITY IN
BROILERS FROM HATCHING TO 21 DAYS OF
AGE***

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1. Introduction

Raw glycerin (GLYC), a byproduct of the biodiesel industry, is an attractive energy source that can replace part of the of cereal grain in poultry diets (Swiatkiewicz and Koreleski, 2009). Raw GLYC contains variable amounts of water, glycerol, and ash and is generally recognized as safe when used in accordance with good manufacturing and feeding practices (Code of Federal Regulations, 2004; EFSA, 2010). The AMEn content of GLYC in poultry ranges from 3,254 to 4,134 kcal/kg (Lammers et al., 2008; Dozier et al., 2011; McLea et al., 2011), values that corresponded to approximately 96% of the gross energy (GE) supplied by the glycerol contained in the commercial GLYC. The maximum level of dietary GLYC for optimal growth performance of broilers has been estimated within the range of 5 to 10% (Simon et al., 1996; Cerrate et al., 2006; Dozier et al., 2008).

Glycerol is an important structural component of triglycerides and phospholipids that is easily absorbed in the gastrointestinal tract (GIT) of poultry and mammals. Glycerol is a precursor of glyceraldehyde 3-phosphate, an intermediate in the lipogenesis and gluconeogenesis pathways, and can yield energy through the glycolytic and tricarboxylic acid pathways (Lin, 1977; Brisson et al., 2001; Lammers et al., 2008). Kerr et al. (2011) indicated that glycerol metabolism largely occurs in the liver of nonruminant animals. Cryer and Bartley (1973) reported that when glycerol is included at high levels in the diet, it induces anatomical, physiological, and biochemical changes in the liver of rats. However, Lin et al. (1976) observed no effects of dietary glycerol on fatty acid synthesis in the liver or relative weight of the liver in chickens. To our knowledge, no information is available regarding the effect of GLYC inclusion in the diet on apparent ileal digestibility (AID) of nutrients and liver variables in modern strains of broilers. The aim of this research was to evaluate the effect of source and level

of raw GLYC in the diet on growth performance, liver variables, and nutrient digestibility in broilers from 1 to 21 d of age.

2. Material and methods

All experimental procedures were approved by the animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish Guidelines for the Care and Use of Animals in Research (Boletín Oficial del Estado, 2007).

2.1. Husbandry

In total, 630 one-day-old Ross 308 chicks were obtained from a commercial hatchery and distributed at random with an equal number of males and females into 63 cages (1.0 m × 0.9 m; Alternative Design, Siloam Springs, AR). The cages were provided with wire flooring and equipped with 2 nipple drinkers and an open trough feeder. Room temperature was kept at 33°C during the first 3 d of life and then it was reduced gradually according to age until reaching 24°C at 21 d. Chicks received a 23L: 1D light program for the first 7 d of life and then 20L: 4D until the end of the experiment. Broilers had free access to feed and water throughout the experiment.

2.2. Raw GLYC, Diets, and Experimental Design

The GLYC sources used were provided by Bio-Oil Huelva, S. L. (Huelva, Spain) and resulted from the process of biodiesel production from soybean oil. A batch of GLYC was divided into 2 portions and heated at either $146 \pm 2^\circ\text{C}$ or $151 \pm 2^\circ\text{C}$ for 30 min. Consequently, the main difference between the 2 sources of GLYC was the amount of water that remained in the commercial product after the process (4.8 vs. 10.7%) and the glycerol content (87.5 vs. 81.6%). The estimated AMEn concentrations of the 2 GLYC sources were 3,631 and 3,474 kcal/kg, respectively, values that corresponded approximately to 96% of the GE of glycerol. There were 9 experimental

diets that consisted of a corn and soybean meal control diet without any GLYC inclusion and 8 additional diets organized as a 2×4 factorial with 2 sources of GLYC (H-GLYC with 87.5% glycerol and L-GLYC with 81.6% glycerol) at 4 levels of inclusion (2.5, 5.0, 7.5, and 10.0%). For the manufacturing of the feeds, the control diet and the 2 diets that contained 10% GLYC were formulated (Fundación Española Desarrollo Nutrición Animal, 2010). Within each of the 2 GLYC set of diets, the intermediate feeds were obtained by judicious mixing in adequate proportions of the summit diets. Each treatment was replicated 7 times, and the experimental unit was a cage with 10 birds. All diets were presented in crumble form and had similar nutritive value (3,000 kcal of AMEn/kg, 21.8% CP, and 1.20% digestible Lys) according to Fundación Española Desarrollo Nutrición Animal (2008). As the level of GLYC in the diet increased, the amount of corn and soybean oil decreased and that of soybean meal increased. Celite, an acidwashed diatomaceous earth (Celite Hispánica S.A., Alicante, Spain), was added at 1% to all diets to increase the acid insoluble ash content of feeds, ileal digesta, and excreta. The chemical composition of the GLYC sources and the ingredient composition and nutritive value of the experimental diets are shown in Tables 1 and 2, respectively.

2.3. Growth Performance and Digestive Trait Measurements

Body weight and feed consumption were determined by cage at 7, 14, and 21 d of age. Feed wastage was recorded daily in pans placed beneath the cages, and mortality was recorded and weighed as produced. From these data, ADG, ADFI, and feed conversion ratio (**FCR**) were determined by week and for the entire experimental period.

At 21 d of age, 2 birds per cage replicate were randomly selected, weighed individually, and slaughtered by CO₂ inhalation. The GIT, from the beginning of the

proventriculus to the cloaca, including digesta content, liver, spleen, and pancreas, was removed aseptically and weighed. Then, the liver was excised and weighed. The weight of the full GIT and liver was expressed as a percentage of BW. The livers were stored in a freezer at -20°C and representative samples were freeze-dried, ground using a pestle and mortar to pass through a 0.5-mm screen, and mixed by replicate. Samples were maintained in airtight containers at 4°C until chemical analyses.

Table 1. Chemical analyses of the glycerin sources (% as-fed basis, unless otherwise stated)

Item	L-GLYC ¹	H-GLYC ²
DM	89.3	95.2
Gross energy (kcal/kg)	3,578	3,820
Glycerol	81.6	87.5
Ash	5.56	5.59
Ether extract	0.25	0.32
Nitrogen	0.13	0.17
Sodium chloride	4.4	3.9
Methanol	0.06	0.05
Non-glyceride organic matter ³	2.1	1.3
AME _n ⁴ (kcal/kg)	3,474	3,631
pH	5.3	5.3

¹ Raw glycerin containing 81.6% glycerol.

² Raw glycerin containing 87.5% glycerol.

³ Defined as the difference between 100 and the percentage of glycerol, water, and ash of the raw glycerin (Hansen et al., 2009).

⁴ Calculated assuming that the AME_n for glycerol was $0.96 \times$ gross energy.

Table 2. Ingredient and chemical composition of the experimental diets (% as-fed basis, unless otherwise stated)

	Control	L-GLYC ¹				H-GLYC ²			
		2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
Ingredient									
Corn	56.14	53.36	50.59	47.81	45.03	53.38	50.63	47.87	45.11
Soybean meal (47.5 % CP)	34.70	35.15	35.60	36.05	36.50	35.15	35.60	36.05	36.50
Soybean oil	3.50	3.43	3.35	3.28	3.20	3.38	3.25	3.13	3.00
Raw glycerin	—	2.50	5.00	7.50	10.00	2.50	5.00	7.50	10.00
Calcium carbonate	0.70	0.70	0.70	0.69	0.69	0.73	0.75	0.78	0.80
Dicalcium phosphate	2.34	2.35	2.35	2.36	2.36	2.35	2.35	2.36	2.36
Sodium chloride	0.46	0.37	0.28	0.19	0.10	0.37	0.28	0.19	0.10
DL-Methionine (99%)	0.31	0.31	0.32	0.32	0.32	0.31	0.32	0.32	0.32
L-Lys-HCl (78.5%)	0.27	0.26	0.25	0.24	0.23	0.26	0.26	0.25	0.24
L-Thr (99%)	0.08	0.08	0.08	0.07	0.07	0.08	0.08	0.07	0.07
Vitamin and mineral premix ³	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Celite ⁴	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calculated analysis ⁵									
AME _n (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Calcium	0.95	0.95	0.95	0.96	0.97	0.95	0.95	0.96	0.97
Available phosphorus	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Digestible Lys	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Digestible Met	0.59	0.59	0.59	0.60	0.60	0.59	0.59	0.60	0.60
Digestible Thr	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Digestible Trp	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Digestible Arg	1.28	1.29	1.29	1.30	1.30	1.29	1.29	1.30	1.30
Determined analysis ⁶									
Gross energy (kcal/kg)	4,051	4,048	4,082	4,102	4,100	4,056	4,093	4,098	4,104
Dry matter	89.1	89.7	89.6	90.4	89.2	90.6	91.0	92.0	89.9
Crude protein	21.2	21.0	20.9	21.3	21.0	20.8	21.1	21.3	21.0
Total ash	6.3	6.6	6.6	6.7	6.9	6.8	6.7	6.9	7.1
Ether extract	7.2	7.1	6.9	7.0	6.9	6.9	7.0	6.9	6.8

¹ Raw glycerin containing 81.6% glycerol.

² Raw glycerin containing 87.5% glycerol.

³ Provided the following (per kilogram of diet): vitamin A (transretinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 2,000 IU; vitamin E (all-rac-tocopherol acetate), 20 IU; vitamin K (bisulphate menadione complex), 3 mg; riboflavin, 5 mg; pantothenic acid (D-calcium pantothenate), 10 mg; nicotinic acid, 30 mg; pyridoxine (pyridoxine-HCl), 3 mg; thiamin (thiamin-mononitrate), 1 mg; vitamin B12 (cyanocobalamine), 12 µg; D-biotin, 0.15 mg; choline (choline chloride), 300 mg; folic acid, 0.5 mg; Se (Na₂SeO₃), 0.1 mg; I (KI), 2.0 mg; Cu (CuSO₄·5H₂O), 10 mg; Fe (FeSO₄·7H₂O), 30 mg; Zn (ZnO), 100 mg; Mn (MnSO₄·H₂O), 100 mg; and ethoxyquin, 110 mg.

⁴ Acid-washed diatomaceous earth (Celite Hispánica, S.A., Alicante, Spain).

⁵ According to Fundación Española Desarrollo Nutrición Animal (2010).

⁶ In triplicate.

2.4. Nutrient Retention and Apparent Ileal Digestibility

At 21 d of age, representative samples of the excreta produced during the previous 48 h were collected daily by replicate, homogenized, oven-dried (60°C for 72 h), ground using a hammer mill (model Z-I, Retsch, Stuttgart, Germany) fitted with a 1-mm screen, and mixed. The total tract apparent retention (**TTAR**) of DM, organic matter (**OM**), GE, and nitrogen (**N**) of the diets was estimated by the indigestible marker method using celite as inert marker. The AME_n content of the diets, adjusted for N retention, was also determined as indicated by Lazaro et al. (2003). After the determination of the TTAR of the nutrients, all the remaining birds (9-10 broilers per cage) were slaughtered by asphyxiation in CO₂ atmosphere and the ileal section of the small intestine from the vitelline Meckel's diverticulum to 2 cm anterior to the ileocecal junction was immediately removed and the digesta collected by gently flushing the contents with distilled water into plastic containers. Samples were pooled, homogenized, frozen at -20°C, and freeze-dried. Then, the dried samples were ground using a pestle and mortar to pass through a 0.5-mm screen and maintained in airtight containers at room temperature until determination of the AID of DM, N, and GE.

2.5. Laboratory Analyses

Samples of GLYC, diets, and excreta were analyzed for moisture (oven-drying, method 930.01), total ash (muffle furnace, method 942.05), and N (combustion method, 990.03) using a Leco equipment (model FP-528, Leco Corporation, St. Joseph, MI) as described by AOAC International (2000). Gross energy was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL). The ether extract of GLYC and diets was determined after 3 N HCl acid hydrolysis (method Am 5-04) as described by AOCS (2004) using an Ankom XT10 Extraction system (Ankom Technology Corp. Macedon, NY). The GLYC sources were analyzed also for

glycerol (method Ea 6-51) and sodium chloride (method Ea 2-38) content as described by AOCS (2000). Methanol content was determined according to method EN 14110 of the European Standard (2003) and the pH using a digital pH meter (model 507, Crison Instruments S. A., Barcelona, Spain). The non-glyceride organic matter of the GLYC, defined as the difference between 100 and the percentage of glycerol, water, and ash, was also calculated (Hansen et al, 2009). Ileal samples were analyzed for DM, N, and GE as indicated for the diets. Acid insoluble ash of diets, excreta, and ileal contents were determined as indicated by De Coca-Sinova et al. (2011). The lipid content of the livers was determined as indicated by Folch et al. (1957). In addition, the color of the liver [lightness (L^*), redness (a^*), and yellowness (b^*)] was measured by spectrophotometry (CM-700d/600d, Konica Minolta Company, Osaka, Japan) at a wavelength range from 400 to 700 nm. All analyses were conducted in triplicate for feed samples and livers and in duplicate for GLYC, excreta, and ileal contents.

2.6. Statistical Analysis

Data on growth performance, GIT weight, liver characteristics, and TTAR and AID of nutrients were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Institute, 1990). For the 8 diets arranged as a 2×4 factorial, the main effects (source and level of GLYC inclusion) and the interaction were studied. In addition, treatment sums of squares for GLYC content of the feeds were partitioned into linear (L) and quadratic (Q) responses to increasing levels of GLYC in the diet (0, 2.5, 5.0, 7.5, and 10.0%). Results in tables are reported as least-square means. When the model was significant, the Tukey test was used to make pairwise comparisons between treatment means. Differences among treatments were considered significant at $P < 0.05$.

3. Results

The determined chemical analyses of the GLYC sources were close to planned values (Table 1). As expected, the main differences between the 2 GLYC sources tested were the moisture (9.7 vs 4.8%) and the glycerol (81.6 vs 87.5%) contents. The methanol concentration of the 2 samples of GLYC was low (600 and 500 mg/kg, respectively) and was not expected to create any metabolic problem in the chicks (Dozier et al., 2011; Jung and Batal, 2011). The determined nutritive values of the experimental diets were close to expected values, confirming that the diets were mixed correctly (Table 2).

3.1. Growth Performance and Digestive Trait Measurements

No interactions between source and level of GLYC in the diet were observed for any of the traits studied and therefore, only main effects are presented (Tables 3 and 4). Type of GLYC did not affect broiler performance or GIT traits in any of the periods considered. However, the inclusion of GLYC in the diet tended to improve ADG (L, $P = 0.108$) and improved FCR (L, $P < 0.01$) from 1 to 21 d of age. Most of the beneficial effects of GLYC inclusion on broiler performance were observed during the first days of life. In this respect, the FCR from d 1 to 7 (L, $P < 0.01$) and from d 7 to 14 (L, $P = 0.095$) were improved as the GLYC content of the diet increased but no benefits were detected from d 14 to 21. The inclusion of GLYC in the diet increased the relative weight of the GIT (L, $P < 0.01$) and the liver (L, $P = 0.01$) but lipid content and color variables of the liver were not affected (Table 4).

3.2. Nutrient Retention and Apparent Ileal Digestibility

No interactions between source and level of dietary GLYC were detected for TTAR or AID of the nutrients and therefore, only main effects are presented (Tables 5 and 6). The TTAR of DM ($P \leq 0.05$), OM ($P = 0.05$), and GE ($P = 0.092$) were higher

for the H-GLYC diets than for the L-GLYC diets. Also, DM (Q, $P \leq 0.05$) and OM (Q, $P \leq 0.05$) retention increased as the level of GLYC in the diet increased with the highest retention values reported with 2.5 to 5.0% GLYC inclusion. Moreover, the AME_n of the diet increased as the level of GLYC increased (L, $P \leq 0.01$). The inclusion of GLYC in the diet increased the AID of DM (L, $P = 0.01$; Q, $P = 0.067$) and GE (L, $P < 0.01$; Q, $P = 0.102$). However, neither TTAR nor AID of N was affected by diet.

4. Discussion

4.1. Growth Performance and Digestive Trait Measurements

In the current experiment, type of GLYC did not affect broiler performance, a finding that was expected because diets were formulated using different AME_n values for the 2 GLYC used. The AME_n content of GLYC in poultry reported in the literature varies widely but most of the differences observed among researchers are due primarily to the glycerol content of the GLYC batch used. The AME_n content of the H-GLYC (87.5% glycerol) and L-GLYC (81.6% glycerol) used in the current experiment was 3,631 and 3,474 kcal/kg, respectively, values that compare well with the value of 3,540 kcal/kg recommended by Fundación Española Desarrollo Nutrición Animal (2010) for a commercial GLYC with 85 % glycerol. In fact, the energy content (per unit of glycerol) used in the current research for the GLYC were slightly lower than the value of 3,621 kcal/kg determined by Dozier et al. (2008) and of 3,487 kcal/kg determined by Mclea et al. (2011) for GLYC samples containing 86.9% and 81.0% glycerol, respectively.

Table 3. Influence of source and level of raw glycerin (GLYC) in the diet on growth performance of broilers

	1-7 days			7-14 days			14-21 days			1-21 days		
	ADG	ADFI	FCR ¹	ADG	ADFI	FCR	ADG	ADFI	FCR	ADG	ADFI	FCR
GLYC source (%)												
L-GLYC ²	21.5	23.2	1.08	42.0	53.4	1.27	47.6	72.9	1.53	37.0	49.2	1.33
H-GLYC ³	21.7	23.4	1.08	41.9	53.2	1.27	47.7	72.5	1.52	37.1	49.2	1.33
GLYC level (%)												
0.0	21.7	23.8	1.10	41.3	53.3	1.29	46.2	71.5	1.55	36.4	49.2	1.35
2.5	21.7	23.4	1.08	42.3	53.8	1.28	47.1	71.9	1.53	37.0	49.3	1.33
5.0	21.8	23.5	1.08	41.9	52.4	1.25	47.9	72.3	1.51	37.2	49.0	1.32
7.5	22.0	23.6	1.08	42.6	54.3	1.28	47.7	72.9	1.53	37.4	49.7	1.33
10.0	21.0	22.4	1.07	41.7	52.8	1.27	49.6	75.0	1.52	37.4	49.0	1.31
SEM ⁴	0.45	0.43	0.01	0.89	0.93	0.01	1.71	2.27	0.02	0.65	0.79	0.01
<i>P-value</i> ⁵												
GLYC source	0.445	0.613	0.660	0.824	0.692	0.777	0.966	0.829	0.600	0.917	0.959	0.637
GLYC level												
Linear	0.213	0.008	0.006	0.601	0.779	0.095	0.057	0.121	0.208	0.108	0.967	0.002
Quadratic	0.160	0.230	0.466	0.225	0.790	0.075	0.841	0.528	0.391	0.476	0.764	0.453

¹ Feed conversion ratio.² Raw glycerin containing 81.6% glycerol.³ Raw glycerin containing 87.5% glycerol.⁴ Standard error of the mean (n = 7 replicates of 10 birds each).⁵ The interaction between source and level of glycerin was not significant (P > 0.10).

The inclusion of up to 10% GLYC in the diet improved growth performance of the broilers with effects being more pronounced for FCR than for ADG and for the first week of life than thereafter, consistent with data of Lima et al. (2013) who reported that young broilers have a higher capacity to utilize the energy content from this ingredient than older broilers. Mclea et al. (2011) and Topal and Ozdogan (2013) reported also that the inclusion of 8 to 10% glycerol in the diet improved ADG and FCR in broilers from 1 to 28 d of age, in agreement with the results of the current experiment. Similarly, Yalçın et al. (2010) observed that the inclusion of 7.5% GLYC in the diet improved FCR in laying hens from 39 to 55 wk of age. In contrast, Simon et al. (1996) and Cerrate et al. (2006) reported that the inclusion of 5 or 10% GLYC in the diet did not affect FCR of broilers although ADG was also improved.

In the current research broilers utilized more efficiently the diets that contained GLYC than the diet containing no GLYC, suggesting that the real contribution of GLYC to the energy content of the diet was higher than calculated based on the estimate of 4,160 kcal AME_n per kg glycerol of the GLYC of the Fundación Española Desarrollo Nutrición Animal (2010) (e.g., 3,540 kcal AME_n/kg for the H-GLYC that contained 85% glycerol). The experimental diets were formulated on metabolizable energy bases rather than on net energy bases. Consequently, the real contribution of glycerol to the energy content of the diet was underestimated because glycerol is absorbed by the bird more rapidly and efficaciously (without any heat loss) than corn (Simon et al., 1997). In addition, because of its physico-chemical properties, GLYC inclusion may affect digesta transit time in the GIT of the birds, improving the utilization of other components of the diet, as has been demonstrated for fats by Mateos and Sell (1980, 1981). The data provided indicate that the coefficient of utilization of the GE of glycerol contained in the GLYC averaged 96% in young broilers, a value that is higher than the

range of 85.0 to 90.3% reported for poultry by Alvarenga et al. (2012) and for nursery and growing pigs by Kerr et al. (2009) and Kovács et al. (2011).

The effects of dietary GLYC on the size and fat content of the liver have not been studied extensively in broilers and in fact, the information available is conflicting. Sehu et al. (2012) observed that the weight of the liver of 42 d-old broilers increased when 10% GLYC was included in the diet, in agreement with the results reported herein. In the liver, glycerol can be converted into glucose and used to yield energy or for synthesis of triglyceride and phospholipid (Robergs and Griffin, 1998). Cryer and Bartley (1973) suggested that the increase in liver size observed in rats fed high levels of GLYC was caused by increased gluconeogenesis that resulted in overloading of liver function. In contrast, Lessard et al. (1993) and Topal and Ozdogan (2013) reported no effects on liver size in broilers of similar age when 5 or 8% GLYC was included in the diet. Moreover, Suchy et al. (2011) reported that the weight of the liver of 40 d-old broilers was reduced when 3% GLYC was included in the diet. All this information suggests that the response of the liver to dietary glycerol might depend on the dose, with increases in liver size when high concentrations of GLYC are used.

Dietary GLYC did not affect fat accumulation in the liver, in agreement with data of Lessard et al. (1993) in chickens and Cryer and Bartley (1973) in rats. In non-ruminants, GLYC is metabolized preferably to glucose at a rate that will depend on the metabolic state of the animal and the amount of glycerol consumed (Kerr et al., 2011). In this respect, Lin et al. (1976) reported that the inclusion in the diet of 42.1% glycerol reduced lipogenic enzyme activity in the liver of the chicken. Liver color was not affected by the inclusion of GLYC in the diet, suggesting that glycerol did not affect the macroscopic characteristics and function of the liver. In this respect, Suchy et al. (2011)

Table 4. Influence of source and level of raw glycerin (GLYC) in the diet on gastrointestinal tract (GIT) weight, and weight, lipid, and color of the liver in broilers at 21 days of age

Item	BW (g)	Relative weight (% of BW)		g lipid/100 g of liver	Liver color ²		
		GIT ¹	Liver		L*	a*	b*
GLYC source (%)							
L-GLYC ³	872	15.0	2.77	12.4	30.1	15.1	10.4
H-GLYC ⁴	864	14.8	2.74	12.4	30.5	15.6	10.8
GLYC level (%)							
0.0	846	14.5	2.64	12.1	30.2	14.8	10.9
2.5	862	14.4	2.67	12.4	30.3	16.2	10.8
5.0	864	15.0	2.82	12.4	30.8	15.2	10.4
7.5	852	14.8	2.75	12.7	30.3	15.2	10.5
10.0	894	15.5	2.79	12.3	29.9	15.3	10.4
SEM ⁵	20.01	0.35	0.01	0.41	1.03	0.69	0.71
<i>P-value</i> ⁶							
GLYC source	0.638	0.326	0.601	0.914	0.500	0.247	0.353
GLYC level							
Linear	0.062	0.003	0.015	0.402	0.769	0.820	0.374
Quadratic	0.482	0.511	0.246	0.367	0.466	0.304	0.883

¹ Weight of the full GIT (from end of the crop to cloaca) including digesta contents and liver, spleen, and pancreas.

² Lightness (L*), redness (a*), and yellowness (b*).

³ Raw glycerin containing 81.6% glycerol.

⁴ Raw glycerin containing 87.5% glycerol.

⁵ Standard error of the mean (n = 7 replicates of 2 birds each).

⁶ The interaction between source and level of glycerin was not significant (P > 0.10).

reported also that the inclusion of 3% GLYC in the diet did not affect the color, shape, structure, or consistency of the liver in broilers. Further research on the effects of level of glycerol consumption on the activity of gluconeogenic enzymes in broilers is needed to evaluate how a metabolic overload of glycerol affects liver size and lipid deposition.

Broilers fed 10% GLYC had heavier digestive tracts than broilers fed the control diet. The authors have not found any full report on the effects of GLYC inclusion in the diet on GIT development in broilers to compare with the results reported herein. In this respect, Topal and Ozdogan (2013) reported no effects on proventriculus and gizzard weight in 42 d-old broilers when 8% GLYC was included in the diet.

Table 5. Influence of source and level of raw glycerin (GLYC) in the diet on total tract apparent retention (%) of nutrients and AME_n of the diet in broilers at 21 days of age

	DM	Organic Matter	Nitrogen	Gross energy	AME _n (kcal/kg)
GLYC source (%)					
L-GLYC ¹	71.1	75.5	64.2	77.0	2,907
H-GLYC ²	71.9	76.2	64.5	77.5	2,927
GLYC level (%)					
0.0	70.6	74.9	64.3	76.5	2,880
2.5	72.0	76.3	64.0	77.6	2,900
5.0	71.9	76.2	64.8	77.6	2,930
7.5	71.6	76.0	64.0	77.2	2,927
10.0	71.5	75.8	64.6	77.4	2,948
SEM ³	0.42	0.39	0.67	0.35	12.88
<i>P</i> -value ⁴					
GLYC source	0.037	0.045	0.626	0.092	0.110
GLYC level					
Linear	0.285	0.188	0.803	0.217	0.006
Quadratic	0.034	0.017	0.908	0.116	0.506

¹ Raw glycerin containing 81.6% glycerol.

² Raw glycerin containing 87.5% glycerol.

³ Standard error of the mean (n = 7 replicates of 10 birds each).

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

Table 6. Influence of source and level of raw glycerin (GLYC) in the diet on the apparent ileal digestibility (%) of nutrients in broilers at 21 days of age

	DM	Nitrogen	Gross energy
GLYC source (%)			
L-GLYC ¹	69.9	77.9	75.5
H-GLYC ²	70.1	77.8	75.4
GLYC level (%)			
0.0	67.0	76.8	72.8
2.5	69.4	77.2	74.9
5.0	70.9	78.1	76.1
7.5	72.2	79.4	77.2
10.0	70.5	78.0	76.2
SEM ³	1.68	1.48	1.44
<i>P-value</i> ⁴			
GLYC source	0.841	0.906	0.918
GLYC level			
Linear	0.011	0.158	0.006
Quadratic	0.067	0.413	0.102

¹ Raw glycerin containing 81.6% glycerol.² Raw glycerin containing 87.5% glycerol.³ Standard error of the mean (n = 7 replicates of 10 birds each).⁴ The interaction between source and level of glycerin was not significant (P > 0.10).

4.2. Nutrient Retention and Apparent Ileal Digestibility

The TTAR of all nutrients, except N that was not affected, tended to be higher in broilers fed the H-GLYC than in broilers fed the L-GLYC diets although the differences reported were of little practical interest. Similar results were observed with increases in the level of GLYC of the diet. Diets were formulated to have similar calculated AME_n concentration according to Fundación Española Desarrollo Nutrición Animal (2010), assuming in all cases a metabolicity of 96% for the GE of the glycerol contained in the GLYC. Kim et al. (2013) reported also that the TTAR of DM and GE, but not of N, increased when 5% glycerol was included in the diet of 31 d-old broilers. Similarly, Swiatkiewicz and Koreleski (2009) did not detect any significant effect on N retention when up to 6% GLYC was included in the diet of laying hens, consistent with the results of the current experiment. Also in pigs, Zijlstra et al. (2009) reported that the

TTAD of GE increased as the level of dietary GLYC increased from 0 to 8%. Moreover, Madrid et al. (2013) reported that nutrient digestibility of OM but not of N, increased when 5% GLYC was included in the diet, although the authors did not provide any explanation for the improvement observed. When GLYC is included in the diet, small variations in ingredient and chemical composition occurred, which might have resulted in the small improvements in nutrient digestibility reported by the different authors.

The AME_n concentration of the diet increased by 2.3% (from 2,880 to 2,948 Kcal/kg) as the level of GLYC increased from 0 to 10%. The reason for the improvement in energy content of the diet as the level of GLYC increased is not apparent but the data are consistent with the higher retention reported for OM and GE. Dozier et al. (2008) reported also a an increase of 1.4% in the AME_n content of the diet in 45 d-old broilers when the GLYC level of the diet increased from 0 to 9%. Similarly, Mclea et al. (2011) reported a 5.9% increase in the energy content of the diet as the GLYC level increased from 0 to 10 % in 28 d-old in broilers. Dietary GLYC might improve nutrient utilization of other ingredients of the diet as has been demonstrated with supplemental fat by Mateos et al. (1982). These authors reported that supplemental fat delays the intestinal transit time of the digesta, allowing a better contact between the components of the digesta and the endogenous enzymes present in the GIT, which in turn could improve nutrient utilization. However, Kim et al. (2013) were not able to detect any benefit of glycerol supplementation on the intestinal transit time of the digesta in 31-d old broilers. Therefore, other mechanisms might be involved. In this respect, some constituents of the GLYC used, others than the glycerol, might account for the differences observed (EFSA, 2010; Alvarenga et al., 2012). For example, Dozier et al. (2011) reported in 17-d old broilers higher energy content per unit of glycerol of

the GLYC originated from soybean oil than of the GLYC originated from tallow or poultry fat.

The AID of GE and DM increased as the level of GLYC in the diet increased, but that of N was not affected, consistent with the TTAR data. In contrast, Mclea et al. (2011) did not detect any influence of the level of dietary GLYC (0 to 10%) on the AID of DM. The authors have not found any other published report on the effects of GLYC inclusion on AID of nutrients in poultry. In the current research, the AID of GE increased as the GLYC content of the diet increased but GE retention was not affected. GLYC is almost completely absorbed in the small intestine of the bird and therefore, GE ileal digestibility was expected to be higher for the GLYC containing diets. On the other hand, the fermentation process that takes place in the large intestine will increase the utilization of the energy of the remaining substrates in all dietary treatments, and consequently the GE retention of all diets will tend to be equalized.

In summary, raw GLYC can be used at levels of at least 10% in substitution of other energy sources in broiler diets without any negative effect on growth performance. An increase in the level of GLYC in the diet increased TTAR and AID of all nutrients, except N, in broilers at 21d of age. Also, GLYC inclusion increased the relative weight of the liver but lipid content and liver color were not affected. Type of GLYC did not affect any of the variables studied except DM, OM, and GE retention that tended to be higher with the H-GLYC diets. In conclusion, the data indicate that broilers might benefit of the inclusion of moderate (up to 10%) amounts of high quality GLYC in the diet.

CHAPTER 3:

***GLYCERIN AND LECITHIN INCLUSION IN
DIETS FOR BROWN EGG-LAYING HENS:
EFFECTS ON EGG PRODUCTION AND
NUTRIENT DIGESTIBILITY***

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1. Introduction

Raw glycerin, a coproduct of the ethanol industry, is an attractive energy source that can replace part of the cereal grain in poultry diets and contribute to reduce feed cost (Lammers et al., 2008; Swiatkiewicz and Koreleski, 2009; Mandalawi et al., 2014). Glycerol, a precursor of glyceraldehyde 3-phosphate, is an intermediate in the lipogenesis and gluconeogenesis pathways and yields energy through the glycolytic and tricarboxylic-acid pathways (Brisson et al., 2001; Lammers et al., 2008). The commercial product, raw glycerin (GLYC), is recognized as safe when used in accordance with good manufacturing and feeding practices (Code of Federal Regulations, 2004). Raw glycerin contains variable amounts of water and ash and its nutritive value depends primarily on the glycerol content. The maximum level of inclusion of GLYC in laying hen diets for optimal production, has been estimated within the range of 50 to 100 g/kg (Lammers et al., 2008; Swiatkiewicz and Koreleski, 2009; Németh et al., 2013). Also, the inclusion of GLYC in broiler diets increased dry matter (DM) and gross energy (GE) retention (Kim et al., 2013; Mandalawi et al., 2014). In spite of the commercial interest for using GLYC as an energy source in the diet, little information is available on the effects of GLYC on total tract apparent retention (TTAR) of dietary components and egg quality in laying hens.

Lecithin is a by-product of the oil refining industry. Soybeans are the largest source of commercially available raw lecithin. Commercial raw soy lecithin is a combination of polar lipids (mostly phospholipids and glycolipids) and oil at an approximate ratio of 60:40 and has a high content of unsaturated fatty acids (FA) (van Nieuwenhuyzen and Tomás, 2008; Mateos et al., 2012). In addition, soy lecithin is rich in phospholipids a fraction of the oil with important emulsifying and anti-oxidant properties. The inclusion of raw lecithin in the diet could improve lipid digestibility and liver

function, effects that should be more pronounced in birds fed diets rich in saturated fats because of the key role of unsaturated FA and phospholipids in micelle formation (An et al., 1997; Huang et al., 2007).

The information available on the effects of raw lecithin inclusion in laying hens diets as a replacement of animal fat is scarce. Moreover, no published report is available on the effects of the inclusion GLYC and lecithin in the diet and their interaction on nutrient digestibility and performance of laying hens. We hypothesized that moderate amounts of GLYC could be used in diets for laying hens without affecting performance and that the inclusion of lecithin in the diet, at the expense of animal fat, could improve FA digestibility, nutrient retention and egg weight because of the easier incorporation into the yolk of dietary phospholipids, as compared with saturated, non-polar fats. The aim of this research was to evaluate the effect of the inclusion of raw glycerin and lecithin in the diet and their interaction on performance and nutrient retention of brown egg-laying hens from 23 to 51 wk of age.

2. Materials and methods

All experimental procedures were approved (PROEX 007/15) by the animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish Guidelines for the Care and Use of Animals in Research (Boletín Oficial del Estado, 2007).

2.1. Glycerin, lecithin and animal fat

The batch of GLYC used was a by-product of the biofuel industry from soybean oil and contained by analyses 810 g glycerol and 590 mg methanol/kg. The batch of raw lecithin was a by-product of the biodiesel industry from soy oil and contained 600 g polar lipids/kg. The animal fat was obtained from non-edible, out of date, pork parts (mostly

hams and shoulders) after grinding and cooking the original materials at 3 bars and 133 °C for 20 min (Woodgate and Van der Veen, 2004). The chemical composition of the GLYC and the lipid sources used is shown in Table 1.

Table 1

Determined chemical analyses (g/kg as-fed basis) of the ingredients tested.

	Raw glycerin ^a	Lecithin ^b	Animal fat ^c
Dry matter	898	990	988
Gross energy (MJ/kg)	14.90	32.57	38.87
Acetone insoluble ^d	-	600	-
Ash	55.7	45.0	-
Nitrogen	1.1	4	-
Ether extract	3.6	923	980
Linoleic acid	-	470	75.0
Oleic acid	-	147	421
Stearic acid	-	37	116
Calcium	0.4	1.7	-
Phosphorus	2.4	18.6	-
Sodium	16.0	0.6	-
AME _n (MJ/kg) ^e	14.54	31.90	33.91
Glycerol	810	-	-
Initial peroxide value (meq/kg)	-	1	20

^a Contained 0.59 g methanol and 21 g non-glyceride organic matter/kg. The pH was 5.3.

^b Contained 21 g inositol and 20 g choline/kg.

^c The content of moisture, impurities and unsaponifiables (MIU) was 20 g/kg.

^d The composition of the phospholipid fraction (450 g/kg acetone insoluble) in g/kg was phosphatidylcholine 150, phosphatidylethanolamine 100, phosphatidylinositol 100, phosphatidic acid 30, lysophosphatidylcholine 10, lysophosphatidylethanolamine 10 and others, 50.

^e Calculated values according to Fundación Española Desarrollo Nutrición Animal (2010).

2.2. Husbandry, diets and experimental design

In total, 480 Lohmann Brown hens were housed in an environmentally controlled barn at 23 wk of age. At arrival to the experimental station, the hens were weighed individually and placed in groups of ten with similar average body weight in 48 enriched cages (40 cm x 80 cm x 68 cm; Facco S. p. A., Padova, Italy) provided with an open trough feeder and two nipple drinkers. The temperature inside the barn was recorded

daily with average values that varied from 26 ± 2 °C in July (first period of the experiment) to 24 ± 2 °C in March (last period of the experiment). The light program consisted of 16 h light per day. All diets were based on maize and soybean meal and included 40 g supplemental fat/kg. The diets were formulated to have similar nutritive value according to Fundación Española Desarrollo Nutrición Animal (2010) and contained, on calculated bases, 11.51 MJ apparent metabolisable energy corrected for nitrogen (AME_n), 165 g crude protein and 7.3 g digestible Lys per kilogram. Diets were fed in mash form. The diets that included 70 g GLYC/kg had less maize and more soybean meal than the diets without GLYC. Lecithin, at 20 or 40 g/kg, was incorporated into the corresponding experimental diets at the expense (wt:wt) of the animal fat. In the formulation of the diets, no attempt was made to equalize the AME_n concentration of the diets, as a consequence of differences in energy content of the two fat sources used, because the AME_n of the lecithin in laying hen diets is not well established, with values ranging from 27.5 MJ/kg (Peña et al., 2014) to 35.5 MJ/kg (Mateos et al., 2012). Two batches of experimental diets were manufactured; the first batch was used for the first four feeding periods and the second batch was used for the last three feeding periods. The ingredient composition and the calculated and determined chemical analysis of the experimental diets are shown in Tables 2 and 3, respectively.

The experimental design was completely randomized with six treatments that resulted from the combination of two levels of GLYC (0 and 70 g/kg) and three animal fat to lecithin ratios (40:0, 20:20 and 0:40 g/kg). Eight replicates per treatment were used and the experimental unit was the cage with ten hens for all measurements.

Table 2

Ingredient composition and calculated chemical analysis of the experimental diets (g/kg as fed basis, unless otherwise stated).

Lecithin ^a , g/kg	Raw glycerin, 0 g/kg			Raw glycerin, 70 g/kg		
	0	20	40	0	20	40
Ingredient						
Maize	565.5	565.5	565.5	486.0	486.0	486.0
Soybean meal (480 g CP/kg)	212.4	212.4	212.4	225.1	225.1	225.1
Sunflower meal (280 g CP/kg)	70.0	70.0	70.0	70.0	70.0	70.0
Raw glycerin (816 g glycerol/kg)	0.0	0.0	0.0	70.0	70.0	70.0
Animal fat	40.0	20.0	0.0	40.0	20.0	0.0
Soy lecithin	0.0	20.0	40.0	0.0	20.0	40.0
Calcium carbonate	91.0	91.0	91.0	91.1	91.1	91.1
Monocalcium phosphate	10.0	10.0	10.0	10.1	10.1	10.1
Sodium chloride	4.7	4.7	4.7	1.5	1.5	1.5
DL-Methionine (990 g/kg)	1.1	1.1	1.1	1.2	1.2	1.2
L-Lysine HCl (780 g/kg)	0.3	0.3	0.3	-	-	-
Vitamin and mineral premix ^b	5.0	5.0	5.0	5.0	5.0	5.0
Calculated analysis^c						
Dry matter	888.6	888.4	888.2	891.1	890.9	890.7
Total ash	125.6	126.5	127.4	126.4	127.3	128.2
AME _n (MJ/kg)	11.5	11.5	11.5	11.5	11.5	11.5
Crude protein	165.0	165.0	165.0	165.0	165.0	165.0
Ether extract	65.1	63.6	62.0	62.6	61.1	59.6
Linoleic acid	15.0	22.2	29.4	13.6	20.8	28.0
Crude fiber	37.5	37.5	37.5	36.0	36.0	36.0
Total phosphorus	5.8	6.1	6.4	5.8	6.2	6.5
Sodium	1.8	1.8	1.8	1.8	1.8	1.8
Calcium	37.9	37.9	38.0	38.0	38.0	38.1
Digestible Lys	7.2	7.2	7.2	7.4	7.4	7.4
Digestible Met	3.6	3.6	3.6	3.6	3.6	3.6
Digestible Met+Cys	5.9	5.9	5.9	5.9	5.9	5.9
Digestible Thr	5.4	5.4	5.4	5.4	5.4	5.4
Digestible Trp	1.7	1.7	1.7	1.7	1.7	1.7

^a Lecithin was included in the diet at expense (wt:wt) of the animal fat.

^b Supplied per kg of diet: vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (dL- α -tocopheryl acetate), 30 mg; vitamin B1, 1 mg; vitamin B2, 4 mg; vitamin B6, 1 mg; vitamin B12 (cyanocobalamin), 15 μ g; vitamin K₃ (bisulfate menadione complex), 2.5 mg; choline (choline chloride), 150 mg; nicotinic acid, 25 mg; pantothenic acid (d-calcium pantothenate), 7.5 mg; folic acid, 0.10, mg; manganese (MnO), 80 mg; zinc (ZnO), 60 mg; iron (FeSO₄ H₂O), 30 mg; copper (CuSO₄ · 5H₂O), 5 mg; iodine (Ca (IO₃)₂), 0.5 mg; selenium (Na₂SeO₃), 0.3 mg; canthaxantin; 2.4 g; ester of β -apo-8-carotenoic, 1.7 g (Lucanmix yellow/red, Basf, Tarragona, Spain), [Endo-1.3(4)- β -glucanase (EC 3.2.1.6), 150 IU/g]; [Endo-1.4- β -xylanase (EC 3.2.1.8), 105 IU/g (Endofeed, GNC Bioferm, Saskatchewan, SK, Canada)], Natuphos 5000 [300 FTU/kg of 6-phytase (EC 3.1.3.26), Basf Española, S.A., Tarragona, Spain].

^c According to Fundación Española Desarrollo Nutrición Animal (2010).

2.3. Hen performance and egg quality

Feed disappearance and egg production were recorded by replicate for seven 28-d periods as well as for the entire experiment (23 to 51 wk of age). All the eggs produced the last day of each week of the experiment were weighed by replicate and the average value of the 4 wk was used to estimate egg weight by period. Also, all the hens were weighed by replicate at the start of the experiment and at the end of each of the 7 experimental periods. Any mortality was recorded and weighed as produced. From these data, egg production, feed intake, average egg weight, egg mass, feed conversion ratio (FCR) per kilogram of eggs and per dozen of eggs and body weight gain (BWG) were calculated by period and cumulatively. Egg quality, including Haugh units, yolk color, shell strength and shell thickness, were measured in 12 eggs collected at random from each replicate the last 2-d of each of the 7 experimental periods. Haugh units and yolk color (Roche color fan) were measured using a Multitester equipment (QCM System, Technical Services and Supplies, Dunnington, York, UK) as indicated by Pérez Bonilla et al. (2012). Egg shell strength, expressed in kg/cm^2 , was evaluated applying increased pressure to the broad pole of the egg using a press meter (Egg Force Reader, Sanovo Technology A/S, Odense, Denmark) as indicated by Safaa et al. (2008). Shell thickness (average of 3 measurements per egg) was measured with a digital micrometer (model IT-014UT, Mitotuyo, Kawasaki, Japan). The proportion of albumen, yolk and shell of the eggs were determined in all the eggs used for egg quality measurements. In addition, the number of under-grades (dirty, broken, fissured and shell-less eggs) was recorded daily in all eggs produced. An egg was considered as dirty when a spot of any kind or size was detected on the shell (Lázaro et al., 2003).

2.4. Total tract apparent retention of nutrients

At 26 wk of age, hens were fed for six days their respective experimental diets to which 20 g of an acid-washed diatomaceous earth (Celite, Celite Hispánica S.A., Alicante, Spain)/kg was added. Excreta samples were collected from six out of the eight replicates from each treatment, exclusively. Representative samples of the excreta produced during the last 48 h were collected daily by replicate, homogenized, oven-dried (60 °C for 72 h) and ground using a hammer mill (model Z-I, Retsch, Stuttgart, Germany) fitted with a 1-mm screen and mixed. The total tract apparent retention (TTAR) of DM, organic matter (OM), gross energy, EE and nitrogen (N) of the diets was estimated using the indigestible marker method (De Coca-Sinova et al., 2011). The AME_n content of the diets was determined as indicated by Lázaro et al. (2003).

Table 3

Determined analysis^a (g/kg as-fed basis, unless otherwise indicated) of the diets.

Lecithin ^b , g/kg	Raw glycerin, 0 g/kg			Raw glycerin, 70 g/kg		
	0	20	40	0	20	40
Batch 1						
Gross energy (MJ/kg)	15.59	15.28	15.27	15.62	15.27	15.22
Dry matter	913	908	912	903	898	892
Crude protein	164	167	166	170	170	172
Ether extract	67.9	63.4	57.0	64.5	62.5	52.8
Total ash	122	123	122	136	126	126
GMD ^c + GSD ^d (µm)	934 ± 2.13	1,027 ± 2.09	997 ± 2.13	1,006 ± 2.12	1,023 ± 2.09	1,002 ± 2.05
Batch 2						
Gross energy (MJ/kg)	15.59	15.50	15.37	15.42	15.29	15.26
Dry matter	902	911	906	913	916	916
Crude protein	162	156	164	159	161	159
Ether extract	68.4	62.1	56.7	65.4	57.3	53.9
Total ash	124	122	118	129	131	121
GMD + GSD (µm)	966 ± 2.15	1,025 ± 2.10	1,010 ± 2.14	992 ± 2.11	1,012 ± 2.09	999 ± 2.09

^aAnalysed in triplicate.

^b Lecithin was included in the diet at expense (wt:wt) of the animal fat, without considering any potential difference in AME_n content.

^cGeometric mean diameter.

^dGeometric standard deviation (Log normal SD).

2.5. Laboratory analysis

Samples of diets and excreta were analysed for moisture by oven-drying (method 930.01), total ash using a muffle furnace (method 942.05) and nitrogen by combustion (method, 990.03) using a Leco equipment (model FP-528, Leco Corporation, St. Joseph, MI) as described by AOAC International (2000). Gross energy was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL) and ether extract was analysed after 3 *N* HCl acid hydrolysis (method Am 5–04) using an Ankom XT10 Extraction system (Ankom Technology Corp. Macedon, NY) as described by AOCS (2004). Samples of GLYC, lecithin and animal fat were analysed for moisture (method, 984.20) as described by AOAC International (2000). Nitrogen was determined using an automatic analyser (2300 Kjeltex, Foss Analytical, Hillerød, Denmark). Ether extract was determined by Soxhlet analysis after 3 *N* HCl acid hydrolysis (method 4.b) as described by Boletín Oficial del Estado (1995). Total ash and gross energy of the fat sources were analysed as indicated for the diet samples. Glycerol, methanol and non-glyceride organic matter content, defined as the difference between 100 and the percentage of glycerol, water and ash and the pH of the GLYC were analysed as indicated by Mandalawi et al. (2014). Lecithin was analysed for insoluble acetone (method Ja 4-46) and the inositol and choline contents were calculated from the phosphatidylinositol and phosphatidylcholine contents (method Ja 7c-07) as described by AOCS (2013). Representative samples of GLYC and lecithin were analysed for phosphorus and calcium (method 985.01) and sodium (method 956.01) as described by AOAC International (2000). Animal fat was analysed for impurities (method 926.12) and unsaponifiable (method 933.08) as indicated by AOAC International (2000). In addition, samples of lecithin and animal fat were analysed for initial peroxide value (method Ja 8-87) as described by AOCS (2013). The FA profile of the animal fat and the lecithin was

analysed as indicated by Grobas et al. (2001) and AOCS (2013) (method Ce 1-62), respectively. The particle size distribution and the geometric mean diameter (GMD) of the diets were determined using a shaker (Retsch, Stuttgart, Germany) equipped with 8 sieves ranging in mesh from 5,000 to 40 μm , according to the methodology outlined by the ASAE (1995). Acid insoluble ash of diets and excreta were determined as indicated by De Coca-Sinova et al. (2011). All analyses were conducted in triplicate for feed samples and in duplicate for ingredient and excreta samples.

2.6. Statistical analysis

Data on egg production and egg quality traits were analyzed as a completely randomized design using the MIXED procedure of (SAS Inst. Inc., Cary, NC) for repeated measures, with GLYC and animal fat to lecithin ratio as fixed effects and laying period as the repeated term (Littell et al., 1998). Several covariance structures were tested and the compound symmetry structure was selected according to the Schwarz Bayesian criterion. The TTAR of nutrients and AME_n of the diets were analysed as a completely randomized design using the GLM procedure of SAS Institute (1990). Diets were arranged as a 2×3 factorial and main effects (GLYC and animal fat: lecithin ratio) and the interactions were studied. When the model was significant, the t-test was used to separate treatment means. Differences among treatments were considered significant at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$. Results in tables are reported as least-square means.

3. Results

3.1. Hen performance and egg quality

Average cumulative mortality was 1.9 % and was not affected by diet. No interactions between GLYC and lecithin content of the diets were detected for any of the traits studied and therefore, only main effects are presented. Hen production and egg weight were not affected by the inclusion of GLYC in the diet but FCR was hindered

(2.071 vs. 2.039; $P \leq 0.05$). The substitution of animal fat by lecithin (40:0, 20:20 and 0:40 g/kg) increased egg weight (60.1, 60.7 and 61.8 g, respectively; $P \leq 0.001$) and egg mass (56.8, 57.5 and 58.8 g/d, respectively; $P \leq 0.01$) and improved FCR per kilogram of eggs (2.072, 2.068 and 2.027, respectively; $P \leq 0.05$) but feed intake, egg production and BWG were not affected (Table 4). The effects of GLYC and lecithin inclusion in the diet on hen performance by period are shown in Figs. 1 and 2, respectively. Most of the negative effects of GLYC inclusion on FCR were detected between the third and the fifth periods of the laying phase (Fig. 1D). The beneficial effects of raw lecithin inclusion on egg weight, egg mass and FCR were consistent throughout all the periods of the laying phase (Fig. 2).

Egg quality traits were not affected by the inclusion of GLYC in the diet (Table 5). However, when the lecithin was used in substitution of the animal fat, yolk color increased ($P \leq 0.001$) and the proportion of shell in the egg tended to be reduced ($P = 0.061$). Data on the effects of GLYC and lecithin inclusion on yolk color and on the proportion of shell in the eggs by period are shown in Figure 3. Most of the beneficial effects of lecithin inclusion in the diet on yolk color were detected during the first five periods of the laying phase (Fig. 3 C). In contrast, most of the negative effects of lecithin inclusion on the proportion of shell in the eggs were detected during the last two periods of the experiment (Fig. 3 D).

Table 4

Influence of the inclusion of raw glycerin (GLYC) and lecithin in the diet on hen production from 23 to 51 wk of age.

	Feed intake (g/d)	Egg production ^a	Egg weight (g)	Egg mass (g/d)	FCR ^b (kg/kg)	FCR (kg/dozen)	BWG ^c (g)
GLYC, g/kg							
0	117.7	0.946	61.1	57.8	2.039	1.501	263.2
70	119.0	0.949	60.6	57.5	2.071	1.513	244.8
S.E.M ^d (n=24)	0.793	0.004	0.235	0.360	0.010	0.009	8.308
Lecithin ^e , g/kg							
0	117.4	0.944	60.1 ^b	56.8 ^b	2.072 ^a	1.493	264.9
20	118.7	0.948	60.7 ^b	57.5 ^{ab}	2.068 ^a	1.512	243.9
40	118.9	0.952	61.8 ^a	58.8 ^a	2.027 ^b	1.517	253.2
S.E.M (n=16)	0.972	0.005	0.288	0.441	0.012	0.011	10.18
<i>P-value</i> ^f							
GLYC	0.256	0.608	0.159	0.597	0.023	0.360	0.155
Lecithin	0.488	0.529	>0.001	0.008	0.019	0.265	0.152

^a Eggs/hen per day.^b Feed conversion ratio.^c Body weight gain.^d Standard error of the mean (10 birds per replicate).^e Lecithin was included in the diet at expense (wt:wt) of the animal fat.^f The interactions between GLYC and lecithin were not significant ($P > 0.10$).

Table 5

Influence of the inclusion of raw glycerin (GLYC) and lecithin in the diet on egg quality from 23 to 51 wk of age.

	Dirty eggs (%)	Broken eggs (%)	Shell-less eggs (%)	Haugh units	Yolk ^a Color	Relative weight (% of the egg)			Shell thickness (mm)	Shell strength (kg/cm ²)
						Albumen	Yolk	Shell		
GLYC, g/kg										
0	2.9	0.3	0.1	85.2	9.3	59.3	26.7	14.0	0.383	4.94
70	2.9	0.4	0.1	84.8	9.3	59.4	26.7	13.9	0.386	4.91
S.E.M ^b (n=24)	0.202	0.042	0.022	0.324	0.040	0.125	0.109	0.058	0.003	0.035
Lecithin ^c , g/kg										
0	2.8	0.3	0.1	85.3	9.2 ^b	59.4	26.6	14.1	0.389	4.94
20	2.7	0.4	0.1	85.1	9.2 ^b	59.2	26.9	14.0	0.384	4.95
40	3.4	0.4	0.1	84.6	9.5 ^a	59.4	26.8	13.9	0.381	4.89
S.E.M (n=16)	0.248	0.052	0.027	0.397	0.049	0.153	0.134	0.072	0.004	0.043
<i>P-value</i> ^d										
GLYC	0.935	0.807	0.796	0.365	0.898	0.457	0.589	0.481	0.451	0.499
Lecithin	0.145	0.167	0.906	0.474	<0.001	0.392	0.204	0.061	0.369	0.590

^a Roche color fan.

^b Standard error of the mean (10 birds per replicate).

^c Lecithin was included in the diet at expense (wt:wt) of the animal fat.

^d The interactions between GLYC and lecithin were not significant ($P > 0.10$).

3.2. Total tract apparent retention

No interactions between GLYC and lecithin content of the diet on nutrient retention were detected and therefore, only main effects are presented (Table 6). The inclusion of GLYC in the diet did not affect TTAR of the nutrients or AME_n of the diets. However, TTAR of DM ($P \leq 0.05$), OM ($P \leq 0.05$), EE ($P \leq 0.01$) and GE ($P \leq 0.001$) increased as the level of lecithin in the diet increased. N retention and AME_n of the diets, however, were not affected by lecithin inclusion.

4. Discussion

The determined chemical composition of the GLYC, lecithin and animal fat was close to expected values. The fatty acid profile of the lecithin was similar to values reported by van Nieuwenhuyzen and Tomás (2008) and Mateos et al. (2012). The high peroxide value of the animal fat was expected because of the nature of the original rendered product, as well as the high pressure applied to extract the fat during the process. The determined nutritive value of the experimental diets of the two batches used, were close to expected values, confirming that the diets were mixed correctly.

4.1. Hen performance and egg quality

The inclusion of 70 g GLYC/kg diet hindered FCR per kilogram of eggs but did not affect any other production trait. Németh et al. (2013) reported also that the inclusion of 100 g/kg of a GLYC batch that contained 86.8 g glycerol/kg hindered FCR in hens from 28 to 37 wk of age without affecting any other production trait, in agreement with the data reported herein. Similar results have been reported by Erol et al. (2009) in laying quails fed diets that containing 100 g GLYC/kg. Swiatkiewicz and Koreleski (2009) and Németh et al. (2014), however, did not detect any effect on production when 60 or 100 g GLYC/kg was included in the diet. The reason for the impairment of FCR observed when

GLYC was included in the diet is not apparent. Cerrate et al. (2006) reported that feeds that included 100 g GLYC/kg diet did not flow well through the feeder system and resulted in reduced feed intake and poor FCR in broilers. However, no visible effects of GLYC on feed flow was observed in the current research and in fact, feed intake increased slightly with GLYC inclusion. Two potential explanations for the poorer FCR observed with GLYC inclusion are a) the GLYC was not well absorbed by the hens because of the high level used and b) the maize used had higher AME_n content than assumed (13.72 MJ/kg; Fundación Española Desarrollo Nutrición Animal, 2010). Under both circumstances, the AME_n content of the diets that contained GLYC could be in relative terms lower than the AME_n content of the diets that did not contain GLYC and thus, GLYC inclusion could result in higher feed intake and poorer FCR than expected. It is worthy to notice that in the current research, daily egg mass production was 0.3 g lower and feed intake was 1.3 g higher in hens fed the GLYC containing diet than in hens fed the non-GLYC supplemented diets, although the differences were not significant. In this respect, Silva et al. (2012) and Romano et al. (2014) observed also an increase in feed intake in broilers fed diets that included 75 g GLYC/kg at the expense primarily of maize. The information provided indicate that well processed GLYC can be included at levels of up to 70 g/kg in diets for laying hens without any major effect on production, although feed efficiency could be slightly reduced.

The information available on the effects of dietary lecithin on egg production is very limited. In broilers, Huang et al. (2007) reported that the substitution of soy oil by soy lecithin improved FCR from 1 to 42 d of age. Similarly, Sibbald et al. (1962) reported an increase in energy utilization when 20 g raw soybean lecithin/kg was added to chick diets containing animal tallow. In contrast, Summers and Leeson (1981) did not detect any effect on BWG or FCR when 10 g lecithin/kg were included in a broiler diet from 1

to 21 d of age. In the current research, the substitution of animal fat by lecithin improved FCR per kilogram of eggs and increased egg weight and egg mass production. Attia et al. (2009) reported that the inclusion of 30 g lecithin/kg diet in dual-purpose crossbred hens improved FCR per kg of eggs and increased egg weight and egg mass production from 47 to 70 wk of age, consistent with the results reported herein. Also, An et al. (1997) observed that the inclusion of 50 g phospholipids from crude safflower/kg diet improved egg mass in hens from 60 to 67 wk of age. However, Wolford and Polin (1975) reported that the inclusion of 16 g lecithin/kg diet did not affect egg production from 31 to 34 wk of age. The reasons for the discrepancies among authors with respect to the effects of dietary lecithin on poultry production are not known but might depend on the type of bird used, as well as on the characteristics of the diet, including level, source and FA profile of the lipid source used. For example, Summers and Leeson (1981) and Donaldson and Ward (1988) reported that the inclusion of lecithin in the diet improved the utilization of saturated animal fats but not of unsaturated vegetable oils.

In the current research, diets were formulated assuming that the two lipid sources (animal fat and lecithin) had the same AME_n content. However, the determined GE (38.9 vs. 32.6 MJ/kg) and the calculated AME_n (33.9 vs. 31.9 MJ/kg; Fundación Española Desarrollo Nutrición Animal, 2010) were higher for the animal fat than for the soy lecithin. Consequently, a higher feed intake (1.5 g/d) was expected in hens fed diets with 40 g lecithin than in hens fed diets with 40 g animal fat/kg. However, no differences in feed intake between fat sources were detected in the current research, suggesting a better utilization of the energy contained in the other ingredients of the diet, namely lipids, with the use of lecithin.

† $P \leq 0.1$; * $P \leq 0.05$; ** $P \leq 0.01$.

Fig. 1. Effect of inclusion of raw glycerin in the diet on feed intake (A), egg production (B), egg weight (C), feed conversion ratio (D), BW gain (E) and egg mass (F) from 23 to 51 wk of age.

† $P \leq 0.1$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Fig. 2. Effect of inclusion of soy lecithin in the diet on feed intake (A), egg production (B), egg weight (C), feed conversion ratio (D), BW gain (E) and egg mass (F) from 23 to 51 wk of age.

† $P \leq 0.1$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Fig. 3. Effect of inclusion of raw glycerin and soy lecithin in the diet on yolk color RCF, (Roche colour fan) (A and C) and proportion of shell in the egg (B and D) from 23 to 51 wk of age.

In this respect, lecithin has higher linoleic acid (LNA), phospholipid and inositol content than animal fats, and all these components might improve nutrient utilization, egg production and hen performance (Couch and Grossie, 1970; An et al., 1997; Attia et al., 2009). For example, Jensen and Shutze (1963), Scragg et al. (1987) and Grobas et al. (1999a) reported increases in egg size with increases in the LNA content of the diet. In the current research, when 40 g lecithin was used in substitution of the animal fat, the LNA content increased from 14.3 to 28.7 g/kg. Probably, a LNA content of 14.3 g/kg diet was sufficient to maximize egg size (Grobas et al., 1999b,c; Safaa et al., 2008) and therefore, no effects of the

LNA content of the diet on egg weight should be expected. On the other hand, soy lecithin and egg yolk lipids have a high phospholipid content (450 and 300 g/kg, approximately) (Lipstein et al., 1977; Attia et al., 2009; Mateos et al., 2012). Hens might utilize with higher efficiency the phospholipids of the lecithin for egg production than the non-polar lipids of the animal fat, resulting in better FCR and increased egg weight. In addition, lecithin contains 21 g inositol/kg, a component of the phospholipid fraction that plays important metabolic roles in the liver and in the reproductive organs of poultry (Reed et al., 1968; Beemster et al., 2002). In addition, inositol might increase insulin activity, which in turn might improve egg production and feed efficiency (Cowieson et al., 2013). In this respect, Żyła et al. (2004) reported that the inclusion of 1 g inositol/kg diet increased BWG and tended to improve FCR in broilers from 1 to 21 d of age. Also, Couch and Grossie (1970) reported that the inclusion of 1 g inositol/kg diet increased egg production and egg size in laying hens from 18 to 28 wk of age. In contrast, Pearce (1972) reported no effects of the inclusion of 5 g inositol/kg diet on hen production.

Raw glycerin did not affect any of the egg quality traits studied, in agreement with data of Swiatkiewicz and Koreleski (2009), Yalçın et al. (2010) and Németh et al. (2014) with diets that included 60, 75 and 100 g GLYC/kg, respectively. Similarly, lecithin inclusion did not affect egg quality traits, except for yolk colour that was improved and for the proportion of egg shell that tended to be reduced. The information available on the effects of lecithin on yolk pigmentation is scarce. Attia et al. (2009) reported that the inclusion of 60 g lecithin/kg diet increased yolk colour in hens from 47 to 70 wk of age, consistent with the results reported herein. The unsaturated FA profile of the lecithin is more favorable for xanthophyll absorption and utilization than that of the more saturated FA profile of the animal fat. As we increased the concentration of lecithin in the diet, fat digestibility improved, favoring the absorption of the xanthophyll responsible for egg yolk color. In addition, the animal fat used

had a high level of peroxides which act as pro-oxidants, reducing the pigmenting capacity of the xanthophyll (Karunajeewa et al., 1984; Baiao and Lara, 2005). The information available on the effects of lecithin on egg quality traits, other than yolk colour, is scarce. Han et al. (2010) reported that the inclusion of 1.5 g lysolecithin, a metabolite that results from the hydrolysis of the lecithin per kg of diet, did not affect Haugh units, egg shell proportion in the egg or egg cleanness. In the current research, however, the inclusion of 40 g lecithin/kg tended to reduce the proportion of shell in the eggs that affected only eggs produced during the second part of the egg cycle. The trend for reduced egg shell proportion of the egg with lecithin inclusion was not expected and we do not have any clear explanation for this effect. It was detected that the ash (and probably Ca) content of the diets fed for the last three periods of the experiment was lower for the two diets that contained 40 g lecithin/kg than for the other diets, a period in which most of the negative effects of lecithin on shell proportion was observed. However, neither shell thickness nor shell strength were affected by the substitution of animal fat by lecithin and consequently, no major effects of lecithin on shell quality is expected under commercial conditions.

4.2. Total tract apparent retention

The inclusion of GLYC in the diet did not affect TTAR of any of the nutrients studied or the AME_n of the diets. Swiatkiewicz and Koreleski (2009) reported also that the inclusion of 60 g GLYC/kg diet did not affect N retention in laying hens. No other information is available regarding the effect of GLYC on TTAR of nutrients in laying hens. In broilers, Mandalawi et al. (2014) did not detect any effect on TTAR of N and GE when up to 100 g GLYC/kg was included in the diet. Similarly, Kim et al. (2013) reported no differences in TTAR of N and EE in broilers fed diets that contained 50 g GLYC/kg but GE retention increased with GLYC supplementation. Also, Peña et al. (2014) reported that the inclusion of 60 g GLYC/kg diet increased the AME_n of diet in 25-d old broilers. In weanling pigs,

Groesbeck et al. (2008) reported that TTAR of GE and N were not affected when up to 60 g GLYC/kg was included in the diet, in agreement with the results reported herein. All this information indicates that the effects of GLYC on nutrient retention are very limited and of little practical interest.

Table 6

Influence of the inclusion of raw glycerin (GLYC) and lecithin in the diet on total tract apparent retention (TTAR) of nutrients and AME_n content of the diets at 26 wk of age.

	TTAR					AME _n ^f (MJ/kg)
	DM ^a	OM ^b	N ^c	EE ^d	GE ^e	
GLYC, g/kg						
0	0.757	0.794	0.571	0.841	0.812	11.25
70	0.757	0.797	0.583	0.836	0.816	11.28
S.E.M ^g (n=18)	0.003	0.004	0.009	0.004	0.003	0.043
Lecithin ^h , g/kg						
0	0.750	0.782 ^b	0.567	0.821 ^b	0.800 ^b	11.26
20	0.762	0.804 ^a	0.574	0.845 ^a	0.821 ^a	11.27
40	0.760	0.801 ^a	0.589	0.849 ^a	0.821 ^a	11.29
S.E.M (n=12)	0.004	0.005	0.011	0.005	0.003	0.053
<i>P-value</i> ⁱ						
GLYC	0.989	0.711	0.360	0.372	0.325	0.691
Lecithin	0.046	0.013	0.352	0.001	<0.001	0.905

^a Dry matter.

^b Organic matter.

^c Nitrogen.

^d Ether extract.

^e Gross energy.

^f Nitrogen corrected, apparent metabolisable energy.

^g Standard error of the mean (10 birds per replicate).

^h Lecithin was included in the diet at expense (wt:wt) of the animal fat.

ⁱ The interactions between GLYC and lecithin were not significant ($P > 0.10$).

The substitution of animal fat by lecithin increased the TTAR of all nutrients, except that of N that was not affected. Attia et al. (2009) reported also that the inclusion of 60 g lecithin/kg diet increased EE digestibility and tended to improved OM retention in dual-purpose crossbred hens, consistent with the results reported herein. In 21-d old broilers, Huang et al. (2007) observed also that the addition of 5 g lecithin/kg to a diet that contained 15 g soy oil improved EE digestibility but did not affect N retention. In piglets, Jin et al. (1998) observed that the inclusion of 10 g lecithin/kg in a diet that contained 90 g tallow/kg

increased GE, EE and N digestibility. Also in piglets, Reis de Souza et al. (1995) reported that the inclusion of 15 g lecithin/kg diet increased fat digestibility but did not affect N or GE digestibility. Lecithin is more unsaturated and has a higher phospholipid content than animal fat. Consequently, the inclusion of lecithin in the diet should improve lipid utilization. In this respect, Soares and Lopez-Bote (2002) suggested that most of the beneficial effects of including lecithin in the diet in substitution of a more saturated fat, on nutrient retention, resulted from the increase in FA unsaturation rather than to a specific emulsifying effect. The substitution of animal fat by lecithin did not affect AME_n of the diet. However, the energy content of the animal fat was expected to be higher than that of the commercial lecithin and therefore, the results suggest a beneficial effect of lecithin on the utilization of the energy of other components of the diet. In this respect, Donaldson and Ward (1988) included 50 g lecithin/kg at the expense of animal fat in diets for broilers and reported also similar AME_n of the two diets. Similarly, Huang et al. (2007) reported equal AME_n content of a control diet with 20 g soy oil and of an experimental diet that contained the same amount of soy lecithin in 42-d old broilers in spite of the higher AME_n content of the soy oil. Also, Peña et al. (2014) reported that the inclusion of 60 g lecithin/kg increased the AME_n of the diet in 25-d old broilers.

5. Conclusions

No interactions between glycerin and lecithin were detected on any of the growth performance, egg quality traits or TTAR of nutrients studied. The inclusion of 70 g GLYC/kg diet reduced feed efficiency slightly, but did not affect any of the other production and egg quality traits or nutrient retention variables studied. The substitution of animal fat by lecithin improved egg weight, egg mass, FCR per kilogram of eggs and yolk color and increased nutrient retention in laying hens. The inclusion of lecithin in the diet might be an useful strategy to increase egg size and egg yolk colour in commercial operations of laying hens.

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CHAPTER 4:

METABOLIZABLE ENERGY CONTENT OF TRADITIONAL AND RE-ESTERIFIED LIPID SOURCES: EFFECTS OF INCLUSION IN THE DIET ON NUTRIENT RETENTION AND GROWTH PERFORMANCE OF BROILERS FROM HATCH TO 21 DAYS OF AGE

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1. Introduction

Fats and oils are used in poultry diets to increase energy concentration, improve feed efficiency and reduce dust formation (Grobas et al., 1999a, b; Safaa et al., 2008). Triacylglycerol (TAG) digestion occurs primarily in the duodenum of the chicken with hydrolysis of the molecule by the pancreatic lipase which results in two free fatty acid (FFA) and a 2-monoacylglyceride (MAG). Fatty acids (FA) are less digestible when present in free form than when present as a part of the TAG molecule (Sklan, 1979). The presence of MAG in the chyme facilitates micelle formation and hence, the absorption of the FFA (Ravindran et al., 2016). Also, unsaturated FA are more polar and therefore more digestible than saturated FA (Renner and Hill, 1961). Consequently, soy oil (SO), a TAG that contains high levels of unsaturated FA, is better digested by the chick than more saturated fats, such as lard or tallow. Acidulated soy soapstocks (ASO) is a co-product of the refining SO industry characterized by its high proportion of FFA. Consequently, the apparent metabolizable energy (AME) of ASO is lower than that of the original SO (Irandoost et al., 2012). Differences in lipid digestibility, because of fat characteristics, are more pronounced in young chicks than in adult birds.

Re-esterification, a process used by the oil industry, is based on the esterification of FFA with hydroxyl groups that remain free in the crude oil or that are added in the form of glycerol (Bhosle and Subramanian, 2005). The process yields a blend of neutral lipids including FFA, MAG, diacylglycerols (DAG) and TAG in variable proportions, depending on the temperature, the catalyst used and the amount of glycerol added (Kombe et al., 2013). During the process of digestion, the amphiphilic molecules act as emulsifying agents, facilitating the incorporation of FFA into the mixed micelles and lipase activity. Therefore, the inclusion of re-esterified oils in the diet might be a sound physiological strategy for the utilization of co-products of the oil industry (Vilarrasa et al., 2014).

The information available comparing the AME content of traditional fat sources and lipids resulting from the re-esterified process, and its influence on the digestibility of other dietary components and growth performance of broilers is scarce. The hypothesis of this research was that the re-esterification of FFA from the soy oil refining and glycerol from the biodiesel industry could result in a new lipid source with a digestibility and an AME similar to those of the original TAG. The aim of this research was to determine and compare the digestibility and AME content of traditional and re-esterified lipid sources in young broilers. In addition, the effects of supplementing a control diet based on maize and soybean meal with 25 or 50 g/kg of these lipid sources on broiler performance was studied from 7 to 21 d of age.

2. Materials and methods

All the experimental procedures were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish Guidelines for the Care and Use of Animals in Research (Boletín Oficial del Estado, 2007).

2.1. Fat sources

The SO, ASO and lard used were obtained from Bunge Ibérica S.A. (Barcelona, Spain), Oleínas y Grasas S.L. (Tarragona, Spain) and Ibergrasa S.A. (Madrid, Spain), respectively. The ASO was a mixture of different batches of soapstocks resulting from the refining of SO for human consumption. The re-esterified oils, monoacylglycerol (RMAG) and triacylglycerol (RTAG), were processed by Norel S.A. (Madrid, Spain) and resulted from a proprietary process based on the esterification reaction between glycerol (pure or diluted in 15% water) and a mixture of acidulated soapstocks from SO and sunflower oil, in the presence of 150 mg/kg of butylated hydroxytoluene, added as an antioxidant. The process was conducted for 10 h approximately, at a temperature of 180 ± 20 °C, without the use of any chemical catalyst, and the acidity was reduced to less than 30 g/kg. The only difference between the RMAG and RTAG used in the current research was the ratio between FA and

glycerol in the molecule (1:1 and 3:1, respectively). The chemical composition and the characteristics of the lipid sources are shown in Table 1.

Table 1

Chemical analyses and quality variables (g/kg as-fed basis unless otherwise indicated) of the experimental lipid sources (Experiments 1 and 2).

	Soy oil	ASO ^a	Lard	RMAG ^b	RTAG ^c
Gross energy (MJ/kg)	39.66	38.88	39.47	38.06	38.56
Moisture	1	27	4	4	3
Insoluble impurities	1	3	1	5	5
Unsaponifiable matter	1.2	-	0.03	-	-
Non eluted material	8	111	36	182	153
Acid value	8	522	3	32	33
Initial peroxide index (meq/kg)	2.2	1.1	3.1	4.2	3.5
Fatty acid profile					
Myristic acid (C14:0)	2	1.2	13	-	-
Palmitic acid (C16:0)	101	179	231	181	173
Palmitoleic acid (C16:1)	2	3	29	2.1	1.9
Stearic acid (C18:0)	41	43	110	75	60
Oleic acid (C18:1)	221	220	501	397	386
Linoleic acid (C18:2)	572	478	97	347	333
Linolenic acid (C18:3)	53	59	6	32	29
Monoacylglycerides	-	54	-	127	92
Diacylglycerides	-	98	-	436	386
Triacylglycerides	970	85	183	278	384
Free fatty acids	7	674	13	36	47

^a Acidulated soy oil soapstocks.

^b 860 g of a mixture of acidulated soapstocks from soy oil and sunflower oil and 140 g of glycerol/kg.

^c 766 g of a mixture of acidulated soapstocks from soy oil and sunflower oil and 234 g of glycerol/kg.

2.2. Experiment 1

2.2.1. Husbandry and experimental design

In total, 240 one-day-old straight-run Ross 308 broilers with an initial body weight (BW) of 40.0 ± 2.1 g were obtained from a commercial hatchery. At arrival at the experimental station, the chicks were placed in a windowless, environmentally controlled room and randomly housed in groups of ten in 24 wire flooring battery cages with similar

average BW. The cages (1.0 m × 0.9 m; Avícola Grau S.A., Madrid, Spain) were equipped with two cup drinkers and an open trough feeder. Room temperature was maintained at 33 ± 2 °C during the first three days of life and then was reduced gradually according to age until reaching 24 ± 2 °C at d 21. For the first week of life, chicks received 23 h light per day and then a continuous light program of 18 h/d until the end of the experiment. Chicks had free access to feed and water throughout the trial. The experiment was completely randomized and treatments consisted in a control diet based on maize and soybean meal without any added fat, and three extra diets that included 25 g/kg of the different fat sources tested (SO, RMAG and RTAG) at the expense (wt:wt) of the control diet. Each treatment was replicated six times and the experimental unit was a cage with ten birds.

2.2.2. Experimental diets

From 1 to 6 d of age, chicks were fed a common standard commercial diet based on maize and soybean meal in crumble form, with 12.77 MJ AME and 12.9 g digestible Lys/kg. At 7 d of age, broilers received their respective experimental diets in mash form for 14 d. The maize, soybean meal and oils used in the formulation of the diets were analyzed for major components before feed manufacturing. The control diet contained 11.30 MJ AME/kg and 12.7 g digestible Lys/kg (FEDNA, 2010) with a AME: digestible Lys ratio of 0.90. All the others indispensable AA were formulated according to the ideal protein concept as indicated by the FEDNA (2008). Celite (Celite Hispánica S.A., Alicante, Spain) an acid-washed diatomaceous earth, was added at 20 g/kg to increase the acid insoluble ash content of feeds and excreta. The ingredients were ground using a hammer mill fitted with a 3-mm screen (Model Z-I, Retsch, Stuttgart, Germany) and mixed. The batch was divided into 4 equal portions. The first portion was used as such (control diet) and the other portions were mixed with 25 g/kg of each of the three fat sources tested (SO, RMAG and RTAG). Consequently, the AME and the AME: digestible Lys ratio of the diets increased with fat supplementation.

The ingredient composition and calculated analyses of the control diet are shown in Table 2 and the determined analyses of the four experimental diets are shown in Table 3.

2.2.3. Growth performance and total tract apparent retention

Body weight and feed disappearance were recorded by cage at 7, 14 and 21 d of age. Plastic pans were placed beneath the cages for evaluation of feed wastage. Any mortality was recorded and weighed as produced. From these data, average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F:G) were calculated per week and for the entire experimental period.

At 21 d of age, representative samples of the excreta produced during the previous 48 h were collected daily by replicate, homogenized, oven-dried (60 °C for 72 h), ground using a hammer mill (model Z-I, Retsch, Stuttgart, Germany) fitted with a 1-mm screen and mixed. The coefficients of total tract apparent retention (CTTAR) of dry matter (DM), organic matter (OM), gross energy (GE), ether extract (EE) and nitrogen (N) of the diets were estimated by the indigestible marker method using celite as inert marker. The CTTAR of the supplemental fat was estimated by correcting the digestibility of the EE of the corresponding diet by the digestibility of the EE of the basal diet (Irandoost et al., 2012). The AME content of the diets was calculated as indicated by Lázaro et al. (2003) and the AME content of the supplemental fats was estimated using two different procedures. In procedure 1, lipids were extracted from diets and faeces and the AME of the experimental fats was estimated by multiplying the GE content of the experimental fat source by the corrected CTTAR of the EE of the corresponding diet. In procedure 2, the AME of the experimental fats was calculated by difference between the AME of the control and the oil containing diet.

2.2.4. Laboratory analysis

The FA profile of the lipid sources was determined by gas-liquid chromatography (GC-14B, Shimadzu, Kyoto, Japan) as indicated by Grobas et al. (2001). Moisture (method 984.20), insoluble impurities (method 926.12) and non-eluted material, which reflects the indigestible portion of the lipid sources (method 977.17), were determined as indicated by AOAC International (2000). Free fatty acid (method Ca 5a-40), acid value (method Cd 3d-63), initial peroxide index (Cd 8-53), MAG and DAG (method Cd 11b-91) and TAG (method Ce 5-86) contents of the fat sources were determined as indicated by AOCS (2009).

Representative samples of diets and excreta were analysed for moisture by oven-drying (method 930.01), total ash using a muffle furnace (method 942.05) and N by combustion (method 990.03) using a Leco equipment (model FP-528, Leco Corporation, St. Joseph, MI) as described by AOAC International (2000). Gross energy was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL) and EE, after 3 *N* HCl acid hydrolysis (method Am 5-04), using an Ankom XT10 extraction system (Ankom Technology Corp. Macedon, NY) as described by AOCS (2004). Acid insoluble ash was determined as indicated by De Coca-Sinova et al. (2011). In addition, the diets were analysed for phosphorus and calcium (method 985.01) as described by AOAC International (2000). All analyses were conducted in triplicate for feed samples and in duplicate for ingredients and excreta samples.

2.2.5. Statistical analyses

Data on growth performance and CTTAR of nutrients were analyzed as a completely randomized design with four treatments, using the GLM procedure of SAS Institute (1990). The individual cage (10 chicks each) represented the experimental unit for all traits. When the model was significant, the Bonferroni test was used to make pairwise comparisons

between sample means. In addition, a non-orthogonal contrast was used to compare data from broilers fed the control diet and the average of broilers fed the supplemental fat diets. Differences among treatments were considered significant at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$. Results in tables are reported as means.

2.3. Experiment 2

2.3.1. Husbandry, experimental diets and controls

In total, 420 one-d-old straight-run Ross 308 broilers with an initial BW of 42 ± 2.4 g were used. Bird management was similar to that indicated in Exp. 1 and growth performance and CTTAR of the diets were determined at the same ages. The experimental diets consisted in a control diet (same than that used in Exp.1) and six extra diets that included 50 g/kg of each of six experimental fat sources (SO, ASO, lard, RMAG, RTAG and SO-RMAG, a 20:80 mixture of SO and RMAG) at the expense of the control diet. The ingredient composition and calculated analyses of the control diet and the determined analyses of the six experimental diets are shown in Tables 2 and 3, respectively. All laboratory analyses were conducted as indicated in Exp. 1.

2.3.2. Experimental design and statistical analyses

The experimental design was completely randomized, with each treatment replicated six times. The experimental unit was a cage with ten chicks. Data on growth performance and CTTAR of the dietary components were analyzed as indicated for Exp. 1. The only difference was that in Exp. 2 seven diets were used.

Table 2

Ingredient composition and calculated analysis (g/kg, as-fed basis unless otherwise indicated) of the control diet used from 7 to 21 days of age (Experiments 1 and 2).

Ingredient	
Maize	552.5
Soybean meal (470 g CP/kg)	383.8
Monocalcium phosphate	14.0
Calcium carbonate	14.9
Sodium chloride	4.3
DL-Methionine (990 g/kg)	2.9
L-lysine HCl (780 g/kg)	2.1
L-threonine (980 g/kg)	0.5
Celite ^a	20.0
Vitamin and mineral premix ^b	5.0
Calculated analysis ^c	
AMEn (MJ/kg)	11.3
Crude protein	226.3
Digestible Lys	12.7
Digestible Met	6.2
Digestible Met + Cys	9.4
Digestible Thr	8.0
Digestible Trp	2.4
Ether extract	26.0
Crude fibre	31.5
Total ash	64.8
Calcium	9.4
Available phosphorus	4.3

^a Acid-washed diatomaceous earth (Celite Hispánica, S.A., Alicante, Spain).

^b Provided the following (per kilogram of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (all-rac-tocopherol acetate), 20 IU; vitamin K (bisulphate menadione complex), 3 mg; riboflavin, 5 mg; pantothenic acid (D-calcium pantothenate), 10 mg; nicotinic acid, 30 mg; pyridoxine (pyridoxine-HCl), 3 mg; thiamin (thiamin-mononitrate), 1 mg; vitamin B12 (cyanocobalamine), 12 µg; D-biotin, 0.15 mg; choline (choline chloride), 300 mg; folic acid, 0.5 mg; Se (Na₂SeO₃), 0.1 mg; I (KI), 2.0 mg; Cu (CuSO₄·5H₂O), 10 mg; Fe (FeSO₄·7H₂O), 30 mg; Zn (ZnO), 100 mg; Mn (MnSO₄·H₂O), 100 mg; and ethoxyquin, 110 mg.

^c According to FEDNA (2010).

Table 3

Determined chemical analysis^a (g/kg, as-fed basis unless otherwise indicated) of the experimental diets used from 7 to 21 d of age (Experiments 1 and 2).

	Control ^d	Experiment 1 ^b			Experiment 2 ^c					
		SO ^e	RMAG ^f	RTAG ^g	SO	ASO ^h	Lard	RMAG	RTAG	SO-RMAG ⁱ
Gross energy (MJ/kg)	15.80	16.42	16.41	16.47	17.22	17.09	17.34	17.12	17.22	17.24
Dry matter	901	888	890	893	887	888	904	894	895	895
Crude protein	226	222	230	225	226	213	218	210	215	225
Ether extract	23.4	43.1	49.0	46.2	67.3	82.0	75.1	78.0	81.0	80.0
Ash	73.9	71.0	70.8	72.5	67.1	69.6	71.2	71.0	67.8	71.5
Calcium	11.2	9.1	9.6	10.1	9.8	10.3	9.5	9.9	10.4	10.5
Phosphorus	6.2	6.1	6.6	5.5	5.5	6.7	6.0	5.4	5.6	5.7

^a Analysed in triplicate samples.

^b Diets included 25 g supplemental fat/kg.

^c Diets included 50 g supplemental fat/kg.

^d The same control diet was used in both experiments.

^e Soy oil.

^f Re-esterified monoacylglyceride.

^g Re-esterified triacylglyceride.

^h Acidulated soy oil soapstocks.

ⁱ SO-RMAG was a mixture of 200 g soy oil and 800 g re-esterified monoacylglyceride/kg.

3. Results

3.1. Experiment 1

The chemical characteristics, FA composition and GE content of the lipid sources were within the range reported in the literature (FEDNA, 2010; Perez-Bonilla et al., 2011; Irandoust et al., 2012; Premier Atlas, 2014), with the highest GE value for soy oil (39.66 MJ/kg and the lowest for the reconstituted fats (38.06 and 38.56 for the RMAG and RTAG, respectively) (Table 1).

3.1.1. Coefficient of total tract apparent retention of nutrients and AME of the lipid sources

Independent of the lipid source used, oil supplementation increased EE retention (0.842, 0.846, 0.840 and 0.800; $P < 0.001$) and AME (11.73, 11.67, 11.72 and 11.06 MJ/kg; $P < 0.001$) of the SO, RMAG, RTAG and control diets, respectively. However, the inclusion of the lipid sources did not affect the CTTAR of the other dietary components (Table 4). The AME of the lipid sources, determined by multiplying their GE times the CTTAR of the EE, was higher for SO than for RMAG, with that of RTAG being intermediate (33.37, 32.21 and 32.38 MJ/kg, respectively; $P < 0.001$). However, when the CTTAR of the EE of the oil supplemental diets was corrected by the digestibility of the EE of the control diet, no differences in EE digestibility or AME among the three oil sources were detected (Table 5). The AME of all the lipid sources was similar when calculated by difference between the control and the oil supplemented diets.

3.1.2. Growth performance

From 7 to 21 d of age, broilers fed the oil supplemented diets had similar ADG and ADFI but better F:G ratio ($P < 0.01$) than broilers fed the control diet (Table 6). Most of the benefits of oil supplementation on broiler growth was observed from 7 to 14 d of age, a

period in which oil supplementation improved ADG ($P < 0.05$) and F:G ratio ($P < 0.01$). No significant differences between SO and the re-esterified oils were observed for any trait at any age.

3.2. Experiment 2

3.2.1. Coefficient of total tract apparent retention of nutrients and AME of the lipid sources

The inclusion of 50 g supplemental fat/kg diet affected in different ways nutrient retention and AME of the diets, with values that were in general higher for the soy oil and lard diets than for the other fat supplemented diets (Table 7). The CTTAR of EE (0.820 for the control diet vs. a range from 0.881 to 0.896; $P < 0.001$) and the AME of the diets (11.10 for the control diet vs. a range from 12.27 to 12.56 MJ/kg; $P < 0.001$) increased with fat supplementation, independent of the lipid source used. The AME of the lipid sources, determined by multiplying their GE by the CTTAR of the EE of their respective diet, after correction for the digestibility of the EE of the control diet, was higher ($P < 0.001$) for the SO than for the RMAG and RTAG with that of the other lipid sources being intermediate (Table 8). When the AME of the lipid sources was calculated by difference between the AME of the oil containing diet and the basal diet, the value was higher ($P < 0.01$) for SO and lard than for RMAG, with all the other sources being intermediate.

3.2.2. Growth performance

From 7 to 21 d of age, fat supplementation increased ADG ($P < 0.05$) and improved F:G ($P < 0.001$) of the birds, independent of the lipid source used (Table 9). Similar results were observed from 7 to 14 d of age. Diet did not affect ADG from 14 to 21 d of age but F:G was improved ($P < 0.001$) with fat supplementation. In all periods, F:G ratio was best with SO inclusion.

Table 4

Influence of the inclusion in the diet of 25 g/kg of the lipid source on the coefficient of total tract apparent retention (CTTAR) of the nutrients and the AME^a content of the diets in broilers at 21 days of age (Experiment 1).

	Added fat (g/kg)	CTTAR					AME (MJ/kg)
		Dry mater	Organic Mater	Gross energy	Crude protein	Ether extract	
Control diet	-	0.722	0.764	0.763	0.681	0.800 ^b	11.06 ^b
Soy oil	25	0.723	0.763	0.771	0.696	0.842 ^a	11.73 ^a
Re-esterified monoacylglyceride	25	0.721	0.763	0.769	0.698	0.846 ^a	11.67 ^a
Re-esterified triacylglyceride	25	0.732	0.772	0.778	0.695	0.840 ^a	11.72 ^a
SEM ^b (n= 6)		0.007	0.007	0.006	0.014	0.007	0.099
<i>P</i> -value		0.627	0.683	0.466	0.812	0.001	0.001
Control vs. supplemental fat diets		0.951	0.806	0.215	0.344	0.001	0.001

^{a,b,c} Value within columns with no common superscript are different ($P < 0.05$).

^a Apparent metabolizable energy.

^b Standard error of the means (6 replicates of 10 birds each per treatment).

Table 5

Estimated and calculated apparent metabolizable energy (AME) of the lipid sources in broilers at 21 days of age (Experiment 1).

	Basal	Fat source				
	diet	Soy oil	RMAG ^a	RTAG ^b	SEM ^c	<i>P</i> -value
Oil source						
Gross energy (MJ/kg)	38.94	39.66	38.06	38.56		
CTTAR ^d	0.800 ^b	0.842 ^a	0.846 ^a	0.840 ^a	0.007	0.001
Corrected CTTAR ^e	-	0.894	0.891	0.883	0.014	0.859
AME of the fat source (MJ/kg)						
Gross energy × CTTAR ^f	31.15 ^c	33.37 ^a	32.21 ^{bc}	32.38 ^{ab}	0.270	0.001
Gross energy × corrected CTTAR ^g	-	35.44	33.89	34.05	0.548	0.122
By difference ^h	-	37.70	35.68	35.83	4.109	0.928

^{a,b,c}Value within lines with no common superscript are different ($P < 0.05$).

^a Re-esterified monoacylglyceride.

^b Re-esterified triacylglyceride.

^c Standard error of the means (n= 6 replicates of 10 birds each per treatment).

^d Coefficient of total tract apparent digestibility of the ether extract fraction of the diet.

^e Coefficient of TTAR of the supplemental oil after correcting by the digestibility of the ether extract fraction of the basal diet (Irandoost et al., 2012).

^f Calculated by multiplying the gross energy of the lipid source by the CTTAR of the supplemented fat of the corresponding experimental diet.

^g Calculated by multiplying the gross energy of the fat by the corrected CTTAR of the supplemented fat of the corresponding diet.

^h Calculated by difference between the AME of the basal and the oil containing diets.

AME = [(AME experimental feed – [97.5 x (AME control feed/100)]) / 2.5] x 100.

Table 6Influence of the inclusion in the diet of 25 g/kg of the lipid source on growth performance of broilers from 7 to 21 days of age^a (Experiment 1).

	7-14 days			14-21 days			7-21 days		
	ADG ^b	ADFI ^c	F: G ^d	ADG	ADFI	F: G	ADG	ADFI	F: G
Control diet	33.4	42.7	1.28 ^a	50.9	74.8	1.47	42.1	58.8	1.39 ^a
Soy oil	37.1	44.1	1.19 ^b	53.6	74.1	1.39	44.8	58.5	1.31 ^b
Re-esterified monoacylglyceride	35.9	44.0	1.22 ^b	51.3	74.1	1.45	43.1	58.1	1.35 ^{ab}
Re-esterified triacylglyceride	34.7	42.6	1.23 ^{ab}	50.4	73.1	1.45	42.1	56.8	1.35 ^{ab}
SEM ^e (n= 6)	1.03	1.12	0.014	1.12	1.47	0.023	0.874	1.236	0.015
<i>P</i> -value	0.096	0.693	0.001	0.233	0.876	0.073	0.125	0.698	0.005
Control vs. supplemental fat diets	0.044	0.531	0.001	0.526	0.557	0.121	0.251	0.513	0.003

^{a,b,c}Value within columns with no common superscript are different ($P < 0.05$).^a The average BW of the broilers at 7 d of age was 182 g.^b Average daily gain (g).^c Average daily feed intake (g).^d Feed to gain ratio.^e Standard error of the means (6 replicates of 10 birds each per treatment).

Table 7

Influence of the inclusion in the diet of 50 g/kg of the lipid source on the coefficient of total tract apparent retention (CTTAR) of the nutrients and the AME content of the diets in broilers at 21 days of age (Experiment 2).

	Added fat (g/kg)	CTTAR					AME (MJ/kg)
		Dry mater	Organic mater	Gross energy	Crude protein	Ether extract	
Control diet	-	0.724 ^{bc}	0.761 ^{abc}	0.757 ^b	0.682	0.820 ^b	11.10 ^c
Soy oil	50	0.740 ^a	0.777 ^{ab}	0.790 ^a	0.710	0.894 ^a	12.56 ^a
Acidulated soy oil soapstocks	50	0.731 ^{ab}	0.769 ^{abc}	0.776 ^{ab}	0.693	0.893 ^a	12.36 ^{ab}
Lard	50	0.740 ^a	0.781 ^a	0.792 ^a	0.708	0.881 ^a	12.54 ^{ab}
Re-esterified monoacylglyceride	50	0.717 ^{bc}	0.758 ^{bc}	0.764 ^b	0.684	0.889 ^a	12.27 ^b
Re-esterified triacylglyceride	50	0.713 ^c	0.754 ^c	0.761 ^b	0.683	0.894 ^a	12.29 ^{ab}
SO-RMG ^a	50	0.723 ^{bc}	0.762 ^{abc}	0.781 ^a	0.708	0.896 ^a	12.46 ^{ab}
SEM ^b (n=6)		0.003	0.004	0.003	0.010	0.007	0.061
P-value		0.001	0.001	0.001	0.159	0.001	0.001
Control vs. supplemental fat diets		0.375	0.868	0.001	0.136	0.001	0.001

^{a,b,c} Value within columns with no common superscript are different ($P < 0.05$).

^a A mixture of 10 g soy oil and 40 g re-esterified monoacylglyceride.

^b Standard error of the means (6 replicates of 10 birds each per treatment).

Table 8

Estimated and calculated apparent metabolisable energy (AME) of the lipid sources in broilers at 21 days of age (Experiment 2).

	Basal	Fat source						SEM ^e	<i>P</i> -value	
	diet	Soy oil	ASO ^a	Lard	RMAG ^b	RTAG ^c	SO-RMAG ^d			
Oil source										
Gross energy (MJ/kg)	38.94	39.66	38.88	39.47	38.06	38.56	39.39			
CTTAR ^f	0.820 ^b	0.894 ^a	0.893 ^a	0.881 ^a	0.889 ^a	0.894 ^a	0.896 ^a	0.007	0.001	
Corrected TTAR ^g	-	0.923	0.915	0.906	0.910	0.914	0.917	0.006	0.461	
AME _n of the fat source (MJ/kg)										
Gross energy × CTTAR ^h	31.93 ^c	35.46 ^a	34.73 ^{ab}	34.77 ^{ab}	33.82 ^b	34.47 ^{ab}	35.25 ^a	0.275	0.001	
Gross energy × Corrected CTTAR ⁱ	-	36.61 ^a	35.57 ^{abc}	35.75 ^{ab}	34.61 ^c	35.23 ^{bc}	36.09 ^{ab}	0.237	0.001	
By difference ^j	-	40.55 ^a	36.62 ^{ab}	40.27 ^a	34.80 ^b	35.24 ^{ab}	38.59 ^{ab}	1.206	0.005	

^{a,b,c} Value within lines with no common superscript are different ($P < 0.05$).

^a Acidulated soy oil soapstocks.

^b Re-esterified monoacylglyceride.

^c Re-esterified triacylglyceride.

^d A mixture of 10 g soy oil and 40 g re-esterified monoacylglyceride.

^e Standard error of the means (n= 6 replicates of 10 birds each per treatment).

^f Coefficient of total tract apparent digestibility of the ether extract fraction of the diet

^g CTTAR of the supplemental oil after correcting by the digestibility for the ether extract fraction of the basal diet (Irandoost et al., 2012).

^h Calculated by multiplying the gross energy of the fat by the CTTAR of the supplemented fat of the corresponding diet.

ⁱ Calculated by multiplying the gross energy of the fat by the corrected CTTAR of the supplemented fat of the corresponding diet

^j Calculated by difference between the AME of the basal and the oil containing diet.

AME = [(AME experimental feed – [95 x (AME control feed/100)]) / 5] x 100.

Table 9

Influence of the inclusion in the diet of 50 g/kg of the lipid source on growth performance of broilers from 7 to 21 days of age^a (Experiment 2).

	7-14 days			14-21 days			7-21 days		
	ADG ^b	ADFI ^c	F: G ^d	ADG	ADFI	F: G	ADG	ADFI	F: G
Control diet	35.9 ^b	43.6	1.22 ^a	54.0	79.8	1.48 ^a	44.9	61.7	1.37 ^a
Soy oil	38.6 ^{ab}	44.9	1.16 ^b	56.2	77.4	1.38 ^b	46.8	60.1	1.28 ^b
Acidulated soy oil soapstocks	39.1 ^{ab}	45.7	1.17 ^b	55.8	78.8	1.41 ^{ab}	46.9	61.1	1.31 ^b
Lard	39.5 ^{ab}	45.6	1.16 ^b	56.1	79.1	1.41 ^{ab}	47.2	61.2	1.30 ^b
Re-esterified monoacylglyceride	40.1 ^a	47.1	1.17 ^b	54.4	78.9	1.45 ^{ab}	46.8	61.9	1.32 ^b
Re-esterified triacylglyceride	39.1 ^{ab}	45.2	1.16 ^b	54.4	77.9	1.43 ^{ab}	46.2	60.5	1.31 ^b
SO-RMAG ^e	39.5 ^{ab}	46.2	1.17 ^b	55.0	76.9	1.40 ^{ab}	46.7	60.5	1.30 ^b
SEM ^f (n=6)	0.84	0.97	0.008	1.11	1.49	0.016	0.806	1.081	0.009
<i>P</i> -value	0.029	0.316	0.001	0.677	0.824	0.003	0.512	0.878	0.001
Control vs. supplemental fat diet	0.001	0.047	0.001	0.286	0.328	0.001	0.041	0.486	0.001

^{a,b,c} Value within columns with no common superscript are different ($P < 0.05$).

^a The average BW of the broilers at 7 days of age was 181 g.

^b Average daily gain (g).

^c Average daily feed intake (g).

^d Feed to gain ratio.

^e A mixture of 10 g soy oil and 40 g re-esterified monoacylglyceride.

^f Standard error of the means (6 replicates of 10 birds each per treatment).

4. Discussion

The determined chemical analyses of the experimental feeds were close to expected values, indicating that the diets were mixed correctly.

4.1. Coefficient of total tract apparent retention and AME of the lipid sources. Experiments 1 and 2

The inclusion of a lipid source affects in different ways the utilization of other components of the diet (Mateos and Sell, 1980b; Jansen et al., 2015). In the current research, EE retention was higher for the supplemental fat diets than for the control diet in both experiments. Jimenez-Moreno et al. (2009) in 15-d-old broilers and Irandoust et al. (2012) in laying hens reported also an increase in EE digestibility with the inclusion of 50 g SO/kg diet, consistent with the results reported herein. Similarly, Mateos et al. (1980c) reported in laying hens that EE digestibility increased with yellow grease inclusion. When the basal diet does not add any supplemental fat, most of the EE is supplied by maize or other dietary ingredients and therefore, a high proportion of the lipid fraction is entrapped within the cell structure, which is less accessible to enzyme activity, especially when the diets are fed in mash form. In contrast, when the diets are supplemented with extra amounts of fat, most of the EE is supplied by the lipid source, which is freely available and of easy access for lipase activity. Consequently, EE digestibility is expected to be lower in the control diet than in the fat supplemented diets.

Fat supplementation reduces the rate of feed passage through the digestive tract of the bird, facilitating the contact between nutrients and enzymes which in turn might improve diet digestibility (Mateos et al., 1981a, 1982). In the current research, added fat increased the retention of most nutrients and the AME content of the diet, with effects that were greater in Exp 2, in which a higher level of supplemented fat was used (50 vs. 25 g /kg). In fact, the

inclusion of 50 g SO/kg increased the CTTAR of DM, OM, GE and CP by 0.016, 0.006, 0.033 and 0.028 units, respectively, values that were within the range reported by other authors (Vieira et al., 2002; Jimenez-Moreno et al., 2009; Jansen et al., 2015). In contrast, Zollitsch et al. (1997) and Zhang et al. (2011) reported that DM and N retention was not affected when different fat sources were included in the diet of 36 and 17-d-old broilers, respectively. Mateos and Sell (1981b), Leeson and Summers (2001) and Ravindran et al. (2016) suggested that most of the contradictory information regarding the effect of supplemental fat on the digestibility of the other constituents of the diet, could be explained by differences in the source and level of fat used, as well as the type of bird and the characteristics of the control diet. The available information, together with the results of the current research, suggest that within practical levels of use, the beneficial effects of fat on nutrient retention increase as the level of supplemental fat increases. Similar, results have been reported recently by Aardsma and Parsons (2016).

The contribution of fats to the energy content of the diet depends on the methodology used, with greater values when calculated by difference between the AME of the control and the oil containing diets than when calculated by multiplying the GE of the fat by the CTTAR of the EE of the experimental diet (Mateos et al., 1980c, 2015; Huyghebaert et al., 1988; Irandoust et al., 2012; Aardsma and Parsons, 2016). In the current research, the difference in AME across fat sources between the two methods of estimation was, as an average, 1.94 MJ/kg in Exp. 1 and 2.04 MJ/kg in Exp. 2. Irandoust et al. (2012) reported also higher AME for SO and ASO (0.87 and 0.48 MJ/kg) when determined by difference between diets than when determined directly from GE and EE digestibility data, in laying hens fed diets supplemented with 30 g soy oil/kg. Huyghebaert et al. (1988) reported also that the AME contents of SO and lard, in diets that contained 90 g fat/kg, were 1.80 and 0.63 MJ/kg higher when calculated by difference than when calculated from GE and EE retention data.

Moreover, Aardsma and Parsons (2016) reported 2.60 MJ/kg higher true metabolizable energy (TME) for maize oil added at 50 g/kg, when calculated by difference than from GE and EE retention data.

In general, the AME of the SO, ASO and lard reported herein were higher than values published by other researchers (Huyghebaert et al., 1988; Zumbado et al., 1999; Mossab et al., 2000; Irandoust et al., 2012) or suggested in most tables on nutrient content of ingredients (FEDNA, 2010; CVB, 2011; Premier Atlas, 2014). Pesti et al. (2002), however, reported in broilers, AME values for seven different fat sources (SO, palm oil, yellow grease, white grease, restaurant grease, poultry grease and animal/vegetable oil blend) in the range of 29.77 to 53.00 MJ/kg (average of 39.15 MJ/kg). Blanch et al. (1996) reported also AME values for different lipid sources in the range of 39.80 to 49.8 MJ/kg (average of 44.04 MJ/kg). Moreover, Aardsma and Parsons (2016) reported that the TME of eight different lipid sources, ranged from 35.31 to 45.36 MJ/kg (average of 41.32 MJ/kg). The reasons for the discrepancies observed among authors on the AME of a given fat source are probably related to variability in the quality of the fats as well as the age of the birds and the type of diets used in the different experiments. In the current research, the AME of the fats increased as the level of fat increased, probably because the ratio between the EE contained in the dietary ingredients and that of the supplemental fat was reduced (Irandoust et al., 2012). In this respect, Vieira et al. (2002) and Peña et al. (2014) reported in broilers that the AME of the experimental ASO source increased by 2.53 and 5.36 MJ/kg, when the level of the oil in the diet increased from 40 to 80 g/kg or from 20 to 60 g/kg, respectively. Aardsma and Parsons (2016) reported also that the TME of SO and maize oil increased by 1.01 and 2.33 MJ/kg, as the level of oil supplementation increased from 50 to 100 g/kg.

In Exp. 2, the AME of the SO and lard were 36.61 and 35.75 MJ/kg when calculated from the GE and the corrected CTTAR of the EE of the diets, but 40.55 and 40.27 MJ/kg

when calculated by difference between the AME of the diets, values that were 2.89 and 4.47 MJ/kg higher than those reported by FEDNA (2010) for these fat sources and 0.89 and 0.80 MJ/kg above their GE values. Numerous authors (Blanch et al., 1996; Mossab et al., 2000; Pesti et al., 2002; Peña et al., 2014; Aardsma and Parsons, 2016) have reported also energy values for different fat sources higher than their corresponding GE value from 0.5 to 3.90 MJ/kg. Biologically, the AME of a given ingredient cannot be higher than its GE and consequently, these high values are caused either by problems related with the methodology used to estimate the energy content of the fat or to the beneficial effects of fat supplementation on the utilization of the non-fiber components of the diet (Mateos and Sell, 1980a, 1981c; Ravindran et al., 2016). In this respect, Mateos and Sell (1982) reported that supplemental fat reduced rate of feed passage, facilitating the contact between nutrients and digestive enzymes, increasing thereby the utilization of other dietary components. However, because of the methodology used for the calculation of the AME of the fat source, the improvement is usually attributed to the supplemental fat. On the other hand, when determining the energy content of a lipid source the amount of fat included in the experimental diets is quite limited and therefore, any small mistake is magnified, resulting in wide confidence intervals for the estimated AME value.

The information available on the effects on nutrient retention and AME content of the supplementation of a diet with re-esterified oils, as compared with more traditional fat sources, in broilers is scarce. Vilarrasa et al. (2014) did not find any difference in EE retention or AME content in 10-d old broilers fed diets that contained 60 g/kg of native or re-esterified palm oil. Similar results have been reported by Vilarrasa et al. (2015a) who observed that the inclusion of 60 g of re-esterified SO or palm oil/kg diet did not affect the AME of the diet as compared with their corresponding native SO or palm oil in 12-d-old broilers. They also reported greater AME for the re-esterified SO than for the re-esterified

palm oil, probably because of differences in the unsaturated to saturated FA ratio of the two lipid sources. In addition, Vilarrasa et al. (2015b) reported that the AME of the diet increased linearly with the inclusion of up to 60 g/kg of re-esterified SO in replacement of re-esterified palm oil in 10-d-old, but not in 38-d-old broilers.

In the current research, EE digestibility was similar for the RMAG and the SO-RMAG mixture, indicating that the inclusion of SO did not improve the digestibility of the re-esterified oil. The proportion of FFA and the FA profile were similar for both types of oils and consequently, no benefits of the combination should be expected. Vieira et al. (2002) reported also that the inclusion of 40 or 80 g/kg of a mixture of SO and ASO (50:50) did not increase the AMEn of the fat as compared with SO or ASO in 30-d-old broilers. In general, the data reported herein indicate that broilers responded in a similar way to diets with re-esterified oils or conventional lipid sources. However, in the current research the GE of the re-esterified lipids was lower than that of lard, SO and ASO and consequently, their AME were slightly lower than those of traditional lipid sources.

4.2. Growth performance. Experiments 1 and 2

Supplemental fat improved broiler performance in both experiments, with more pronounced effects in Exp 2, in which the level of fat used was higher. Broilers fed the control diet had similar feed intake than broilers fed the fat supplemented diets, a result that was unexpected because diets had different energy concentration and birds eat to satisfy their energy requirements (Plavnik et al., 1997; Saldaña et al., 2015). The ingredients of the basal diet used in the 2 experiments were ground using a 3 mm-screen and probably, the presence of fine particles reduced voluntary feed intake (Huang and de Beer, 2010). The inclusion of fat, however, reduced dust formation and increased the palatability of the fat supplemented diets, resulting in an increase in feed intake (Herrera et al., 2016). In addition, the data suggest that the requirements in indispensable AA of the birds were satisfied when fed the

high fat diets, in spite of their higher AME to digestible Lys ratio. In this respect, Pesti et al. (2002) observed that the inclusion of 30 to 60 g of eight sources of fat/kg, at the expense of the control diet, improved ADFI and F:G in broilers. Similar improvements in F:G ratio have been reported by Bartov (1987), Barbour et al. (2006) and Pekel et al. (2013) when SO was included in the diet of broilers. The data reported herein, suggest that supplemental fat improves growth performance of broilers, provided that their AA requirements are satisfied.

In both experiments, the inclusion of SO was more effective in improving ADG and F:G than the inclusion of the other fat sources, consistent with the higher digestibility of the more unsaturated lipid sources (Vanschoubroek et al., 1971; Wiseman et al., 1991; Dvorin et al., 1998). Zhang et al. (2011) and Sharifi et al. (2012) reported also greater ADG and better F:G in broilers when 30 g of tallow or ASO/kg was substituted by SO, consistent with the results reported herein. The data confirm that TAG and unsaturated lipid sources are better absorbed by the chick than FFA and saturated lipid sources.

The information available on the inclusion of re-esterified oils in substitution of conventional fat sources on growth performance of broilers is very limited. In the two experiments of the current research, broilers fed the re-esterified oils grew faster and had better F:G ratio than broilers fed the control diet, with most of the differences occurring from 7 to 14 d of age. Moreover, growth performance was similar to that of broilers fed the more traditional fat sources. Vilarrasa et al. (2015a) reported also that the substitution of 60 g/kg SO or palm oil by their corresponding re-esterified oils did not affect growth performance of broilers from 0 to 20 d of age. These authors observed also that broilers fed the native or the re-esterified SO diets grew faster and had better F:G than broilers fed the native or the re-esterified palm oil, consistent with differences in the FA profile of the two lipid sources. Also, Vilarrasa et al. (2014) reported similar growth performance in broilers fed diets with 60 g/kg of native or re-esterified palm oil. Moreover, Vilarrasa et al. (2015b) reported that the

substitution of 60 g/kg re-esterified palm oil by re-esterified SO or a 25:75 combination of both, improved F:G without affecting ADG or ADFI of broilers from 0 to 41 d of age. In a second paper, Vilarrasa et al. (2015c) reported similar results in weanling pigs fed diets with 100 g/kg of native or re-esterified palm oil. The authors had not found any report comparing these two re-esterified lipid sources in broilers.

5. Conclusions

The AME of the experimental fats were higher when determined by difference between the AME of the diets than when calculated from the GE and the corrected TTAR of the supplemented fat, suggesting that supplemental fat improved the utilization of other dietary components. Supplemental fat increased EE retention and improved F:G ratio in broilers from 7 to 21 d of age in both experiments. Also, the AME of the fats and its effects on the digestibility of other dietary constituents increased as the level of fat supplementation increased.

The inclusion of RMAG and RTAG, lipid sources that resulted from the re-esterification process of two co-products of soy oil refining industry, were well digested by the bird and did not have any negative effect on nutrient retention or growth performance of broilers as compared with more traditional lipid sources. Consequently, RMAG and RTAG can be used as alternative fat sources in broilers diets.

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CHAPTER 5:
GENERAL DISCUSSION AND CONCLUSIONS

1. General discussion

The aim of this thesis was to gain new scientific insights on the use of co-products of the bioenergy industry from soy oil, such as glycerol, lecithin, and re-esterified lipids in poultry diets. In this chapter, the conclusions from the three studies of the thesis were combined into a general discussion.

1.1. Animal performance

1.1.1. Influence of glycerin

According to the literature, crude glycerin can replace as much as 10% of the carbohydrates (mostly starch) in broiler feeds, without compromising and even improving the growth performance of the bird (Cerrate et al. 2006; Lammers et al., 2008b; Swiatkiewicz and Koreleski, 2009). The energy value of crude glycerol for poultry depends primarily on its glycerine content and hence, on the quality of the process to produce biodiesel. Results from chapter 2 of the present thesis indicate that the AMEn content of glycerol close to 96% of the GE of a product containing 81-87% glycerol, less than 0.4% free fatty acids, 5% ash, 0.06% methanol and less than 10% water. This estimation is close to the predictive value of 97% of the GE obtained by Kerr et al. (2011) for glycerol containing less than 0.5% free fatty acids, but higher than the range of 86.0 to 92% reported by Lima et al. (2013) in products with lower glycerin and higher fat residues. Differences in the composition of the fat residue, might explain the lower values reported by these authors.

Studies with laying hens have shown that glycerin can be included at levels of up to 15% in the diet without affecting egg productivity. Data in chapter 3 of this thesis showed that laying hens fed 7% glycerol in isonutritive diets showed the same performance than hens fed diets without glycerol. It is estimated that the AMEn of glycerol in laying hens is close to 98% of its GE in products containing 86% glycerin and 0.49% free fatty acids (Németh et al. 2013).

In the study of Németh et al. (2013) feed efficiency was reduced in hens fed diets containing 10% glycerol. Also, in this thesis feed efficiency of the hens diminished slightly when fed diets

containing 7% glycerol. Therefore, care must be taken when high inclusion levels of crude glycerol, as potential substitute of cereals is used because a slight decrease in glycerin absorption might occur.

1.1.2. Influence of source of fat

The increased use of cereals, by the bioenergy industry to produce ethanol from starch and biodiesel from oils has reduced the availability of these ingredients in poultry diets, leading to explore other co-products, such as lecithins, and reconstituted fats as a cost-effective alternative to increase the energy concentration of the feeds (Ravindran 2016). Fats and oils are complex mixtures of mixed glycerides with smaller amounts of polar lipids, sterols, and fat-soluble vitamins. Despite their wide use in poultry diets, the complex chemical composition of lipid sources, together with methodological aspects related to the age of the bird, made fats a highly variable energy source, yielding a wide range of AME in the literature (reviewed by Ravindran 2016). Consequently, it is important to improve our knowledge on all these factors to increase the accuracy of AME reference values for the different lipid sources.

The chemical composition of the different fat sources is one of the major factors that limits the digestion and the AME of supplemental fats. It is well known that chain length and degree of saturation of the fatty acids are important factors explaining the digestibility of fats with lower digestibility values for saturated fats with a high percentage of long chain fatty acids. Animal fats are rich in long-chain saturated fatty acids, resulting generally in lower AME values for poultry than vegetable oils rich in long-chain saturated fatty acids, such as soy or sunflower oils (Ravindran 2016). Lecithins from the soy oil industry are mixtures of phospholipids with emulsifying properties that facilitate further fat digestion and absorption. Also, lecithins provide linoleic acid and inositol to the diet with an important metabolic role in laying hens. Both effects, highly unsaturated FA and phospholipid content result in an overall increase in digestibility compared to animal fat in the current study.

In the present thesis, fat sources varying in fatty acid and lipid profile were tested, using different methodological approaches to determine its AME content. In chapter 3, the use of 4% lecithins at expenses of animal fat in the diet improved FCR per kilogram of eggs, suggesting a better utilization of the energy contained in this lipid source compared with calculated values. Similar results were reported by (An et al. 1997 and Attia et al. 2009) with inclusion levels of up to 7%.

Chapter 4 explored the AME content of traditional and re-esterified lipid sources in broilers from hatch to 21 d of age. As previously indicated, fats and oils are complex mixtures of mixed glycerides composed mainly by TAG. This lipid class is digested by the animal lipases yielding MAG and free fatty acids. Among all these lipid classes free fatty acids are the less digestible. In chapter 4, two experiments were conducted. In experiment 1 a control diet without added fat and 3 extra diets that included 2.5% of soy oil (SO), re-esterified monoacylglycerol (RMAG) or re-esterified triacylglycerol (RTAG) were tested. Diets that included any of the three lipid sources showed better feed to gain ratio than the control diet. Birds fed the soy oil performed better than birds fed the other oils. Experiment 2 had the same experimental design but we used a higher inclusion level of fat (5%) and included acidulated soapstocks, lard, or a 20:80 mixture of SO and RMAG (SO-RMAG) as extra fats in addition for SO, RMAG and RTMG. Results were similar to those of experiment 1, with better feed to gain ratio in broilers fed diets with added fat and SO out performing in terms of feed to gain ratio the other fat sources. These two experiments confirm previous results reported in the literature, showing that lipid sources rich in TAG and unsaturated fatty acids are better digested than lipid sources containing high levels of saturated free fatty acids. Also, the data showed that based on broiler performance, of up to 5% re-esterified fats from the soy oil refining industry can be used in the diet as a replacement of SO or lard.

The determination of the true energy contribution of added fats to a practical diet in poultry is not an easy task. Two common methodologies used consist in 1) the difference between the AME of a control diet without added fat and the experimental diet to which the test fat was added, and 2) calculating the AME of the test fat by multiplying its GE t by the digestibility of the lipid of the diet (Mateos et al., 1980c; Irandoust et al., 2012). In the present thesis, the method based on differences between a control and a test fat diet gave on average 2 MJ/kg higher AME values. This is in agreement with previous studies from our group (Irandoust et al., 2012) and with data reported by others (Huyghebaert et al., 1988; Aardsma and Parsons, 2016). Moreover, the AME of the SO and lard, estimated by using the difference method, was higher than that estimated from the GE and EE digestibility values, suggesting that supplemental fat improved the utilization of other dietary components. These results do not support the basic idea of this method assuming that lipid inclusion in the diet does not affect the metabolic use of other dietary components and that lipid digestion is independent of diet composition. An important drawback of this method is the low inclusion level of fat in the test diets (2.5-5% under practical conditions). The low level of fat in these diets results in important variability of the final data because any small error is magnified in the evaluation of the AME content of the lipid sources. This problem results in wide confidence intervals for the estimated AME values of the lipid sources.

1. 2. Egg quality

1.2.1. Influence of glycerin

The influence of the GLYC content of the diet on egg quality was studied in chapter 3. The inclusion of 7% GLYC did not affect any of the egg quality traits studied including albumen, yolk colour, and shell strength and thickness from 23 to 51 wk of age. These data are agreement with data reported in the literature by several authors with inclusion levels of

glycerol of up to 10% (Swiatkiewicz and Koreleski, 2009; Yalçin et al., 2010; Németh et al., 2014).

1.2.2. Influence of source of fat

There is evidence in the literature that the inclusion of fat in laying hens diets results in heavier eggs with increased yolk and albumen weights. This benefit effect is more pronounced when the lipid source is rich in polyunsaturated fatty acids such as corn, sunflower, or soy oil (Whitehead et al. 1993; Grobas et al. 2001). Although the mechanisms of these effects remain to be fully elucidated it is proposed that, part is due to the a positive effect of fat inclusion on dietary energy digestibility. Also, certain unsaturated fatty acids, such as oleic and linoleic acid have a direct effect on estrogen production, favoring lipid and albumen secretion and their deposition in the egg (Whitehead et al. 1993). Under some circumstances, supplemental fat can interfere in the mineral metabolism of the hen, reducing Ca absorption and egg shell quality (Atteh and Leeson 1985a). It was suggested that dietary fats, especially those rich in saturated fatty acids form soaps in the GIT of the chick reducing Ca and fat absorption. However, the practical interest of this potential mechanism might have a limited impact in laying hens (Atteh and Leeson 1985b; Bregendahl, 2006). The influence of lecithin on egg quality traits was studied in chapter 3. In this research, the substitution of animal fat by lecithin did not affect egg quality traits, except yolk colour that was improved. Similar effect on egg yolk has been observed by Lipstein et al. (1970) and Attia et al. (2009). Probably, dietary lipid digestibility improves dietary with the inclusion of lecithin in the feed favoring the absorption of the xanthophyll, responsables for egg yolk color. On the other hand, animal fats with a high level of peroxides, as that used in the current experiment, may act as pro-oxidants, reducing the pigmenting capacity of the xanthophyll (Karunajeewa et al., 1984; Baião and Lara, 2005). In the current research, the inclusion of 4% lecithin in the diet tended to reduce the proportion of shell in the eggs, an effect that was observable only in eggs

produced during the second part of the egg cycle. This negative effect was not apparent in the research of Dagaas et al. (2015) in a study using 2% soy lecithins. Attia et al. (2009), however, reported that the inclusion of 6% lecithin in laying hens diet reduced the proportion of shell in the eggs from 47 to 70 wk of age. According to the literature fats and calcium sources can interact and form insoluble soaps, decreasing the digestibility of both fatty acids and minerals. This effect depend on the fatty acid profile of the lipid source with saturated fatty acids more prone to promote such effects. However, neither shell thickness nor shell strength were affected by the substitution of animal fat by lecithin and consequently, no major effects of lecithin on shell quality is expected under commercial conditions.

1.3. Total tract apparent retention

1.3.1. Influence of glycerin

The effect of GLYC inclusion in the diet on TTAR and AMEn were studied in two chapters. In chapter 2, broilers fed the crude glycerol with a higher glycerine content tended to have higher TTAR of all nutrients except N that was not affected. Similar results were observed with increases in the level of GLYC of the diet. This effect has been reported by others with inclusion levels of glycerol up to 10% (Kim et al. 2013; Zavarize et al. 2014). According to this authors when GLYC is included in the diet, small variations in ingredient and chemical composition take place, which might have resulted in the small improvements in nutrient digestibility reported.

Consistent with a higher retention reported for OM and GE the AMEn concentration of the diet increased by 2.3% as the level of GLYC increased from 0 to 10%. Similar results have been reported by Dozier et al. (2008), Gianfelici et al. (2011), and Peña et al. (2014) in broilers. As previously indicated not only the glycerine content but the amount and profile of the free fatty acids together with the methanol and phosphorus, potassium and sodium

chloride concentration within the crude glycerol has to be taken into account and might be behind the AMEn differences.

In chapter 3, the inclusion of 7% GLYC in laying hens diet did not affect TTAR of any of the nutrients studied or the AMEn of the diet. In agreement with the results reported by Lammers et al. (2008) and Swiatkiewicz and Koreleski (2009) when 6 or 15% GLYC was included in the diet of laying hens. The available information regarding to the effect of GLYC inclusion on TTAR of nutrients and AMEn of the diets in laying hens is scarce.

1.3.2. Influence of source of fat

In general, the results of this thesis showed that fat sources rich in unsaturated fatty acids are better utilized by poultry than saturated ones. The improvement in fat utilization depends on type and level of fats, age and type of birds, diet ingredients, and unsaturated to saturated fatty acids ratio (Mateos and Sell, 1981b; Ravindran et al., 2016; Aardsma and Parsons, 2016).

In chapter 3, the substitution of animal fat by lecithin increased the TTAR of all nutrients, except that of N that was not affected. Attia et al. (2009) reported also that the inclusion of 6% lecithin in the diet increased EE digestibility and tended to improve OM retention in hens. The authors have not found any other report comparing the effect of lecithin on TTAR of nutrients in laying hens. In broilers, Polin (1980) reported that fat digestibility was increased by 13% when 2% of lecithin was added to the diet that contained animal tallow at 21 d of age. Similar results have been reported by Huang et al. (2007) with addition of 0.5% lecithin. Most of the beneficial effects of including lecithin in the diet in substitution of saturated fat, on nutrient retention, resulted from the increase in bile acids excretion (Lindsay et al., 1969) and FA unsaturation rather than to a specific emulsifying effect (Soares and Lopez-Bote, 2002).

In chapter 4, the effect of the inclusion of traditional and re-esterified lipid sources in the diet on TTAR and AME of the lipid were studied in two experiments. In both experiments, EE retention was higher for the supplemental fat diets than for the control diet without added fat. However, the CTTAR of all the other nutrients were only improved in experiment 2 with higher dietary fat incorporation. According to the literature, fat supplementation reduced the rate of feed passage through the digestive tract of the bird, facilitating the contact between nutrients and enzymes which in turn might improve diet digestibility. Moreover, in experiment 2 the AME of the fats increased as the level of fat increased, probably because the ratio between the EE contained in the dietary ingredients and that of the supplemental fat was reduced (Irandoost et al., 2012). Similar increased in AME of fats have been reported by (Vieira et al., 2002; Peña et al., 2014; Aardsma and Parsons, 2016) with increased level of supplemented fat in diet of broilers.

The CTTAR of the nutrients was similar in the traditional sources of fat (lard, SO and ASO) than in the re-esterified oils. However, in the current research the GE of the re-esterified lipids was lower than that of lard, SO and ASO and consequently, their AME were slightly lower than those of traditional lipid sources. The information available on the effects on nutrient retention and AME content of the supplementation of a diet with re-esterified oils, as compared with more traditional fat sources, in broilers is scarce. Studies performed by Vilarrasa et al. (2014 and 2015) with re-esterified oils from palm and soy oil showed a similar EE retention and AME content than the control diet (their respective native palm and soy oil). However, the AME content was higher for the re-esterified oil from soy oil and in younger birds (10 vs. 38 d old), therefore it seems that the unsaturation index of the oil used in the re-esterification process and the age of the bird have to be taken into account when using the re-esterified oils in broiler diets.

2. General conclusions

- Glycerin, lecithin, and re-esterified oils, co-products from the soy oil industry can be used efficiently, as a source of energy for poultry diets without any negative effect on nutrient retention, growth performance, and egg quality.
- Raw glycerin can be used in broiler diets at inclusion levels of at least 10%, with increased nutrient retention and AMEn of the diet and improvement in feed conversion ratio from 0 to 21 d of age.
- Raw glycerin can be included at levels of up to 7% in diets for laying hens without any major effect on production, although feed efficiency could be slightly reduced. However, total tract apparent retention, AMEn of the diet, and egg quality were not effected by the inclusion of glycerin in the diet.
- The AMEn of the glycerol in commercial products was 95 to 97 % of its GE value.
- The inclusion of 2 or 4% lecithin in substitution of animal fat, increased nutrient retention in laying hens, and improved egg weight, egg mass production, feed conversion ratio per kilogram of eggs, and yolk color of the eggs. Consequently, the use of up to 4% lecithin is recommended in diets for laying hens to increase egg size and egg yolk color in commercial operations.
- Re-esterified monoacylglycerol and triacylglycerol oils, that resulted from the re-esterification process of two co-products of soy oil refining industry, can be included at levels of up to 5% in broiler diets without any negative effect on total tract apparent retention or growth performance. Consequently, the use of up to 5% re-esterified monoacylglycerol and triacylglycerol oils might be recommended as alternative fat sources in broilers diets.
- The addition of 2.5 or 5% fat supplementation in broilers diet increased EE retention and AME of the diet and improved feed conversion ratio from 7 to 21 d of age.

- The AME of the fats tested and its effects on the digestibility of other dietary constituents increased as the level of fat supplementation increased from 2.5 to 5%.
- The AME of the supplemental fats were higher when determined by difference between the AME of the diets than when calculated from the GE and the corrected total tract apparent retention of the supplemented fat.

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