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Glycerin and lecithin inclusion in diets for brown egg-laying hens: Effects on egg production and nutrient digestibility

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ABSTRACT

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.

Abbreviations: AME_n, apparent metabolisable energy, corrected for nitrogen; BWG, body weight gain; CP, crude protein; DM, dry matter; EE, ether extract; FA, fatty acid; FCR, feed conversion ratio; GMD, geometric mean diameter; GSD, geometric standard deviation; GE, gross energy; LNA, linoleic acid; MIU, moisture, impurities and unsaponifiables; N, nitrogen; OM, organic matter; GLYC, raw glycerin; RCF, Roche colour fan; TTAR, total tract apparent retention.

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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 50–100 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.

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 × 80 cm × 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 (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

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).

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.

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 (23–51 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 6 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 2
Ingredient composition and calculated chemical analysis of the experimental diets (g/kg as fed basis, unless otherwise stated).

	Raw glycerin, 0 g/kg			Raw glycerin, 70 g/kg		
Lecithin[a], 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), 3000 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 μg; vitamin K_3 (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 ($FeSO_4$ H_2O), 30 mg; copper ($CuSO4{\cdot}5H_2O$), 5 mg; iodine ($Ca(IO_3)_2$), 0.5 mg; selenium (Na_2SeO_3), 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).

Table 3
Determined analysis[a] (g/kg as-fed basis, unless otherwise indicated) of the diets.

	Raw glycerin, 0 g/kg			Raw glycerin, 70 g/kg		
Lecithin[b], 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

[a] Analysed 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.
[c] Geometric mean diameter.
[d] Geometric 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 Kjeltec, 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 5000 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 analysed 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 Fig. 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. 3C). 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. 3D).

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

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
P-value[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 ($P > 0.10$).

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.

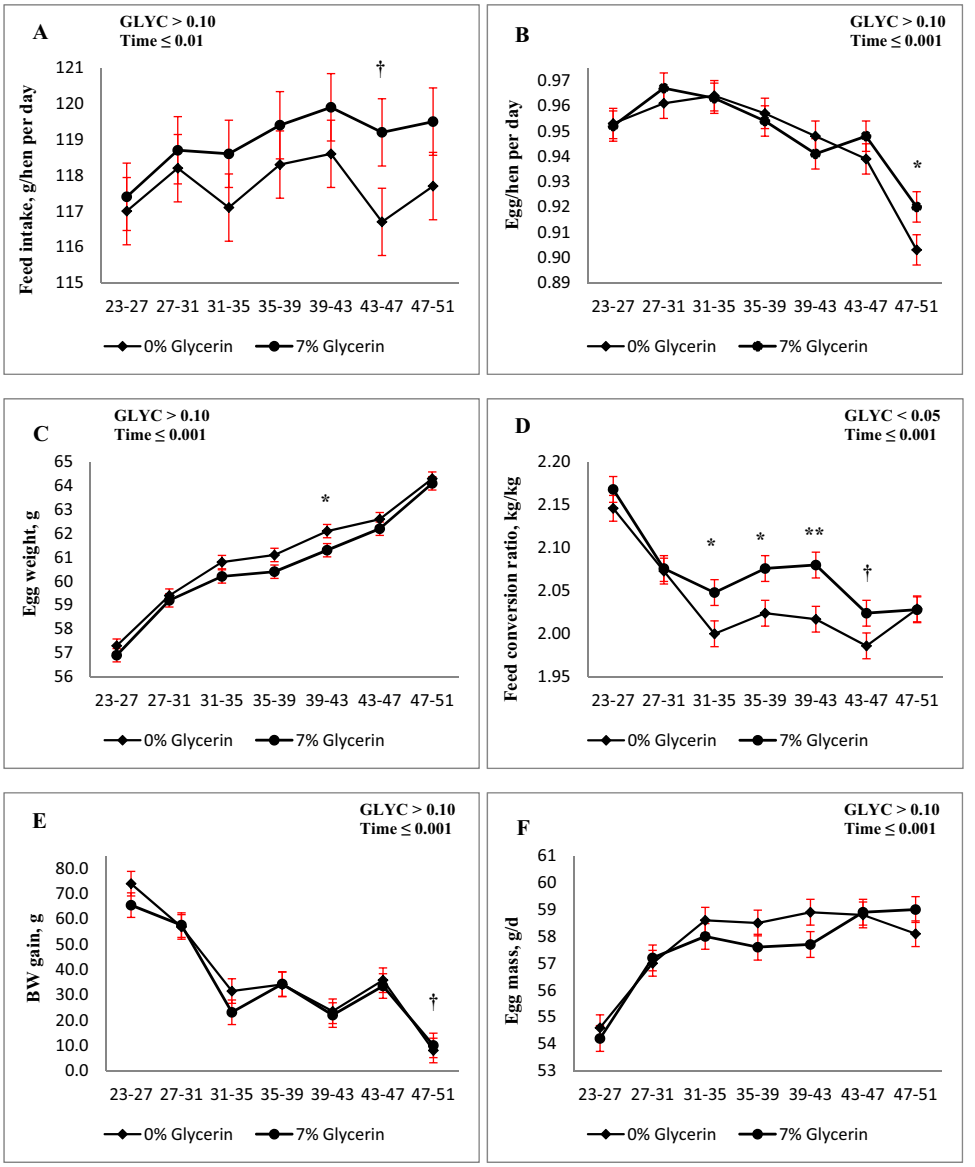
4.1. Hen performance and egg quality

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

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
P-value[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 ($P > 0.10$).



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

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.

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

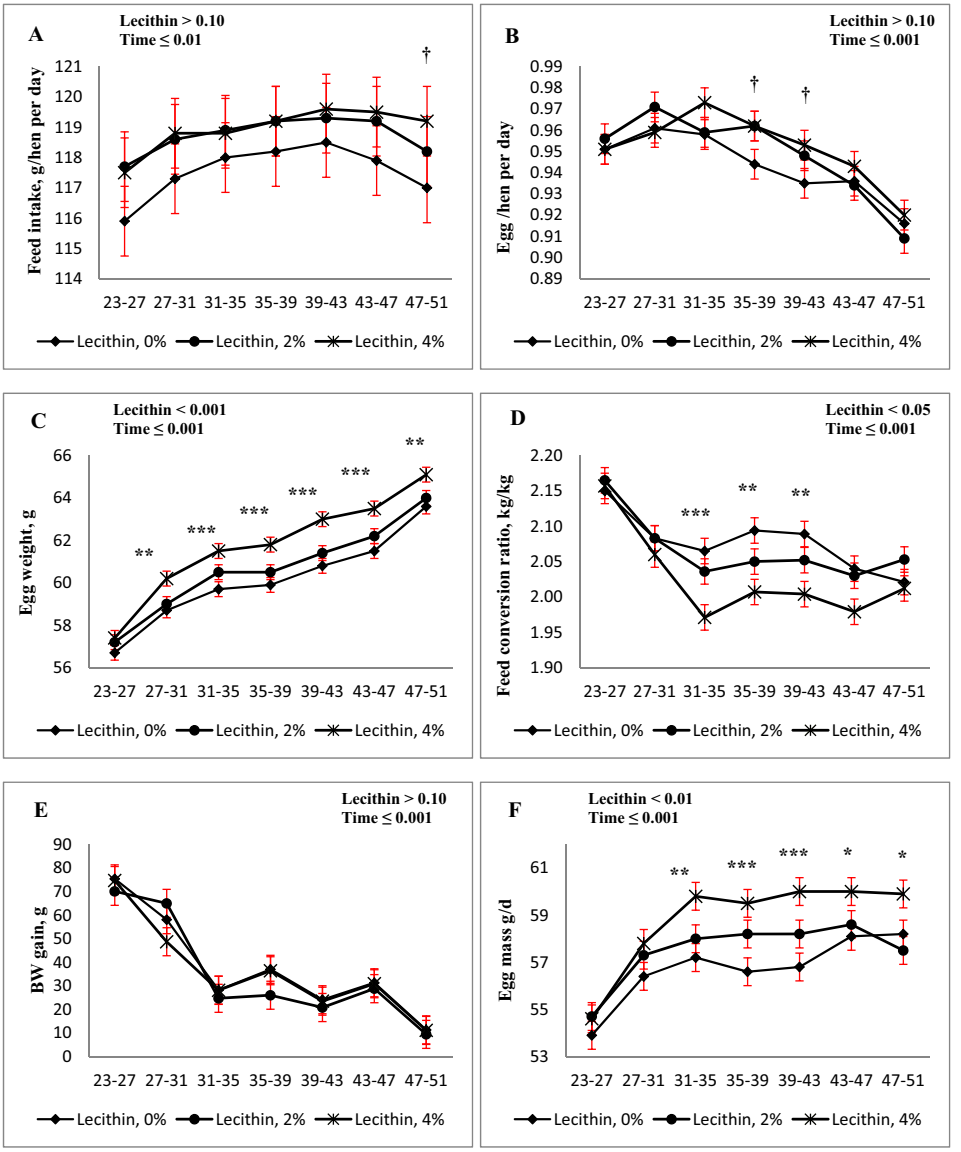
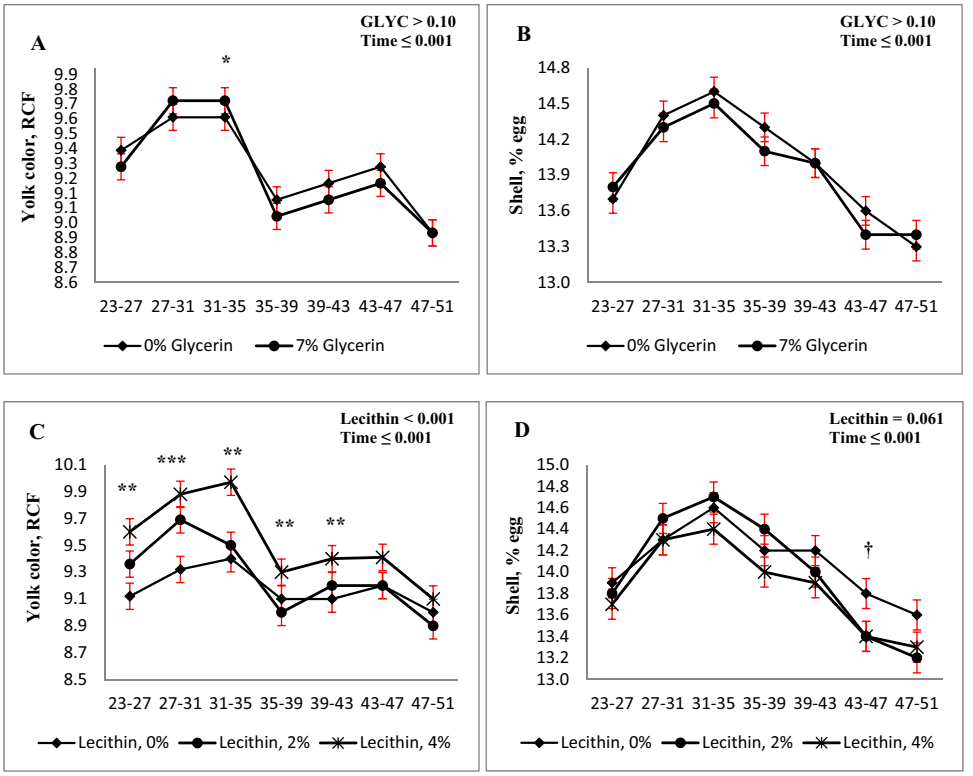


Fig. 2. Effect of inclusion of soy lecithin in the diet on feed intake (A), egg production (B), egg weight (C), feed conversion ratio (D), BW gain (E) and egg mass (F) from 23 to 51 wk of age.

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



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

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

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
P-value[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 ($P > 0.10$).

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. 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; Baião 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 hydrolysation 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.

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.

Conflict of interest

The authors confirm that there are no conflict of interest in this research

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