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Effect of feeding diets containing barley, wheat and corn distillers dried grains with solubles on the carcass traits and meat quality in growing-fattening rabbits

Gilbert Alagón[1], Mariam Pascual [2*], Paula Serrano[2], Juan José Pascual[3], Concepción Cervera[3]

[1]Facultad de Agronomía y Zootecnia, Universidad Nacional de San Antonio Abad del Cusco. Perú.

[2] Centro de Investigación y Tecnología Animal (CITA-IVIA), Polígono de la Esperanza s/n, 12400, Segorbe, Spain.

[3]Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Camino de Vera s/n, Valencia 46022, Spain.

* Corresponding author: Te.: ; Fax: ; Email address: .

Abstract

The effect of dried distillers grains with solubles (DDGS) of barley (20%), wheat (20%) and corn (20 and 40%) included in the diet of rabbits on some carcass characteristics, meat quality, chemical composition and fatty acid composition of *Longissimus* muscle was studied. No effect of the inclusion of DDGS on the hot carcass weight, cold carcass weight, drip loss percentage, percentage full digestive tract percentage, liver weight percentage, dressing-out percentage and color of the carcass was found. The fat percentage in the different fat depots was affected by the diet, resulting in a obtaining a higher dissectible fat percentage when including of barley and corn DDGS. No effect of DDGS on texture parameters, cooking loss, water holding capacity and intramuscular fat of the loin meat was found. Instead, the redness of the meat, pH, protein content and the concentration of saturated and polyunsaturated fatty acids in the loin meat depending on the diet. The polyunsaturated:saturated and saturated:unsaturated fatty ratios and the atherogenic and thrombogenic indexes values were improved form the health point of view when including a 40% of corn DDGS in the diet.

Keywords: distillers dried grains with solubles; carcass traits; meat quality; rabbits

1. Introduction

The distillers dried grains with solubles (DDGS) of barley, wheat and corn are co-products of the industry bioethanol used in livestock feed. These products have high potential to be included in formulation and manufacture of diets for rabbits because they are characterized by being good sources of digestible energy (11.9 - 15.7 MJ kg DM), digestible protein (16.8 - 26.3%), fat (7.2 - 14.4%) and soluble fiber (20 - 21.7%) (De Blas, Mateos, & García-Rebollar, 2010; Alagón, Arce, Martínez-Paredes, Ródenas, Moya, Blas, Pascual, & Cervera, 2013a) and by improving growth performance (Youssef, Soha, Abd El-Gawad, Eman, & Ali, 2012; Alagón, Arce, Martínez-Paredes, Ródenas, Blas, Cervera, & Pascual, 2013b).

The determination of optimal levels of DDGS in diets for feeding farm animals, is usually based on the evaluation of production and economic performance. However, the use of DDGS may affect the quality of the carcass and meat. Typically, DDGS contain 7 to 15% of fat, with 70 to 80% of mono and polyunsaturated fatty acids (Xu, Baidoo, Johnston, Bibus, Cannon, & Shurson, 2010; Alagón et al. 2013a;) and according to some studies the monogastrics show a fatty acid profile in the meat similar to the profile of the diet (Bee, Gebert, & Messikomer, 2002; Dalle Zotte, 2002).

In pigs, the use of DDGS has shown a reduction in dressing-out percentage in some studies (Cook,Paton, & Gibson, 2005; Thacker, 2006; Whitney,Shurson, Johnston, Wulf, & Shanks, 2006; Gaines, Spencer, Petersen, Augspurger, & Kitt, 2007; Weimer, Stevens, Schinckel, Latour, & Richert, 2008;), and increased levels of corn DDGS 20-30% in growing-finishing diets reduced pork fat firmness (Whitney et al., 2006), while others found no change in dressing-out percentage due to the use of these co-products (McEwen, 2006; Xu, Shurson, Hubby, Miller, & de Rodas, 2007; Drescher, Johnston, Shurson, & Goihl, 2008). In chickens, levels above 12% corn DDGS increased the level

of fatty acids in the thigh meat, increasing the oxidation during storage (Schilling, Battula, Loar, Jackson, Kin, & Corzo, 2010). In steers, feeding with diets that included levels of 20 and 40% of wheat and corn DDGS did not lead to differences in carcass and meat quality (Aldai, Aalhus, Dugan, Robertson, McAllister, Walter, & McKinnon, 2010). However, no information is available about the effect of DDGS in diets on carcass and meat quality in rabbits.

Therefore, the objective of the present study was to evaluate the effect of the inclusion of 20% DDGS for barley, wheat and corn and 40% corn DDGS in diets for growing-finishing rabbits on carcass and meat quality.

2. Material and methods

2.1. Diets

Five isoproteic, isoenergetic and isofibrous diets were formulated according to the nutritional requirements for growing and fattening rabbits (De Blas and Mateos, 2010), with the inclusion of dried distillers grains and solubles (DDGS) as follows: diet C (control diet, including 0% of DDGS), diet Db_{20} (with 20% of barley DDGS), diet Dw_{20} (with 20% of wheat DDGS), diet Dc_{20} (with 20% of corn DDGS) and diet Dc_{40} (with 40% of corn DDGS). From each diet, both medicated and unmedicated feed were prepared. The ingredients, chemical composition, nutritive value and fatty acid composition are shown in Tables 1 and 2.

The diets were analyzed according to the methods of AOAC (2000): 934.01 for dry matter (DM), 942.05 for ash, 976.06 for crude protein (CP) and 920.39 for ether extract (EE). Previous acid–hydrolysis of samples was carried out in the analysis of EE. Starch content was determined according to Batey (1982). The aNDFom (assayed with a thermo–stable amylase and expressed exclusive of residual ash), ADFom (expressed exclusive of residual ash) and lignin (determined by solubilisation of cellulose with sulfuric acid, sa) were analyzed sequentially (Van Soest, and Roberston, Lewis, 1991). The neutral detergent soluble fibre content was determined according to Hall, Lewis, Van Soest, & Chase (1997), adapting the method to the nylon filter bag system and with the modifications proposed by Martínez-Vallespín, Navarrete, Martínez-Paredes, Ródenas, Cervera & Blas (2011). Insoluble hemicelluloses and cellulose were determined by difference (aNDFom–ADFom and ADFom–Lignin (sa), respectively). Finally, the digestible protein and digestible energy of the experimental diets were calculated using an apparent digestibility assay with pools of feces, measured in 5

rabbits per experimental diet, according to the European Reference method (Pérez, Lebas, Gidenne, Maertens, Xiccato, Parigi-Bini, Dalle Zotte, & Cossu et al., 1995).

The amino acid content was determined after acid hydrolysis with HCL 6N at 110 ºC for 23 h as previously described by Liu, H. J., Chang, B. Y., Yan, H. W., Yu, F. H. & Liu, X. X. (1995), using a Waters (Milford, Massachusetts, USA) HPLC system consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717, Waters), a fluorescence detector (Mod. 474, Waters) and a temperature control module. Aminobutyric acid was added as internal standard after hydrolysation. The amino acids were derivatised with AQC (6–aminoquinolyl–N–hydroxysuccinimidyl carbamate) and separated with a C–18 reverse-phase column Waters AcQ Tag (150mm×3.9mm). Methionine were determined separately as methionine sulphone after performic acid oxidation followed by acid hydrolysis.

The content of methyl esters of fatty acids was determined in samples of the five experimental diets, using a gas chromatograph Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless inlet and flame ionization detector. The separation was performed on a capillary column SPTM 2560 (Supelco, PA, USA) (100m×0.25mm×0.2mm film thickness) with a flow rate of 1.1 mL Helium min^{-1}, according to the following temperature gradient: 140°C initial temperature for 5 min, increasing in a linear gradient of 4°C min^{-1} until 240°C, which temperature was maintained for 30 min, to finally return to initial conditions. The injector and detector were maintained at 260ºC. Fatty acids were identified by comparing their retention times with those of a pattern of fatty acid methyl esters (47885–U) from Supelco® (Pennsylvania, USA) and quantified using C13:0 as internal standard (O´Fallon, Busboom, Nelson, & Gaskins, 2007).

111

112 *2.2. Animals*

113 The experimental protocol followed both the Spanish Royal Decree 1201/2005 on
114 protection of animals used for scientific purposes (Boletín Oficial del Estado, 2005) and
115 the recommendations for applied nutrition research in rabbits described by the European
116 Group on Rabbit Nutrition (Fernández-Carmona, Blas, Pascual, Maertens, Gidenne,
117 Xiccato & García, 2005), being approved by the Committee of Ethics and Animal
118 Welfare of the Universidad Politécnica de Valencia.

119 A total of 475 weaned rabbits 28 days old of both sexes from a three ways cross were
120 used in the experiment. Animals were reared in 5 rounds. Rabbits were allocated in
121 individual cages and fed until 59 days-old with one of the 5 experimental diets. Diets
122 were medicated from 28 to 48 days-old and unmedicated from 49 to 59 days-old.

123 *2.3. Slaughter traits and carcass composition*

124 At 59 days of age, 100 rabbits (4 per diet and round) were weighed (SW), electrically
125 stunned and slaughtered at the abattoir in the farm. No fasting was applied. The
126 slaughtering and carcass dissection procedures followed the World Rabbit Science
127 Association (WRSA) recommendations described by Blasco and Ouhayoun (1996).

128 The slaughtered rabbits were bled, and the skin, genitals, urinary bladder,
129 gastrointestinal tract and the distal part of the legs were removed. The full gastro
130 intestinal tract was weighed and expressed as percentage with respect to SW (FGTP).
131 The hot carcasses obtained were weighed (HCW) and then chilled at +4 °C for 24 h in a
132 ventilated room. The chilled carcasses were weighed (CCW) and the dressing out
133 percentage was calculated as CCW×100/ SW. The drip loss percentage (DLP) was
134 calculated as (HCW-CCW)/HCW × 100. Liver, inguinal fat, perirenal fat and scapular

fat were removed, weighed and expressed as percentage with respect to CCW (LvP, IfaP, PfaP and SfaP, respectively). Dissectible fat percentage (DfaP) was calculated as the sum of the inguinal, perirenal and scapular fat weight, expressed as percentage with respect to CCW. Both sides of the *Longissimus* muscles were excised from the carcass and used to determine the meat quality parameters.

2.4. Meat quality

2.4.1. Color measurements

Color measurements in the CIELAB space (Lightness, L*; redness, a* and yellowness, b*) (CIE, 1976) were measured at 24 h post-mortem using a Minolta Chromameter (Minolta CR-300, Osaka, Japan), which gives L*, a* and b* values at each point. Carcass color was determined on the surface of the right *Longissimus* muscle, at the level of the fourth lumbar vertebra (Pla, Hernández, & Blasco, 1995). Meat color was measured in the transversal section of the *Longissimus* muscle at the level of the 7th lumbar vertebra.

2.4.2. pH measurement

Meat pH was measured at 24 h post-mortem (pH24h) in the right *Longissimus* muscle at the level of the fourth lumbar vertebra at 20 ºC and penetrating 3 mm, with a digital pH meter (Basic 20+ Crison Instruments S.A., Barcelona, Spain).

2.4.3. Water holding capacity

A sample of 300±5 mg of meat from the left *Longissimus* muscle, corresponding to the sixth lumbar vertebra, was weighed (G) (0.1 mg accuracy) and deposited on a previously desiccated and weighed (P) 7-cm disk of Whatman No. 1 filter paper. Then the sample on the paper was placed between two Plexiglass plates and a load of 2.25 kg was applied. After 5 min, the load was removed and the damp paper filter was weighed

(D) after removing the compressed meat. The mean of two replicates was used in the analysis. Water-holding capacity (WHC) was calculated as (D - P) × 100/G.

2.4.4. Cooking losses

The left *Longissimus* muscle of each animal were weighed (F), vacuum packed in plastic bags and frozen at -20 ºC. When required, *Longissimus* muscles were thawed at 4 ºC for 24 h and cooked vacuum packed in the plastic bags at 80 ºC for 1 h by immersion in a water bath. Cooked samples were cooled by immersion in water for 10 min. After cooling, samples were removed from the bags and weighed (C). Cooking losses were calculated as (F-C) × 100/F.

2.4.5. Texture measurements

A Warner-Bratzler shear test was performed with the left *Longissimus* muscle cooked for the CL determination. Two to three rectangles of 1cm × 1cm × 2cm of cross section, from each *Longissimus* muscle were extracted, parallel to the muscle fibers direction. The Texture Analyzer Model TA-XT Plus (Stable Micro Systems, UK) was used for test and all the samples were cut perpendicular to the muscle fiber direction. The samples were completely cut using a Warner-Bratzler shear blade with an angular triangular slot cutting edge. Three parameters were measured: the maximum shear force (kg/cm^2), which represents the connective tissue component of tenderness (Moller, 1980); shear firmness ($kg/s\ cm^2$) as the slope of a line drawn from the origin of the curve to the maximum shear forces (Brady & Hunecke, 1985), and the total work performed to cut the sample or the area under the curve (kg s/cm2). The average value for each *Longissimus* muscle sample was recorded (mean of two to three replicates).

2.4.6. Chemical and fatty acids composition determined

The right *Longissimus* muscle was fascia removed, ground, packed in a petri plate and stored at -80°C. The samples were freeze-dried, ground and scanned between 1100 and 2498 nm with a monochromator (Model 5000, NIRSystem INC., Silver Spring, MD, USA) equipped with a transport module using ISI software, version 3.10 from Infrasolft International (Infrasoft International LLC, State College, PA, USA). Absorbance data were recorded at 2 nm and stored as log (1/reflectance). Sample measurements were taken in circular cups with quartz windows of 3.8 cm diameter. A sample cup was filled, placed in the NIRS unit and two spectra, rotating 90 degrees the sample cup were obtained. The sample cup was refilled with the same sample and procedure was repeated to obtain four spectra of each sample. The similarity between the four reflectance spectra was studied using Root Mean Squared (RMS) statistics. Then, four spectra were averaged. The chemical and fatty acid composition of the samples were predicted using the equations developed by Zomeño, Juste & Hernández (2012). The saturation (S/U), atherogenic (AI) and thrombogenic (TI) indexes were calculated according to Ulbricht & Southgate (1991) using equations presented by Peiretti & Meineri (2008) and Volek & Marounek (2011):

$$S/U = (14:0 + C16:0 + C18:0) / \sum MUFA + \sum PUFA$$

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / [\sum MUFA + \sum(n\text{-}6) + \sum(n\text{-}3)]$$

$$TI = (14:0 + C16:0 + C18:0) / [0.5 \times \sum MUFA + 0.5 \times \sum(n\text{-}6) + 3 \times \sum(n\text{-}3) + \sum(n\text{-}3) / \sum(n\text{-}6)]$$

where MUFA and PUFA are monounsaturated and polyunsaturated fatty acids, respectively. The C:12 was not included in the AI calculation as the content in *Longissimus* muscle is not detectible.

2.5. Statistical analysis

206 Carcass composition and meat quality characteristics were analyzed using the GLM
207 procedure of Statistical Analysis System (SAS, 2008). The model included as fixed
208 effects the experimental diet [C, Db_{20}, Dw_{20}, Dc_{20} and Dc_{40}] and the round (1 to 5).
209 Preliminary analysis showed that the diet × round interaction was not significant;
210 therefore it was not included in the model.

211 Linear and quadratic effects of including different levels corn DDGS in the diets (C,
212 Dc_{20} and Dc_{40}) were studied using orthogonal polynomial contrasts. In addition,
213 orthogonal contrasts were used to compare the mean of all diets with a 20% of DDGS
214 ($DDGS_{20}$) with the C diet. All reported means are least squares means.

215

3. Results

3.1. Carcass Characteristics

The carcass composition of the rabbits fed with the experimental diets is shown in table 3. The use of diet Db_{20} led to higher values of IFaP, SFaP and DFaP than when feeding with the C diet (+21, +23 and +17 percentage points, respectively). The use of Dc_{20} also turns out the DFaP (+11 percentage points) with respect to C, and Dc_{40} led to higher SFaP and DFaP (+17 and +15 percentage points, respectively). Rabbits fed with DDGS20 diets showed higher values of IFaP, SFaP, DFaP than those fed with C. The use corn DDGS in the diet increased linearly PFaP, SFaP and DFaP.

3.2. Meat quality

The effect of the experimental diets on color, pH, water holding capacity, cooking losses and the parameters of the texture in the meat of the *Longissimus* muscle is shown in Table 4.

No statistical differences ($P > 0.05$) in the color of the carcasses of rabbits fed with the experimental diets were found. In relation to the color of the meat, no effect of the experimental diets on parameters L* was found, while diets differed in a* ($P < 0.05$). The Dc_{20} diet had higher Chroma and Dw_{20} diet had higher a*, compared with the other experimental diets. Also, diets that included 0, 20 and 40% corn DDGS reported a quadratic effect on a*. Similarly, there was a linear effect on b* of the meat, decreasing as corn DDGS level was increased.

Db_{20} and Dw_{20} diets led to higher pH values (5.52 and 5.53, respectively) than diets with Dc_{20} diet (5.44; $P<0.05$), while the rest showed intermediate values.

No differences in WHC, CL and the texture parameters (shear force, shear firmness and area) in the longissimus muscle depending on the diet were found ($P > 0.05$).

Table 5 shows the chemical and fatty acids composition in the *Longissimus* muscle of rabbits fed with the experimental diets. The level of protein decreased as the corn DDGS level in the diet increased (22.15% for control diet, decreasing -1.5% and -2.9% in diets Dc_{20} and Dc_{40}, respectively). In general, diets that included 20% DDGS decreased (-0.26%, $P < 0.05$) protein content of the meat, with respect to the control diet. No differences ($P > 0.05$) in intramuscular fat content of the *Longissimus* muscle where found depending on the diet.

Meat from rabbits fed with the different diets did not affect most of the fatty acid percentages, except C16:0, which was higher when feeding with Db_{20} and Dw_{20} the when feeding with diet C, and C17:0, which showed a positive linear effect when increasing the corn DDGS in the diet. MUFA and PUFA values did not differ between diets. Differences were found in the SFA concentration, as a percentage of total fatty acids, in the meat of rabbits fed with the diets evaluated, reporting +0.86% and +1.75% with diet Dw_{20} than with diet Dc_{20} and Dc_{40} diets, respectively. The Db_{20} and C diets had an intermediate effect on the concentration of SFA. A linear effect of corn DDGS level in the diets was found, so that the concentration of SFA in meat rabbits decreased with greater inclusion of corn DDGS in diets evaluated.

No differences were found in n-3, n-6 and n-6/n-3 between diets. Ratios P/S, AI and TI were higher in Dc_{40} than in Db_{20} and Dw_{20}. The inclusion of increasing corn DDGS levels in the diets led to a reduction of the ratio S/U.

Discussion

Effects of the DDGS on the carcass traits of rabbits

The use of DDGS co-products of the bioethanol industry in animal feeding have shown to reduce dressing out percentage in pigs in some studies (Cook et al., 2005; Thacker, 2006; White, Richert, Radcliffe, Schinckel, & Latour, 2007; Weimer, et al., 2008; Bregendahl, 2008), although no effect was found by other authors (McEwen, 2006; Xu et al., 2007; Drescher et al., 2008). In the present study the mean values obtained of hot carcass weight (HCW, 1216 ± 9g), cold carcass weight (CCW, 1171 ± 9g), drip loss (DLP, 3.71 ± 0.12%) and the dressing out percentage (DoP, 56.06 ± 0.21% CCW) were not affected by the use of DDGS and correspond to those expected by weight, age and genetics (Pla, & Cervera, 1997; Pla, 1999; Hernández, Ariño, Grimal & Blasco, 2006). Thus, carcass yield, economically important for the rabbit manufactures, is not affected when using DDGS for rabbit nutrition at these levels.

An effect observed in some species when including DDGS in the diet is an increase of fat deposition (Benz, Linneen, Tokach, Dritz, Nelssen, DeRouchey, Goodband, Sulabo, & Prusa, 2010). This is a negative consequence for the consumers' acceptance, which lately tend to low fat diets. The rabbit carcass is considered as a low fat carcass (Dalle Zotte & Szendrö, 2011), but the results found in this study show that rabbits also increase the fat in the carcass when including DDGS in the diets. The higher fat percentage of inguinal, perirenal and scapular depots and in dissectible fat percentage (Table 3) when feeding with some diets that included DDGS could be due to higher concentrations and higher intakes of crude fat with the Db_{20} (9.15 g/d), Dw_{20} (7.51 g/d), Dc_{20} (8.25 g/d) and Dc_{40} (8.90 g/d) vs C (6.25 g/d) diets, as observed in other studies (Fernández & Fraga, 1996; Pla & Cervera, 1999). In fact, positive correlations were found between intake of fat per day and dissectible fat (% CCW) in the carcasses studied ($r = 0.62$, 0.58, 0.56 and 0.71, for IFaP, PFaP, SFaP and DFP, respectively, $P < 0.0001$, results not shown).

The variation in crude fat depending on the diet was not only an effect of intake but also because of the diet composition. Diets were formulated isoenergetic, isoproteic, and isofibrosous, but differ in both fat content (57 g/kg DM in C, vs 68 to 82 g/kg DM in diets with DDGS) and starch content (186 g/kg DM in C vs. 129 to 159 in DDGS). These differences in chemical composition could have affect not only to the fat

deposition, as observed, but also to the liver percentage in the carcass, which is the organ responsible of the reserve of glycogen. Nevertheless, in this study the liver percentage did not differ between diets.

On the other hand, the difference in the deposition of fat in the carcass could be also associated to differences in composition of fatty acids in the experimental diets (Table 2) and the higher intake of PUFA (Db_{20}, 2.72 g/d; Dw_{20}, 2.07 g/d; Dc_{20}, 2.70 g/d and Dc_{40}, 3.2g g/d, vs C, 1.84 g/d) and especially the AG C18:2 (linoleic) (Db_{20}, 1.92 g/d; Dw_{20} ,1.83 g/d, Dc_{20}, 2.46 g/d and $Dc_{40,}$ 3.11 g/d, vs C, 1.62 g/d), as the long chain is more easily deposited in the dissectible fat (Dalle Zotte, 2002). Nevertheless, the higher fat percentage in the carcasses was observed when using barley and corn DDGS but not wheat DDGS, and despite the fat increase, the carcasses can still considered as lean compared to other species.

Effects of the DDGS on the meat quality

Carcass and meat color are important characteristics that could affect acceptability of the consumers. In the present study, rabbits fed with the different diets did not differ in the color parameters of the carcass, reporting average values of 52.80±0.5 for brightness, 5.27±0.33 for redness and -1.57±0.40 for yellowness. The lightness and yellowness values were comparable to those reported by Pascual & Pla (2007), Hernández, Aliaga, Pla, & Blasco, (2004) and Ramírez, Oliver, Pla, Guerrero, Ariño, & Blasco et al. (2004) (53.96, 54.90 and 54.0 for L*, and 0.90, -1.03 and -0.54, for b*, respectively). These authors found lower redness values (3.22, 2.46 and 2.84, respectively) than those found in this study. Furthermore, the parameters of brightness (49.56 ± 0.54) and yellowness (1.54 ± 0.2) of the meat longissimus muscle were not affected by the experimental diets and are within the averages reported by other authors (Liu, Zhou, Tong, & Vaddella, V., 2012, Carrilho, Golf, Olleta, Beltran & Lopez, 2009; Hernandez, Aliaga, Pla, Blasco, 2004). The only parameter affected by the diet was the redness, higher in the meat of rabbits fed with wheat DDGS at 20% ($P < 0.05$) than with the other diets. This which could be due to a higher content of myoglobin, which is the pigment responsible for meat color (Dalle-Zotte & Ouhayoun, 1993). Other authors reported the influence of diet on the color of rabbit meat (Dalle Zotte, Ouhayoun, Parigi Bini, & Xiccato, 1996). Widmer, McGinnis, Wulf, & Stein (2008) and Rickard, Wiegand, Pompeu, Hinson, Gerlemann, Disselhorst, Briscoe, Evans, & Allee (2012) in swine diets including corn DDGS up to 20%, and Xu et al., (2010) using corn DDGS up

to 30%, found no differences in color parameters in the *Longissimus* muscle of pigs at 24 hours post mortem. Schilling et al., (2010) using corn DDGS up to 24% in broiler diets, reported no differences in color parameters of the breast meat.

The pH is an important indicator of the meat quality, as it is related to the water holding capacity and tenderness (Huff-Lonergan & Lonergan, 2005). The pattern of decrease of pH and ultimate pH in the meat affect to the catepsines activity, responsible of the proteolisis post-mortem in the meat which ends the rigor mortis. The overcoming break of the muscle structure affect to the capacity of the meat to retain the water, and the level of proteolysis affects to the tenderness of the meat. Regarding to the effect of the DDGS, Schilling et al. (2010) found differences in the pH of the breast meat when feeding with corn DDGS between 6-24% inclusion in chicken, but were within the normal values of breast meat at 24 hours post mortem. In pig, Widmer et al. (2008), Xu et al. (2010) and Rickard et al. (2012), including 20%, 30% and 20% of corn DDGS, respectively, found no differences in pH in the meat loin. In the present study, the values of pH, water holding capacity and tenderness were similar to those obtained in other studies (pH 5.5 to 5.7, Liu et al., 2012; Dal Bosco, Mourvaki, Cardinali, Servili, Sebastiani, Ruggeri, Mattioli, Taticchi et al., 2012; Pascual & Pla, 2007). In rabbit, Dalle Zotte (2002) reports that diet has little effect on the pH of the meat, being more important factors as the type of muscle, age, method of slaughter and handling of the carcass. In this study, although the pH was higher when using diets with 20% of wheat and barley DDGS than with 20% of corn DDGS, values did not differ with the control diet. Moreover, the texture parameters and the water holding capacity did not differ between animals fed with the different diets, showing the DDGS at these levels do not affect to these characteristics in rabbit meat.

With regard to the chemical composition of *Longissimus* muscle meat, the mean values were within the range obtained by other authors (Pla, Pascual, & Ariño, 2004; Hernández & Gondret, 2006; Hernández & Dalle Zotte, 2010). The higher fat deposition in the carcass associated to the DDGS was not observed in the fat content of the *Longissimus* muscle, although the amount of ingested fat differed depending on the diet (6.25, 9.15, 7.51, 8.25 and 8.90 g / d for C, Db_{20}, Dw_{20}, Dc_{20} and Dc_{40}, respectively, $P<0.0001$, data not shown) and there was a correlation of 0.50 ($P <0.0001$, data not shown) between the amount of fat consumed and the percentage of fat in the *Longissimus* muscle. An increase in lipid deposition in rabbit meat with fat intake

increase was observed by Christ, Lange, & Jeroch, (1996) and Pla & Cervera (1997). Moreover, Pla & Cervera (1997) also observed a decrease of the protein when increasing fat intake, which is in concordance with the lower protein contents observed in this study when including corn DDGS in the diets. In a study of beef, Aldai et al. (2010) observed a decrease in meat protein in the *Longissimus* muscle when including wheat DDGS at a level of 20 and 40%.

The differences in percent of meat protein would not be due to restrictions in energy, protein and amino acids of diets, since dietary intake of these nutrients was within the requirements (De Blas & Mateos, 2010). Moreover, the inclusion of these DDGS in the diets did not reduce growth in a larger experiment which included the animals used in this study (Alagón et al., 2013b). A problem observed in pigs when feeding with DDGS is a low digestibility and availability of lysine after subjecting the product to high temperatures in the process of obtaining bioethanol (Almeida, Petersen, & Stein, 2011). However, the apparent digestibility of DDGS lysine used in this study was adequate (Alagón et al., 2013a) probably due to the formation of microbial lysine at caecum level (Belenguer, Abecia, Belanche, Milne, Balcells, 2012), which is subsequently ingested during caecotrophy.

The fatty acid composition of the *Longissimus* muscle meat of this study, in MUFA (26.7 ± 0.3%), PUFA (38.7 ± 0.3%) and SFA (34.6 ± 0.1%) differ with those reported by other authors in rabbits (Kouba, Benatmane, Blochet, & Mourot, 2008; Dal Bosco et al., 2012) who found higher values in SFA than in PUFA. The variability in the percentage of MUFA, PUFA and SFA is high, as observed Zotte Hernandez & Dalle (2010) in a review of 21 references (28.0 ± 4.1, 32.5 ± 6.1 and 38.9 ± 4.4, respectively) for *Longissimus* muscle meat. This could be because the rabbit, as monogastric, is able to incorporate directly from the diet, the long chain fatty acids in the adipose tissue and intramuscular lipids (Dalle Zotte, 2002), so that the observed change in the fatty acid profile of the loin meat from rabbits respond to the fatty acid composition of the experimental diets. In this way, differences in the SFA (Table 5) in the loin meat would be in direct relation to the differences in the contents of SFA C17:0 ($P < 0.001$) and especially of C16:0 ($P < 0.016$), and respond to differences in the composition of SFA in the diets (Table 2).

Diets had an effect on PUFA, not when expressed as percentage of total fatty acids but as mg/100g of the *Longissimus* muscle. Values obtained were of 295, 314 and 325

393 mg/100g of loin for C, Dc_{20} and Dc_{40} diets, describing a linear effect ($P < 0.05$, results
394 not shown), due to the higher contribution of linoleic with 180, 197 and 205 mg/100g of
395 loin, respectively. The linoleic acid is deposited directly into the fat of the animal
396 (Wood, Enser, Fisher, Nute, Sheard, Richardson, et al., 2008). This fatty acid was
397 higher in corn DDGS diets (Table 2) and consequently the incorporation into muscle fat
398 was directly proportional to its intake. On the other hand, the values of n-3 (mg/100 g
399 loin) showed differences ($P<0.027$, results not shown) between the experimental diets,
400 with superiority in corn DDGS (54.4, 55.8 and 52.5 for Dc_{20}, Dc_{40} and C, respectively),
401 probably due to the greater relative abundance of linoleic acid in the diets (14.7, 15.1
402 and 12.7 for Dc_{20}, Dc_{40} and C, respectively).

403 The DDGS inclusion in diets did not alter the abundance of long chain eicosapentaenoic
404 acid (EPA, C20: 5n-3) and docosahexaenoic (DHA, C22: 6n-3) in the rabbit meat,
405 which are derived from the acid α-linolenic and considered functional nutrients that play
406 important metabolic roles (Dalle Zotte & Szendrö, 2011; Hernandez & Dalle Zotte,
407 2010) and together with the other n-3, have been related to the prevention of
408 cardiovascular disease (Ulbricht & Southgate, 1991).

409 The fatty acid ratios studied are also used as criteria to describe the value of dietary fat
410 from the point of view of cardiovascular health. The British Nutritional Foundation
411 (1999) points out the need to consume food with n-6/n-3 ratios lower or equal to 6. The
412 Department of Health & Social Security UK (1994) recommends ratios for P/S and S/U
413 above 0.45 and below 4.5, respectively, for a balanced diet. The AI and TI values,
414 which are directly related to the saturation of the fatty acids, should be as low as
415 possible in the diets, and Ulbricht & Southgate (1991) reported values of AI and TI of
416 0.50 and 0.95, respectively, for chicken meat. The means obtained in the current study
417 are within the recommended values, and the ratios of fatty acids obtained in the
418 *Longissimus* muscle indicate that the use of corn DDGS at 40% in diets leads to the
419 deposition of a healthier fat in the meat. Although the n-6:n-3 ratio did not differ when
420 using the different diets, P/S was increased and S/U, AI, and TI were lower than in the
421 control diet. It has to be highlight that, although high levels of PUFA could increase the
422 rancidity and the color deterioration of the meat during storage, it is also associated to
423 an improvement of the flavor development of the meat during cooking (Wood et al.,
424 2003).

425

Conclusions

The inclusion barley, wheat and corn DDGS in the diet of rabbits did not affect most of the carcass and meat quality traits. The use of barley and wheat DDGS increased the carcass fat percentage, but still maintaining the rabbit carcass within leaner considered. The use of DDGS improved fatty acid profile of the meat form the health point of view, especially with the use of corn DDGS at 40% level.

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Table 1. Ingredient composition of the experimental diets evaluated (g /kg dry matter).

	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}
Barley grain	150	160	150	160	170
Wheat bran	270	150	190	135	0
Soybean meal 44%	120	30	0	60	0
Alfalfa hay	220	250	200	160	100
Defatted grape seed	90	130	100	97	104
Beet pulp	33	0	0	16.5	0
Oat hulls	30	0	90	95	160
Soybean hulls	34	0	0	17	0
Soybean oil	35	49	32	22.8	10.6
Beet molasses	0	9.4	10	12.5	25
DDGS evaluated	0	200	200	200	400
Calcium carbonate	4.2	5	5	4.6	5
Dicalcium phosphate	0	0	5	4.5	9
Sodium chloride	4	4	4.2	4	4
L-Lysine HCL	0.3	2.7	3.4	1.7	3.2
L-Threonine	0.5	0.9	1.4	0.4	0.2
Vitamin/trace element premix[1]	5	5	5	5	5
Coccidiostac[2]	1	1	1	1	1
Antibiotics[3]	3	3	3	3	3

C: control diet, 0% DDGS; Db_{20}: diet with 20% of barley DDGS; Dw_{20}: diet with 20% of wheat DDGS; Dc_{20} and Dc_{40}: diets with 20 and 40% of corn DDGS, respectively.

[1] Supplied per kg of feed: Vitamin A: 8375 IU; Vitamin D3: 750 IU; Vitamin E: 20 mg; Vitamin K3: 1 mg; Vitamin B1: 1 mg; Vitamin B2: 2 mg; Vitamin B6: 1 mg; Nicotinic acid: 20 mg; Choline chloride: 250 mg; Magnesium: 290 mg; Manganese: 20 mg; Zinc: 60 mg; Iodine: 1.25 mg; Iron: 26 mg; Copper: 10 mg; Cobalt: 0.7 mg; Butyl hydroxylanysole and ethoxiquin mixture: 4 mg.

[2] Cycostat (66 ppm of robenidine).

[3] Dinco-spectim (29 ppm dincomicina + 29 ppm spectinomicyn), 120 ppm neomicin, Apsamix Tiamulin (50 ppm tiamulin), normally used in rabbit farms whit high incidence of mucoid enteropathy (ME).

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647

Table 2. Chemical composition, nutritive value and fatty acids composition of the experimental diets.

	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}
Determined chemical composition(g/kg DM)					
Dry matter	907	911	908	909	903
Ash	61	61	59	60	55
Crude protein, CP	169	167	167	180	182
CP bound to NDF	43	48	44	55	49
Starch	186	154	149	159	129
Ether extract, EE	57	81	68	75	82
Neutral detergent fibre, NDF	370	410	396	390	389
Acid detergent fibre, ADF	191	216	196	189	184
Acid detergent lignin, ADL	50	74	63	54	56
Insoluble hemicelluloses	179	194	200	201	206
Cellulose	141	142	133	135	128
Neutral detergent soluble fibre	84	88	117	104	107
Lysine	10.3	10.6	9.5	8.7	9.4
Methionine	2.1	2.2	2.5	3.0	3.1
Threonine	7.1	7.7	8.0	8.7	7.6
Determined nutritive value					
Digestible energy, DE (MJ/kg DM)[1]	11.2	11.9	11.3	11.7	11.9
Digestible protein, DP[1] (g/Kg DM)	133	132	133	140	148
Ratio DP/DE (g/MJ)	11.9	11.1	11.8	11.9	12.4
Determined fatty acids composition (g/kg DM)					
C14:0 (myrístic)	0.4	0.6	0.4	0.3	0.2
C16:0 (palmític)	12.7	15.5	12.5	13.2	11.8
C16:1 (palmitoleic)	0.9	1.2	1.1	0.8	0.3
C17:1 (heptadecanoic)	0.1	0.1	0.1	0.0	0.0
C18:0 (stearic)	3.8	4.6	3.3	3.5	2.2
C18:1 n-9 (oleico)	16.3	19.4	14.8	19.1	17.2
C18:1 n-7 (vaccenic)	2.6	2.8	2.2	1.8	1.6
C18:2 n-6 (linoleic)	14.7	17.0	16.6	22.3	28.7
C20:0 (arachidic)	0.1	0.1	0.1	0.1	0.0
C20:1 (eicosenoic)	0.3	0.5	0.3	0.4	0.2
C18:3 n-3 (linolenic)	1.6	1.7	1.7	1.6	1.3
C20:2 (eicosadienoic)	0.5	1.0	0.5	0.6	0.4
SFA	17.0	20.8	16.3	17.1	14.2
MUFA	20.2	24.1	18.5	22.2	19.3
PUFA	16.8	19.7	18.7	24.6	30.3
P/S	1.0	0.9	1.1	1.4	2.1
n-3	1.6	1.7	1.7	1.6	1.3
n-6	14.7	17.0	16.6	22.3	28.7
n-6/n-3	9.3	10.0	10.0	13.9	22.7

C: diet control, 0% DDGS; Db20: diet 20% barley DDGS; Dw20: diet 20% wheat DDGS; Dc20 and Dc40: diets 20 and 40% corn DDGS.

[1] Calculated from pooled faeces in a digestibility trial.

SFA, saturated fatty acids [C14:0+C16:0+C18:0+C20:0]; MUFA, monounsaturated fatty acids [C16:1+C17:1+C18:1n-9+C18:1n-7+C20:1; PUFA, poliunsaturated fatty acids [C18:2n-6+C18:3n-3+C20:2].

Table 3. Main traits of the carcass composition of rabbits fed with diets with no DDGS (C), 20% of barley DDGS (Db_{20}), 20% of wheat DDGS (Dw_{20}), 20% of corn DDGS (Dc_{20}) and 40% of corn DDGS (Dc_{40}).

Items	Diets							$DDGS_{20}$-C
	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}	SEM	P-Value	
SW, g	2066	2134	2082	2089	2070	13	0.504	36± 34
FDTP, % SW	20.2	20.2	19.8	18.6	20.1	0.2	0.134	-0.7±0.6
HCW, g	1190	1233	1211	1237	1208	9	0.492	37± 24
CCW, g	1142	1186	1172	1190	1163	9	0.489	40± 24
DLP, %	3.98	3.82	3.18	3.81	3.77	0.12	0.259	-0.37± 0.3
DoP, % CCW	55.32	55.57	56.31	56.95	56.16	0.21	0.125	0.95± 0.5
LvP, % CCW	6.41	6.64	6.71	6.38	6.26	0.12	0.751	0.17± 0.3
IFaP, % CCW	1.47^{a}	1.86^{b}	1.57^{a}	1.58^{a}	1.65^{ab}	0.04	0.014	0.20± 0.10*
PFaP, % CCW[1]	2.05	2.29	2.23	2.36	2.42	0.05	0.184	0.24± 0.13
SFaP, % CCW [1]	0.64^{a}	0.83^{b}	0.66^{a}	0.73^{ab}	0.77^{b}	0.02	0.003	0.1±0.04*
DFaP, % CCW [1]	4.16^{a}	4.99^{c}	4.46^{abc}	4.67^{bc}	4.88^{bc}	0.08	0.015	0.54± 0.2*

[1] Linear or [2] quadratic effect of level inclusion of corn DDGS (P<0.05).
$DDGS_{20}$-C: mean ±standard error of the contrast between DDGS at 20% and the C diet.
* Diets containing DDGS at 20% of level differ from the C diet (P<0.05).
SEM: Standard error of the means.
[a, b, c]: Means in the same row with no common superscripts differ significantly (P<0.05).
SW: Slaugther weight; FDTP: Full digestive tract percentage, HCW: Hot carcass weight; CCW: Chilled carcass weight; DLP: Drip loss percentage; DoP: Dressing-out percentage; LvP: Liver weigth percentage; IFaP: Inguinal fat percentage; PFaP: Perirenal fat percentage; SFaP: Scapular fat percentage; DFaP: Dissectible fat percentage.

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Table 4. Carcass and meat color, pH, water holding capacity (WHC), cooking losses (CL) and texture parameters in the *Longissimus* muscle of rabbits fed with diets with no DDGS (C), 20% of barley DDGS(Db_{20}), 20% of wheat DDGS (Dw_{20}), 20% of corn DDGS (Dc_{20}) and 40% of corn DDGS (Dc_{40}).

Items	Diets					SEM	P-Value	$DDGS_{20}$-C
	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}			
Carcass color								
L*	52.79	52.32	52.95	53.42	52.54	0.23	0.609	0.10± 0.60
a*	5.04	5.56	4.94	5.07	5.75	0.15	0.399	0.15± 0.4
b*	-1.81	-0.85	-2.24	-1.67	-1.32	0.18	0.164	0.22± 0.5
Meat color								
L*	49.74	49.1	49.94	49.03	50.02	0.25	0.569	-0.40±0.60
a* [2]	6.42[a]	6.36[a]	7.81[b]	6.44[a]	6.02[a]	0.16	0.005	0.45±0.4
b* [1]	1.86	1.55	1.42	1.71	1.16	0.09	0.157	-0.30±0.20
pH24h	5.49[ab]	5.52[b]	5.53[b]	5.44[a]	5.49[ab]	0.011	0.095	0.004± 0.03
WHC, %	33.28	33.43	33.57	34.19	33.02	0.238	0.611	0.45±0.6
CL, %	32.87	32.49	33.14	33.42	33.14	0.208	0.69	0.15± 0.5
Texture parameters								
Shear force	3.22	3.09	3.35	3.34	3.44	0.056	0.337	0.03±0.14
Shear firmness	1.46	1.43	1.5	1.49	1.49	0.025	0.918	0.01±0.06
Area	5.04	4.69	5.04	5.18	5.51	0.105	0.182	-0.07±0.3

Linear or [2] quadratic effect of level inclusion of corn DDGS (P<0.05).
$DDGS_{20}$-C: mean ±standard error of the contrast between DDGS at 20% and the C diet.
* Diets containing DDGS at 20% of level differ from the C diet (P<0.05).
SEM: Standard error of the means.
[a, b, c]: Means in the same row with no common superscripts differ significantly (P<0.05).
L: lightness; a: redness; b*:yellowness.

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Table 5. Chemical and fatty acid composition of *Longissimus* muscle of rabbits fed with diets with no DDGS (C), 20% of barley DDGS(Db_{20}), 20% of wheat DDGS (Dw_{20}), 20% of corn DDGS (Dc_{20}) and 40% of corn DDGS (Dc_{40}).

Items	Diets							$DDGS_{20}$-C
	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}	SEM	P-Value	
Chemical composition (g/100 g)								
Protein [1]	22.15^{c}	21.89^{bc}	21.97^{bc}	21.81^{b}	21.50^{a}	0.043	0.001	-0.26±0.1*
Fat	1.18	1.23	1.17	1.25	1.26	0.024	0.631	0.04± 0.06
Fatty acids composition (% total fatty acids)								
C14:0	1.81	1.87	1.73	1.81	1.78	0.028	0.556	-0.01± 0.07
C15:0	0.55	0.54	0.54	0.54	0.55	0.03	0.739	-0.01± 0.01
C16:0	22.29^{ab}	23.07^{b}	23.29^{b}	22.37^{ab}	21.49^{a}	0.179	0.016	0.62± 0.46
C16:1	1.81	1.94	1.73	1.9	1.65	0.077	0.762	0.04±0.19
C17:0 [1]	0.73^{a}	0.73^{a}	0.75^{a}	0.76^{ab}	0.80b	0.006	0.001	0.02± 0.01
C18:0	9.24	8.83	8.98	8.94	8.93	0.080	0.564	-0.32± 0.21
C18:1n-7	1.85	1.79	1.82	1.83	1.83	0.015	0.744	-0.04± 0.04
C18:1n-9	22.62	23.58	22.61	23.09	23.51	0.206	0.388	0.48± 0.53
C18:2n-6	23.63	23.5	24.05	24.02	24.77	0.203	0.317	0.23± 0.52
C18:3n-3	1.62	1.67	1.7	1.74	1.8	0.033	0.491	0.08±0.08
C20:2n-6	0.34	0.33	0.35	0.34	0.33	0.006	0.776	-0.0± 0.0
C20:3n-6	0.68	0.64	0.71	0.66	0.61	0.018	0.423	-0.01±0.05
C20:4n-6	5.2	4.94	4.93	4.9	4.98	0.124	0.945	-0.27±0.32
C20:5n-3	2.23	1.84	2.05	2.01	1.89	0.066	0.376	-0.26±0.17
C22:4n-6	2.36	2.15	2.31	2.2	2.11	0.049	0.449	-0.13± 0.13
C22:5n-3	0.72	0.61	0.66	0.68	0.68	0.023	0.657	-0.07±0.06
C22:6n-3	2.75	2.51	2.53	2.64	2.78	0.094	0.849	-0.19±0.24
SFA [1]	34.63^{bc}	35.04^{bc}	35.29^{c}	34.43^{b}	33.54^{a}	0.129	0.001	0.29±0.33
MUFA	26.28	27.30	26.16	26.82	26.99	0.25	0.556	0.48± 0.64
PUFA	39.09	37.65	38.55	38.75	39.46	0.311	0.428	-0.77±0.79
n-3	7.07	6.09	6.20	6.64	6.8	0.133	0.127	-0.76±0.36*
n-6	32.2	31.56	32.35	32.13	32.78	0.245	0.627	-0.19± 0.63
n-6/n-3	4.84	5.42	5.49	4.88	5.10	0.097	0.119	0.4±0.2
P/S	1.14^{ab}	1.08^{a}	1.10^{a}	1.13^{ab}	1.18^{b}	0.013	0.098	-0.04±0.03
S/U [1]	0.51^{b}	0.52^{b}	0.53^{b}	0.51^{ab}	0.48^{a}	0.003	0.001	0.01±0.01
AI	0.45^{ab}	0.47^{b}	0.47^{b}	0.45^{ab}	0.43^{a}	0.005	0.034	0.01±0.01
TI	0.67^{ab}	0.71^{b}	0.71^{b}	0.68^{ab}	0.64^{a}	0.007	0.004	0.03±0.02

Linear or [2] quadratic effect of level inclusion of corn DDGS (P<0.05).

$DDGS_{20}$-C: mean ±standard error of the contrast between DDGS at 20% and the C diet.

* Diets containing DDGS at 20% of level differ from the C diet (P<0.05).

SEM: Standard error of the means.

[a, b, c]: Means in the same row with no common superscripts differ significantly (P<0.05).

SFA, saturated fatty acids [C14:0+C15:0+C16:0+C17:0+C18:0]; MUFA, monounsaturated fatty acids [C16:1+ C18:1n-7+ C18:1n-9]; PUFA, poliunsaturated fatty acids [C18:2n-6+C18:3n-3+C20:2n-6+C20:3n-6+C20:4n-6+ C20:5n-3+C22:4n-6+ C22:5n-3+C22:6n-3]; n-3: Omega-3 fatty acids [C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3]; n-6:Omega-6 fatty acids [C18:2n-6+C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6]; P/S: ratio PUFA/SFA; S/U: ratio SFA/(MUFA+PUFA); AI, atherogenic index; TI, thrombogenic index.

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