Use of barley, wheat and corn distiller's dried grains with solubles in diets for growing rabbits: nutritive value, growth performance and meat quality.

Ph.D. Thesis

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Ciencia es todo aquello sobre la cual siempre cabe discusión.

José Ortega y Gasset

Con todo mi amor:

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ABSTRACT

The aim of the present thesis was to evaluate the potential use of distillers dried grains with solubles (DDGS) of barley, corn and wheat available in the Iberian Peninsula in the feeding of growing rabbits. For this task, it has been determined DDGS' nutritive value, as well as their effect on growth performance, carcass characteristics and quality of meat of growing rabbits. In the first experiment the chemical, amino acid and fatty acid composition of eight DDGS batches (2, 2 and 4 from barley, corn and wheat grains, respectively) was analized. Five diets were formulated to determine the nutritive value of DDGS in growing rabbits: a control diet and 4 experimental diets containing 200 g of the DDGS/kg dry mater (DM) [DDGS from national barley, national corn, Brazilian corn and national wheat grains]. Sixty three-way crossbred fattening rabbits aged 42 days were used in the digestibility trial. DDGS can be characterized as a raw material rich in crude protein (CP), neutral detergent fibre and neutral detergent soluble fibre (on av. 318, 352 and 208 g/kg DM, respectively). Barley DDGS had higher fibre and lower protein contents than wheat DDGS (+25 g of acid detergent fibre and –91 g of CP/kg DM, respectively; $P<0.05$). Corn DDGS had intermediate fibre and protein values between barley and wheat DDGS, but were the richest in ether extract (on av. +72 g/kg DM). DDGS' protein was richer in proline, phenylalanine, valine and arginine for barley DDGS, in leucine, alanine and histidine for corn DDGS, and in glutamic acid for wheat DDGS. Barley DDGS was richer in saturated (SFA; 267 g/kg total fatty acids), corn DDGS in monounsaturated (MUFA; 278 g/kg total fatty acids) and wheat DDGS in polyunsaturated fatty acids (PUFA; 615 g/kg total fatty acids). Barley DDGS had the lowest nutritive value traits for rabbits (11.9 MJ of digestible energy (DE) and 168 g digestible protein (DP)/kg DM). No significant differences for the nutritive value between both corn DDGS were observed (on av. 15.3 MJ DE and 208 g DP/kg DM), and wheat

DDGS might be considered as the DDGS with the highest nutritive value (15.7 MJ DE and 263 g DP/kg DM). In the second experiment, and to evaluate how the dietary inclusion of DDGS could affect the performance and caecal environment of growing rabbits, four experimental diets were formulated from a control diet without DDGS (C), including 20% of barley DDGS (Db_{20}), 20% of wheat DDGS (Dw_{20}) and 20 (Dc_{20}) or 40% (Dc_{40}) of corn DDGS. Performance trial was done using 475 three-way crossbred weaned rabbits of 28 d of age individually housed. Caecal fermentation traits were determined on 20 animals per diet and age at 42 d (using 200 rabbits housed in collective cages) and at 59 d of age (from the performance trial). In the whole period and respect to the control group, animals fed with Db_{20} showed higher DM and DE intake (+6 and +12%, respectively; $P<0.05$), but similar daily weight gain (DWG) and increased feed conversion ratio (+9%; $P<0.05$). In this same way, and independently of its inclusion level, the increase on DE intake on animals fed with corn DDGS (+9 kJ/d, respectively; $P<0.05$) did not result in a significant increase of DWG. In the contrary, higher DM and DE intake of animals feed with Dw_{20} (+8; $P<0.05$) resulted in the highest DWG registered (+2.8 g/d; $P<0.05$) than the control group. Although inclusion of DDGS at 20% did not affected main caecal parameters controlled at 42 d, caecum of animals fed with the diet Dc_{40} was characterized by greater N-NH_3 and valeric acid, and lower total volatile fatty acids and acetic acid concentrations ($P<0.05$). Increased values of caecum DM, propionic and valeric acids and reduced values of total volatile fatty acids and acetic/propionic rate were observed at 59 d for DDGS inclusion at 20% ($P<0.05$) and for the linear inclusion of corn DDGS ($P<0.05$). Animals given Dc_{40} were also characterized for a greater caecum N-NH_3 content ($P<0.05$) at 59 d of age. Finally in the third experiment, the effect of diets of the second experiment (C, Db_{20}, Dw_{20}, Dc_{20} and Dc_{40}) on some carcass characteristics, meat quality, chemical composition and fatty acid composition of *Longissimus* muscle of 100 growing rabbits at 59 days of age was studied.

No effect of the inclusion of DDGS on the hot carcass weight, cold carcass weight (CCW), drip loss 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 higher dissectible fat percentage when including of barley and corn DDGS (on av. +0.7% CCW; $P<0.05$). 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 SFA and PUFA in the loin meat depended on the diet. The PUFA:SFA and SFA:unsaturated fatty ratios and the atherogenic and thrombogenic indexes were improved from the health point of view when including corn DDGS 40%. The results of the present thesis reveal that the inclusion of DDGS up to 20% in balanced diets for growing rabbits, independently of their grain source (barley, wheat or corn), could be an interesting alternative, allowing an adequate growing performance without any negative consequence on the carcass and meat quality.

Keywords: distillers dried grains with solubles; chemical composition; digestibility; growing rabbits, cacecal environment, meat quality.

RESUMEN

El objetivo de esta tesis fue evaluar la utilización de los granos secos de destilería con solubles (DGGS) de cebada, maíz y trigo, co-productos de la industria del bioetanol disponibles en la Península Ibérica, en la alimentación de conejos en crecimiento. Para ello, se determinó el valor nutritivo de los DDGS y el efecto de su inclusión en la dieta sobre el rendimiento productivo, características de la canal y calidad de la carne del conejo. En el primer experimento se determinó la composición química, composición de aminoácidos y ácidos grasos de ocho lotes de DDGS (2, 2 y 4 de cebada, trigo y maíz, respectivamente). Posteriormente, se formularon cinco dietas para determinar el valor nutritivo de los DDGS: una dieta control y cuatro dietas con 200 g/kg materia seca (MS) de los diferentes DDGS (DDGS de cebada nacional, maíz nacional, maíz brasileño y trigo nacional). El ensayo de digestibilidad se realizó con un total de 60 conejos de 42 días de edad provenientes de un cruce a tres vías. Los resultados mostraron que los DDGS se caracterizan como una materia prima rica en proteína bruta (PB), fibra neutro detergente y fibra soluble en detergente neutro (con valores medios de 318, 352 y 208 g/kg MS, respectivamente). Los DDGS de cebada mostraron mayor contenido en fibra y menor en proteína que los DDGS de trigo (+25 g de fibra ácido detergente y –91 g de PB/kg MS, respectivamente; $P<0,05$). En los DDGS de maíz se obtuvieron valores de fibra y proteína intermedios a los obtenidos en los DDGS de cebada y maíz; sin embargo, mostraron el mayor valor en extracto etéreo (en promedio +72 g/kg MS). La proteína con mayor contenido en prolina, fenilalanina, valina y arginina fue la del DDGS de cebada, la proteína con mayor contenido en leucina, alanina y histidina fue la del DDGS de maíz, y la proteína de mayor contenido en glutámico, la del DDGS de trigo. El DDGS de cebada fue el de mayor contenido en ácidos grasos saturados (SFA, 267 g/kg ácidos grasos totales), el DDGS de maíz en mono insaturados (MUFA, 278 g/kg

ácidos grasos totales) y el DDGS de trigo el de mayor contenido de ácidos grasos poliinsaturados (PUFA, 615 g/kg ácidos grasos totales). El DDGS de cebada mostró el menor valor nutritivo para conejo (11.9 MJ de energía digestible (ED) y 168 g de proteína digestible (PD)/kg MS). El valor nutritivo de los dos DDGS de maíz no difirió significativamente (valores medios de 15.3 MJ ED y 208 g PB/kg MS), y el DDGS de mayor valor nutritivo fue el de trigo (15.7 MJ ED y 263 g PB/kg MS). En el segundo experimento se evaluó el efecto de la inclusión de los DDGS sobre el rendimiento productivo y el ambiente cecal de los conejos de engorde. Se formularon cuatro dietas a partir de una dieta control sin DDGS (C), que incluían un 20% de DDGS de cebada (Db_{20}), un 20% de DDGS de trigo (Dw20), y un 20% (Dc_{20}) o 40% (Dc_{40}) de DDGS de maíz. En el estudio de rendimiento productivo se utilizaron un total de 475 conejos recién destetados de 28 días de edad provenientes de un cruce a tres vías y alojados individualmente. Los parámetros de fermentación cecal fueron determinados en 20 conejos por dieta y edad, a los 42 días (a partir de 200 conejos alojados en jaulas colectivas) y a los 59 días de edad (a partir de los 475 conejos del estudio de rendimiento productivo). Los animales alimentados con Db_{20} mostraron mayor ingestión de MS y ED (+6 y + 12%, respectivamente; $P<0,05$), similar ganancia de peso diaria (GPD) y mayor índice de conversión (+9%; $P<0,05$) que los animales alimentados con el pienso control. El incremento en la ingestión de ED de los animales que consumieron pienso con DDGS de maíz, tanto al 20% como al 40% de inclusión (+9 kJ/d; $P<0,05$), no se tradujo en un aumento significativo de la GPD con respecto a los animales alimentados con el pienso control. Por otro lado, la mayor ingestión de MS y de ED de los animales alimentados con la dieta Dw20 llevó a que estos animales tuvieran mayor GPD (+2.8 g/d; $P<0,05$) que el grupo control. La inclusión de los DDGS al 20% no afectó a los principales parámetros del ambiente cecal evaluados a los 42 días, sin embargo, la inclusión de DDGS de maíz al 40% se caracterizó por una mayor concentración de $N\text{-}NH_3$ y de ácido valérico, y un menor contenido en ácidos grasos volátiles y concentración de ácido acético (P<0,05). A los 59 días de edad, el efecto de la inclusión de DDGS al 20% y la inclusión lineal de DDGS de maíz llevó a valores superiores de MS en contenido cecal, ácido propiónico y ácido valérico, y a una reducción de ácidos grasos volátiles y del ratio acético/propiónico (P<0,05). Los animales que fueron alimentados con dietas con un 40% de DDGS de maíz mostraron además un mayor contenido de $N\text{-}NH_3$ en el ciego a los 59 días de edad (P<0,05). En el tercer experimento se estudió el efecto de las dietas utilizadas en el segundo experimento (C, Db_{20}, Dw_{20}, Dc_{20} y Dc_{40}) sobre la composición de la canal y calidad de la carne en el músculo *Longissimus* a los 59 días de edad. Se utilizaron un total de 20 animales por pienso. No se encontraron diferencias en el peso de la canal caliente, peso de la canal fría (CCW), porcentaje de pérdidas por goteo, peso en porcentaje de tracto digestivo lleno, peso en porcentaje de hígado, rendimiento de la canal y color de la canal. El porcentaje de grasa disecable fue superior en los animales alimentados con DDGS de cebada y maíz (en promedio +0.7% CCW; P<0,05). No se encontraron diferencias significativas entre los animales alimentados con las diferentes dietas en textura, pérdidas por cocción, capacidad de retención de agua y grasa intramuscular en el lomo. Sin embargo, el tipo de DDGS incorporado a la dieta tuvo efecto sobre el índice de rojez de la carne, el pH, y el contenido en proteína y la concentración de SFA y PUFA de la carne. Los animales alimentados con las dietas con DDGS de maíz al 40% obtuvieron valores de PUFA/SFA, SFA/PUFA+MUFA, índice aterogénico e índice trombogénico mejores desde el punto de vista de la salud cardiovascular del consumidor que los animales que fueron alimentados con las dietas Db20 y Dw20. En conclusión, los resultados de la tesis muestran que la inclusión de DDGS de cebada, trigo o maíz en la dieta de conejos de engorde hasta un nivel del 20% puede ser una alternativa interesante, ya que se obtienen rendimientos productivos adecuados sin tener consecuencias negativas sobre la calidad de la canal y de la carne.

Palabras clave: Granos secos de destilería y solubles; composición química; digestibilidad; crecimiento; conejos; ambiente cecal ; calidad de carne.

RESUM

L'objectiu d'esta tesi va ser avaluar la utilització dels grans secs de destil·leria amb solubles (DGGS) d'ordi, dacsa i blat, co-productes de la indústria del bioetanol disponibles en la Península Ibèrica, en l'alimentació de conills en creixement. Per a això, es va determinar el valor nutritiu dels DDGS i l'efecte de la seua inclusió en la dieta sobre el rendiment productiu, característiques de la canal i qualitat de la carn del conill. En el primer experiment es va determinar la composició química, composició d'aminoàcids i àcids grassos de huit lots de DDGS (2, 2 i 4 d'ordi, blat i dacsa, respectivament). Posteriorment, es van formular cinc dietes per a determinar el valor nutritiu dels DDGS: una dieta control i quatre dietes amb 200 g/kg matèria seca (MS) dels diferents DDGS (DDGS d'ordi nacional, dacsa nacional, dacsa brasiler i blat nacional). L'assaig de digestibilitat es va realitzar amb un total de 60 conills de 42 dies d'edat provinents d'un encreuament a tres vies. Els resultats van mostrar que els DDGS es caracteritzen com una matèria primera rica en proteïna bruta (PB), fibra neutre detergent i fibra soluble en detergent neutre (amb valors mitjans de 318, 352 i 208 g/kg MS, respectivament). Els DDGS d'ordi van mostrar major contingut en fibra i menor en proteïna que els DDGS de blat (+25 g de fibra ácid detergent i -91 g de PB/kg MS, respectivament; $P<0,05$). En els DDGS de dacsa es van obtindre valors de fibra i proteïna intermedis als obtinguts en els DDGS d'ordi i dacsa; no obstant això, van mostrar el major valor en extracte eteri (en mitjana +72 g/kg MS). La proteïna amb major contingut en proleta, fenilalanina, valina i arginina va ser la del DDGS d'ordi, la proteïna amb major contingut en leucina, alaneta i histidina va ser la del DDGS de dacsa, i la proteïna de major contingut en glutámico, la del DDGS de blat. El DDGS d'ordi va ser el de major contingut en àcids grassos saturats (SFA, 267 g/kg àcids grassos totals), el DDGS de dacsa en mona insaturada (MUFA, 278 g/kg àcids grassos totals) i el DDGS de blat el de major contingut d'àcids

grassos insaturats (PUFA, 615 g/kg àcids grassos totals). El DDGS d'ordi va mostrar el menor valor nutritiu per a conill (11.9 MJ de energia digestible (ED) i 168 g de proteïna digestible (PD)/kg MS). El valor nutritiu dels dos DDGS de dacsa no va diferir significativament (valors mitjans de 15.3 MJ ED i 208 g PB/kg/MS), i el DDGS de major valor nutritiu va ser el de blat (15.7 MJ ED i 263 g PB/kg MS). En el segon experiment es va avaluar l'efecte de la inclusió dels DDGS sobre el rendiment productiu i l'ambient cecal dels conills d'engreixament. Es van formular quatre dietes a partir d'una dieta control sense DDGS (C), que incloïen un 20% de DDGS d'ordi (Db20), un 20% de DDGS de blat (Dw20) , i un 20% (Dc20) o 40% (Dc40) de DDGS de dacsa. En l'estudi de rendiment productiu es van utilitzar un total de 475 conills acabats de deslletar de 28 dies d'edat provinents d'un encreuament a tres vies i allotjats individualment. Els paràmetres de fermentació cecal van ser determinats en 20 conills per dieta i edat, als 42 dies (a partir de 200 conills allotjats en gàbies col·lectives) i als 59 dies d'edat (a partir dels 475 conills de l'estudi de rendiment productiu). Els animals alimentats amb Db20 van mostrar major ingestió de MS i ED (+6 i + 12%, respectivament; $P<0,05$), semblant guany de pes diària (GPD) i major índex de conversió (+9%; $P<0,05$) que els animals alimentats amb el pinso control. I'increment en la ingestió d'ED dels animals que van consumir pense amb DDGS de dacsa, tant al 20% com al 40% d'inclusió (+9 kJ/d; $P<0,05$), no es va traduir en un augment significatiu de la GPD respecte als animals alimentats amb el pinso control. D'altra banda, la major ingestió de MS i d'ED dels animals alimentats amb la dieta Dw20 va portar que estos animals tingueren la major GPD que la dieta control (+2.8 g/d; $P<0,05$). La inclusió dels DDGS al 20% no va afectar els principals paràmetres de l'ambient cecal avaluats als 42 dies, no obstant això, la inclusió de DDGS de dacsa al 40% es va caracteritzar per una major concentració de $N-NH_3$ i d'àcid valérico, i un menor contingut en àcids grassos volàtils i concentració d'àcid acètic ($P<0,05$) . Als 59 dies d'edat, l'efecte de la inclusió de DDGS al 20% i la inclusió lineal de DDGS de dacsa va portar a valors superiors de MS en contingut cecal, àcid propiónico i àcid valérico, i a una reducció d'àcids grassos volàtils i del ràtio acético/propiónico ($P<0,05$). Els animals que van ser alimentats amb dietes amb un 40% de DDGS de dacsa van mostrar a més un major contingut de $N\text{-}NH_3$ en el cec als 59 dies d'edat ($P<0,05$). En el tercer experiment es va estudiar l'efecte de les dietes utilitzades en el segon experiment (C, Db20, Dw20, Dc20 i Dc40) sobre la composició de la canal i qualitat de la carn en el múscul Longissimus als 59 dies d'edat. Es van utilitzar un total de 20 animals per pinso. No es van trobar diferències en el pes de la canal calenta, pes de la canal freda (CCW), percentatge de pèrdues per goteig, pes en percentatge de tracte digestiu ple, pes en percentatge de fetge, rendiment de la canal i color de la canal. El percentatge de greix disecable va ser superior en els animals alimentats amb DDGS d'ordi i dacsa (valors mitjans de +0.7% CCW; $P<0,05$). No es van trobar diferències significatives entre els animals alimentats amb les diferents dietes en textura, pèrdues per cocció, capacitat de retenció d'aigua i greix intramuscular en el llom. No obstant això, el tipus de DDGS incorporat a la dieta va tindre efecte sobre l'índex de rojor de la carn, el pH, i el contingut en proteïna i la concentració de SFA i PUFA de la carn. Els animals alimentats amb les dietes amb DDGS de dacsa al 40% van obtindre valors de PUFA/SFA, SFA/PUFA+MUFA, índex aterogénico i índex trombogénico millors des del punt de vista de la salut cardiovascular del consumidor que els animals que van ser alimentats amb les dietes Db20 y Dw20. En conclusió, els resultats de la tesi mostren que la inclusió de DDGS d'ordi, blat o dacsa en la dieta de conills d'engreixament fins un nivell del 20% pot ser una alternativa interessant, ja que porten a uns rendiments productius adequats sense tindre conseqüències negatives sobre la qualitat de la canal i de la carn.

Paraules clau: Grans secs de destil·leria i solubles; composició química; digestibilitat; creixement; conills; ambient cecal; qualitat de carn.

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ABBREVIATIONS

ADF: acid detergent fiber
ADL: acid detergent lignin
AI: atherogenic index
AOAC: Association of Official Analytical Chemists
CCW: chilled carcass weigh
CF: crude fiber
CL: cooking losses
CP: crude protein
CV: coefficient of variation
DDGS: Distillers dried grains with solubles
DE: digestible energy
DFaP: dissectible fat percentage
DFI: daily feed intake
DLP: drip loss percentage
DM: dry matter
DoP: dressing-out percentage
DP: digestible protein
DWG: daily growth gain
EE: ether extract
EGRAN: European Group on Rabbit Nutrition
ERE: Epizootic Rabbit Enteropathy
FCR: feed conversion rate
FDTP: full digestive tract percentage
GE: gross energy
GIT: full gastro-intestinal tract
HCW: hot carcass weigh
IFaP: inguinal fat percentage
LvP: liver weigh percentage
LW: live weight
ME: metabolisable energy
MJ: mega Julius
MUFA: monounsaturated fatty acids
NDF: neutral detergent fibre
NDSF: neutral detergent soluble fibre
$N\text{-}NH_3$: Ammonia nitrogen
PFaP: perirenal fat percentage
PUFA: polyunsaturated fatty acids
SAS: statistical analysis system
SEM: standard error of the mean
SFA: saturated fatty acids

SFaP: scapular fat percentage
SW: slaughter weight
TI: thrombogenic index
UFA: unsaturated fatty acids
VFA: volatile fatty acids
WHC: water holding capacity

I. GENERAL INTRODUCTION

1.1. INTRODUCTION

World production of bioethanol for use as fuel has increased by 22% since 2004 (Hayes, 2008) and reached 109 billion litres in 2011, with 50 and 4% of the total corresponding to the USA and the European Union, respectively. In 2011, the USA produced 35.7 million tons of distillers' grains for animal feed, exporting 25% to 50 countries, the most important destination being China, followed by Mexico, Canada, South Korea and Vietnam (Renewable Fuels Association, 2011). Brazil mainly produces ethanol from sugar cane and the USA uses corn, whereas in Europe and Canada the main raw material used is wheat grain. In Spain, the three major bioethanol plants process 1.23 million tons of cereal grains annually (mainly wheat, corn and barley), producing 546 million litres of ethanol and 0.36 million tons of distillers' grain and dried solubles, also known as DDGS (Distillers' Dried Grains with Solubles), which are mainly used as raw material in animal feed (Abengoa, 2011).

The use of cereal grains to obtain ethanol has given rise to important changes in the worldwide feedstuffs market, on one hand raising the price of raw materials and in consequence feed prices, while on the other hand providing new raw materials or co-products mainly corresponding to the non-starchy grain fraction, as the starch is hydrolysed and fermented to produce ethanol. Although dry mill ethanol plants produce a great variety of co-products, corn DDGS are the most important marketed worldwide for the use of raw materials in feed formulation and preparation. Some 48% is for beef cattle feed, 32% for dairy cattle, 11% for swine, 8% for poultry and 1% for other species (US Grains Council, 2012; Renewable Fuels Association, 2012).

Rabbit production is also no stranger to this phenomenon. The increasing price of raw materials and the market launch of these new materials opens up the possibility of their inclusion in diets. In fact, Table 1.1.1 shows the average composition in commercial feed ingredients for rabbits in Spain (de Blas and Mateos, 2010), where we see how cereal co-products (among them DDGS) can make up an important part of the formulas (up to 35%).

Table 1.1.1. Usual range of ingredient composition of feeds for rabbits in Spain (g/kg) given by De Blas and Mateos (2010).

Ingredient	Amount
Cereal grains[a]	100-200
Animal and vegetal fats	5-30
Molasses	0-30
Beet, apple and citrus pulp, soy hulls	0-100
Cereal co-products[b]	150-350
Lucerne hay	150-300
Lignified fibrous co-products[c]	50-150
Protein concentrates[d]	120-220

[a] Mainly barley and wheat.
[b] Mainly wheat bran, corn gluten feed and distiller’s co-products.
[c] Mainly wheat straw, olive and grape co-products.
[d] Mainly sunflower, soybean, rapeseed and palm kernel meal.

Co-products of this type are characterised by their high variability and by serious problems of accurate typification. Their nutritional value for the animals may thus vary considerably depending on the source grain, processing plant, manufacturing process, season of the year, etc. For instance, Blas *et al.* (2000) concluded that the classification of wheat bran in leaves and thin stems is quite hard to justify on the basis of their chemical composition, and that their nutritional value seems to be more related with the fibre and protein fractions than to other components.

So, considering their possible inclusion in feed at high levels and the possible nutritive variability they may present, appropriate characterisation of cereal co-products would be recommendable prior to their use. In this sense, the information available on the nutritional value of corn DDGS is very scarce

(Villamide *et al.*, 1989), and non-existent in the case of wheat and barley DDGS (the only values available are from some reference Tables; De Blas *et al.*, 2010). Moreover, there is not information available on the possible effect of including DDGS in rabbit diets on their production performance, digestive parameters, carcass features and meat quality. It is reasonable to suppose that the rabbit's digestive peculiarities could mean that some of the nutritive losses observed in other monogastrics are not all that relevant, and that given their chemical composition (rich in fibrous fractions) and market availability DDGS could be an interesting feedstuff for use in rabbit feed.

This doctoral thesis is intended to help improve the knowledge on the possible use of these co-products in rabbit feeds. However, to be able to determine the current state of knowledge of this co-product as the basis for this thesis, it is necessary to find out how the product is obtained and the consequences of the manufacturing process on its nutritive value, as well as the existing information on its use in animal feedstuffs to date.

1.1.1. DDGS extraction process

DDGS, which are co-products of the bioethanol industry, are obtained by drying out the residues from the ethanol extraction process from cereals rich in starch such as corn, wheat, barley and sorghum. The process per se consists of converting the starches and sugars from the cereals into ethanol, so that the end product has a drastically reduced non-structural carbohydrate content and the remaining nutrient percentage is proportionally concentrated (De Blas *et al.*, 2010)

The industrial process designated dry milling (dry-grind) is the most widely used procedure in bioethanol plants, and as shown in Figure 1, consists of several phases: a) grain particle size reduction; b) cooking (from 90 to

165ºC) and saccharification or starch-to-glucose step using the appropriate enzymes (thermostable amylases); c) fermentation of glucose with yeasts (*Saccharomyces cerevisiae*) to produce ethanol, where 94% of the glucose is converted to ethanol and CO_2 (each molecule of glucose produces 2 molecules of ethanol and 2 of CO_2), 1% for yeast cell maintenance and 4% mainly for glycerol production; d) ethanol distillation in column systems; e) corn oil extraction from thin stillage is sometimes carried out after the fermentation and distillation and before drying to produce DDGS, with a system currently being adopted in ethanol plants; and f) the water and solids are remaining after ethanol distillation are designated whole stillage, which mainly consists of water, fibre, protein and fat. This mixture is centrifuged to remove coarse solids from the liquid. The liquid (thin stillage) passes through an evaporator to remove the additional moisture and results in condensed distillers' solubles (syrup), which contains approximately 30% dry matter. Coarse solids may be supplied in dry or wet format, with or without addition of condensed distillers' solubles. DDGS are mainly composed of a mixture of distillers' dry grains (DDG) and solubles (DDS, vinasse or thin stillage), in a ratio of 3:1 (US Grains Council, 2012). DDGS represent between 27 and 30% of the corn grain's original weight, containing approximately 88 % dry matter (DM), 25- 28 % of crude protein (CP), 11% of ether extract (EE) and 7-9% crude fibre (CF) (US Grains Council, 2007).

Some plans have the facility to carry out the process designated 'elusive' that consists of fractionating the DDGS to extract the fibre by sifting and air aspiration. This results in a product with more than 40% CP, 15% EE and 20% detergent neutral fibre (NDF) (Srinivasan *et al*., 2005; Srinivasan *et al*., 2009). The reduction of oil in corn DDGS would affect the nutritional profile of the DDGS, mainly by lowering the fat and energy contents and increasing the protein concentration. For some monogastric species such as swine, poultry and fish, fat and energy-rich DDGS are very interesting, whereas in dairy and beef cattle the reduced oil DDGS can be used more effectively (Schingoethe *et al.*, 2009).

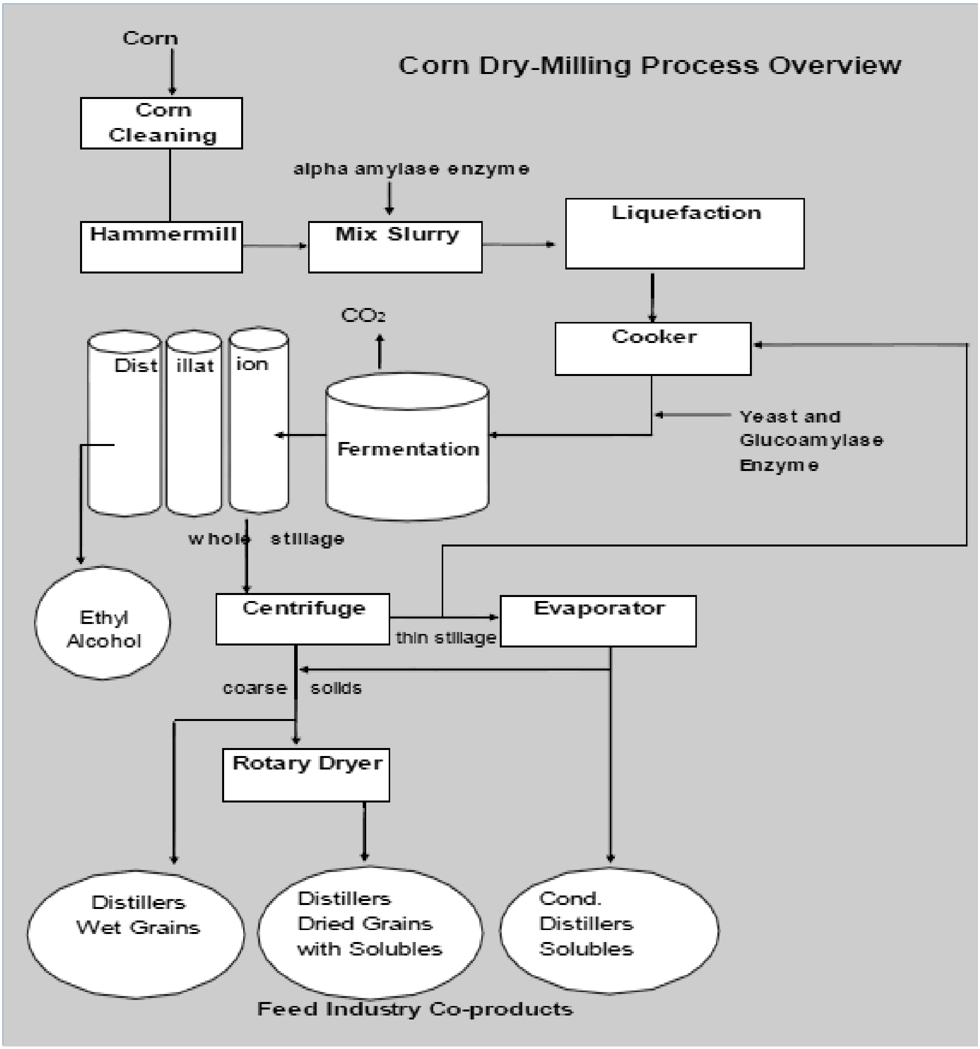

Figure 1.1. Ethanol and DDGS extraction process by dry milling system (Shurson, 2005).

1.1.2. Chemical composition of DDGS

The chemical composition and nutritive value of DDGS varies depending on the different sources consulted. Olentine (1986) put forward 35 causes as a responsible for this variability, among them variations in the raw materials *per se* (Reese and Lewis, 1989), processing factors in the plant and between plants (Spiehs *et al*., 2002; Knott *et al*., 2004; Noll *et al.,* 2006), changes in ethanol production processes (Spiehs *et al*., 2002; Shurson and Alghamdi, 2008), variations in the mixture ratio of the DDGS components in the plant (Noll *et al*., 2006, Kim *et al.,* 2008) and drying time and temperature differences (US Grains Council, 2007).

Spiehs *et al.* (2002) assessed the variability in the nutrient content of corn DDGS, analysing a total of 118 samples from 10 bioethanol plants in 1997, 1998 and 1999. They found that the average values for CP, EE, ash, CF, detergent acid fibre (ADF) and NDF were 30.2, 10.9, 5.8, 8.8, 16.2, and 42.1% DM, respectively. The coefficient of variation (CV) varied from 6.4% for CP to 28.4% for ADF (Table 1.1.2). Belyea *et al.* (2004) analysed 235 samples of corn DDGS from an ethanol fuel plant in Minnesota, and found that the mean values of CP, EE, ash, starch, CF and FAD were 31.4, 12.0, 4.6, 5.3, 10.2, and 16.8% DM, respectively. Thus, Belyea *et al.* (2004) provided higher average values for CP, EE and CF, and a lower ash value, as well as lower CVs compared to Spiehs *et al.* (2002).

On the other hand, Cozannet *et al*. (2010) analysed 10 samples of DDGS from wheat and reported average values of 36.1, 4.6, 5.2, 4.1, 8.3, 12.0 and 29.2% DM for CP, EE, ash, starch, CF, ADF and NDF, respectively. So, the wheat DDGS seem to show higher CP values, and mostly low in EE, ADF and NDF compared to corn DDGS (Table 1.1.2; Spiehs *et al.,* 2002 and Belyea *et al.,* 2004).

However, information on the composition of DDGS from barley is quite scarce. To the author's best knowledge, no research works have analysed in detail the use of DDGS from barley grains, and our only references are the values given in certain reference tables (De Blas *et al*., 2010).

Table 1.1.2. Chemical composition (% dry matter) of corn and wheat DDGS from different plants, years, and sources reported in several publications.

	Spiehs *et al.*[1]			Belyea *et al.*[1]			Cozannet *et al.*[2]	
	Mean	Range[a]	CV	Mean	Range[b]	CV	Mean	Range
Nº samples	118	10	118	235	5	5	10	10
Dry matter	88.9	87.2 - 90.2	1.7				96.2	89.3 - 94.4
Crude protein	30.2	28.7 - 31.6	6.4	31.4	28.3 - 33.3	6.3	36.1	32.6 - 38.9
Ether extract	10.9	10.2 - 11.4	7.8	12.0	10.9 - 12.6	5.6	4.6	3.6 - 5.6
Ash	5.8	5.2 - 6.7	14.7	4.6	4.3 - 5.0	5.7	5.2	4.3 - 6.7
Starch				5.3	4.7 - 5.9	9.7	4.1	2.5 - 9.5
Crude fibre	8.8	8,3 - 9.7	8.7	10.2	9.6 - 10.6	3.7	8.3	6.2 - 10.9
Acid detergent fibre	16.2	13.8 - 18.5	28.4	16.8	15.4 - 19.3	9.3	12.0	7.7 - 17.9
Neutral detergent fibre	42.1	36.7 - 49.1	14.3				29.2	25.1 - 33.8

CV, Coefficient of variation (%). [a]Range values for means of 10 sample origins (locations). [b]Range and CV (%) values for means of 5 sample groups (by year).

[1] Corn DDGS.

[2] Wheat DDGS.

Due to the relatively high protein content of this co-product, its amino acid composition becomes especially interesting in characterising its potential use. However, most studies show that the amino acid content of DDGS is also quite variable, presenting the typical imbalances of the source grains. Among these studies, Spiehs *et al.* (2002) analysed 10 essential amino acids in 118 samples of DDGS from corn, finding a lysine mean of 0.85% (from 0.72 to 1.02%) as the most variable among the 10 amino acids measured, with an average CV of 17.3% (Table 1.1.3). These results are comparable to the lysine values of 0.78% (between 0.48 and 0.97%; CV, 18.7%) reported by Cromwell *et al.* (1993) in 9 samples of corn DDGS, and those provided by Batal and Dale (2006) with 7.1% (CV, 22.5%) in another 8 samples. Methionine is the second most variable amino acid (CV, 13.6%) reported by Spiehs *et al.* (2002), and agrees with a CV of 11% published by Batal and Dale (2006). Stein *et al.* (2006) analysed the amino acid content of 10 samples of corn DDGS and found mean value similar to those indicated by Spiehs *et al.* (2002), and in general presented lower variability (Table 1.1.3), although tryptophan showed a higher CV (12.3%), followed by lysine (8.1%) and methionine (6.4%).

Bandegan *et al.* (2009) analysed 5 samples of DDGS from wheat and found lower CV values compared to those reported for amino acids in corn DDGS (Table 1.1.3). Likewise, better average values were also observed for arginine (1.61%), isoleucine (1.37%), phenylalanine (1.81%), threonine (1.18%) and valine (1.7%), but were lower in lysine (0.74%) and leucine (2.63%) and similar in methionine (0.61%), compared to the average values of the amino acids in corn DDGS.

As with the chemical composition, variability in the content of the individual amino acids also depends on the process whereby the DDGS were obtained and the source of the samples.

The phosphorus content in DDGS varies from 0.60 to 0.70% and their apparent digestibility in swine is 59.1%, values much higher than those observed for corn grain (Pedersen *et al.*, 2007). So, when DDGS were included in swine diets, the use of organic phosphorus was improved and the need for organic phosphorus supplementation was reduced (Stein and Shurson, 2009), although phosphorus retention remained constant as the inclusion levels of DDGS in the diet increased (McDonnell *et al.*, 2011).

Table 1.1.3. Essential amino acid composition (% dry matter) of corn and wheat DDGS reported in different sources.

	Spiehs *et al.*[1]			Stein *et al.*[1]			Bandegan *et al.*[2]		
	Mean	Range[a]	CV	Mean	Range	CV	Mean	Range	CV
Nº sample	118	10	118	10	10	10	5	5	5
Arginine	1.20	1.11 - 2.17	9.1	1.24	1.01 - 1.37	5.5	1.61	1.53 - 1.67	3.7
Histidine	0.76	0.72 - 0.82	7.8	0.87	0.69 - 0.93	4.4	0.82	0.78 - 0.85	3.4
Isoleucine	1.12	1.05 - 1.17	8.7	1.15	0.94 - 1.19	4.4	1.37	1.30 - 1.41	3.4
Leucine	3.55	3.51 - 3.81	6.4	3.51	2.79 - 3.60	4.6	2.63	2.51 - 2.68	3.7
Lysine	0.85	0.72 - 1.02	17.3	0.89	0.74 - 0.98	8.1	0.74	0.69 - 0.79	5.4
Methionine	0.55	0.49 - 0.69	13.6	0.69	0.54 - 0.78	6.4	0.61	0.59 - 0.62	2.1
Phenylalanine	1.47	1.41 - 1.57	6.6	1.50	1.19 - 1.57	4.3	1.81	1.72 - 1.90	4.1
Threonine	1.13	1.07 - 1.21	6.4	1.11	0.89 - 1.19	4.0	1.18	1.13 - 1.22	2.8
Tryptophan	0.25	0.21 - 0.27	6.7	0.19	0.16 - 0.22	12.3			
Valine	1.50	1.43 - 1.56	7.2	1.59	1.28 - 1.63	4.4	1.7	1.63 - 1.74	2.5

CV: Coefficient of variation (%).[a]Range values for means of 10 sample origins (locations).
[1] Corn DDGS.
[2]Wheat DDGS.

The calcium, potassium, sulphur and sodium content in cereals is quite low (De Blas *et al.*, 2010). As a result of disappearance of the amylaceous fraction, the quantities of calcium and potassium may be tripled in the DDGS; however, the sulphur and sodium content in the DDGS is much greater than the proportion expected due to the concentration process. The extra source of sulphur in DDGS comes from sulphur present in the yeasts, in the water and from the sulphuric acid added during ethanol production processes. Sulphuric acid is added in several stages to adjust the pH to optimum levels for enzyme and yeast activity. Depending on the water quality and the need to adjust the pH, the sulphur content in DDGS can vary from 0.3 to 1.0% (Spiehs *et al.*, 2002; Batal and Dale, 2003). Broiler chickens can support levels of up to 0.5%, and laying hens tolerate even higher levels, so there would seem to be no problem in feeding poultry with high sulphur content DDGS. Nevertheless, sulphur can interfere with the absorption of calcium and some oligoelements in the small intestine and thus affect bone hardness and eggshell quality (Leeson and Summers, 2005; Bregendahl, 2008).

Moreover, the sodium content in DDGS is high and variable, ranging from 0.09 to 0.52% (Spiehs *et al.*, 2002; Batal and Dale, 2003). Although the source of the extra sodium that appears in DDGS is unclear, it could be attributed to the sodium content in the water used in the ethanol extraction process. Poultry can tolerate high dietary sodium levels (Klasing and Austic, 2003). Nevertheless, the sodium content in poultry diets must be monitored and adjusted when using DDGS with high sodium content. High sodium content diets increase water consumption and may give rise to an increase in the rate of wet bedding and dirty eggs (Klasing and Austic, 2003; Leeson *et al.*, 2005).

1.1.3. Nutritive value of DDGS

Current information available on the digestibility and nutritional value of the main constituents of DDGS is also relatively scarce (Stein *et al.*, 2006; Pedersen *et al.*, 2007; De Blas *et al.*, 2010).

Regarding one of the most important fractions of this co-product, protein, the standardised ileal digestibility of the CP of the DDGS in swine was 72.2 and 72.8 % for wheat and corn DDGS, respectively (Stein and Shurson, 2009). Due to the high susceptibility to heat damage, the lysine content and its digestibility are the main concern in the use of DDGS as raw material for feedstuffs. During drying, the Maillard reactions can bind the free NH_2 group of lysine to the reducing sugars, which may be responsible for the variability in the ileal digestibility of lysine of the DDGS (Fastinger and Mahan, 2006; Stein *et al.*, 2006). The reaction may result in the destruction of a significant amount of lysine if heated to excess. Only the lysine which does not bind to the reducing sugars (reactive lysine) is bioavailable for the animal, whereas bonded lysine (non-reactive lysine) is unusable. In fact, Pahm *et al.* (2008) observed how reactive lysine content is correlated with the standard ileal digestibility of lysine of DDGS in swine.

Thus, Cromwell *et al.* (1993) observed that the lysine concentration tended to be lower in darker coloured DDGS and higher in lighter ones. Indeed, they observed a significant correlation between the score of the Hunter colour parameters L* (luminosity), a* (redness), b* (yellowness) and the lysine content of DDGS. This was confirmed by Fastinger and Mahan (2006), who reported that the lysine content in six sources varied from 0.48 to 0.76%, with the lowest lysine content in the dark DDGS.

Stein *et al.* (2006) showed ranges in the coefficients of true lysine digestibility for swine of 43.9 to 63.0%. Fastinger *et al.* (2006) in adult roosters reported values of 38.2 to 61.5% of lysine ileal digestibility in the protein of five sources of DDGS, and that the apparent and true digestibility of lysine was significantly lower for those of a darker colour. At the same time, Batal and Dale (2006) observed differences in the true digestibility of amino acids among the samples, noting that the lysine content and digestibility were significantly lower in samples of darker DDGS.

It is also suggested that colour analysis may be a swift and reliable method to estimate the digestibility of the amino acids of DDGS and of lysine in particular for broiler poultry and swine. However, an analytical procedure has been established which gauges the reactive lysine concentration in DDGS, through the furosine procedure (Pahm *et al.* 2008), which may be more useful to determine the degree of damage suffered by the co-product during the manufacturing process and its potential nutritive value.

In general, the amino acids in DDGS display average digestibility and, except for a lysine, variability between different samples is within the normal range of variation observed in feedstuff ingredients.

The next fraction of particular interest in DDGS is their fat. The major cause of variation in the quantity of EE in DDGS is the amount of condensed solubles added to them (Noll *et al.*, 2007). When the fatty acid composition of the fat in DDGS is expressed as a relative percentage (Moureau *et al.*, 2011; Díaz-Royón, 2012), linoleic acid is the most abundant (54.0 - 56.5%), followed by oleic acid (25.3 - 27.2%) and palmitic acid (13.3 - 16.4%), with low levels in stearic (1.8 - 2.3%) and linolenic (1.2 - 1.4%).

The gross energy content (GE) of corn DDGS is approximately 22.7 MJ/kg DM. This value is higher than the GE concentration of corn (18.8 MJ/kg DM). However, GE digestibility in pigs, measured as a percentage of the GE (76.8%), is lower in DDGS than in corn grain, obtaining digestible energy (DE) and metabolisable energy (ME) values in 10 samples of DDGS of 17.3 and 16.3 MJ/kg DM, respectively, than is similar at corn. (Pedersen *et al.*, 2007).

Same authors indicate values of 12.0 MJ of apparent ME, 12.5 MJ of true ME and 11.7 MJ of true ME per kg for DDGS in turkeys (Noll *et al.*, 2006), broiler chickens (Lumpkins *et al.*, 2004) and laying hens (Lumpkins *et al.*, 2005), respectively, and no negative effects on feed conversion at inclusion levels of 10 % was observed.

Most of the starch in the grain is converted into ethanol during the fermentation process, unlike the corn grain fibre, and as a result the DDGS contain 42.1% (from 31.2 to 46.3) of total dietary fibre, and the ADF (9.9% from 7.2 to 17.3%) and NDF (25.3% from 20.1 to 32.9%) concentrations are three times higher than those of corn grain. The apparent digestibility of dietary fibre in swine is 43.7% (from 23.4 to 55%), which results in a reduction of the digestibility values of DM and GE (Stein and Shurson, 2009).

1.1.4. Use of DDGS in monogastrics

1.1.4.1. Use of DDGS in growing-finishing swine diets

Stein and Shurson (2009) recently reviewed the use of DDGS in swine, concluding that they may be included in diets for pigs in all production phases. Piglets 2-3 weeks post-weaning can be fed on diets containing up to 30% DDGS with no negative impact on growth and weight gain (Whitney y Shurson, 2004; Gaines *et al.*, 2006; Barbosa *et al.*, 2008; Burkey *et al.*, 2008). Research results in the growth and finishing stage establish acceptable levels of up to 30% DDGS in diets without affecting performance (Cook *et al.*, 2005; Gaines *et al.*, 2007a; Xu *et al.*, 2007; Linneen *et al.*, 2008; Weimer *et al.*, 2008). However, at these inclusion levels the carcass fat reaches a higher iodine index than in carcasses from pigs fed without DDGS (Whitney *et al.*, 2006c).

Most of the work carried out showed how inclusion of corn DDGS in the diet did not affect pig carcass performance (McEwen, 2006; Xu *et al.*, 2007; Drescher *et al.*, 2008), although other studies observed a reduced carcass performance (Cook *et al.*, 2005; Whitney *et al.*, 2006c; Gaines *et al.*, 2007a, b; Weimer *et al.*, 2008). On the other hand, pigs fed with wheat DDGS presented reduced carcass performance (Thacker, 2006). It has been suggested that the inclusion of fibre-rich ingredients in swine diets can undermine carcass performance due to the increased intestinal volume and mass (Kass *et al.*, 1980). This may explain the reduced carcass performance reported in pigs fed on DDGS in some experiments, but why this effect has not been observed in other experiments remains unknown (Stein and Shurson, 2009). It is likely that the variability in the nutritional value between DDGS displayed in the previous section may be behind this discrepancy.

The carcass fat of pigs fed on diets containing more than 20% corn DDGS has a higher iodine index than carcass fat from pigs fed whitout DDGS, due to a higher linoleic acid concentration in the fat of these DDGS (Whitney *et al.,* 2006c; Xu *et al.*, 2007; Hill *et al.*, 2008; Linneen *et al.*, 2008). In any case, withdrawal of these DDGS from the diets of fattening pigs in the final 3 to 4 weeks before slaughter is recommended, or the addition of conjugated linolenic acid (CLA) to reduce the iodine index values in the carcass fat (White *et al.*, 2007; Hill *et al.*, 2008; Xu *et al.*, 2008b). CLA can reduce activity of the Δ9-desaturase enzyme, which is responsible for fatty acid of novo-synthesis desaturation, improving the SFA/UFA ratio (Gatlin *et al.*, 2002).

There is some evidence that feeding pigs in growth phase with diet containing DDGS can improve intestinal health, reducing the incidence, severity and duration of lesions caused by a moderate challenge infection of *Lawsonia intracellularis* (Whitney *et al., 2006a*). The action mode of this response is unknown, but it seems that the compounds of the condensed solubles fraction of the DDGS can improve the villus height: crypt depth ratio in the proximal portion of the small intestine (Whitney *et al.*, 2006b). It is not known whether diets containing DDGS are effective to reduce the negative effects of other enteric diseases (Stein and Shurson, 2009).

Manure volume increases when DDGS are included in diets due to the reduced DM digestibility of DDGS. Nitrogen excretion may also increase, but this can be avoided by maintaining an appropriate balance through the use of synthetic amino acids in diets containing DDGS. In contrast, phosphorus excretion can be diminished in diets containing DDGS if the total dietary phosphorus concentration is reduced to compensate the greater digestibility of phosphorus in DDGS (Whitney *et al.*, 2001).

Feeding pigs in growth-finishing stage with 20% DDGS has no effect on the emissions of H_2S, NH_3 and odour levels during a 10-week manure storage period, compared to diets based on corn and soybean meal (Spiehs *et al.*, 2000).

1.1.4.2. Use of DDGS in poultry

In past decades, the use of DDGS in poultry diets was around 5%, due to its usage limitations in these animals, among others their fibre content and variability in the composition and digestibility of nutrients (Noll *et al.*, 2001). They were included in poultry diets mainly as a source of unidentified factors which promoted growth, production and egg incubability (Manley *et al.*, 1978; US Grains Council, 2007), having attributed to DDGS the ability to provide vitamins and perhaps oligoelements lacking in poultry diet formulas. This is currently unlikely to occur, as the essential nutrient requirements in poultry diets have been established and a variety of commercial nutrient supplements is available.

Corn DDGS may contain up to 40 ppm of xanthophylls, significantly increasing egg yolk colour when included in diets for laying hens and enhancing skin colour when included at levels of 10% in broiler chicken diets (Roberson *et al.*, 2005; US Grains Council, 2007). However, the true xanthophyll content may be lower due to heat damage during drying. Roberson *et al.* (2005) analysed two samples of DDGS and observed 30 ppm of xanthophylls in one of the samples, but only 3 ppm in the other, darker coloured heat-damaged sample.

Feed studies on DDGS in broiler chicken feed recommend including up to 6% in diets for chickens in starter phase and 12 – 15% in the growth and finishing stages (Lumpkins *et al.*, 2004). Wang *et al.* (2007a) indicated that when corn DDGS is included up to 25% in balanced digestible amino acid diets for male chickens weight gain is not affected, noting a greater total feed consumption than for the control diet; they suggest that arginine would be a limiting amino acid in diet with 25% DDGS. In another study, Wang *et al.* (2007b) found better responses with diets of 30% DDGS in finishing phase (35 to 42 days of age), 15% DDGS in growth diets (14 to 35 days of age) and 0% DDGS in diets for starter phase (1-14 days of age).

With DDGS in starter chickens, the response is limited by immaturity of the digestive system until day 14 of age (Batal and Parsons, 2002), due to the higher fibre content and low digestibility of amino acids in the DDGS. Inclusion of DDGS up to 30% in poultry feed diminishes grain quality and this could contribute to the low performance of chickens when high levels of DDGS are incorporated (Bregendahl, 2008).

When broiler chickens were fed on diets including up to 15% (Wang *et al.*, 2007a) and 12% of corn DDGS (Schilling *et al.*, 2010), no effect was seen on the carcass quality or the quality of breast and leg meat. Levels higher than 12% of corn DDGS in chicken feed increases the polyunsaturated acids in leg meat, increasing oxidation during storage, but does not affect the instrumental and sensorial quality of the leg meat (Schilling *et al.*, 2010).

Depending on the extent of the DDGS inclusion in poultry diets, nitrogen consumption and excretion follows a linear response due to low digestibility of the amino acids in DDGS, as well as the higher protein content levels in diets formulated with DDGS (Roberts *et al.*, 2007; Bregendahl, 2008). There is a direct relation between nitrogen excretion and ammonia emissions from manure. However, some studies have observed an attenuating effect on ammonia emission in diets including DDGS (Roberts *et al.*, 2007).

The fibre is not digestible by the birds, although a small fraction is fermented by microorganisms in the large intestine, producing short chain fatty acids which lower the manure's pH, favouring the conversion of ammonia to ammonium ion (NH_4^+), which is less volatile. Thus, poultry fed with DDGS may excrete more nitrogen, but since the pH of the manure is lower, the nitrogen does not evaporate. This effect of dietary fibre on the acidification of fertilisers and NH_3 emissions was first demonstrated in swine by Canh *et al.* (1998) and later in laying hens by Roberts *et al.* (2007) with diets containing DDGS. So, initially there is usually an increase in the CP content of diets containing DDGS, which may have adverse effects on air and environmental quality due to greater nitrogen excretion. However, the nitrogen remains in the manure, which when correctly applied in fields has no negative environmental impact, and may increase the fertility and economic value of the fertiliser (Bregendahl, 2008).

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II. OBJECTIVES

2.1. OBJECTIVES

Throughout the introduction we observed how DDGS may be a raw material of special interest for use in rabbit feed, characterised by providing the feed with energy and protein content, whose high fibre content may be better received in rabbit than other monogastrics due to the digestive particulars of this species. In fact, recent decades have seen their use gradually spreading in swine and even broiler chickens.

However, due to the variability of their chemical composition and nutritional value, especially linked to the source grain and manufacturing process, these products require appropriate characterisation before defining recommendations for their use and inclusion in rabbit diets. Moreover, as seen in other species, the possible impact of their high content in protein, and especially fat, on the carcass and meat characteristics of rabbits fed on diets including DDGS should be studied.

So, the main aim of this thesis was to study the possible use of DDGS from barley, wheat and corn produced in the Iberian Peninsula in feeds fattening rabbits.

The specific objectives were:

1. To characterise the chemical, amino acid and fatty acid composition of DDGS of barley, corn and wheat, and determine their nutritional value in growing rabbits.
2. To evaluate the incorporation of DDGS of barley (20%), wheat (20%) and corn (20 and 40%), in growing rabbit diets and the effect on productive performance, caecal environment, carcass features and meat quality.

III.EXPERIMENTS

3.1. NUTRITIVE VALUE OF DISTILLERS DRIED GRAINS WITH SOLUBLES FROM BARLEY, CORN AND WHEAT FOR GROWING RABBITS

3.1.1. Abstract

The distillers dried grains with solubles (DDGS) from the bioethanol industry could be considered as an interesting feedstuff for rabbit nutrition due to their fibrous nature. To characterize the DDGS available in the Iberian Peninsula, chemical, amino acid and fatty acid composition of eight DDGS batches (2, 2 and 4 from barley, corn and wheat grains, respectively) was analized. Five diets were formulated to determine the nutritive value of DDGS in growing rabbits by the substitution method: a control diet and four experimental diets containing 200 g of the DDGS/kg dry mater (DM) [DDGS from national barley, national corn, Brazilian corn and national wheat grains]. The digestibility trial was performed using 60 three-way crossbred fattening rabbits (12 per diet), aged 42 days with average live weight of 1.49 kg (± 0.033 kg). DDGS can be characterized as a raw material really rich in crude protein (CP), neutral detergent fibre and neutral detergent soluble fibre on av. 318, 352 and 208 g/kg DM, respectively. Barley DDGS had higher fibre and lower protein contents than wheat DDGS (+25 g of acid detergent fibre and –91 g of CP/kg DM, respectively; $P<0.05$), as well as the highest ash content (on av. +16 g/kg DM; $P<0.05$). Corn DDGS had intermediate fibre and protein values between barley and wheat DDGS, but were the richest in ether extract (on av. +70 g/kg DM). DDGS' protein was richer in proline, phenylalanine, valine and arginine for barley DDGS (107, 55, 54 and 51 g/kg CP, respectively), in leucine, alanine and histidine for corn DDGS (114, 75 and 27 g/kg CP, respectively), and in glutamic acid for wheat DDGS (290 g/kg CP). Barley

DDGS was richer in saturated (236 g/kg total fatty acids), corn DDGS in monounsaturated (278 g/kg total fatty acids) and wheat DDGS in polyunsaturated fatty acids (615 g/kg total fatty acids). Barley DDGS had the lowest nutritive value traits for rabbits (11.9 MJ digestible energy (DE) and 168 g digestible protein (DP)/kg DM). No significant differences for the nutritive value of both corn DDGS were observed (on av. 15.3 MJ DE and 208 g DP/kg DM) in spite of higher protein and lower fibre content of the Brazilian (+1.7 g CP and –31 g neutral detergent fibre/kg DM), and Wheat DDGS might be considered as the DDGS with the highest nutritive value (15.7 MJ DE and 263 g DP/ kg DM).

Keywords: distillers dried grains with soluble; chemical composition; amino acids; digestibility; rabbits.

3.1.2. Introduction

Distillers dried grains with solubles (DDGS), are the most important co-products of the bioethanol manufacture industry (0.3 tones per cereal processed ton), composed mostly by mixing distillers grains (DDG) and solubles (DDS, thin stillage) in a 3:1 ratio (Erickson *et al.*, 2005) and are mainly address to animal nutrition (US Grains Council, 2007). However, DDGS are characterized by a relative high variability in their chemical composition and nutritive value. Olentine (1986) describes even 35 causes for this variability, highlighting grains source (Reese and Lewis, 1989), technology used in their manufacture (Spiehs *et al.*, 2002; Noll *et al.*, 2006), efficiency in the ethanol manufacture process (Spiehs *et al.*, 2002; Shurson and Alghamdi, 2008), mixing ratio of the final components obtained (Noll *et al.*, 2006; Kim *et al.*, 2008), and drying time and temperature (US Grains Council, 2007).

Literature about chemical composition and nutritive value of corn DDGS is extensive. However, the available knowledge about DDGS from other cereal grains, as wheat (Nyachoti *et al.*, 2005; Widyaratne and Zijlstra, 2007; Nuez Ortín and Yu, 2009; Avelar *et al.*, 2010) and especially barley and sorghum (Waller, 2004), is more limited.

De Blas and Mateos (2010), describing the usual range of ingredients composition of feeds for rabbits in Spain, indicated that rabbit diets usually include even 350 g/kg of cereal co-products (mainly wheat bran, maize gluten feed and DDGS). Considering the fibrous nature of DDGS and that their availability has been exponentially increased in the last decade (Renewable Fuel Association, 2012), DDGS inclusion on rabbit diets could have been promoted. However, the nutritive value of corn DGGS for rabbits has been poorly studied (Villamide *et al.*, 1989), and until authors' knowledge there is not scientific knowledge about the nutritive value of other cereal grains DDGS for rabbit, as those from wheat and barley grains which production in the rabbit production areas are not negligible.

Therefore, the present study has been address to characterize the chemical composition of the barley, corn and wheat DDGS available in the Iberian Peninsula, as well as their nutritive value for growing rabbits.

3.1.3. Materials and methods

3.1.3.1. Samples of DDGS

Eight batches of DDGS from the three major bioethanol plants in Spain were used in the present study. These factories process the cereal grains by dry grind technology, rendering ethanol and DDGS as product and co-product, respectively (Abengoa, 2011). Batches were obtained during the last quarter of 2010, differing in their cereal source (both type and origin: two from national barley grains, one from national and one from Brazilian corn grains, and three from British and one from national wheat grains). A total of 200 kg, in pellets of 0.86 ± 0.04 cm in diameter, were sampled from each DDGS. Representative samples of 1 kg of each DDGS were milled with a 1 mm sieve and stored for their subsequent chemical, amino acid and fatty acids analyses.

3.1.3.2. Digestibility trial

Four of the eight DDGS analyzed were selected, considering the grain source and within grain source composition variability (mainly protein and fibre), for their subsequent nutritive evaluation by the substitution method. Five diets were formulated from a basal mixture (Table 3.1.1 and Table 3.1.2).

Table 3.1.1. Ingredients (g/kg DM) of the basal diet (C) and the experimental diets (D) including the distillers dried grains with solubles (DDGS) evaluated.

	C	D
DDGS evaluated	-	*200*
Basal mixture	*982.4*	*782.4*
Barley grain	290	231
Alfalfa hay	270	215
Wheat bran	170	135
Sunflower meal 30%CP	85	68
Defatted grape seed	65	52
Soybean hulls	33	26
Oat hulls	33	26
Soybean oil	20	16
Beet molasses	10	8
L-Lysine HCL	3	2.6
DL-Methionine	0.6	0.5
L-Threonine	1.8	1.5
L-Tryptophan	1	0.8
Vitamin-mineral premix	*17.6*	*17.6*
Calcium carbonate	4.6	4.6
Sodium chloride	5	5
Vitamin-micromineral mixture	5	5
Antibiotics[3]	3	3

CP: Crude protein
Vitamin-micromineral mixture supplies per kg of feed: Vitamin A: 8.375 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.
Antibiotics: Linco-spectin (29 ppm lincomicyn + 29 ppm spectinomicyn), 120 ppm neomicin, Apsamix Tiamulina (50 ppm tiamulina), normally used in rabbit farms with high incidence of mucoid enteropathy.

Table 3.1.2. Chemical, amino acid and fatty acids composition (g/kg DM) of the experimental diets [control diet (C) and diets including distillers dried grains with solubles (DDGs) from national barley (Dnb), national corn (Dnc), brazilian corn (Dbc) and national wheat (Dnw)].

	C	Dnb	Dnc	Dbc	Dnw
Chemical composition					
Dry matter, DM	908	915	914	912	911
Ash	74	73	70	67	67
Ether extract	44	48	63	64	49
Starch	192	162	164	166	164
Crude protein, CP	143	164	170	177	183
CP bound to NDF	22	32	28	26	38
Neutral detergent fibre, NDF	397	392	393	365	379
Acid detergent fibre, ADF	208	196	186	174	184
Lignin	58	48	48	44	51
Insoluble hemicelluloses, NDF–ADF	189	196	208	191	195
Celluloses, ADF–Lignin	150	148	137	130	133
Neutral detergent soluble fibre	132	154	110	132	127
Gross energy, MJ/kg DM	19.0	19.1	19.7	19.6	19.5
Amino acid composition					
Alanine	6.9	7.5	10.2	10.7	8.6
Arginine	8.4	8.6	9.2	9.5	9.7
Aspartic acid	13.4	13.5	15.3	15.4	14.6
Cysteine	2.1	2.3	2.8	2.7	3.0
Glutamic acid	26.0	32.1	33.0	32.8	43.4
Glycine	8.3	8.5	9.4	9.2	9.4
Histidine	3.5	3.6	4.5	4.5	4.4
Isoleucine	5.3	5.8	6.5	6.6	6.8
Leucine	9.3	10.5	14.0	14.8	12.4
Lysine	9.1	8.7	9.2	9.4	9.7
Methionine	2.7	3.2	3.5	3.4	3.3
Phenylalanine	6.3	7.0	7.8	7.7	7.4
Proline	10.2	12.5	12.5	13.6	15.0
Serine	6.8	7.5	9.1	8.8	9.1
Threonine	7.1	7.2	8.3	8.3	8.3
Tyrosine	2.8	3.2	4.1	4.2	3.5
Valine	7.1	7.9	8.8	8.9	8.9

Table follows in the next page…

…continuation of Table 3.1.2

Fatty acid composition					
C14:0	0.2	0.1	0.1	0.1	0.1
C16:0	7.2	8.1	9.1	9.7	8.2
C18:0	1.3	1.2	1.4	1.5	1.1
C18:1n-7	0.6	0.6	0.7	0.8	0.6
C18:1n-9	7.4	7.6	12.1	14.4	7.8
C18:2	22.9	25.7	32.7	35.0	27.4
C18:3	2.5	2.5	2.3	2.4	2.6
C20:0	0.1	0.1	0.2	0.3	0.1
C22:0	0.1	0.1	0.1	0.1	0.1

Amino acid content was that rendered after acid hydrolysis.
Only fatty acids with a sample quantification of at least 0.1 g/kg DM were presented.

The control diet (C) contained 982.4 g of the basal mixture by kg dry matter (DM) and 17.6 g of a vitamin–mineral premix/kg DM, and the four experimental diets (D) contained 782.4 g of the basal mixture by kgDM, 17.6 g of a vitamin–mineral premix by kg DM and 200 g of the DDGS being evaluated kg/DM [Dnb, DDGS from national barley (nb) grains; Dnc, from national corn (nc) grains; Dbc, from Brazilian corn (bc) grains; Dnw, from national wheat (nw) grains]. Feed formulation (basal mixture and DDGS level of inclusion) was performed to avoid undesirable nutritional deviations of the recommendations for fattening rabbits (De Blas and Mateos, 2010), and following the guidelines for the evaluation of raw materials by the substitution method described by Villamide *et al.* (2001).

The digestibility trial was performed using sixty three-way crossbred fattening rabbits, aged 42 days with average live weight of 1.49 kg (S.E.: 0.033 kg). Twelve rabbits per diet were randomly housed in metabolic cages of 52 × 44 × 32 cm, and feed and water were offered *ad libitum* during the experimental period. Following an adaptation period of 14 days the consumption control and faeces collection period was 4 days (Pérez *et al.*, 1995). Faeces were stored in identified sealed plastic bags and frozen at –20ºC until their dehydrated and analyses. Apparent digestibility coefficients for DM, CP, gross energy (GE), neutral detergent fibre (NDF), acids detergent fibre (ADF) were determined for each animal, while those for neutral detergent soluble fibre (NDSF), starch, ether extract (EE), CP bound to NDF, amino acids and fatty acids were obtained from pooled faeces of animals within-diet.

The apparent digestibility coefficients (d) of the main nutrients of each DDGS were calculated by differences, assuming additivity, e.g. for the digestible energy (DE) of the $DDGS_x$:

$$DE_{DDGS_x} = \frac{(DE_D - B\ DE_C)}{D}$$

where DE_D is the DE of diet that includes the $DDGS_x$ being evaluated, B the substitution rate of the basal mixture in the diet (0.80), DE_C the DE in the control diet and D the substitution rate of the $DDGS_x$ (0.2).

3.1.3.3. Analytical methods

DDGS samples, diets and faeces were analyzed according to the methods of AOAC (2000): 934.01 for DM, 942.05 for ash, 976.06 for CP and 920.39 with previous acid–hydrolysis of samples for EE. Starch content was determined according to Batey (1982). The NDF (assayed with a thermo–stable amylase and expressed exclusive of residual ash,), ADF (expressed exclusive of residual ash) and lignin (determined by solubilisation of cellulose with sulfuric acid) were analyzed sequentially (Van Soest *et al.*, 1991). The NDSF content was determined according to Hall *et al.* (1997), adapting the method to the nylon filter bag system and with the modifications proposed by Martinez–Vallespín *et al.* (2011). Insoluble hemicelluloses and cellulose were determined by difference (NDF–ADF and ADF–Lignin, respectively). Other fibre fraction (RES) that corresponds to a mix of soluble fibre, that includes that part of pectins not solubilised by the NDF solution and sugars, was estimated as (100–Ash–CP–EE–NDF–Starch) and used in the multivariate analyses performed with the literature data. Finally, GE was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, Loughborough, UK).

The content of methyl esters of fatty acids and total amino acids were determined in the eight DDGS, the five experimental diets and the five pools of faeces obtained from each diet during the digestibility trial. The methyl esters of the fatty acids of the samples were analyzed in 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, to pass from the time a linear gradient of 4°C min^{-1} to 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 *et al.*, 2007). Total saturated, monounsaturated and polyunsaturated fatty acids were calculated as [SFA: C14:0+C16:0+C17:0+C18:0+C20:0+C22:0, MUFA: C16:1+C18:1n-9+ C18:1n-7+C20:1+C22:1n-9 and PUFA: C18:2+C18:3n-6+C18:3n-3+C20:2+C22:2, respectively].

The amino acid content was determined after acid hydrolysis with HCL 6N at 110 ºC for 23 h as previously described Liu *et al*. (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 6–aminoquinolyl–N– hydroxysuccinimidyl carbamate (AQC) and separated with a C–18 reverse-phase column Waters AcQ Tag (150mm×3.9mm). Methionine and Cystine were determined separately as methionine sulphone and cysteic acid respectively after performic acid oxidation followed by acid hydrolysis.

3.1.3.4. Statistical analysis

Data were analysed according to the general lineal model (GLM) procedure of SAS (Statistical Analysis System, 2008), as a completely randomised design with a model accounting for the fixed effect of the DDGS grain source (barley, corn or wheat) for DDGS' chemical composition data, the experimental diet (C, Dnb, Dnc, Dbc and Dnw) for the performance traits and apparent digestibility coefficients, or the DDGS type (nb, nc, bc and nw) for the DDGS' nutritive value data. Multivariate analyses were performed to determine the main parameters involved in the characterization and differentiation of DDGS. A principal component analysis including the chemical composition data [Ash, starch, EE, CP, NDF, ADF, Lignin, lysine, methionine, SFA, MUFA and PUFA] from the eight samples DDGS was done, using the PRINCOMP procedure of SAS (2008), to decrease information and determine the main parameters responsible of data variability. Finally, a discriminate analyse of chemical composition data [DM, ash, CP, EE, NDF, ADF and RES] from the DDGS available in the literature was performed (a total of 53 samples: 1, 4, 18 and 30 from sorghum, barley, wheat and corn DDGS, respectively; see Figure 1b), using the DISCRIM procedure of SAS (2008), to determine the main chemical parameters involved in the discrimination between DDGS by grain source.

3.1.4. Results

3.1.4.1. Chemical composition of the DDGS evaluated

Table 3.1.3 shows the main chemical composition of the DDGS in function of grain source. Barley DDGS had higher fibre and lower protein contents than wheat DDGS (+25 g of ADF and –91 g of CP/kg DM, respectively; P<0.05), as well as the highest ash content (on av. +16 g/kg DM; P<0.05). Corn DDGS had intermediate fibre and protein values between barley and wheat DDGS, but were the richest in EE (on av. +72 g/kg DM).

DDGS' protein was richer in proline, phenylalanine, valine and arginine for barley DDGS (on av. +22, +10, +7 and +6 g/kg CP, respectively; P<0.05), in leucine, alanine and histidine for corn DDGS (on av. +44, +33 and +4 g/kg CP, respectively; P<0.05), and in glutamic acid for wheat DDGS (+107 and +46 g/kg CP respect to corn and barley DDGS, respectively; P<0.05). Protein of both barley and corn DDGS had also higher content on aspartic acid and threonine than that of wheat DDGS (on av. +13 and +7 g/kg CP, respectively; P<0.05).

In general, fat of DDGS's was characterized by a high content in PUFA, especially C18:2 (on av. 588 and 566 g/kg total fatty acids), but grain source had a significant effect on fatty acid composition of DDGS. Therefore, barley DDGS was richer in SFA (+35 and +79 g/kg total fatty acids than wheat and corn DDGS; P<0.05), corn DDGS in MUFA (on av. +128 g/kg total fatty acids; P<0.05) and wheat DDGS in PUFA (on av. +55 g/kg total fatty acids; P<0.05). Fat of corn DDGS was characterized for a higher UFA/SFA ratio than barley and wheat DDGS (31 vs. 17 and 18 g/g, respectively; P<0.05).

Table 3.1.3. Chemical, amino acid and fatty acids composition of the distillers dried grains with solubles (DDGS) samples evaluated (g/kg DM) by grain source.

	DDGS				
Grain source	Barley	Corn	Wheat	SEM	*P*-value
No. of samples	2	2	4		
Chemical composition (g/kg DM):					
Dry matter, DM	918	930	917	3.1	0.262
Ash	61[b]	45[a]	46[a]	1.4	0.012
Ether extract	72[a]	141[b]	67[a]	0.9	<0.001
Starch	9[a]	25[b]	14[ab]	1.4	0.018
Crude protein, CP	262[a]	305[b]	353[c]	2.7	<0.001
CP bound to NDF	86	100	76		0.267
Neutral detergent fibre, NDF	401[b]	374[b]	317[a]	9.4	0.024
Acid detergent fibre, ADF	129[b]	116[ab]	104[a]	3.3	0.057
Lignin	9	10	19	1.9	0.123
Insoluble hemicellulose, NDF−ADF	273[b]	257[ab]	213[a]	8.7	0.057
Celluloses, ADF−Lignin	119[b]	106[b]	85[a]	2.3	0.003
Neutral detergent soluble fibre	210	217	203	19.6	0.947
Amino acids (g/kg of crude protein):					
Alanine	45[a]	75[b]	39[a]	1.0	<0.001
Arginine	51[b]	43[a]	46[a]	0.7	0.022
Aspartic acid	66[b]	68[b]	54[a]	1.0	0.002
Cysteine	16	16	20	1.5	0.489
Glutamic acid	244[b]	183[a]	290[c]	2.9	<0.001
Glycine	47	43	45	0.8	0.255
Histidine	23[a]	27[b]	22[a]	0.5	0.007
Isoleucine	39[b]	36[ab]	35[a]	0.4	0.048
Leucine	74[a]	114[b]	66[a]	2.3	<0.001
Lysine	31	31	24	1.6	0.193
Metionine	15	18	15	1.5	0.718
Phenylalanine	55[b]	47[a]	44[a]	1.4	0.035
Proline	107[c]	78[a]	92[b]	1.2	<0.001
Serine	50	52	50	0.4	0.173
Threonine	39[b]	40[b]	33[a]	0.4	<0.001
Tyrosine	25[a]	32[b]	24[a]	0.7	0.014
Valine	54[c]	49[b]	45[a]	0.5	0.002

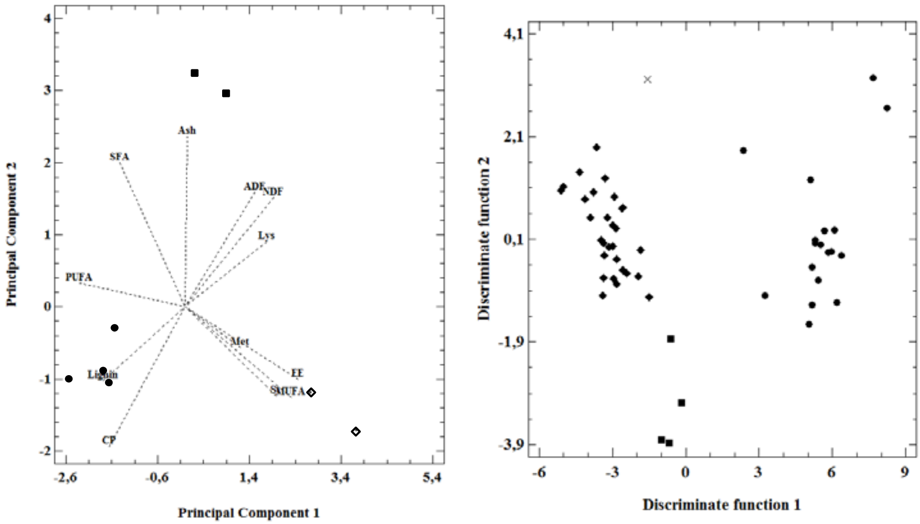
Table follows in the next page...

…continuation of Table 3.1.3

Main fatty acids (g/kg total fatty acids):					
C16:0	236^{c}	160^{a}	211^{b}	3.5	0.001
C18:0	22^{b}	22^{b}	16^{a}	1.0	0.038
C18:1 n-9	131^{a}	260^{b}	132^{a}	6.3	<0.001
C18:1 n-7	8^{a}	12^{c}	11^{b}	0.1	<0.001
C18:2	548^{ab}	522^{a}	581^{b}	8.4	0.065
C18:3 n-3	36^{b}	10^{a}	32^{b}	1.1	<0.001
SFA	267^{c}	188^{a}	232^{b}	4.4	0.003
MUFA	148^{a}	278^{b}	152^{a}	6.4	<0.001
PUFA	586^{ab}	534^{a}	615^{b}	9.3	0.033

SEM: standard error of the means.
Amino acid content was that rendered after acid hydrolysis.
Only fatty acids with a sample quantification of at least 0.1 g/kg DM were presented.
SFA, saturated fatty acids [C14:0+C16:0+C17:0+C18:0+C20:0+C22:0]; MUFA, monounsaturated fatty acids [C16:1+C18:1n-9+C18:1n-7+C20:1+C22:1n-9]; PUFA, poliunsaturated fatty acids [C18:2+C18:3n-6+C18:3n-3+C20:2+C22:2].
^{a,b,c} Means not sharing the same superscript withtin a row were significantly different at P<0.05.

Graphic representation of the first two principal components obtained from the main chemical composition of the analyzed DDGS samples (which explained 75.6% of total variability) clearly placed them in function of their grain source (Figure 3.1a). Thus, barley DDGS were placed in the area characterized for high fibre, ash and SFA contents, wheat DDGS in that with high CP, Lignin and PUFA, and corn DDGS in that with high EE, starch and MUFA. In fact, the discriminate analysis by grain source, from the available common chemical composition of DDGS in the literature, allowed the right classification of the 96.2 percent of the samples. Representation of samples by their values for the first two discriminate functions clearly clustered them by grain source (Figure 3.1b).



(a) (b)

Figure 3.1. (a) Distribution of the evaluated distillers dried grains with solubles (DDGs) by grain source [■ barley, ◆ corn, ● wheat and × sorgum] in the first two principal components obtained from their main chemical composition [Ash; S, starch; EE, ether extract; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; Lignin; Lys, Lysine; Met, methionine; SFA: saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids]. **(b)** Distribution of DDGs described in the literature by grain source in the first two discriminate functions from their main available chemical composition [dry matter; ash, CP, EE, NDF, ADF and RES (as 100–Ash–CP–EE–NDF–Starch)].

Literature DDGS samples sources: Villamide *et al.*, 1989; Cromwell *et al.*, 1993; NRC, 1994; Spiehs *et al.*, 2002; Sauvant *et al.*, 2004; Shurson *et al.*, 2004; Waller, 2004; Nyachoti *et al.*, 2005; Stein *et al.*, 2006; US Grains Council, 2007; Widyaratne and Zijlstra, 2007; Babcock *et al.*, 2008; Emiola *et al.*, 2009; Nuez-Ortin *et al.*, 2009; Avelar *et al.*, 2010; Cozannet *et al.*, 2010; De Blas *et al.*, 2010; Liu, 2011; present study.

3.1.4.2. Nutritive value of DDGS

Table 3.1.4 shows the daily intake and the apparent digestibility coefficients of main nutrients for the experimental diets evaluated from the individual faeces analysis. Average apparent digestibility coefficients for the nutrients determined from faeces pool can be looked up at the Table 3.1.5.

Table 3.1.4. Daily feed intake and apparent digestibility coefficients of main nutrients for the experimental diets [control diet (C) and diets including distillers dried grains with solubles (DDGs) from national barley (Dnb), national corn (Dnc), brazilian corn (Dbc) and national wheat (Dnw)].

	Diets						
	C	Dnb	Dnc	Dbc	Dnw	SEM	*P*-value
No. of animals	12	12	12	12	12		
Feed intake (g/d)	141	144	133	135	139	4	0.8753
Apparent digestibility coefficients:							
dDM	0.582^{a}	0.583^{ab}	0.597^{bc}	0.592^{abc}	0.600^{c}	0.002	0.0468
dCP	0.714	0.701	0.706	0.710	0.732	0.005	0.2668
dGE	0.579^{a}	0.582^{ab}	0.606^{c}	0.598^{bc}	0.609^{c}	0.003	0.0015
dNDF	0.244^{ab}	0.263^{b}	0.295^{c}	0.234^{a}	0.264^{b}	0.005	0.0001
dADF	0.094^{a}	0.134^{b}	0.136^{b}	0.083^{a}	0.090^{a}	0.006	0.0020
dHemicelluloses	0.409^{bc}	0.393^{ab}	0.437^{c}	0.371^{a}	0.428^{c}	0.006	0.0008
dCellulose	0.140^{a}	0.181^{b}	0.183^{b}	0.131^{a}	0.135^{a}	0.006	0.0017

SEM: standard error of the means.
d, apparent digestibility coefficient; DM, dry matter; CP, crude protein; GE, gross energy; NDF, neutral detergent fibre; ADF, Acid detergent fibre; Hemicelluloses, NDF−ADF; Celluloses, ADF−Lignin.

Table 3.1.5. Apparent digestibility coefficients (d) of nutrients calculated using faeces pools for the experimental diets [control diet (C) and diets including distillers dried grains with solubles (DDGS) from national barley (Dnb), national corn (Dnc), brazilian corn (Dbc) and national wheat (Dnw)].

	Diets				
	C	Dnb	Dnc	Dbc	Dnw
dNDSF	0.646	0.706	0.571	0.648	0.710
dStarch	0.985	0.977	0.991	0.987	0.989
dEE	0.854	0.864	0.884	0.881	0.865
dCP-NDF	0.251	0.477	0.466	0.393	0.577
Grouped fatty acids[1]:					
dSFA	0.804	0.777	0.775	0.793	0.762
dMUFA	0.921	0.913	0.937	0.946	0.920
dPUFA	0.965	0.960	0.970	0.975	0.967
Amino acids[2]:					
dAlanine	0.702	0.629	0.776	0.771	0.786
dArginine	0.899	0.864	0.887	0.898	0.888
dAspartic acid	0.778	0.700	0.794	0.814	0.819
dCysteine	0.778	0.758	0.770	0.780	0.815
dGlutamic acid	0.850	0.843	0.861	0.864	0.912
dGlycine	0.733	0.660	0.727	0.747	0.784
dHistidine	0.835	0.846	0.844	0.849	0.880
dIsoleucine	0.735	0.685	0.765	0.771	0.809
dLeucine	0.756	0.711	0.797	0.797	0.826
dLysine	0.852	0.784	0.854	0.864	0.882
dMethionine	0.867	0.791	0.814	0.831	0.854
dPhenylalanine	0.796	0.750	0.788	0.805	0.845
dProline	0.807	0.803	0.820	0.821	0.899
dSerine	0.759	0.729	0.782	0.797	0.834
dThreonine	0.761	0.691	0.774	0.759	0.797

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dTyrosine	0.750	0.737	0.788	0.812	0.838
dValine	0.742	0.679	0.757	0.770	0.804
dAA (contrast D vs. C)[3]		–0.044[a]	+0.020[b]	+0.012[b]	+0.051[c]

NDSF: neutral detergent soluble fibre; EE: ether extract; CP-NDF: crude protein bound to neutral detergent fibre; SFA: saturated fatty acids (as C14:0+C16:0+C18:0+C20:0+C22:0); MUFA: monounsaturated fatty acids (as C16:1+C18:1n-7+C18:1n-9+C20:1+C22:1n-9); PUFA: polyunsaturated fatty acids (as C18:2+C18:3 n-3+C22:2).

[1] Only fatty acids with a sample quantification of at least 0.1 g/kg DM were presented.

[2] Amino acid content rendered after acid hydrolysis.

[3] Contrast of diets including DDGS respect to diet C obtained for the average difference for each amino acid digestibility (dAA). Standad error of the mean, 0.007; P<0.001.

The inclusion of the national barley DDGS (Dnb) did not significantly affect dDM and dGE, but increased both dADF and dCellulose values (+4.0 points of percentage respect to diet C; P<0.05). Although no significant differences were observed for dCP variable, contrast between Dnb and Dnw showed a relative lower value for Dnb (–3.2 points of percentage; P<0.05), perhaps related to their higher proportion of CP bound to NDF (0.33 and 0.22 of the total CP for barley and wheat DDGS, respectively). In fact, difference respect to diet C for the individual apparent digestibility coefficients of main amino acids (dAA) showed an average reduction in the diet Dnb (–4.4 points of percentage), while they were increased respect to diet C in the other diets including DDGS (+2.0, +1.2 and +5.1 points of percentage for Dnc, Dbc and Dnw, respectively; P<0.001).

On the contrary, the inclusion of the national wheat DDGS (Dnw) increased both dDM and dGE values (+2 and +3 points of percentage respect to C diet, respectively; P<0.05), being the diet with the highest values registered for dCP and main dAA. Finally, the inclusion of both corn DDGS (Dnc and Dbc) increased the dDM and dGE (on av. +2 points of percentage respect to diet C, respectively; P<0.05), showing also the highest values observed for dEE

(Table 3.1.5). Dnc also showed higher dNDF, dADF 55 and dCellulose values (+5, +4 and +4 points of percentage respect to C diet, respectively; P<0.05).

Nutritive value of the evaluated DDGS is presented in the Table 3.1.6. DDGS from national barley had lower dDM, dCP, dGE and DP values than DDGS from national wheat (–11, –11, –17 points of percentaje and –95 g DP/kg DM, respectively; P<0.05), having both DDGS from corn intermediate values. DDGS from national barley had significant lower DE value than those obtained for corn and wheat DDGS (on av. –3.56 MJ DE/kg DM; P<0.05).

Table 3.1.6. Apparent digestibility coefficients (d) of dry matter (DM), crude protein (CP) and gross energy (GE), and nutritive value [digestible protein (DP) and digestible energy (DE) values] for the evaluated distillers dried grains with solubles (DDGS) in growing rabbits.

	DDGS					
	National barley	National Corn	Brazilian corn	National wheat	SEM	P-value
dDM	0.647[b]	0.722[ab]	0.684[ab]	0.754[a]	0.014	0.0547
dCP	0.635	0.656	0.704	0.748	0.016	0.0827
dGE	0.582[c]	0.718[ab]	0.653[cb]	0.750[a]	0.014	0.0006
DP, g/kg DM	168[c]	195[bc]	221[b]	263[a]	5	0.0001
DE, MJ/kg DM	11.87[a]	15.89[b]	14.72[b]	15.69[b]	0.30	0.0001

SEM: standard error of the means.
[a,b,c] Means not sharing the same superscript were significantly different at P<0.05.

3.1.5. Discussion

3.1.5.1. Chemical composition of the DDGS evaluated

In general, DDGS can be characterized as a raw material really rich in fibre (NDF and NDSF) and CP (on av. 352, 208 and 318 g/kg DM, respectively). These values, initially places DDGS as an interesting raw material for rabbit nutrition respect to other monogastric species for its high fibre content, especially soluble fibre with attributed properties for gut health in weaned rabbits (Gómez-Conde *et al*., 2007; Martínez-Vallespín, 2011), although its high protein content leads to a warily inclusion in this same way (Carabaño *et al*., 2009).

Main differences between the analyzed DDGS seem to be mainly related to the differences on chemical composition of their original grains (De Blas *et al*., 2010), especially in fibre and protein content, and no great differences respect to the values given by the literature were found. Therefore, ADF and CP values obtained for barley DDGS were similar to those previously reported (on av. 131 and 286 g/kg DM, respectively; De Blas and Mateos, 2010; Waller, 2004). However for wheat DGGS, the obtained values were clearly lower to the average reported by the recent literature in ADF (on av. 104 vs. 150 g/kg DM) and CP (353 vs. 384 g/kg DM) (Nyachoti *et al*., 2005; Widyaratne and Zijlstra, 2007; Emiola *et al*., 2009; Nuez-Ortin *et al*., 2009; Avelar *et al*., 2010; Cozannet *et al*., 2010; De Blas *et al*., 2010), although similar to those presented by Emiola *et al*. (101 and 344 g/kg DM, respectively) in 2009. Ethanol industries have improved their efficiency along the way (efficacy on the starch extraction process, the level of inclusion and recovery of the yeast used in the fermentation…) that could contribute to the observed variability on DDGS composition, especially in protein content.

In fact, the EE value obtained for the corn DDGS was relatively higher to the average given by the literature (141 vs. 107 g/kg DM), although within the range (58 to 165 g/kg DM; Villamide *et al*., 1989; Cromwell *et al*., 1993; NRC, 1994; Spiehs *et al*., 2002; Stein *et al*., 2006; US Grains Council, 2007; Belyea *et al*., 2004; Shurson *et al*., 2004; Widyaratne and Zijlstra, 2007; Nuez-Ortin *et al*., 2009; De Blas *et al*., 2010). These differences on EE content could be related to differences on the laboratory analysis method (use or not of acid hydrolysis; AOAC, 2000), and/or to the level of inclusion of solubles during manufacture of the DDGS that some authors have related to EE content increase (Noll *et al.*, 2007; Ganesan *et al.*, 2008).

On the other hand, DDGS' protein can be considered can be considered poor in three of the most limiting amino acids in rabbit diets (lysine, sulpphur-cointaining amino acids and arginine; Xiccato and Trocino, 2010) respect to other protein concentrates frequently used in rabbit nutrition, as soya and sunflower meals (Villamide *et al.*, 2010). Although the available information for DDGS from barley is scarce (De Blas *et al.*, 2010), amino acid composition obtained for corn and wheat DDGS were similar to that widely reported for these products in the literature (recently, Widyaratne and Zijlstra, 2007; Stein and Shurson, 2009; Avelar *et al.*, 2010; Yang *et al.*, 2010; Kim *et al.*, 2008). The variability and the relative unbalance observed in DDGS' amino acid composition could be mainly attributed to their protein of origin, but also to differences in the fermentation effectiveness, the drying temperature, as well as the amounts of solubles added to dried distillers grains (Martínez-Amezcua *et al.*, 2007; US Grains Council, 2007; Han and Liu, 2010).

Finally, DGGS had a relative high fat content, especially in corn DDGS that was characterized for a higher UFA/SFA ratio than barley and wheat DDGS (31 vs. 17 and 18 g/g, respectively; $P<0.05$), which besides its higher EE content may lead to a higher oxidation and rancidity potential (Cromwell *et al.*, 2011).

3.1.5.2. Nutritive value of DDGS

As it was expected, barley DDGS was characterized by the lowest nutritive value traits for rabbits, although comparable to other commonly used cereal by-products as corn gluten feed (on av. 11.6 MJ DE, 162 g DP and 406 g NDF/kg DM; De Blas et al, 2010). The inclusion of barley DDGS had as main consequence the increase soluble fibre content and cellulose digestibility of the experimental diet. Soluble fibre has been frequently related with a general promotion of caecal fermentative activity (including cellulase), increasing cellulose digestibility (Falçao-Cunha *et al*., 2004). In addition and in spite of the difficulties in the determination of fiber digestibility of cereals in rabbits, Villamide *et al*. (1989), with diets including until 60% of cereal grain, have described a high dADF for the barley grain (0.30).

No significant differences for the nutritive value of both corn DDGS were observed (on av. 15.3 MJ DE and 208 g DP/kg DM), in spite of higher protein and lower fibre content of the Brazilian (+1.7 g CP and –31 g NDF/kg DM). The high energy content of corn DDGS, even greater to that reported for corn grain (14.6 MJ DE/kg DM; Villamide *et al*., 2010), seems to be mainly related to its high fat content rich in MUFA highly digestible (0.94). In general, these values were a few higher to those reported for rabbits (14.1 MJ DE and 198 g DP/kg DM; Villamide *et al.*, 2010), but closer to those usually given for extra quality corn DDGS (14.6 MJ DE and 207 g DP/kg DM). Literature values for DE were closer to those reported for the Brazilian corn DDGS. The inclusion of national corn DDGS led to a higher digestibility of the fibre (respect to both C and Dbc diets), which could contribute to explain this higher value.

On the other hand, wheat DDGS might be considered as the DDGS with the highest nutritive value of those evaluated, placing them close to some oil meals (as rapeseed meal, 14.4 MJ DE and 273 g DP/kg DM) and legume seeds (as Australian lupin, 15.2 MJ DE and 274 g DP/kg DM). The protein value obtained agrees with that report for the Spanish wheat DDGS (262 g PD/kg DM; De Blas *et al.*, 2010), however the energy value was greater to that previously reported (13.2 MJ DE/kg DM; De Blas *et al*., 2010). This discrepancy can be partially explained for the high EE content of the evaluated wheat DDGS (67 g/kg DM), which usually range from 32 to 67 g/kg DM (Widyaratne and Zijlstra, 2007; Nuez-Ortín *et al*., 2009), as well as for its higher dCP to that reported for wheat DDGS in rabbits (0.75 vs 0.71, De Blas *et al*., 2010).

Finally, some authors have described (Stein *et al*., 2006; Pahm *et al*., 2008), as the main limitation for the use of DDGS in pigs, the possible negative effect of heat treatment performed during DDGS manufacture on amino acids digestibility (especially on lysine). Faecal digestibility for the main amino acids in the diets including corn and wheat DDGS was similar to that observed in the control diet, only the diet with barley DDGS showed a generalized decrease on dAA. Pahm *et al.* (2008), comparing corn DDGS with great differences on heat damage, proposed that Lys:CP ratio can be considered as an indicator of heat damage, being considered undamaged corn DDGS those with a ratio higher than 2.9 (as in the present 3.1). On the other hand, end result of the bacterial activity in the rabbit cacecum leads to a substantial change in the amino acid composition of the protein that enters the caecum, this may lead to an enrichment in lysine, methionine and threonine, comparing the total faecal excretion hard and soft (Garcia *et al*., 2005). No information is available about the digestibility coefficients of main amino acids for DDGS in rabbits. The literature values of apparent faecal digestibility for lysine, methionine and threonine in rabbit diets given for protein concentrates, cereals and cereal products (Garcia *et al*, 2005; Llorente *et al.*, 2006, 2007) did not show large variations from those found in the present study.

3.1.6. Conclusion

From the results of the present work can be concluded that, DDGS could be considered as an interesting raw material due to their high content on digestible protein and energy, comparable to other sources of protein frequently used in rabbit nutrition. On the other hand, their high fibre content (on av. 570 g of total fibres/kg DM; NDF+NDSF) makes this co-product especially interesting for rabbit nutrition respect to other monogastric species.

3.1.7. References

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3.2. EFFECT OF DIETARY INCLUSION OF DISTILLERS DRIED GRAINS WITH SOLUBLES FROM BARLEY, WHEAT AND CORN ON THE PERFORMANCE AND CAECAL ENVIRONMENT OF GROWING RABBITS

3.2.1. Abstract

To evaluate how the dietary inclusion of distillers dried grains with solubles (DDGS) could affect the performance and caecal environment of growing rabbits, four experimental diets were formulated from a control diet without DDGS (C), including 20% of barley DDGS (Db_{20}), 20% of wheat DDGS (Dw_{20}) and 20 (Dc_{20}) or 40% (Dc_{40}) of corn DDGS. Animals had free access to medicated versions of the diets until 49 d, and then to unmedicated until 59 d of age. Performance trial was done using 475 three-way crossbred weaned rabbits of 28 d of age individually housed. Caecal fermentation traits were determined on 20 animals per diet and age at 42 d (using other 200 rabbits housed in collective cages) and at 59 d of age (from the performance trial). No significant effect of the growing diet on mortality, morbidity and sanitary risk index was observed. In the whole period and respect to the control group, animals fed with Db_{20} showed higher dry matter (DM) and digestible energy (DE) intake (+6 and +12%, respectively; $P<0.05$), but similar daily weight gain (DWG) and increased feed conversion ratio (+9%; $P<0.05$). In this same way, and independently of its inclusion level, the increase on DE intake on animals fed with corn DDGS (+9 kJ/d, respectively; $P<0.05$) did not result in a significant increase of DWG. On the contrary, higher DM and DE intake of animals feed with Dw_{20} (+8; $P<0.05$) resulted in the highest DWG registered (+2.8 g/d; $P<0.05$) than the control group. Although inclusion of DDGS at 20% did not affected main caecal parameters controlled at 42 d, caecum of animals fed with the diet Dc_{40} was characterized by greater $N\text{-}NH_3$ and valeric acid, and lower total volatile fatty acids and acetic acid concentrations (on av. +5.2±1.7 mmol/L, +0.29±0.07 mol/100 mol, –17.17±4.41 µmol/L and –2.60±0.99 mol/100 mol, respectively; P<0.05). Increased values of caecum DM, propionic and valeric acids and reduced values of total volatile fatty acids and acetic/propionic rate were observed at 59 d for DDGS inclusion at 20% (+1.6±0.5%, +0.95±0.44 mol/100 mol, +0.21±0.07 mol/100mol, –9.3±4.3 µmol/L and –2.7±1.2, respectively; P<0.05) and linear inclusion of corn DDGS (+4.0±0.4%, +2.27±0.41 mol/100 mol, 0.65±, –21.37±3.9 µmol/L and –5.6±1.1, respectively for Dc_{40} respect to C; P<0.05). Animals given Dc_{40} were also characterized for a greater caecum N-NH_3 content (on av. +8.7±1.7 mmol/L; P<0.05) at 59 d of age. The results of the present work reveal that the inclusion of DDGS up to 20% in balanced diets for growing rabbits, independently of their grain source (barley, wheat or corn), could be an interesting alternative to other raw materials.

Keywords: Dried distillers grain with solubles, daily weight gain, feed intake, caecal environment, growing rabbits.

3.2.2. Introduction

The production of bioethanol from cereal grains has increased dramatically the availability of distillers dried grains with solubles (DDGS) in the world market (Renewable Fuels Association, 2012). As a results of their high content on energy, protein and fibre (Spiehs *et al*, 2002; Widyaratne and Zijlstra, 2007; Liu, 2011; Alagón *et al* ., 2013), DDGS have been frequently included in the formulation and manufacture of feeds in many species, especially in pigs (Linneen *et al.*, 2008; Stein and Shurson, 2009; Avelar *et al.*, 2010; Cromwell *et al.*, 2011), but also in poultry (Bregendahl, 2008), dairy (Anderson *et al.*, 2006) and beef cattle (Erickson *et al*., 2005).

In weanling pigs, main of the studies addressed to evaluate the effect of corn and sorghum DDGS on growing performance have not reported negative effects when included up to 30% (Senne *et al.*, 1995; Whitney and Shurson, 2004; Gaines *et al*, 2006; Spencer *et al.*, 2007; Linneen *et al.*, 2008), although in other studies have been observed a reduction of performance when DDGS were included before day 21 postweaning (Burkey *et al*. 2008; Senne *et al.*, 1996; Feoli *et al.*, 2008). Stein and Shurson, (2009) attributed performance differences to the different quality of the DDGS or to differences on diets' balance in the formulation. Recommendations for DDGS inclusion are more restrictive in broilers (up to 6% in the startup phase and 12-15% in the growing and finishing phases; Lumpkins *et al*., 2004).

Limitation for the dietary inclusion of DDGS in both weanling pigs and broilers have been attributed to their high fibre content (especially for poultry) and to a marginal lysine deficiency associated to heat damage of this amino acid during the DDGS manufacture (Stein *et al.*, 2006). Therefore, it could be hypothesized that DDGS could be considered a priori as a raw material of especial interest for growing rabbits due to their digestive particularities, that allow higher dietary levels of fibre and amino acids deficiencies could be partially solved because of the contribution of recycled microbial protein with the caecotrophy (Alagón *et al.*, 2013). However, the knowledge available about the effect dietary inclusion of DDGS in the performance of growing rabbits is still scarce (only with corn DDGS; Youssef *et al.*, 2012).

The aim of this study was to evaluate how the inclusion of DDGS, from different grain sources (barley, wheat and corn) at 20% and the lineal inclusion of corn DDGS until 40%, could affect the performance and caecal environment of growing rabbits.

3.2.3. Materials and methods

3.2.3.1. Diets

Three batches of DDGS from the major bioethanol plants in Spain were used in the present study. Batches of 200 kg, in granules of 0.86 ± 0.04 cm in diameter, were obtained during the last quarter of 2010, differing in their cereal source (barley, corn and wheat grains).

From a control diet (C), formulated according to the requirements for growing rabbits recommended by De Blas and Mateos (2010), four experimental diets were also formulated including 20% of barley DDGS (Db_{20}), 20% of wheat DDGS (Dw_{20}) and 20 (Dc_{20}) or 40% (Dc_{40}) of corn DDGS to be evaluated (Table 3.2.1). The five diets were designed trying to be isoenergetic, isoproteic and isofibrous, on av. 11.6 MJ of digestible energy (DE), 137 g digestible protein (DP) and 195 g of acid detergent fibre (ADF) per kg dry matter (DM). Although the differences on determined DE, DP and ADF among C, Db_{20}, Dw_{20}, Dc_{20} were lower than 6%, the diet including corn DDGS at 40% (Dc_{40}) was characterized for a higher DP content (on av. +13 g/kg DM). To compensate possible unbalances, synthetic amino acids were added if needed (Table 3.2.2). The diets were prepared in two batches, one including robenidine (66 mg/kg), neomycin (120 g/kg), lincomycin (29 g/kg), spectinomycin (29 g/kg) and tiamulina (50 g/kg) to minimize the effects of coccidiosis and epizootic rabbit enteropathy (ERE) during the first 3 weeks of the growing period (28 to 49 d of age), and another unmedicated for the finishing period (49 to 59 d of age).

Table 3.2.1. Ingredients (g/kg dry matter) of the experimental diets [C: control diet; Db_{20}: diet including 20% of barley distillers dried grains with solubles (DDGS); Dw_{20}: diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}: diets including 20 and 40% of corn DDGS, respectively].

	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
Barley DDGS	0	200	0	0	0
Wheat DDGS	0	0	200	0	0
Corn DDGS	0	0	0	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

[1] Supplied per kg of feed: Vitamin A: 8.375 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] Linco-spectin (29 ppm lincomicyn + 29 ppm spectinomicyn), neomicin (120 ppm) and apsamix tiamulina (50 ppm tiamulina), recommended in rabbit farms with high incidence of epizootic rabbit enteropathy.

Table 3.2.2. Chemical composition and nutritive value (g/kg dry matter) of the experimental diets [C, control diet; Db_{20}, diet including 20% of barley distillers dried grains with solubles (DDGS); Dw_{20}, diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}, diets including 20 and 40% of corn DDGS, respectively].

	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}
Chemical composition					
Dry matter, DM	907	911	908	909	903
Ash	61	61	59	60	55
Ether extract, EE	57	81	68	75	82
Starch	186	154	149	159	129
Crude protein, CP	169	168	168	179	184
CP bound to NDF	43	48	44	55	49
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, NDF-ADF	179	194	200	201	206
Cellulose, ADF-ADL	141	142	133	135	128
Neutral detergent soluble fibre, NDSF	84	88	117	104	107
Lysine	10.3	10.6	8.7	9.5	9.4
Methionine+Cystine	5.7	5.5	6.4	5.9	6.6
Threonine	7.1	7.7	8.7	7.9	7.6
Arginine	10.8	9.8	11.6	9.6	8.4
Nutritive value[1]					
Digestible energy (DE; MJ/kg DM)	11.2	11.9	11.3	11.7	11.9
Digestible protein (DP)	133	132	133	140	148
Ratio DP/DE (g/MJ)	11.9	11.1	11.8	11.9	12.4

[1]Determined from pooled faeces of 5 rabbits in a digestibility trial according to Pérez *et al*. (1995).

DP and DE of the experimental diets were determined throughout a digestibility trial, using 5 three-way crossbred fattening rabbits by experimental diet, according to the recommendations of Pérez *et al*. (1995). In brief, following an adaptation period of 10 d the consumption control and faeces collection period was 4 d. Pooled faeces of animals' within-diet were stored in identified sealed plastic bags and frozen at –20ºC until their analyses.

Chemical analyses of diets and faeces were done following the methods of AOAC (2000): 934.01 for DM, 942.05 for ash, 976.06 for crude protein (CP) and 920.39 for ether extract (EE) with previous acid hydrolysis of samples. Starch content was determined according to Batey (1982), by 2-step enzymatic procedure with solubilisation and hydrolysis to maltodextrins with thermostable α-amylase followed by complete hydrolysis with amyloglucosidase (both enzymes from Sigma-Aldrich, Steinheim, Germany), and the resulting glucose being measured by the hexokinase/glucose-6 phosphate dehydrogenase/NADP system (R-Biopharm, Darmstadt, Germany). Neutral detergent fibre (NDF), ADF and acid detergent lignin (ADL) fractions were analysed sequentially (Van Soest *et al.*, 1991) with a thermo-stable α-amylase pre-treatment and expressed exclusive of residual ash, using a nylon filter bag system (Ankom, Macedon, NY, USA). Neutral detergent soluble fibre (NDSF) content was determined according to Hall *et al*. (1997) and modified by Martinez-Vallespín *et al.* (2011). The content of insoluble hemicellulose and cellulose were calculated by difference (NDF-ADF and ADF-ADL, respectively). Gross energy was determined by combustion in adiabatic calorimetric pump, according to the European Group on Rabbit Nutrition (EGRAN, 2001) recommendations.

The content on main limiting amino acids (lysine, methionine+cysteine, threonine and arginine) was determined after acid hydrolysis with HCL 6N at 110 ºC for 23 h as previously described Liu *et al*. (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 and cystine were determined separately as methionine sulphone and cysteic acid respectively after performic acid oxidation followed by acid hydrolysis.

3.2.3.2. Animals and housing

Housing, husbandry and slaughtering conditions followed the current recommendations on principles of ethical care and protection of animals used for experimental purposes in the European Union (2003) and all trials were subject to approval by the Animal Protocol Review Committee of the Polytechnic University of Valencia. The experiment was also carried out following the recommendations for applied nutrition research in rabbits described by EGRAN (Fernández-Carmona *et al.*, 2005).

A total of 475 three-way crossbred weaned rabbits, of 28 d old and average live weight of 610 ± 5 g, were randomly distributed into the five experimental diets in five series (from January to July, 2012), but blocking by litter. All the animals were individually housed under a controlled environment, and had free access to the medicated diet until 49 d, and then the unmedicated until 59 d of age. Mortality was daily recorded, and animals showing diarrhea, constipation, weight loss or decreased feed intake was classified as morbid. The sanitary risk was calculated as the sum of morbidity and mortality (Bennegadi *et al.*, 2000). Live weight (LW) was recorded at 28, 49 and 59 d of age. Daily feed intake (DFI) was controlled from 28 to 49 and 49 to 59 d of age. DWG and feed convertion ratio (FCR) were calculated. The analysis of the performance traits was only done with values from healthy animals in each period.

Another group of 200 three-way crossbred rabbits (40 rabbits per diet), housed in collective cages of 8 animals (50 × 80 × 32 cm) in five series (from January to July, 2012), were used to study the effect of the experimental diets (in their unmedicated version) in the caecal fermentation parameters of growing rabbits at 42 d of age. Caecal fermentation traits were determined on 20 animals per diet and age at 42 d (using rabbits housed in collective cages) and at 59 d of age using rabbits (from the performance trial).

3.2.3.3. Caecal traits

A total of 200 hundred healthy animals, 100 from the collective group at 42 d of age and 100 from the individual trial at 59 d age (4 rabbits per diet age and serie), were slaughtered without previous fasting between 11:00 and 13:00 h. Animals were weighed, electrically stunned (90 V, 6 s, 50 Hz) and slaughtered by intra-cardiac injection of sodium thiopental (75 mg/kg LW).

Thereafter, full gastro-intestinal tract (GIT), full stomach and full caecum were separated and weighed. The pH of stomach content was recorded at the fundus area (pH-meter, Consort C533 model, Belgium). After measuring the pH of caecal content, aliquots of approximately 1 g of caecal content were weighed and 3 mL of a solution of 2% sulphuric acid or 2 mL of 2% ortho-phosphoric acid was added for further analysis of ammonia nitrogen (N-$NH_{3)}$ and volatile fatty acids (VFA), respectively. Samples for VFA analysis were centrifuged at 10,000 rpm for 10 min and the liquid phase was collected into Eppendorf vials of 0.5 mL. Finally, all samples were stored at −80ºC until analysis. The remaining caecal content was stored at −20 ºC until DM analysis.

The DM and N-NH_3 concentrations in the caecal contents were determined according to AOAC (2000) procedures (methods 934.01 and 984.13, respectively). For VFA analysis, samples were previously filtered through a cellulose filter (0.45) and 250 mL were transferred to the injection vials. Two microliters from each sample were injected into the gas chromatograph (FISONS 8000 series, Milan, Italy) equipped with an AS800 automatic injector. The column used was a BD-FFAP of 30 m length × 0.25 mm internal diameter × 0.25 mm film thickness. The injector and detector temperatures were maintained at 220 and 225ºC, respectively.

3.2.3.4. Statistical analysis

Mortality, morbidity and sanitary risk index during the growing period were analyzed using logistic regression, by the GENMOD procedure of the Statistical Analysis System (SAS, 2008), considering a binomial distribution. The results were transformed from the logic scale. All data are presented as least-squares means.

Data from performance traits were analyzed using a GLM procedure of SAS (2008). The model included as fixed effects the diet (C, Db_{20}, Dw_{20}, Dc_{20}, Dc_{40}), the series (1, 2, 3, 4, 5) and their interaction, being included the litter as blocking effect and LW at 28 d of age as a covariate. Data from digestive tract and caecal traits were also analyzed using a GLM procedure for each age, with a model including the diet, the series and their interaction as main effect. The effect of DDGS inclusion at 20% was tested by orthogonal contrasts [⅓ (Db_{20} + Dw_{20} + Dc_{20}) – C]. Linear and quadratic effects for the corn DDGS inclusion (0, 20 and 40%) were analyzed by polynomial contrasts.

3.2.4. Results

The dietary effect on mortality, morbidity and sanitary risk index is presented in the Table 3.2.3. No significant effect (P> 0.05) of the growing diet on the three health traits was observed. Average values for mortality, morbidity and sanitary risk index were 35.8, 10.5 and 46.3%, respectively. From 28 to 49 d of age, a period in which rabbits were fed with medicated diets, average cumulative mortality was 13.5%, increasing dramatically to 35.8% (Figure 3.2) when no-medicated diets were provided during the finishing period (49 to 59 d).

Table 3.2.3. Mortality, morbidity and sanitary risk index of rabbits during the growing period when fed with the experimental diets [C, control diet; Db_{20}, diet including 20% of barley distillers dried grains with solubles (DDGS); Dw_{20}, diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}, diets including 20 and 40% of corn DDGS, respectively].

	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}	*P*-value
No. of rabbits	95	95	95	95	95	
Mortality, %	31.5	37.9	32.6	35.8	41.2	0.638
Morbidity[2], %	10.5	15.8	9.5	8.4	8.4	0.473
Sanitary risk index[3], %	41.9	53.7	41.9	44.1	49.4	0.409

[1] Animals showing diarrhea, constipation, weight loss or decreased feed intake.
[2] Sanitary risk index: mortality + morbidity.

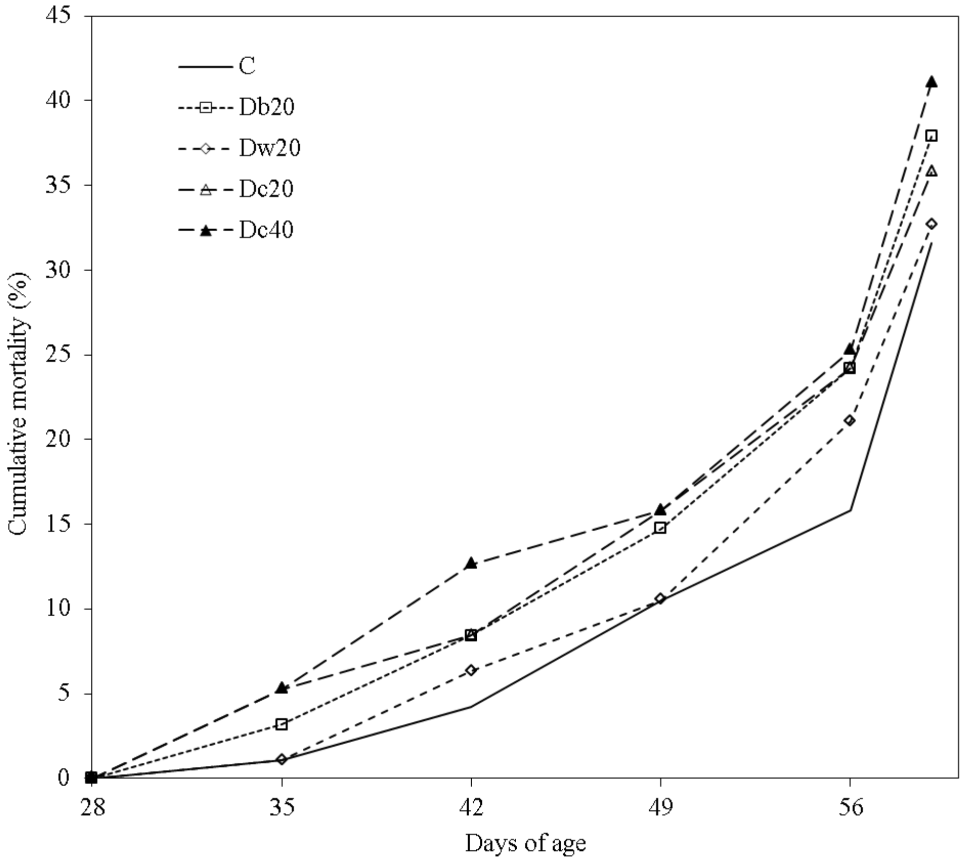

Figure 3.2. Cumulative mortality of growing rabbits given the different experimental diets (see text or explain) medicated from 28 to 49 d and no medicated from 49 to 59 d of age.

The results of the DDGS inclusion on growing performance traits are presented in Table 3.2.4. From 28 to 49 d of age, DDGS inclusion at 20% increased the DE and DP intake (+10 and +8%, respectively; P<0.05) compared with the control diet, which led to a higher DWG and LW at 49 d of age –especially with the Dw_{20} (+8 and +5%, respectively; P<0.05)–, no being affected feed conversion ratio (FCR). Higher inclusion of corn DDGS (40%)

provoked a quadratic response (P<0.05) on DE intake and DWG during this period, showing animals fed with Dc_{40} a similar LW at 49 d than those with C diet. From 49 to 59 d, DDGS inclusion at 20% also led to higher DE and DP intake (+9 and +7%, respectively; P<0.05) but they not allowed to higher DWG, being the difference on LW at 49 maintained at 59 d of age (+56 and +57 g, respectively; P<0.05). In the same way, linear increase of DE and DP intake observed corn DDGS inclusion during this finishing period was not translated to DWG.

In the whole period and respect to the control group, animals fed with Db_{20} showed higher DM and DE intake (+6 and +12%, respectively; P<0.05), but similar DWG and worse feed conversion ratio (FCR) (+9%; P<0.05). In this same way, and independently of its inclusion level, the increase on DE and DP intake on animals fed with corn DDGS (+9 and +12%, respectively; P<0.05) did not result in a significant increase of DWG. In the contrary, higher DM and DE intake of animals feed with Dw_{20} (+8, +9; P<0.05) resulted in the highest DWG registered (+2.8 g/d; P<0.05).

The effect of dietary inclusion of DDGS on digestive tract and caecal parameters of growing rabbits at 42 and 59 d of age is presented in the Table 3.2.5. At 42 days of age, no significant differences (P>0.05) on full digestive tract, full stomach and full caecum weights were observed, but the stomach pH was higher with diets C and Dw_{20} than with the Db_{20} (on av. +0.27±0.11 points; P<0.05). Although inclusion of DDGS at 20% did not affected main caecal parameters controlled at 42 d, caecum of animal fed with the diet Dc_{40} was characterized by greater $N\text{-}NH_3$ and valeric acid, and lower total VFA and acetic acid concentrations (on av. +5.2±1.7 mmol/L, +0.29±0.07 mol/100 mol, –17.17±4.41 µmol/L and –2.60±0.99 mol/100 mol, respectively; P<0.05).

At 59 days of age, although no differences were observed for full digestive tract and full stomach weight, full caecum weight was lower for growing rabbits given the diets with 20% of DDGS inclusion, especially with Dc_{20} (–1.2±0.3 points of percentage respect to diet C; P<005). Increased values of caecum DM, propionic and valeric acids and reduced values of total VFA and acetic/propionic rate were observed at 59 d for DDGS inclusion at 20% (+1.6±0.5%, +0.95±0.44 mol/100 mol, +0.21±0.07 mol/100 mol, –9.3±4.3 µmol/L and –2.7±1.2, respectively; P<0.05) and linear inclusion of corn DDGS (+4.0±0.4%, +2.27±0.41 mol/100 mol, +0.65± , –21.27±3.9 µmol/L and –5.6±1.1, respectively for Dc_{40} respect to C; P<0.05). Animals given Dc_{40} were also characterized for a greater caecum $N\text{-}NH_3$ content (on av. +8.7±1.7 mmol/L; P<0.05) at 59 d of age.

Table 3.2.4. Growth performance of rabbits fed with the experimental diets [C, control diet; Db_{20}, diet including 20% of barley distillers dried grains with solubles (DDGS); Dw_{20}, diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}, diets including 20 and 40% of corn DDGS, respectively].

	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}	SEM	*P*-value	D_{20}–C[1]
No of rabbits	55	44	55	53	48			
Live weight (g) at:								
28 d of age	599	592	600	610	611	5	0.849	2 ± 14
49 d of age[3]	1581[a]	1624[ab]	1662[b]	1623[ab]	1588[a]	8	0.011	56 ± 21*
59 d of age[3]	2053[a]	2086[ab]	2137[b]	2110[ab]	2066[a]	10	0.085	57 ± 27*
28-49 d of age:								
Weight gain (g/d)[3]	46.6[a]	48.6[ab]	50.5[b]	48.6[ab]	46.9[a]	0.4	0.011	2.7±1.0*
DM intake (g/d)	92.8[a]	99.1[b]	100.5[b]	96.3[ab]	93.5[a]	0.7	0.003	5.8±1.8*
DE intake (kJ/d)[3]	1039[a]	1179[c]	1136[bc]	1127[bc]	1111[b]	8	0.001	108±21*
DP intake (g/d)[2]	12.3[a]	13.1[b]	13.4[bc]	13.5[bc]	13.8[c]	0.1	0.001	1.0±0.3*
Feed conversion	2.26	2.30	2.25	2.26	2.23	0.01	0.593	0.00±0.03
49-59 d of age:								
Weight gain (g/d)	47.3	46.1	47.4	48.6	47.8	0.6	0.850	0.1±1.7
DM intake (g/d)[2]	134.3	139.7	142.8	140.3	142.1	1.4	0.322	6.7±3.6
DE intake (kJ/d)[2]	1504[a]	1663[b]	1614[b]	1642[b]	1689[b]	16	0.005	135±41*
DP intake (g/d)[2]	17.9[a]	18.4[ab]	19.0[ab]	19.6[b]	21.0[c]	0.2	0.001	1.2±0.5*
Feed conversion	3.26	3.56	3.44	3.31	3.41	0.04	0.129	0.18±0.09
28-59 d of age:								
Weight gain (g/d)[3]	46.8[a]	47.8[ab]	49.5[b]	48.6[ab]	47.2[a]	0.3	0.083	1.8±0.9*
DM intake (g/d)	106.2[a]	112.3[b]	114.2[b]	110.6[ab]	109.2[a]	0.8	0.017	6.1±2.0*
DE intake (kJ/d)[2,3]	1190[a]	1336[b]	1291[b]	1294[b]	1298[b]	9	0.001	117±23*
DP intake (g/d)[2]	14.1[a]	14.8[ab]	15.8[b]	15.5[bc]	16.1[c]	0.1	0.001	1.0±0.3*
Feed conversion	2.27[a]	2.35[b]	2.31[ab]	2.26[a]	2.31[ab]	0.01	0.095	0.04±0.03

[1] Contrast D_{20}–C, [(Db_{20}+Dw_{20}+Dc_{20})/3]–C, given as mean±standard error; * P<0.05.
[2] linear or [3] quadratic effect (P<0.05) of corn DDGS inclusion (0, 20 and 40%).
[a,b,c] Least square means in the same row not sharing the same superscript differ significantly at P<0.05.
SEM: standard error of the mean.
DM: dry matter. DE: digestible energy. DP: digestible protein.

Table 3.2.5. Live weight, digestive tract and caecal parameters of growing rabbit at 42 and 59 d of age fed with the experimental diets [C, control diet; Db_{20}, diet including 20% of barley distillers dried grains with solubles (DDGS); Dw_{20}, diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}, diets including 20 and 40% of corn DDGS, respectively].

	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}	SEM	*P*-value	D_{20}–C[1]
42 d of age:								
No. of rabbits	20	19	20	20	20			
Live weigth (LW, g)	970	1016	1110	996	995	19.5	0.195	71 ± 53
Full digestive tract, % LW	27.6	26.6	26.0	26.8	27.3	0.3	0.443	–1.1 ± 0.7
Full stomach, % LW	8.1	8.1	7.8	7.9	8.2	0.15	0.882	–0.1 ± 0.4
pH stomach	1.62[b]	1.36[a]	1.64[b]	1.55[ab]	1.43[ab]	0.03	0.058	–0.1±0.4
Full caecum, % LW	9.6	9.2	8.7	9.0	9.7	0.2	0.544	–0.6± 0.5
Caecal parameters:								
Dry matter (%)	22.2	23.5	21.8	22.5	23.6	0.31	0.273	0.4±0.8
N-NH3 (mmol/L)	10.67[ab]	8.56[a]	6.58[a]	9.07[a]	13.93[b]	0.69	0.018	–2.60±1.80
pH	6.12	6.12	6.24	6.08	6.2	0.03	0.354	0.04±0.07
Total VFA (umol/L)[2,3]	77.36[b]	73.18[b]	68.71[b]	78.52[b]	57.27[a]	1.76	0.002	–3.9±4.6
Acetic acid (mol/100 mol)[3]	83.51[ab]	83.38[ab]	84.43[b]	84.59[b]	81.38[a]	0.39	0.095	0.6±1.0
Propionic acid (mol/100 mol)	4.44	4.65	4.13	4.13	5.10	0.22	0.613	–0.1±0.6
Butyric acid (mol/100 mol)	9.75	9.59	9.15	9.65	10.90	0.28	0.373	–0.3±0.7
Valeric acid (mol/100 mol)[2,3]	0.55[a]	0.49[a]	0.46[a]	0.44[a]	0.77[b]	0.03	0.004	–0.1±0.1
Acetic/propionic rate	23.5	20.3	22.6	22.7	18.9	0.89	0.473	–1.6±1.2
59 d of age:								
No. of rabbits	20	20	20	20	20			
Live weigth (g)	2066	2134	2082	2089	2070	13.3	0.504	36±34
Full digestive tract, % LW[3]	20.2	20.2	19.8	18.6	20.1	0.22	0.134	–0.7±0.6
Full stomach, % LW	4.4	4.5	4.9	4.5	4.4	0.09	0.402	0.2±0.2
pH stomach[2]	1.51[b]	1.42[ab]	1.50[ab]	1.54[b]	1.35[a]	0.02	0.109	–0.02±0.06
Full caecum, % LW[2,3]	7.2[b]	6.8[b]	6.6[b]	6.0[a]	6.5[ab]	0.11	0.014	–0.7±0.3*

Table follows in the next page…

…continuation of Table 3.2.5

Caecal parameters:								
Dry matter (%)[2]	23.2[a]	25.0[b]	24.5[b]	24.8[b]	27.2[c]	0.20	<0.001	1.6±0.5*
$N-NH_3$ (mmol/L)[2,3]	8.68[a]	9.01[a]	9.69[a]	8.93[a]	17.78[b]	0.64	<0.001	0.53±1.65
pH	6.10	6.12	6.19	6.19	6.30	0.04	0.555	0.06±0.10
Total VFA (umol/L)[2]	77.26[c]	66.23[b]	68.17[bc]	69.41[bc]	55.89[a]	1.68	0.004	–9.3±4.3*
Acetic acid (mol/100 mol)[2]	77.04	75.67	75.81	74.57	74.12	0.40	0.179	–1.69±1.04
Propionic acid (mol/100mol)[2]	4.66[a]	5.46[ab]	5.76[b]	5.63[ab]	6.93[c]	0.17	0.002	0.95±0.44*
Butyric acid (mol/100 mol)	16.14	15.95	16.69	17.49	15.40	0.33	0.340	0.58±0.86
Valeric acid (mol/100 mol) [2]	0.64[a]	0.81[b]	0.79[ab]	0.95[b]	1.29[c]	0.27	<0.001	0.21±0.07*
Acetic/propionic rate [2]	17.2[c]	14.8[bc]	14.6[bc]	14.1[ab]	11.6[a]	0.45	0.006	–2.7±1.2*

[1] Contrast D_{20}–C, [(Db_{20}+Dw_{20}+Dc_{20})/3]–C, given as mean±standard error; * P<0.05.
[2] Linear or [3] quadratic effect (P<0.05) of corn DDGS inclusion (0, 20 and 40%).
[a,b,c] Least square means in the same row not sharing the same superscript differ significantly at P<0.05.
SEM: standard error of the mean.
N-NH3: ammonia nitrogen.
VFA: volatile fatty acids.

3.2.5. Discussion

As mentioned above, although experimental diets were formulated to be isoenergetic and isoproteic, DDG's inclusion at high levels (200 and especially 400 g/kg DM) led to a dietary protein content in the upper limit recommended (178 g CP/kg DM; De Blas and Mateos, 2010) probably due to DDGS' high protein content (from 262 to 353 g CP/kg DM; Alagón *et al*., 2013).

The dietary inclusion of DDGS at 20% seems to allow an adequate performance of growing rabbits. Even though isoenergetic diets, growing rabbits given the diets including 20% of DDGS showed higher feed and DE energy intake as well as growth rate, especially with the diet including wheat DDGS, than those given the control diet during the first 3 wk of the growing period. Higher feed intake could be partially explained by the dietary composition changes caused by the inclusion of DDGS in the formula, although other factors related to the own product may not be dismissed. Composition of diets including DDGS were characterized for low starch (–3 and –6 percentage points for 20 and 40% of DDGS inclusion) and high EE (+2 percentage points) and NDSF values (especially Dw_{20} diet; +3 percentage points), mainly due to the low starch and high oil and fibre content of DDGS (Spiehs *et al.*, 2002; Belyea *et al.*, 2004; Widyaratne and Zijlstra, 2007; Alagón *et al.*, 2013).

Although all the diets were within the range of DE (9 to 12 MJ/kg) and ADF content (10 to 25%) which allow the regulation of energy intake (Gidenne and Lebas, 2005), other previous works have observed how low starch and high ADF/starch ratios could lead to higher intake to that expected from chemiostatic regulation of the voluntary consumption (Pérez *et al.*, 2000; Xiccato *et al.*, 2008; Pinheiro *et al.*, 2009), although other studies have not found any effect of starch level on consumption (Xiccato *et al.*, 2002, 2011). In that respect, it has been also described how diets with a higher fat content led growing rabbits to higher DE intake to that expected from the DE content (Maertens *et al.*, 1989; Fernández and Fraga, 1996; Cervera *et al.*, 1997). Moreover, the highest consumption was recorded for diets including DDGS at 20%, especially for Dw_{20}, probably as response of their greater total fiber content (NDSF+NDF: 454, 498, 513, 494 and 496 g/kg DM for C, Db_{20}, Dw_{20}, Dc_{20}, Dc_{40}, respectively), which could promote the transit rate through the digestive tract encouraging greater consumption, as previously reported (Pérez *et al.*, 2000; Xiccato *et al*, 2008; Martínez-Vallespín *et al*, 2011). In fact, Gidenne and Lebas (2005) have proposed that dietary intake of growing rabbits is more correlated to the ADF than to DE content of the diet (r = +0.93 and –0.81, respectively).

In general, DWG of growing rabbits was a response of their DE intake, no being affected FCR when wheat and corn DDGS were included in the diet. However, animals receiving the diet with 20% of barley DDGS presented a lower DWG to that expected from their feed and DE intake, being FCR significantly worsen respect to the control group. This result could be partially explained by the higher fibre content of the diet Db_{20}, especially ADL (+2.4 percentage points), frequently related with a FCR increase (Maertens, 2010). Another fact, which could contribute to explain this increased FCR, is the reduced digestibility of the amino acids of barley DDGS protein (on av. −4.4 percentage points when included at 20%), especially for some limiting amino acids (−7 percentage points for lysine, methionine and threonine) in growing rabbits (Alagón *et al*., 2013).

In growing pigs, inclusion of DDGS in the diet has been frequently associated with a reduced performance due to lower digestibility and availability of lysine (Fastinger and Mahan, 2006; Stein *et al.*, 2006; Linneen, 2008; Pahm *et al*., 2008; Almeida *et al*., 2011). This fact has not been observed in growing rabbits, with our balanced amino acid diets, perhaps due to the digestive particularities of rabbit. Through the caecotrophy, microbial lysine represents a quarter of the absorbed lysine (Belenguer *et al*., 2005), and up to 37% of lysine present in the liver has a microbial origin (Belenguer *et al*., 2012). Although there is no knowledge of the true ileal lysine digestibility of DDGS until now, Alagón *et al*. (2013) found high apparent faecal digestibility coefficients for the lysine in diets containing 20% corn DDGS and wheat DDGS (0.85 to 0.88, respectively), but lower for diets including 20% of barley DDGS (0.78).

Increasing levels of low lignified fibre have been frequently related with favored caecal contents weight and VFA concentration in caecum (Garcia *et al*., 2002), which could be an indirect estimation of microbial activity (Gidenne *et al*., 2010). In fact, Alagón *et al*. (2013) observed how the substitution of the basal diet by 20% of DDGS led to an increase of different fibre fractions digestibility in function of the DDGS source, mainly related to their differences on fibre nature. However, the inclusion of DDGS led to lower full caecum weight, higher caecal DM and reduced caecal VFA concentration in the present work. Formulation of balanced diets (on DE, DP and ADF) including DDGS also involved the inclusion of other raw materials (as defatted grape seed and oat hulls) that altogether increased ADL content (from +0.4 to +2.4 percentage points), which could partly explain this reduced fermentative activity. Some studies have shown that increase of dietary ADL caused shorter mean retention time (Gidenne *et al*., 2001), lower weight of caecal contents (Nicodemus *et al.*, 1999) and reduced caecal VFA concentration (Garcia *et al*., 2002). In fact, these differences on ADL can contribute to explain the higher feed intake observed for growing rabbits fed with DDGS diets, as lower accumulation of digesta in the caecum could promote feed intake capacity. Nicodemus *et al*. (1999) observed how a similar increase on dietary ADL (+2.6 percentage points) resulted in similar reduced full caecum weight (–1.1 percentage points on LW basis), as well as increased feed intake (+12 g DM/d) and growth rate (+2.1 g/d).

Respect to the caecal VFA profile, it might be highlighted that DDGS inclusion affected the proportion of caecum acetic and propionic acid that decreased and increased, respectively. Caecal propionic acid proportion was highly correlated (r=+0.79) with the dietary content on high digestible fibre (NDSF+NDF–ADF). It is well known that caecum proportion of acetic acid is increased when fibre level increases (Gidenne *et al*., 2010), and that propionic acid proportion is positive correlated with the dietary concentration on uronic acids (García *et al.*, 2002).

As consequence of the promotion of feed intake on animals giving the diets including DDGS at 20%, daily ingestion of DP was also increased (+1.0 ± 0.3 g/d) that, together the increased DE intake, contributed to the improved rabbits' growth rate. Even though this higher supply of protein, none effect on caecal N-NH_3 concentration and pH of caecum was observed at 42 and 59 d of age.

On the contrary, animals given Dc_{40} diet, with higher DP content and DP/DE ratio and greater proportion of dietary protein coming from the same source (two thirds from corn DDGS), had higher caecal N-NH_3 concentration and valeric acid proportion both at 42 and 59 d of age. Xiccato *et al.* (2011), comparing diets with similar protein content range (169 and 180 g CP/kg DM) but with lower DP/DE ratio (10.5 to 11 g/MJ), did not reported any effect of dietary protein on these caecal traits in growing rabbits. However, when protein intake exceeds the nutritional requirements or amino acid composition is not well balanced, the amount of ileal N flow (Gutiérrez *et al.* , 2003) and recycled urea from blood to cecum could be increased (Villamide *et al.,* 2010), rising caecal ammonia and promoting proteolytic microflora activity. In fact, the increase of valeric acid proportion in the caecum, which comes from the bacterial metabolisation of proline (Amos *et al.*, 1971), has been associated with the higher activity of the proteolytic microflora (Padhila *et al.*, 1995). Under a Epizootic Rabbit Enteropathy (ERE) context, with mortality rate overcoming 30% when medicated diets are removed, these caecal conditions could contribute to increase the intestinal health risk (Gutiérrez *et al.*, 2003; Chamorro *et al.,* 2007). In fact, although no significant differences were found, animals fed with Dc_{40} diet presented the highest figures of mortality.

3.2.6. Conclusion

In conclusion, the results of the present work reveal that the inclusion of DDGS up to 20% in balanced diets for growing rabbits, independently of their grain source (barley, wheat or corn), could be an interesting alternative to other raw materials. DDGS provide a considerable amount of energy, protein and fibres to the diet without negative effects on the performance of growing rabbit. However, a greater dietary inclusion might be done with care to avoid a distortion of the adequate healthy caecal environment.

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3.3. EFFECT OF FEEDING DIETS CONTAINING BARLEY, WHEAT AND CORN DISTILLERS DRIED GRAINS WITH SOLUBLES ON CARCASS TRAITS AND MEAT QUALITY IN GROWING RABBITS

3.3.1. Abstract

The effect of the dietary inclusion of distillers dried grains with solubles (DDGS) on carcass characteristics, meat quality, chemical composition and fatty acid composition of *Longissimus* muscle of 100 growing rabbits at 59 days of age was studied. Four experimental diets from a control diet without DDGS (C), including 20% of barley DDGS (Db_{20}), 20% of wheat DDGS (Dw_{20}) and 20 (Dc_{20}) or 40% (Dc_{40}) of corn DDGS were formulated. Animals were fed with the experimental diets from 28 to 59 days of age. No effect of dietary inclusion of DDGS on the hot carcass weight, cold carcass weight (CCW), drip loss 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, obtaining a higher dissectible fat percentage when including barley and corn DDGS (on av. +0.7% CCW; $P<0.05$). No effect of DDGS on texture parameters, cooking loss, water holding capacity and intramuscular fat of the loin meat was found. However, meat of animals fed with Dw_{20} had a higher redness value compared with the rest of groups (on av. +1.5 for diet Dw_{20} respect to other diets), and higher pH was obtained with Dw_{20} and Db_{20} than with Dc_{20} (5.53, 5.52 and 5.44, respectively; $P<0.05$). Protein content of loin decreased as dietary corn DDGS level increased (-0.34 and -0.65 g/100g for diets Dc_{20} and Dc_{40} respect to C, respectively). Saturated fatty acids concentration in the meat linearly decreased with the inclusion of corn DDGS ($P<0.05$). Finally, meat of animals given corn DDGS at 40% presented the lowest saturated/unsaturated fatty ratio, atherogenic and

trombogenic indexes of the diets evaluated (on av. -0.04, -0.03 and -0.05, respectively; P<0.05). It could be concluded that dietary inclusion of DDGS of barley, wheat and corn at 20% did not affect most of the carcass and meat quality traits in rabbits.

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

3.3.2. Introduction

The distillers dried grains with solubles (DDGS) of barley, wheat and corn are co-products of the industry of bioethanol frequently used in livestock feeding. 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 fibre (20 - 21.7%) (De Blas *et al*., 2010; Alagón *et al*., 2013a), allowing an adequate growth performance when they are included up to 20% in the diet (Youssef *et al*., 2012; Alagón *et al*., 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 both carcass and meat. Typically, DDGS contain 7 to 15% of fat, with 70 to 80% of mono and polyunsaturated fatty acids (Xu *et al*., 2010; Alagón *et al*. 2013a) and monogastrics tend to show a fatty acid profile in the meat similar to the profile of the diet (Bee *et al*., 2002; Dalle Zotte, 2002).

In pigs, the use of DDGS has led to a reduction in dressing out percentage in some studies (Cook *et al*., 2005; Thacker, 2006; Whitney *et al*., 2006; Gaines *et al*., 2007; Weimer *et al*., 2008), and increased levels of corn DDGS at 20-30% in growing-finishing diets reduced pork fat firmness (Whitney *et al*., 2006), while other authors found no change in dressing out percentage due to the use of these co-products (McEwen, 2006; Xu *et al.*, 2007; Drescher *et al.*, 2008). In chickens, dietary levels above 12% corn DDGS increased the level of fatty acids in the thigh meat, increasing the oxidation during storage (Schilling *et al.*, 2010). In steers, feeding with diets that included levels of 20 or 40% of wheat and corn DDGS did not lead to differences in carcass and meat quality (Aldai *et al.*, 2010). However, no information is available about the effect of dietary inclusion of DDGS on carcass and meat quality in rabbits.

Therefore, the objective of the present study was to evaluate the effect of the dietary inclusion of barley, wheat and corn DDGS at 20% and corn DDGS at 40% on carcass and meat quality of growing rabbits.

3.3.3. Material and methods

3.3.3.1. Diets

Five isoproteic, isoenergetic and isofibrous diets were formulated according to the nutritional requirements for growing rabbits (De Blas and Mateos, 2010), including distillers dried grains with solubles (DDGS) as follows: diet C (control diet, 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 (Cycostat®, 66 ppm of robenidine; Linco-spectin®, 29 ppm lincomicyn + 29 ppm spectinomicyn; 120 ppm neomycin; and Apsamix Tiamulina®, 50 ppm tiamulina, normally used in rabbit farms with high incidence of mucoid enteropathy) and unmedicated versions of the feeds were prepared. The ingredients, chemical composition, nutritive value and fatty acid composition are shown in Tables 3.3.1 and 3.3. 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 NDF (assayed with a thermo–stable amylase and expressed exclusive of residual ash), ADF (expressed exclusive of residual ash) and lignin (determined by solubilisation of cellulose with sulfuric acid) were analyzed sequentially (Van Soest *et al.*, 1991). The neutral detergent soluble fibre content was determined according to Hall *et al.*(1997), adapting the method to the nylon filter bag system and with the modifications proposed by Martínez-Vallespín *et al.*(2011). Insoluble hemicelluloses and cellulose were determined by difference (ND–ADF and ADF–Lignin, respectively). Finally, the digestible protein and digestible energy of the experimental diets were calculated using an apparent digestibility assay with pools of faeces, measured in 5 rabbits per experimental diet, according to the European Reference method (Pérez *et al.*, 1995).

The amino acid content was determined after acid hydrolysis with HCL 6N at 110 ºC for 23 h as previously described Liu *et al.* (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 was determined separately as methionine sulphone after performic acid oxidation followed by acid hydrolysis.

Table 3.3.1. 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] Only in medicated versions of feed: Linco-spectin (29 ppm lincomicyn + 29 ppm spectinomicyn), 120 ppm neomicin, Apsamix Tiamulina (50 ppm tiamulina), normally used in rabbit farms with high incidence of mucoid enteropathy.

Table 3.3.2. Chemical composition, nutritive value and fatty acids composition of the experimental diets.

	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}
Chemical composition(g/kg DM)					
Dry matter, DM	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
Nutritive value[1]					
Digestible energy, DE (MJ/kg DM)	11.2	11.9	11.3	11.7	11.9
Digestible protein, DP (g/Kg DM)	133	132	133	140	148
Ratio DP/DE (g/MJ)	11.9	11.1	11.8	11.9	12.4
Fatty acids composition (g/kg DM)					
C14:0 (myristic)	0.4	0.6	0.4	0.3	0.2
C16:0 (palmitic)	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 (heptadecenoic)	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

Table follows in the next page…

…continuation of Table 3.3.2

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
PUFA/SFA	1.0	0.9	1.1	1.4	2.1
n-6/n-3	9.3	10.0	10.0	13.9	22.7

C: diet control, 0% DDGS; Db20: diet with 20% of barley DDGS; Dw20: diet with 20% of wheat DDGS; Dc20 and Dc40: diets with 20 and 40% of corn DDGS.
[1] Calculated from pooled faeces of 5 rabbits/diet in a digestibility trial (Pérez *et al.*, 1995).
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].
n-6/n-3: linoleic/linolenic

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 *et al.,* 2007).

3.3.3.2. Animals

The experimental procedure followed both the Spanish Royal Decree 1201/2005 on protection of animals used for scientific purposes (Boletín Oficial del Estado, 2005) and the recommendations for applied nutrition research in rabbits described by the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005), being approved by the Committee of Ethics and Animal Welfare of the Universidad Politécnica de Valencia.

A total of 475 weaned rabbits 28 days old of both sexes from a three way cross were used in the experiment. Animals were reared in 5 rounds. Rabbits were allocated in individual cages and fed until 59 days-old with one of the 5 experimental diets. Diets were medicated from 28 to 49 days-old and unmedicated from 49 to 59 days-old. Daily feed intake in the white period is available at Alagón *et al.* (2013b), which was used to determine EE and fatty acid intake of the animals.

3.3.3.3. Slaughter traits and carcass composition

At 59 days of age, 100 rabbits (4 per diet and round) were weighed (SW), electrically stunned and slaughtered at the abattoir in the farm. No fasting was applied. The slaughtering and carcass dissection procedures followed the recommendations given by Blasco and Ouhayoun (1996).

The slaughtered rabbits were bled, and the skin, genitals, urinary bladder, gastrointestinal tract and the distal part of the legs were removed. The full gastro intestinal tract was weighed and expressed as percentage with respect to SW (FGTP). The hot carcasses obtained were weighed (HCW) and then chilled at +4 °C for 24 h in a ventilated room. The chilled carcasses were weighed (CCW) and the dressing out percentage was calculated as CCW×100/SW. The drip loss percentage (DLP) was 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 IFaP, PFaP and SFaP, 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.

3.3.3.4. Meat quality

3.3.3.4.1. 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 *et al.*, 1995). Meat color was measured in the transversal section of the *Longissimus* muscle at the level of the 7th lumbar vertebra.

3.3.3.4.2. 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).

3.3.3.4.3. 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.

3.3.3.4.4. 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 (CL) were calculated as (F-C) × 100/F.

3.3.3.4.5. 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 and 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).

3.3.3.4.6. Chemical and fatty acids composition of meat

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 *et al.*, (2012). The saturation (S/U), atherogenic (AI) and thrombogenic (TI) indexes were calculated according to Ulbricht and Southgate (1991) using equations presented by Peiretti and Meineri (2008) and Volek and Marounek (2011):

$$S/U = \frac{C14:0 + C16:0 + C18:0}{\sum MUFA + \sum PUFA}$$

$$P/S = \frac{\sum PUFA}{C14:0 + C16:0 + C18:0}$$

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

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

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.

3.3.3.5. Statistical analysis

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

Linear and quadratic effects of dietary inclusion of corn DDGS (C, Dc_{20} and Dc_{40}) were determined using orthogonal polynomial contrasts. In addition, orthogonal contrasts were used to compare DDGS inclusion at 20% ($DDGS_{20}$, average of Db_{20}, Dw_{20} and Dc_{20}) with the C diet. All reported means are least squares means.

3.3.4. **Results**

3.3.4.1. Carcass Characteristics

The carcass composition of the rabbits fed with the different experimental diets is shown in Table 3.3.3. The use of diet Db_{20} led to higher values of IFaP, SFaP and DFaP than when feeding with the C diet (on av. +0.39, +0.19 and +0.83 percentage points, respectively; $P<0.05$). The use of Dc_{20} also turns out the DFaP (on av. +0.51 percentage points; $P<0.05$) with respect to C, and Dc_{40}

led to higher SFaP and DFaP (on av. +0.13 and +0.72 percentage points, respectively; P<0.05). Rabbits fed with $DDGS_{20}$ diets showed higher values of IFaP, SFaP, DFaP than those fed with C. The inclusion of corn DDGS in the diet increased linearly PFaP, SFaP and DFaP (P<0.05).

Table 3.3.3. Carcass traits of rabbits fed with diets without 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}).

	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	0.10±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 effect of level inclusion of corn DDGS (P<0.05).
$DDGS_{20}$-C: mean ±standard error of the contrast between ⅓[Db_{20}+Dw_{20}+Dc_{20}] and 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.

3.3.4.2. Meat quality

The effect of the experimental diets on carcass and meat color, as well as on pH, WHC, CL and texture parameters in the meat of the *Longissimus* muscle is shown in Table 3.3.4. No statistical differences (P>0.05) in the color of the carcasses of rabbits fed with the different experimental diets were found. In relation to the color of the meat, Dw_{20} diet had higher a* value compared with the other experimental diets (on av. +1.50±0.17 points; P<0.05). Also, dietary inclusion of corn DDGS reported a quadratic effect on a* value (P<0.05). Similarly, there was a linear decrease of b* value of the meat with corn DDGS level inclusion.

Table 3.3.4. Carcass and meat color, pH, water holding capacity (WHC), cooking losses (CL) and texture parameters in the *Longissimus* muscle of rabbits fed with diets without 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}).

	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}	0.01	0.095	0.00± 0.03
WHC, %	33.28	33.43	33.57	34.19	33.02	0.24	0.611	0.45±0.6
CL, %	32.87	32.49	33.14	33.42	33.14	0.21	0.69	0.15± 0.5
Texture parameters								
Shear force	3.22	3.09	3.35	3.34	3.44	0.06	0.337	0.03±0.14
Shear firmness	1.46	1.43	1.5	1.49	1.49	0.03	0.918	0.01±0.06
Area	5.04	4.69	5.04	5.18	5.51	0.11	0.182	-0.07±0.3

[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 ⅓[Db_{20}+Dw_{20}+Dc_{20}] and 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.

Db_{20} and Dw_{20} diets led to higher meat pH values (5.52 and 5.53, respectively) than 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 in function of the diet were found (P> 0.05).

Table 3.3.5 shows the chemical and fatty acids composition in the *Longissimus* muscle of rabbits fed with the different experimental diets. The level of protein decreased as the corn DDGS level in the diet increased (-0.34 and -0.65 g/100g for diets Dc_{20} and Dc_{40} respect to control, respectively; P<0.05). In general, diets that included 20% DDGS led to decrease the protein content on the meat respect to C diet (-0.26 g/100g; P<0.05). 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. However, C16:0 was higher when feeding with Db_{20} and Dw_{20} than with diet Dc40 (on av. +1.69 points of percentage; P<0.05), and C17:0 showed a linear increase with corn DDGS inclusion in the diet (P<0.05). MUFA and PUFA content of meat did not differ between diets. Differences were found in the SFA concentration of rabbit meat with the different diets, reporting +0.86 and +1.75% of total fatty acids with diet Dw_{20} than with diet Dc_{20} and Dc_{40} diets, respectively (P<0.05). In addition concentration of SFA and S/U ratio in the rabbit meat linearly decreased with the inclusion of corn DDGS (P<0.05).

No differences were found in n-3, n-6 and n-6/n-3 content of meat between animals fed with the different diets. PUFA/SFA, AI and TI ratios were different in the meat of rabbit with Dc_{40} than with Db_{20} or Dw_{20} (on av. +0.09, -0.04, -0.07, respectively; P<0.05). The inclusion of corn DDGS at 40% in the diet led to a reduction of the S/U ratio (on av. -0.04; P<0.05).

Table 3.3.5. Chemical and fatty acid composition of *Longissimus* muscle of rabbits fed with diets without 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}).

	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]	0.04	0.001	-0.26±0.1*
Fat	1.18	1.23	1.17	1.25	1.26	0.02	0.631	0.04± 0.06
Fatty acids composition (% total fatty acids)								
C14:0	1.81	1.87	1.73	1.81	1.78	0.03	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]	0.18	0.016	0.62± 0.46
C16:1	1.81	1.94	1.73	1.9	1.65	0.08	0.762	0.04±0.19
C17:0 [1]	0.73[a]	0.73[a]	0.75[a]	0.76[ab]	0.80[b]	0.01	0.001	0.02± 0.01
C18:0	9.24	8.83	8.98	8.94	8.93	0.08	0.564	-0.32± 0.21
C18:1n-7	1.85	1.79	1.82	1.83	1.83	0.02	0.744	-0.04± 0.04
C18:1n-9	22.62	23.58	22.61	23.09	23.51	0.21	0.388	0.48± 0.53
C18:2n-6	23.63	23.5	24.05	24.02	24.77	0.20	0.317	0.23± 0.52
C18:3n-3	1.62	1.67	1.7	1.74	1.8	0.03	0.491	0.08±0.08
C20:2n-6	0.34	0.33	0.35	0.34	0.33	0.01	0.776	-0.0± 0.0
C20:3n-6	0.68	0.64	0.71	0.66	0.61	0.02	0.423	-0.01±0.05
C20:4n-6	5.2	4.94	4.93	4.9	4.98	0.12	0.945	-0.27±0.32
C20:5n-3	2.23	1.84	2.05	2.01	1.89	0.07	0.376	-0.26±0.17
C22:4n-6	2.36	2.15	2.31	2.2	2.11	0.05	0.449	-0.13± 0.13
C22:5n-3	0.72	0.61	0.66	0.68	0.68	0.02	0.657	-0.07±0.06
C22:6n-3	2.75	2.51	2.53	2.64	2.78	0.09	0.849	-0.19±0.24
SFA [1]	34.63[bc]	35.04[bc]	35.29[c]	34.43[b]	33.54[a]	0.13	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	0.31	0.428	-0.77±0.79
n-3	7.07	6.09	6.20	6.64	6.8	0.13	0.127	-0.76±0.36*
n-6	32.2	31.56	32.35	32.13	32.78	0.25	0.627	-0.19± 0.63
n-6/n-3	4.84	5.42	5.49	4.88	5.10	0.10	0.119	0.4±0.2

Table follows in the next page…

…continuation of Table 3.3.5

P/S	1.14[ab]	1.08[a]	1.10[a]	1.13[ab]	1.18[b]	0.01	0.098	-0.04±0.03
S/U [1]	0.51[b]	0.52[b]	0.53[b]	0.51[ab]	0.48[a]	0.00	0.001	0.01±0.01
AI	0.45[ab]	0.47[b]	0.47[b]	0.45[ab]	0.43[a]	0.01	0.034	0.01±0.01
TI	0.67[ab]	0.71[b]	0.71[b]	0.68[ab]	0.64[a]	0.01	0.004	0.03±0.02

[1]Linear effect of level inclusion of corn DDGS (P<0.05).
$DDGS_{20}$-C: mean ±standard error of the contrast between ⅓[Db_{20}+Dw_{20}+Dc_{20}] and 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.

3.3.5. Discussion

3.3.5.1. 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 of pigs in some studies (Cook *et al.*, 2005; Thacker, 2006; White *et al.*, 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 genetic (Pla and Cervera, 1997; Pla, 1999; Hernández *et al.*, 2006). Thus, carcass yield, economically important for the rabbit manufacturers, seemed to be not affected when using DDGS for rabbit nutrition at these levels.

Other effect frequently observed in some species when including DDGS in the diet was an increase of fat deposition (Benz *et al.*, 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 and 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 IFaP, PFaP, SFaP and DFaP (Table 3.3.3) when feeding with some diets that included DDGS could be due to higher dietary concentrations and higher intakes of fat (9.15, 7.51, 8.25 and 8.90 vs. 6.25 g/d for Db_{20}, Dw_{20}, Dc_{20}, Dc_{40} and C diets, respectively; results no shown), as observed in other studies (Fernández and Fraga, 1996; Pla and Cervera, 1997). In fact, positive correlations were found between EE intake 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; $P<0.001$). The variation in EE intake 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 EE (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).

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 3.3.2) 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; results not shown) and especially 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; results not shown), as long chain fatty acids are 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.

3.3.5.2. 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 lightness, 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 and Pla (2007), Hernández *et al.*, (2004) and Ramírez *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 lightness (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 *et al.*, 2012, Carrilho *et al.*, 2009; Hernández, *et al.*, 2004). The only parameter affected by the diet was the redness, higher in the meat of rabbits fed with diet Dw_{20} ($P<0.05$) than with the other diets. This which could be due to a change an myoglobin presentation, which is the pigment responsible for meat color (Ouhayoun and Dalle-Zotte, 1993).

Although myoglobin was not measured in this work, Aldai *et al.* (2010) described an increase of chroma $[(a^{*2} + b^{*2})^{0.5}]$ on meat and hue [arctan (b*/a*)] on retail, as well as metmyoglobin in the retail of steers when feeding with 20 and 40% of both corn or wheat DDGS. Widmer *et al.*, (2008) and Rickard *et al.*, (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 WHC and tenderness (Huff-Lonergan and 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 of chicken when feeding with corn DDGS included between 6 and 24%, 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 between 20 and 30% of corn DDGS, found no differences in pH in the meat loin. In the present study, the values of pH, WHC and tenderness were similar to those obtained in other studies (pH 5.5 to 5.7; Liu *et al.,* 2012; Dal Bosco *et al.*, 2012; Pascual and 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, texture parameters and WHC did not differ between animals fed with the different diets, showing that 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 *et al.*, 2004; Hernández and Gondret, 2006; Hernández and 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 and there was a positive correlation of 0.50 ($P<0.001$, 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 *et al.*, (1996) and

Pla and Cervera (1997). Moreover, Pla and 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 on 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 and Mateos, 2010). Moreover, the inclusion of these DDGS in the diets did not reduce growth in a larger experiment which also 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 *et al.*, 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 *et al.*, 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 *et al.*, 2008; Dal Bosco *et al.*, 2012) who found higher values in SFA than in PUFA. The variability in the saturation index is high, as observed Hernandez and Dalle Zotte (2010) in a recent review including 21 references (28.0 ± 4.1, 32.5 ± 6.1 and 38.9 ± 4.4 for MUFA, PUFA and SFA, 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 (the current diets were rich in dietary fat coming from DDGS or soybean oil). In this way, differences in SFA (Table 3.3.5) 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 3.3.2).

Corn DDGS inclusion had an effect on PUFA meat content, when expressed as mg/100g of the *Longissimus* muscle. Values obtained were of 295, 314 and 325 mg/100g of loin for C, Dc_{20} and Dc_{40} diets, describing a linear effect ($P<0.05$, results not shown), due to the higher contribution of linoleic with 180, 197 and 205 mg/100g of loin, respectively. The linoleic acid is deposited directly into the fat of the animal (Wood *et al.*, 2008). This fatty acid was higher in corn DDGS diets (Table 3.3.2) and consequently the incorporation into muscle fat could be directly proportional to its intake. On the other hand, the values of n-3 in the loin was higher with corn DDGS (54.4, 55.8 and 52.5 mg/100g loin for Dc_{20}, Dc_{40} and C, respectively; $P<0.05$), probably due to the greater relative abundance of linoleic acid in the diets (14.7, 15.1 and 12.7 for Dc_{20}, Dc_{40} and C, respectively).

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

3.3.6. Conclusions

The inclusion barley, wheat and corn DDGS at 20% in the diet of rabbits did not affect most of the carcass and meat quality traits. The use of barley and corn DDGS increased the carcass fat percentage, but still maintaining their carcasses in a lean range.

The use of barley, corn and wheat DDGS at 20% did not change the fatty acid profile of the rabbit meat, and the slight modification produced by the inclusion of corn DDGS at 40% lead to better indexes from a health point of view.

3.3.7. References

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IV. GENERAL DISCUSSION

4.1. GENERAL DISCUSSION

In general, DDGS can be characterized as a raw material really rich in fibre (NDF and NDSF) and CP (on av. 352, 208 and 318 g/kg DM, respectively). Main differences between the analyzed DDGS seem to be mainly related to the differences on chemical composition of their original grains (De Blas *et al.*, 2010). Therefore, barley DDGS had higher fibre and lower protein contents than wheat DDGS (+25 g of ADF and –91 g of CP/kg DM, respectively; P<0.05), and corn DDGS had intermediate fibre and protein values between barley and wheat DDGS, but were the richest in EE (on av. +72 g/kg DM). This fat of corn DDGS was characterized by a higher UFA/SFA ratio than barley and wheat DDGS (31 vs. 17 and 18 g/g, respectively; P<0.05), which besides its higher EE content may lead to a higher oxidation and rancidity potential (Cromwell *et al.*, 2011). On the other hand, DDGS' protein can be considered poor in three of the most limiting amino acids in rabbit diets (lysine, sulpphur-cointaining amino acids and arginine; Xiccato and Trocino, 2010) respect to other protein concentrates frequently used in rabbit nutrition, as soya and sunflower meals (Villamide *et al.*, 2010).

Because of its higher fibre content, barley DDGS was characterized by the lowest nutritive value traits of DDGS evaluated (11.9 MJ DE and 168 g DP/kg DM), although comparable to other commonly used cereal by-products as corn gluten feed (De Blas *et al.*, 2010). No significant differences for the nutritive value of both corn DDGS were observed (on av. 15.3 MJ DE and 208 g DP kg/DM). The high energy content of corn DDGS, even greater to that reported for corn grain (14.6 MJ DE/kg DM; Villamide *et al.*, 2010), seems to be mainly related to its high fat content (141 g EE/kg DM). Finally, wheat DDGS might be considered as the DDGS with the highest nutritive value of those evaluated (15.7 MJ DE and 263 g DP/kg DM), placing them close to some oil meals and legume seeds (De Blas *et al.*, 2010).

The dietary inclusion of DDGS at 20% seems to allow an adequate performance of growing rabbits. Even though isoenergetic diets, growing rabbits given the diets including 20% of DDGS showed higher feed and DE energy intake as well as growth rate, especially with the diet including wheat DDGS, than those given the control diet during the first 3 wk of the growing period. Higher feed intake could be partially explained by the dietary composition changes caused by the inclusion of DDGS in the formula (lower starch and higher EE, NDSF and ADL), although other factors related to the own product may not be dismissed. DWG of growing rabbits was a response of their DE intake, no being affected FCR when wheat and corn DDGS were included in the diet. However, animals receiving the diet with 20% of barley DDGS presented a lower DWG to that expected, being FCR significantly worsen respect to the control group. This result could be partially explained by the higher fibre content of the diet Db_{20}, especially ADL (+2.4 %), frequently related with a FCR increase (Maertens, 2010).

Respect to the caecal environment, DDGS inclusion affected the proportion of caecum acetic and propionic acid that decreased and increased, respectively. Caecal propionic acid proportion was highly correlated (r=+0.79) with the dietary content on high digestible fibre of DDGS. It is well known that caecum proportion of acetic acid is increased when fibre level increases (Gidenne *et al.*, 2010), and that propionic acid proportion is positive correlated with the dietary concentration on uronic acids (García *et al.*, 2002). On the other hand, animals given the diet including corn DDGS at 40%, with higher DP content, DP/DE ratio and proportion of dietary protein coming from the same source, had higher caecal $N\text{-}NH_3$ concentration and valeric acid proportion both at 42 and 59 d of age. When protein intake exceeds the nutritional requirements or amino acid composition is not well balanced, the amount of ileal N flow (Gutiérrez *et al.* , 2003) and recycled urea from blood to cecum could be increased (Villamide *et al.*, 2010), rising caecal ammonia and promoting proteolytic microflora activity. It must be considered that these caecal conditions could contribute to increase the intestinal health risk (Gutiérrez *et al.*, 2003; Chamorro *et al.*, 2007).

The carcass yield is an important trait from the economic point of view for both the producer and the manufacturer industry. No effect (McEwen, 2006; Drescher *et al.*, 2008) or a reduction (Cook *et al.*, 2005; Weimer *et al.*, 2008) of dressing out percentage was observed in pigs when using DDGS in the diet formulation. The results of the present study show that the inclusion of DDGS of barley, wheat or corn at 20%, or corn DDGS at 40% does not affect the carcass yield of the rabbits.

A negative effect of the use of DDGS in the diet was the high fat percentage in the carcass when including barley DDGS at 20% or corn DDGS at 20 and 40%. This effect could be avoid using wheat DDGS at 20% in the diet, as the rabbits fed with this diets showed similar fat percentages in the carcass than rabbits fed with the control diet.

Most of the meat quality traits studied was not affected by the inclusion of DDGS in the diets. Carcass and meat colour parameters, which are important for the consumer acceptability, did not differ depending on the use of the different DDGS except when using wheat DDGS, which led to higher although irrelevant values of redness than when feeding with a control diet. The pH, which plays an important rule in the post-mortem conversion of muscle to meat and the consequent tenderness and capacity of the muscle fibers to retain water (Huff-Lonergan and Lonergan, 2005), differed depending on the DDGS included in the diet. However, the instrumental texture analysis, the water holding capacity and the cooking losses did not differ between rabbits fed with the different diets.

The use of DDGS reduced the protein in the meat of the *Longissimus* muscle and, although fat percentage was similar for all the diets, fat ingestion differed between diets and there was a positive correlation between the fat ingestion and fat percentage in the meat. The fatty acid composition was not affected when including DDGS at a level of 20%. However, the diet with corn DDGS at 40% led to a different fatty acid profile than the control diet. Differences obtained in meat corresponded to differences in the fatty acid composition of the diet because the rabbit, as a monogastric, is able to incorporate the long chain fatty acids from the diet to the fat and intramuscular tissue (Dalle Zotte, 2002). The use of corn DDGS at 40% increased the PUFA content of the meat, and ameliorated the PUFA/SFA ratio, SFA/UFA ratio, atherogenic index and thrombogenic index, which improvement is associated to a reduction of the cardiovascular diseases.

From the results of the present thesis could be concluded that, DDGS can be considered as an adequate co-product to be use in the formulation of diets for growing rabbits. They provide important quantities of nutrients of especial interest for rabbits (soluble fibre, lignified fibre, protein and energy), and some of the limitations detected for the use of this raw material in other species (fibre content, unreactive lysine…) have a lower impact in rabbits due to there digestive particularities. Therefore, DDGS can be included up to 20% in the diet of rabbits without any relevant negative effect on growing performance, caecal environment, carcass traits and meat quality. From the different DDGS evaluated, it might be highlighted the possible especial interest of wheat DDGS for growing rabbits, as it was characterized by the highest values of DE and DP, as well as the best performance traits during the growing period (feed intake and growth rate), without any negative effect on the caecal environment, dissectable fat deposition and meat quality traits evaluated.

4.2. REFERENCES

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