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EFFECT OF INCLUSION OF DISTILLERS DRIED GRAINS AND SOLUBLES FROM BARLEY, WHEAT AND CORN IN ISONUTRITIVE DIETS ON THE PERFORMANCE AND CAECAL ENVIRONMENT OF GROWING RABBITS

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Abstract: To evaluate how dietary inclusion of distillers dried grains and 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% barley DDGS (Db_{20}), 20% wheat DDGS (Dw_{20}) and 20 (Dc_{20}) or 40% (Dc_{40}) corn DDGS. Animals had free access to medicated versions of the diets until 49 d, and then to unmedicated diets until 59 d of age. The performance trial was done using 475 three-way crossbred weaned rabbits of 28 d of age, individually housed in 5 batches. Caecal fermentation traits were determined in 20 animals per diet and at 42 d and at 59 d of age. No significant effect of the growing diet on mortality, morbidity or sanitary risk index was observed. In the whole period and compared 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%; $P<0.05$). Similarly, and regardless of its inclusion level, the increase in DE intake of animals fed with corn DDGS (+9, respectively; $P<0.05$) did not increase DWG. In contrast, higher DM and DE intake of animals fed with Dw_{20} (+8; $P<0.05$) resulted in a higher DWG (+2.8 g/d; $P<0.05$) than those fed with C. Although inclusion of DDGS at 20% did not affect main caecal parameters recorded at 42 d, caecum of animals fed with the diet Dc_{40} was characterised by greater $N\text{-}NH_3$ and valeric acid and lower total volatile fatty acids and acetic acid concentrations than the average of the other groups (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 d of age, higher caecal DM, and propionic and valeric acid concentration and lower values of total volatile fatty acids and acetic/propionic rate were observed for DDGS inclusion at 20% compared to the control (+1.6±0.5%, +0.95±0.44 mol/100 mol, –9.3±4.3 µmol/L and –2.7±1.2, respectively; $P<0.05$). Linear inclusion of corn DDGS increased caecal DM, propionic acid proportion and total volatile fatty acids concentration and reduced acetate/propionic rate (+4.0±0.4%, +2.27±0.41 mol/100 mol, –21.27±3.9 µmol/L and –5.6±1.1, respectively for Dc_{40} compared to C; $P<0.05$). Rabbits given Dc_{40} were also characterised for a greater caecum N-NH3 content than the other groups (on av. –8.7±1.7 mmol/L; $P<0.05$) at 59 d of age. The results of the present work reveal that inclusion of DDGS up to 20%, independently of the grain source (barley, wheat or corn), could be an interesting alternative in balanced diets for growing rabbits.

Key Words: caecal environment, dried distillers grain with solubles, growing rabbits, growth performance.

INTRODUCTION

The production of bioethanol from cereal grains has increased the availability of distillers' dried grains and solubles (DDGS) in the world market (Renewable Fuels Association, 2012). As a result of their high energy, protein and fibre content (Widyaratne and Zijlstra, 2007; Liu, 2011; Alagón, 2013), DDGS have frequently been included in the formulation of feeds for many species, especially in pigs (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).

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In weanling pigs, most of the studies addressing the effect of corn and sorghum DDGS on growing performance have reported no negative effects when they were included up to 30% (Whitney and Shurson, 2004; Gaines *et al.*, 2006; Spencer *et al.*, 2007), although other studies observed a reduction of performance when DDGS were included before day 21 postweaning (Burkey *et al.,* 2008; Feoli *et al.*, 2008). Stein and Shurson, (2009) attributed performance differences to the different quality of the DDGS or to differences in the diets' balance in the formulation.

Limitation for the dietary inclusion of DDGS in weanling pigs has been attributed to their high fibre content and to a marginal lysine deficiency associated with heat damage of this amino acid during DDGS manufacture (Stein *et al.*, 2006). Therefore, it could be hypothesised that DDGS might be considered *a priori* as a raw material of special interest for growing rabbits due to their digestive particularities, as their fibre requirements are much higher and possible amino acids deficiencies associated with heat damage could be partially solved because of the contribution of recycled microbial protein with the caecotrophy (Villamide *et al.*, 2010). However, the knowledge available about the effect of dietary inclusion of DDGS on the performance of growing rabbits is still scarce (only with corn DDGS; Soliman *et al.*, 2010; Bernal-Barragán *et al.*, 2010; Youssef *et al.*, 2012).

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

MATERIALS AND METHODS

Diets

Three batches of DDGS from the major bioethanol plants in Spain were used in this study. Batches of 200 kg, in granules of 0.86±0.04 cm in diameter, were obtained during the last quarter of 2010, from different cereal sources (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), 4 experimental diets were also formulated for evaluation, including 20% barley DDGS (Db_{20}), 20% wheat DDGS (Dw_{20}) and 20 (Dc_{20}) or 40% (Dc_{40}) corn DDGS (Table 1). The 5 diets were designed to be isoenergetic, isoproteic and isofibrous, with on average 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 in 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 characterised by a higher DP content (on av. +13 g/kg DM). To compensate possible unbalances, synthetic amino acids were added if needed. The diets were prepared in 2 batches, one including robenidine (66 mg/kg), neomycin (120 g/kg), lincomycin (29 g/kg), spectinomycin (29 g/kg) and tiamulin (50 g/kg) to minimise the effects of coccidiosis and epizootic rabbit enteropathy (ERE) during the first 3 wk of the growing period (28 to 49 d of age), and another unmedicated for the finishing period (49 to 59 d of age).

DP and DE of the experimental diets were determined throughout a digestibility trial (10 d of adaptation and 4 d of consumption control and faeces collection), using 5 three-way crossbred fattening rabbits per diet, aged 42 d.

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, 28 d old and average live weight of 610±5 g, were blocked by litter and randomly distributed into the 5 experimental diets in each batch (a total of 5 batches from January to July, 2012). All the animals were individually housed and had free access to the medicated diet until 49 d, and then the unmedicated diet until 59 d of age. Mortality was recorded daily. Animals showing diarrhoea, constipation

Table 1: Ingredients (g/kg dry matter) of the experimental diets.

	Diets[1]				
	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[2]	5	5	5	5	5
Coccidiostac[3]	1	1	1	1	1
Antibiotics[4]	3	3	3	3	3

[1] C: control diet; Db_{20}: diet including 20% of barley distillers dried grains and solubles (DDGS); Dw_{20}: diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}: diets including 20 and 40% of corn DDGS, respectively.

[2] 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.

[3] Cycostat (66 ppm of robenidine).

[4] Linco-spectin (29 ppm lincomycin+29 ppm spectinomycin), neomycin (120 ppm) and Apsamix tiamulin (50 ppm tiamulin), recommended in rabbit farms with high incidence of epizootic rabbit enteropathy.

or immobility (daily controlled), as well as weight loss or decreased feed intake compared with animals to the same group (under 1.5 interquartile ranges; SAS, 2002), were classified as morbid. 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. Daily weight gain (DWG) and feed conversion ratio (FCR) were calculated. 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 5 batches (from January to July, 2012), were used to study the effect of the experimental diets (in their unmedicated version) on the caecal fermentation parameters of growing rabbits at 42 d of age. Caecal fermentation traits were determined only in 20 healthy animals per diet and age at 42 d (using 100 rabbits from the 200 housed in collective cages) and at 59 d of age (using rabbits from the performance trial).

Caecal traits

A total of 200 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 series), were slaughtered between 11:00 and 13:00 h without previous fasting. 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%

Table 2: Chemical composition and nutritive value (g/kg dry matter) of the experimental diets.

	Diets[1]				
	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	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
Hemicelluloses, NDF-ADF	179	194	200	201	206
Cellulose, ADF-ADL	141	142	133	135	128
Neutral detergent soluble fibre	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[2]					
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] C: control diet; Db_{20}: diet including 20% of barley distillers dried grains and solubles (DDGS); Dw_{20}: diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}: diets including 20 and 40% of corn DDGS, respectively.

[2] Determined from pooled faeces of 5 rabbits in a digestibility trial.

sulphuric acid or 2 mL of 2% ortho-phosphoric acid was added for further analysis of ammonia nitrogen ($N\text{-}NH_3$) and volatile fatty acids (VFA), respectively. Samples for VFA analysis were centrifuged at 10000 rpm for 10 min and the liquid phase was collected into Eppendorf vials of 0.5 mL. Finally, all samples were stored at –80°C until analysis. The remaining caecal content was stored at –20°C until DM analysis.

Chemical analysis

Chemical analyses of diets and faeces were performed following the AOAC methods (2000): 934.01 for DM, 942.05 for ash, 976.06 for crude protein (CP), and 920.39 with previous acid hydrolysis of samples for ether extract (EE). 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 was 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 [Mertens (2002), the AOAC (2000; procedure 973.187) and Van Soest *et al.* (1991), respectively] 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). Hemicellulose and cellulose contents 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) recommendations (2001).

The content of the main limiting amino acids (lysine, methionine+cysteine, threonine and arginine) was determined after acid hydrolysis with HCL 6N at 110°C for 23 h as previously described Liu *et al.* (1995), using a Waters (Milford, Massachusetts, USA) HPLC system consisting of 2 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 (150 mm×3.9 mm). Methionine and cystine were determined separately as methionine sulphone and cysteic acid respectively after performic acid oxidation followed by acid hydrolysis.

Table 3: Mortality, morbidity and sanitary risk index of individually housed rabbits during the growing period (28 to 59 d of age) when fed with the experimental diets.

	Diets[1]					
	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] C: control diet; Db_{20}: diet including 20% of barley distillers dried grains and solubles (DDGS); Dw_{20}: diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}: diets including 20 and 40% of corn DDGS, respectively.

[2] Animals showing diarrhoea, constipation, weight loss or decreased feed intake.

[3] Sanitary risk index: mortality + morbidity.

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 was transferred to the injection vials. Two microlitres 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 225°C, respectively.

Statistical analysis

Mortality, morbidity and sanitary risk index during the growing period were analysed using a logistic regression, by the GENMOD procedure of the Statistical Analysis System (SAS, 2002), considering a binomial distribution. The results were transformed from the logit scale. Data on performance traits were analysed using a GLM procedure from SAS (2002). The model included as fixed effects the diet (C, Db_{20}, Dw_{20}, Dc_{20}, Dc_{40}), the batch (1, 2, 3, 4, 5) and their interaction, the litter being included as a block and LW at 28 d of age as a covariate. Data on digestive tract and caecal traits were also analysed using a GLM procedure for each age, with a model including the diet, the batch 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 analysed by polynomial contrasts.

All data were presented as least-squares means, and for mean comparison t-test was used.

RESULTS

No significant effect (*P*>0.05) of the growing diet on morbidity and sanitary risk index was observed, being on av. 35.8, 10.5 and 46.3%, respectively (Table 3). From 28 to 49 d of age, a period in which rabbits were fed medicated diets, average cumulative mortality was 13.5%, increasing dramatically to 35.8% (Figure 1) when non-medicated diets were provided during the finishing period (49 to 59 d). In both periods, clinical symptoms of dead and morbid animals revealed an outbreak compatible with ERE (colic pain, borborygmus, and diarrhoea with mucus).

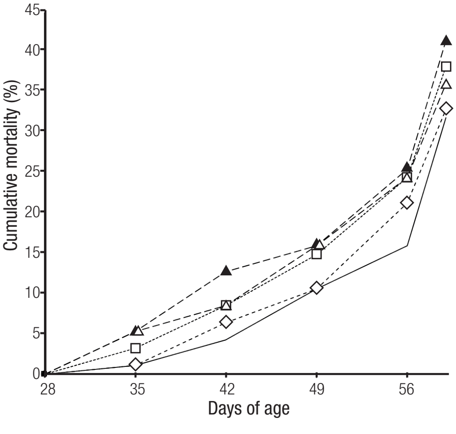

Figure 1: Cumulative mortality of growing rabbits given the different experimental diets [—— C, control diet; ··□·· Db_{20}, diet including 20% of barley distillers dried grains and solubles (DDGS); -◇- Dw_{20}, diet including 20% of wheat DDGS; –△– Dc_{20} and –▲– Dc_{40}, diets including 20 and 40% of corn DDGS, respectively] medicated from 28 to 49 d [robenidine (66 mg/kg), neomycin (120 g/kg), lincomycin (29 g/kg), spectinomycin (29 g/kg) and tiamulin (50 g/kg)]and no medicated from 49 to 59 d of age.

Table 4: Growth performance of rabbits fed with the experimental diets.

	Diets[1]							
	C	Db$_{20}$	Dw$_{20}$	Dc$_{20}$	Dc$_{40}$	SEM	*P*-value	D$_{20}$–C[2]
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[4]	1581^{a}	1624^{ab}	1662^{b}	1623^{ab}	1588^{a}	8	0.011	56±21*
59 d of age[4]	2053^{a}	2086^{ab}	2137^{b}	2110^{ab}	2066^{a}	10	0.085	57±27*
28-49 d of age								
Weight gain (g/d)[4]	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)[4]	1039^{a}	1179^{c}	1136^{bc}	1127^{bc}	1111^{b}	8	0.001	108±21*
DP intake (g/d)[3]	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)[3]	134.3	139.7	142.8	140.3	142.1	1.4	0.322	6.7±3.6
DE intake (kJ/d)[3]	1504^{a}	1663^{b}	1614^{b}	1642^{b}	1689^{b}	16	0.005	135±41*
DP intake (g/d)[3]	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)[4]	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)[3,4]	1190^{a}	1336^{b}	1291^{b}	1294^{b}	1298^{b}	9	0.001	117±23*
DP intake (g/d)[3]	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] C: control diet; Db$_{20}$: diet including 20% of barley distillers dried grains and solubles (DDGS); Dw$_{20}$: diet including 20% of wheat DDGS; Dc$_{20}$ and Dc$_{40}$: diets including 20 and 40% of corn DDGS, respectively.

[2] Contrast D$_{20}$–C, [(Db$_{20}$+Dw$_{20}$+Dc$_{20}$)/3]–C, given as mean±standard error; * $P<0.05$.

[3] Linear or [4] 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.

From 28 to 49 d of age, DDGS inclusion at 20% increased the DE and DP intake (+10 and +8%, respectively; $P<0.05$), which led to a higher DWG and LW at 49 d of age compared to the control [especially with the Dw$_{20}$ diet (+8 and +5%, respectively; $P<0.05$)], with no effect on FCR (Table 4). Higher inclusion of corn DDGS (40%) provoked a quadratic response ($P<0.05$) in DE intake and DWG during this period, showing rabbits fed with Dc$_{40}$ a similar LW at 49 d than those with C diet. From 49 to 59 d, 20% DDGS inclusion also led to higher DE and DP intake (+9 and +7%, respectively; $P<0.05$) but DWG was not affected, the difference in LW at 49 being maintained at 59 d of age (+56 and +57 g compared to the control, respectively; $P<0.05$). Likewise, the linear increase of DE and DP intake observed for corn DDGS inclusion during this finishing period was not translated to DWG.

In the whole period and compared 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 increased FCR (+9%; $P<0.05$). Similarly, and independently of its inclusion level, the increase in DE and DP intake in animals fed with corn 20% DDGS (+9 and +12%, respectively; $P<0.05$) did not result in a significant increase of DWG. In contrast, higher DM and DE intake of animals fed with Dw$_{20}$ (+8; $P<0.05$) resulted in a higher DWG registered (+2.8 g/d; $P<0.05$) than those fed with C.

At 42 d of age, no effect of DDG inclusion at a 20% ($P>0.05$) 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; $P<0.05$; Table 5). Although inclusion of 20% DDGS did not affect main caecal parameters controlled at 42 d, caecum of rabbits fed with the diet Dc$_{40}$ was characterised by greater N-NH$_3$ and valeric acid, and lower total VFA and acetic acid concentrations compared to the average of the rest of dietary groups (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; $P<0.05$).

Table 5: Live weight, digestive tract and caecal parameters of growing rabbit at 42 and 59 d of age fed with the experimental diets.

	Diets[1]							
	C	Db_{20}	Dw_{20}	Dc_{20}	Dc_{40}	SEM	*P*-value	D_{20}–C[2]
42 d of age								
No. of rabbits	20	19	20	20	20			
Live weight (LW, g)	970	1016	1110	996	995	20	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	0.2	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	0.3	0.273	0.4±0.8
N-NH3 (mmol/L)	10.7^{ab}	8.6^{a}	6.6^{a}	9.1^{a}	13.9^{b}	0.7	0.018	–2.6±1.8
pH	6.12	6.12	6.24	6.08	6.2	0.03	0.354	0.04±0.07
Total VFA (µmol/L)[3,4]	77.4^{b}	73.2^{b}	68.7^{b}	78.5^{b}	57.3^{a}	1.8	0.002	–3.9±4.6
Acetic acid (mol/100 mol)[4]	83.5^{ab}	83.4^{ab}	84.4^{b}	84.6^{b}	81.4^{a}	0.4	0.095	0.6±1.0
Propionic acid (mol/100 mol)	4.4	4.7	4.1	4.1	5.1	0.2	0.613	–0.1±0.6
Butyric acid (mol/100 mol)	9.8	9.6	9.2	9.7	10.9	0.3	0.373	–0.3±0.7
Valeric acid (mol/100 mol)[3,4]	0.6^{a}	0.5^{a}	0.5^{a}	0.4^{a}	0.8^{b}	0.0	0.004	–0.1±0.1
Acetic/propionic rate	23.5	20.3	22.6	22.7	18.9	0.9	0.473	–1.6±1.2
59 d of age								
No. of rabbits	20	20	20	20	20			
Live weight (g)	2066	2134	2082	2089	2070	13	0.504	36±34
Full digestive tract (% LW)[4]	20.2	20.2	19.8	18.6	20.1	0.2	0.134	–0.7±0.6
Full stomach (% LW)	4.4	4.5	4.9	4.5	4.4	0.1	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)[3,4]	7.2^{b}	6.8^{b}	6.6^{b}	6.0^{a}	6.5^{ab}	0.1	0.014	–0.7±0.3*
Caecal parameters								
Dry matter (%)[3]	23.2^{a}	25.0^{b}	24.5^{b}	24.8^{b}	27.2^{c}	0.2	<0.001	1.6±0.5*
$N\text{-}NH_3$ (mmol/L)[3,4]	8.7^{a}	9.0^{a}	9.7^{a}	8.9^{a}	17.8^{b}	0.6	<0.001	0.5±1.7
pH	6.10	6.12	6.19	6.19	6.30	0.04	0.555	0.06±0.10
Total VFA (µmol/L)[3]	77.3^{c}	66.2^{b}	68.2^{bc}	69.4^{bc}	$55.\ 9^{a}$	1.7	0.004	–9.3±4.3*
Acetic acid (mol/100 mol)[3]	77.0	75.7	75.8	74.6	74.1	0.4	0.179	–1.7±1.0
Propionic acid (mol/100 mol)[3]	4.7^{a}	5.5^{ab}	5.7^{b}	5.6^{ab}	6.9^{c}	0.2	0.002	1.0±0.4*
Butyric acid (mol/100 mol)	16.1	16.0	16.7	17.5	15.4	0.3	0.340	0.6±0.9
Valeric acid (mol/100 mol)[3]	0.6^{a}	0.8^{b}	0.8^{ab}	1.0^{b}	1.3^{c}	0.3	<0.001	0.2±0.1*
Acetic/propionic rate[3]	17.2^{c}	14.8^{bc}	14.6^{bc}	14.1^{ab}	11.6^{a}	0.446	0.006	–2.7±1.2*

[1] C: control diet; Db_{20}: diet including 20% of barley distillers dried grains and solubles (DDGS); Dw_{20}: diet including 20% of wheat DDGS; Dc_{20} and Dc_{40}: diets including 20 and 40% of corn DDGS, respectively

[2] Contrast D_{20}–C, [(Db_{20}+Dw_{20}+Dc_{20})/3]–C, given as mean±standard error; * $P<0.05$.

[3] Linear or [4] 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; $N\text{-}NH_3$: ammonia nitrogen; VFA: volatile fatty acids.

No differences were observed at 59 d of age in full digestive tract and full stomach weight; full caecum weight was higher for growing rabbits given the diets with 20% of DDGS inclusion compared to diet C, especially with Dc_{20} (–1.2±0.3 points of percentage; $P<005$). Increased values of caecal DM, propionic and valeric acids and reduced values of total VFA concentration and acetic/propionic rate were observed at 59 d for 20% DDGS inclusion compared to the control (+1.6±0.5%, +0.95±0.44 mol/100 mol, –9.3±4.3 µmol/L and –2.7±1.2, respectively; $P<0.05$). Linear inclusion of corn DDGS increased caecal DM, propionic acid proportion and total volatile fatty acids concentration and reduced acetate/propionic rate (+4.0±0.4%, +2.27±0.41 mol/100 mol, –21.27±3.9 µmol/L and –5.6±1.1, respectively for Dc_{40} compared to C; $P<0.05$). Animals given Dc_{40} were also characterised for a greater caecal $N\text{-}NH_3$ content (on av. –8.7±1.7 mmol/L; $P<0.05$) at 59 d of age compared to the average of the rest of the dietary groups.

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 (126 g DP/kg DM; De Blas and Mateos, 2010) probably due to DDGS' high protein content (from 262 to 353 g CP/kg DM; Alagón, 2013).

The dietary inclusion of DDGS at 20% seems to allow an adequate performance of growing rabbits, as previously reported Bernal-Barragán *et al.* (2010) when included up to 30%. Growing rabbits given the diets including 20% of DDGS showed slightly 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 product per se cannot be ruled out. Composition of diets including DDGS were characterised for low starch (–3 and –6% for 20 and 40% of DDGS inclusion) and high EE (+2%) and NDSF values (especially Dw_{20} diet; +3%) compared to the control, mainly due to the low starch and high oil and fibre content of DDGS (Belyea *et al.*, 2004; Widyaratne and Zijlstra, 2006; Alagón, 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 chemostatic 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 this respect, it has been also described how diets with a higher fat content led to growing rabbits to higher DE intake than 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 a response of their greater total fibre 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; Trocino *et al.*, 2013). In fact, the higher amount of insoluble and indigestible fibre (oat hulls and defatted grape seed) of diets including DDGS at 20% compared to the control, which promotes lower mean retention time (García *et al.*, 2002), could be related with their lower caecal weight and higher consumption. Gidenne and Lebas (2005) proposed that dietary intake of growing rabbits is more correlated to the fibre (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, with no effect on 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, FCR being significantly worsened compared 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 an FCR increase (Maertens, 2010). Another fact, which could help explain this increased FCR, is the reduced digestibility of the amino acids of barley DDGS protein (on av. –4.4 points of percentage when included at 20% compared to a basal balanced diet), especially for some limiting amino acids (–7 points of percentage for lysine, methionine and threonine) in growing rabbits (Alagón, 2013).

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

Increasing levels of low lignified fibre have frequently been related with favoured caecal contents weight and VFA concentration in caecum (García *et al.*, 2002), which could be an indirect estimation of microbial activity (Gidenne *et al.*, 2010). In fact, Alagón (2013) observed how substitution of the basal diet by 20% of DDGS led to an increase in digestibility of different fibre fractions depending on the DDGS source, mainly related to differences in the nature of the fibre. 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 (such 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 (García *et al.*, 2002). In fact, these differences in ADL can help 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 in 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).

Regarding the caecal VFA profile, it should be highlighted that DDGS inclusion affected the proportion of caecal acetic and propionic acid concentrations, which decreased and increased, respectively. Caecal propionic acid proportion was highly correlated (r=+0.79; *P*=0.011) with the dietary content in highly digestible fibre (NDSF+NDF-ADF). It is well known that caecal proportion of acetic acid is increased when fibre level increases (Gidenne *et al.*, 2010), and that propionic acid proportion is positively correlated with the dietary concentration in uronic acids (García *et al.*, 2002).

As a consequence of the promotion of feed intake in animals given the diets including DDGS at 20%, daily ingestion of DP was also increased (+1.0±0.3 g/d) and, together with the increased DE intake, contributed to the rabbits' improved growth rate. Despite this higher supply of protein, no effect on caecal N-NH_3 concentration and pH of caecum was observed at 42 and 59 d of age. In contrast, animals given Dc_{40} diet, with higher DP content and DP/DE ratio and a 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 proportions both at 42 and 59 d of age. Xiccato *et al.* (2011), comparing diets with a similar range of protein content (169 and 180 g CP/kg DM) but with lower DP/DE ratio (10.5 to 11 g/MJ), did not report 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 caecum could be increased (Villamide *et al.*, 2010), raising 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 an ERE context, with mortality rate exceeding 30% when medicated diets are removed (Casado *et al.*, 2013), these caecal conditions could contribute to increased intestinal health risk (Gutiérrez *et al.*, 2003; Chamorro *et al.*, 2007).

In conclusion, the results of the present work reveal that the inclusion of DDGS up to 20%, regardless of their grain source (barley, wheat or corn), could be an interesting alternative in balanced diets for growing rabbits. DDGS can provide a non negligible amount of energy, protein and fibres to the diet without negative effects on the performance of growing rabbits. However, greater dietary inclusion must be carried out with care to avoid any distortion of the suitably healthy caecal environment.

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