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Additional Information

1 **Nutritive value of distillers dried grains with solubles from barley, corn and wheat for**  
2 **growing rabbits**

3 Alagón G.<sup>1</sup>, Arce O.N.<sup>2</sup>, Martínez-Paredes E.<sup>3</sup>, Ródenas L.<sup>3</sup>, Moya V.J.<sup>3</sup>, Blas E.<sup>3</sup>, Cervera C.<sup>3</sup>,  
4 Pascual J.J.<sup>3</sup>

5 <sup>1</sup>Facultad de Agronomía y Zootecnia, Universidad Nacional de San Antonio Abad del Cusco. Perú.

6 <sup>2</sup>Facultad de Ciencias Agrarias y Veterinarias, Universidad Técnica de Oruro. Bolivia.

7 <sup>3</sup>Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Camino de Vera, 14, Valencia 46071,  
8 Spain.

9

10

## 11 Abstract

12 Due to their fibrous nature, distillers dried grains with solubles (DDGS) from the bioethanol  
13 industry could be considered an interesting feedstuff for rabbit nutrition. To characterize the  
14 DDGS available in the Iberian Peninsula the chemical, amino acid and fatty acid composition of  
15 eight DDGS batches (2, 2 and 4 from barley, corn and wheat grains, respectively) was  
16 performed. Five diets were formulated to determine the nutritive value of DDGS in growing  
17 rabbits by the substitution method: a control diet and four experimental diets containing 200 g  
18 of the DDGS being evaluated  $\text{kg}^{-1}$  dry mater (DM) [DDGS from national barley, national corn,  
19 Brazilian corn and national wheat grains]. The digestibility trial was performed using 60 three-  
20 way crossbred fattening rabbits (12 per diet), aged 42 days with average live weight of 1.49 kg  
21 (S.E.: 0.033 kg). DDGS can be characterized as a raw material really rich in fibre (neutral  
22 detergent fibre and neutral detergent soluble fibre) and crude protein (CP, on av. 352, 208 and  
23 318  $\text{g kg}^{-1}$  DM, respectively). Barley DDGS had higher fibre and lower protein contents than  
24 wheat DDGS (+25 g of acid detergent fibre and  $-91$  g of CP  $\text{kg}^{-1}$  DM, respectively;  $P < 0.05$ ), as  
25 well as the highest ash content (on av. +16  $\text{g kg}^{-1}$  DM;  $P < 0.05$ ). Corn DDGS had intermediate  
26 fibre and protein values between barley and wheat DDGS, but were the richest in ether extract  
27 (on av. +70  $\text{g kg}^{-1}$  DM). DDGS' protein was richer in proline, phenylalanine, valine and  
28 arginine for barley DDGS (107, 55, 54 and 51  $\text{g kg}^{-1}$  CP, respectively), in leucine, alanine and  
29 histidine for corn DDGS (114, 75 and 27  $\text{g kg}^{-1}$  CP, respectively), and in glutamic acid for  
30 wheat DDGS (290  $\text{g kg}^{-1}$  CP). Barley DDGS was richer in saturated (236  $\text{g kg}^{-1}$  total fatty  
31 acids), corn DDGS in monounsaturated (278  $\text{g kg}^{-1}$  total fatty acids) and wheat DDGS in  
32 polyunsaturated fatty acids (615  $\text{g kg}^{-1}$  total fatty acids). Barley DDGS was characterized by the  
33 lowest nutritive value traits of DDGS evaluated (11.9 MJ digestible energy (DE) and 168 g  
34 digestible protein (DP)  $\text{kg}^{-1}$  DM). In spite of higher protein and lower fibre content of the  
35 Brazilian corn (+1.7 g CP and  $-31$  g neutral detergent fibre  $\text{kg}^{-1}$  DM), no significant differences  
36 for the nutritive value of both corn DDGS were observed (on av. 15.3 MJ DE and 208 g DP  $\text{kg}^{-1}$

37 DM). Wheat DDGS might be considered as the DDGS with the highest nutritive value of those  
38 evaluated (15.7 MJ DE and 263 g DP kg<sup>-1</sup> DM).

39

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

42

## 43 **Introduction**

44 Distillers dried grains with solubles (DDGS), the most important by-products of the bioethanol  
45 manufacture industry (0.3 tones per ton processed cereal) composed mostly by mixing distillers  
46 grains (DDG) and solubles (DDS, thin stillage) at a 3:1 ratio (Erickson et al., 2005), are mainly  
47 used for animal nutrition (US Grains Council, 2007). However, DDGS are characterized by a  
48 relatively high variability in their chemical composition and nutritive value. Olantine (1986)  
49 even describes 35 causes for this variability, highlighting grain source (Reese and Lewis, 1989),  
50 technology used in manufacturing processes (Spiehs et al., 2002; Knott et al., 2004; Noll et al.,  
51 2006), efficiency in the ethanol manufacture process (Spiehs et al., 2002; Shurson and  
52 Alghamdi, 2008), mixing ratio of the final components obtained (Noll et al., 2006; Kim et al.,  
53 2008), and drying time and temperature (US Grains Council, 2007).

54 Literature about chemical composition and nutritive value of corn DDGS is extensive.  
55 However, the available knowledge about DDGS from other cereal grains, such as wheat  
56 (Nyachoti et al., 2005; Widyaratne and Zijlstra, 2007; Nuez Ortín and Yu, 2009; Avelar et al.,  
57 2010) and especially barley and sorghum (Waller, 2004), is more limited.

58 De Blas and Mateos (2010), describing the usual range of ingredients composition of feeds for  
59 rabbits in Spain, indicated that rabbit diets usually include even 350 g kg<sup>-1</sup> of cereal co-products  
60 (mainly wheat bran, maize gluten feed and DDGS). Considering the fibrous nature of DDGS  
61 and that their availability has exponentially increased in the last decade (Renewable Fuel  
62 Association, 2012), DDGS inclusion in rabbit diets could have been promoted. However, the  
63 nutritive value of corn DGGs for rabbits has been poorly studied (Villamide et al., 1989), and as  
64 far as the author is aware there is no scientific knowledge about the nutritive value of other  
65 cereal grains DDGS for rabbit, such as those from wheat and barley grains whose production in  
66 rabbit production areas is not negligible.

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

70

71

72 **2. Materials and methods**

73 *2.1. Samples of DDGS*

74 Eight batches of DDGS from the three major bioethanol plants in Spain were used in the  
75 present study. These factories process the cereal grains by dry grind technology, rendering  
76 ethanol and DDGS as product and co-product, respectively (Abengoa, 2011). Batches were  
77 obtained during the last quarter of 2010, differing in their cereal source (both type and origin:  
78 two from national barley grains, one from national and one from Brazilian corn grains, and  
79 three from British and one from national wheat grains). A total of 200 kg, in pellets of  $0.86 \pm$   
80  $0.04$  cm in diameter, were harvested from each DDGS. Representative samples of 1 kg of each  
81 DDGS were milled with a 1-mm sieve and stored for their subsequent chemical, amino acid  
82 and fatty acids analyses.

83 *2.2. Digestibility trial*

84 Four of the eight DDGS analyzed were selected, considering the grain source and within-grain  
85 source composition variability (mainly protein and fibre), for their subsequent nutritive  
86 evaluation by the substitution method. Five diets were formulated from a basal mixture (Table  
87 1). The control diet (C) contained 982.4 g of the basal mixture  $\text{kg}^{-1}$  dry matter (DM) and 17.6 g  
88 of a vitamin–mineral premix  $\text{kg}^{-1}$  DM, and the four experimental diets (D) contained 782.4 g  
89 of the basal mixture  $\text{kg}^{-1}$  DM, 17.6 g of a vitamin–mineral premix  $\text{kg}^{-1}$  DM and 200 g of the  
90 DDGS being evaluated  $\text{kg}^{-1}$  DM [Dnb, DDGS from national barley (nb) grains; Dnc, from  
91 national corn (nc) grains; Dbc, from Brazilian corn (bc) grains; Dnw, from national wheat (nw)  
92 grains]. Feed formulation (basal mixture and DDGS level of inclusion) was performed to avoid  
93 undesirable nutritional deviations from the recommendations for fattening rabbits (Blas and  
94 Mateos, 2010), and following the guidelines for the evaluation of raw materials by the  
95 substitution method described by Villamide et al. (2001).

96 The digestibility trial was performed using sixty three-way crossbred fattening rabbits, aged 42  
97 days with average live weight of 1.49 kg (S.E.: 0.033 kg). Twelve rabbits per diet were

98 randomly housed in metabolic cages of 52 × 44 × 32 cm, and feed and water were offered ad  
99 libitum during the experimental period. Following an adaptation period of 10 days the  
100 consumption control and faeces collection period was 4 days (Pérez *et al.*, 1995). Faeces were  
101 stored in identified sealed plastic bags and frozen at –20°C until analysis. Apparent digestibility  
102 coefficients for DM, CP, gross energy (GE), neutral detergent fibre (aNDFom), acids detergent  
103 fibre (ADFom) and amino acids of diets were determined for each animal as:

$$104 \quad dNutrient = 1 - \frac{Nutrient\ fecal\ excretion}{Nutrient\ intake}$$

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

$$108 \quad DE_{DDGS_x} = \frac{(DE_D - B DE_C)}{D} \quad \text{and} \quad dGE_{DDGS_x} = \frac{DE_{DDGS_x}}{GE_{DDGS_x}}$$

109 where  $DE_D$  is the DE of diet that includes the  $DDGS_x$  being evaluated,  $B$  the substitution rate of  
110 the basal mixture in the diet (0.7824),  $DE_C$  the DE in the control diet and  $D$  the substitution rate  
111 of the  $DDGS_x$  (0.200).

### 112 2.3. Analytical methods

113 DDGS samples, diets and faeces were analyzed according to the methods of AOAC (2000):  
114 934.01 for DM, 942.05 for ash, 976.06 for CP and 920.39 for EE. Previous acid–hydrolysis of  
115 samples was carried out in the analysis of EE. Starch content was determined according to  
116 Batey (1982). The aNDFom (assayed with a thermo–stable amylase and expressed exclusive of  
117 residual ash), ADFom (expressed exclusive of residual ash) and lignin (determined by  
118 solubilisation of cellulose with sulfuric acid, sa) were analyzed sequentially (Van Soest *et al.*,  
119 1991). The NDSF content was determined according to Hall *et al.* (1997), adapting the method  
120 to the nylon filter bag system and with the modifications proposed by Martínez–Vallespín *et al.*  
121 (2011). Insoluble hemicelluloses and cellulose were determined by difference  
122 (aNDFom–ADFom and ADFom–Lignin (sa), respectively). Other fibre fraction (RES) that



123 corresponds to a mix of soluble fibre, that includes that part of pectins not solubilized by the  
124 NDF solution and sugars, was estimated as  $(100 - \text{Ash} - \text{CP} - \text{EE} - \text{aNDFom} - \text{Starch})$  and used in  
125 the multivariate analyses performed with the literature data. Finally, GE was determined by  
126 adiabatic bomb calorimetry (Gallenkamp Autobomb, Loughborough, UK).

127 The content of fatty acid methyl esters and total amino acids were determined in the eight  
128 DDGS, the four experimental diets and the four pools of faeces obtained from each diet during  
129 the digestibility trial. The fatty acid methyl esters of the samples were analyzed in a gas  
130 chromatograph Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless  
131 inlet and flame ionization detector. Separation was performed on a capillary column SPTM  
132 2560 (Supelco, PA, USA) (100m×0.25mm×0.2mm film thickness) with a flow rate of 1.1 mL  
133 Helium min<sup>-1</sup>, according to the following temperature gradient: 140°C initial temperature for 5  
134 min, gradually increasing along a linear gradient of 4°C min<sup>-1</sup> to 240°C, maintaining this  
135 temperature for 30 min, to finally return to the initial conditions. The injector and detector were  
136 maintained at 260°C. Fatty acids were identified by comparing their retention times with those  
137 of a pattern of fatty acid methyl esters (47885-U) from Supelco® (Pennsylvania, USA) and  
138 quantified using C13:0 as internal standard (O'Fallon et al., 2007). Total saturated,  
139 monounsaturated and polyunsaturated fatty acids were calculated as [SFA:  
140 C14:0+C16:0+C17:0+C18:0+C20:0+C22:0, MUFA: C16:1+C18:1w9+C18:1w7+C20:1+  
141 C22:1w9 and PUFA: C18:2+C18:3w6+C18:3w3+C20:2+C22:2, respectively].

142 The amino acid content was determined after acid hydrolysis with HCL 6N at 110 °C for 23 h as  
143 previously described by Liu et al. (1995), using a Waters (Milford, Massachusetts, USA) HPLC  
144 system consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717, Waters), a  
145 fluorescence detector (Mod. 474, Waters) and a temperature control module. Aminobutyric acid  
146 was added as internal standard after hydrolyzation. The amino acids were derivatized with  
147 AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and separated with a C-18  
148 reverse-phase column Waters AcQ Tag (150mm×3.9mm). Methionine and Cystine were

149 determined separately as methionine sulphone and cysteic acid respectively after performic acid  
150 oxidation followed by acid hydrolysis.

#### 151 *2.4. Statistical analysis*

152 Data were analysed according to the general lineal model (GLM) procedure of SAS (Statistical  
153 Analysis System, 2002), as a completely randomised design with a model accounting for the  
154 fixed effect of the DDGS grain source (barley, corn or wheat) for DDGS' chemical composition  
155 data, the experimental diet (C, Dnb, Dnc, Dbc and Dnw) for the performance traits and apparent  
156 digestibility coefficients, or the DDGS type (nb, nc, bc and nw) for the DDGS' nutritive value  
157 data. Multivariate analyses were performed to determine the main parameters involved in the  
158 characterization and differentiation of DDGS. A principal component analysis including the  
159 chemical composition data [Ash, starch, EE, CP, aNDFom, ADFom, Lignin (sa), lysine,  
160 methionine, SFA, MUFA and PUFA] from the eight samples DDGS was done, using the  
161 PRINCOMP procedure of SAS (2002), to decrease information and determine the main  
162 parameters responsible for data variability. Finally, a discriminate analyse of chemical  
163 composition data [DM, ash, CP, EE, NDF, ADF and RES] from the DDGS available in the  
164 literature was performed (a total of 53 samples: 1, 4, 18 and 30 from sorghum, barley, wheat  
165 and corn DDGS, respectively; see Figure 1b), using the DISCRIM procedure of SAS (2002), to  
166 determine the main chemical parameters involved in the discrimination between DDGS by grain  
167 source.

168

169 **Results**

170 *Chemical composition of the DDGS evaluated*

171 Table 3 shows the main chemical composition of the DDGS by grain source. Barley DDGS had  
172 higher fibre and lower protein contents than wheat DDGS (+25 g of ADFom and -91 g of CP  
173 kg<sup>-1</sup> DM, respectively; P<0.05), as well as the highest ash content (on av. +16 g kg<sup>-1</sup> DM;  
174 P<0.05). Corn DDGS had intermediate fibre and protein values between barley and wheat  
175 DDGS, but were the richest in EE (on av. +70 g kg<sup>-1</sup> DM).

176 DDGS' protein was richer in proline, phenylalanine, valine and arginine for barley DDGS (on  
177 av. +22, +10, +7 and +6 g kg<sup>-1</sup> CP, respectively; P<0.05), in leucine, alanine and histidine for  
178 corn DDGS (on av. +44, +33 and +4 g kg<sup>-1</sup> CP, respectively; P<0.05), and in glutamic acid for  
179 wheat DDGS (+107 and +46 g kg<sup>-1</sup> CP respect to corn and barley DDGS, respectively; P<0.05).  
180 Protein of both barley and corn DDGS also had higher content of aspartic acid and threonine  
181 than that of wheat DDGS (on av. +13 and +7 g kg<sup>-1</sup> CP, respectively; P<0.05).

182 In general, fat in DDGS was characterized by a high content in PUFA, especially C18:2 (on av.  
183 578 and 550 g kg<sup>-1</sup> total fatty acids), but grain source had a significant effect on fatty acid  
184 composition of DDGS. Therefore, barley DDGS was richer in SFA (+35 and +79 g kg<sup>-1</sup> total  
185 fatty acids than wheat and corn DDGS, respectively; P<0.05), corn DDGS in MUFA (on av.  
186 +128 g kg<sup>-1</sup> total fatty acids; P<0.05) and wheat DDGS in PUFA (on av. +55 g kg<sup>-1</sup> total fatty  
187 acids; P<0.05). Fat in corn DDGS was characterized for a higher UFA/SFA ratio than barley  
188 and wheat DDGS (4.32 vs. 2.75 and 3.31 g g<sup>-1</sup>, respectively; P<0.05).

189 Graphic representation of the first two principal components obtained from the main chemical  
190 composition of the analyzed DDGS samples (which explained 75.6% of total variability) clearly  
191 placed them according to grain source (Figure 1a). Thus, barley DDGS were placed in the area  
192 characterized for high fibre, ash and SFA contents, wheat DDGS in that with high CP, lignin  
193 (sa) and PUFA, and corn DDGS in that with high EE, starch and MUFA. In fact, the  
194 discriminate analysis by grain source, from the available common chemical composition of  
195 DDGS in the literature, allowed the right classification of 96.2 percent of the samples.

196 Representation of samples by their values for the first two discriminate functions clearly  
197 clustered them by grain source (Figure 1b).

#### 198 *Nutritive value of DDGS*

199 Table 4 shows the daily performance and the apparent digestibility coefficients of the main  
200 nutrients in the experimental diets evaluated from the individual faeces analysis.

201 Inclusion of the national barley DDGS (Dnb) did not significantly affect dDM, dEE and dGE,  
202 but increased both dADF and dCellulose values (+4.0 points of percentage compared to diet C;  
203  $P<0.05$ ). Although no significant differences were observed for dCP variable, contrast between  
204 Dnb and Dnw showed a relatively lower value for Dnb (-3.2 points of percentage), perhaps  
205 related to their higher proportion of CP bound to NDF (0.33 and 0.22 of the total CP for barley  
206 and wheat DDGS, respectively). In fact, Dnb diet had the lower digestibility coefficients for  
207 main of amino acids (significantly lower for alanine, histidine, isoleucine, leucine, lysine,  
208 serine, threonine and valine, respect to diet C).

209 In the case of corn DDGS, animals fed with Dnc had greater dDM, dEE, dGE (+2.7 points of  
210 percentage), daNDFom, dADFom and dCellulose than those obtained for C animals ( $P<0.05$ ),  
211 and although no differences were observed for dCP, digestibility coefficients for alanine and  
212 serine were also significantly higher ( $P<0.05$ ). However, in the case of Dbc, although  
213 digestibility of GE (+2.2 points of percentage) and alanine were also significantly increased  
214 ( $P<0.05$ ), digestibility coefficients of histidine and valine were even reduced respect to C diet  
215 ( $P<0.05$ ).

216 Finally, the inclusion of the national wheat DDGS (Dnw) increased both dDM and dGE values  
217 (+3.0 points of percentage compared to C diet;  $P<0.05$ ), presented the highest values for dCP  
218 and digestibility of main amino acids, significant in the case of glutamic acid, proline and  
219 serine ( $P<0.05$ ).

220 The nutritive value of the evaluated DDGS is shown in Table 5. DDGS from national barley had  
221 lower dDM, dEE and DP values than DDGS from national wheat (-11, -17 percent and -95 g

222 DP kg<sup>-1</sup> DM, respectively; P<0.05), having both DDGS from corn intermediate values. DDGS  
223 from national barley had a significantly lower dEE (on av. -16 points of percentage; P<0.05)  
224 and DE values (on av. -3.56 MJ DE kg<sup>-1</sup> DM; P<0.05) than the values obtained for corn and  
225 wheat DDGS.

226

## 227 **Discussion**

### 228 *Chemical composition of the DDGS evaluated*

229 In general, DDGS can be characterized as a raw material very rich in fibre (aNDFom and  
230 NDSF) and CP (on av. 352, 208 and 318 g kg<sup>-1</sup> DM, respectively). These values, initially place  
231 DDGS as an interesting raw material for rabbit nutrition compared to other monogastric species  
232 for its high fibre content, especially soluble fibre with attributed properties for gut health in  
233 weaned rabbits (Gómez-Conde et al., 2007; Martínez-Vallespín et al., 2011), although its high  
234 protein content means caution must be exercised when including it in these diets (Carabaño et  
235 al., 2009).

236 The principal differences between the analyzed DDGS seem to be mainly related to the  
237 differences in the chemical composition of the original grain sources (De Blas et al., 2010),  
238 especially in terms of fibre and protein content, although no great differences compared to the  
239 values given by the literature were found. Therefore, ADF and CP values obtained for barley  
240 DDGS were similar to those previously reported (on av. 131 and 286 g kg<sup>-1</sup> DM, respectively;  
241 De Blas and Mateos, 2010; Waller et al., 2010). However for wheat DDGS, the obtained values  
242 were clearly lower in ADF (on av. 104 vs. 150 g kg<sup>-1</sup> DM) and CP (353 vs. 384 g kg<sup>-1</sup> DM) than  
243 the averages reported in recent literature (Distillers Grains Technology Council, 2005; Nyachoti  
244 et al., 2005; Widyaratne and Zijlstra, 2007; Emiola et al., 2009; Nuez-Ortín et al., 2009; Avelar  
245 et al., 2010; Cozannet et al., 2010; De Blas et al., 2010), although similar to those presented by  
246 Emiola et al. (101 and 344 g kg<sup>-1</sup> DM, respectively) in 2009. Ethanol industries have improved  
247 their efficiency over time (better efficacy in the starch extraction process, the level of inclusion  
248 and recovery of the yeast used in the fermentation...) which could contribute to the observed  
249 variability in DDGS composition, especially in protein content.

250 In fact, the EE value obtained for the corn DDGS was relatively higher than the average given  
251 by the literature (141 vs. 107 g kg<sup>-1</sup> DM), although within the range (58 to 165 g kg<sup>-1</sup> DM;  
252 Villamide et al., 1989; Cromwell et al., 1993; NRC, 1998; Spiels et al., 2002; Sauvant et al.,  
253 2004; Stein, 2006; US Grains Council, 2007; Belyea et al., 2004; Shurson et al., 2004;

254 Widyaratne and Zijlstra, 2007; Nuez-Ortin et al., 2009; De Blas et al., 2010). These differences  
255 in EE content could be related to differences in the laboratory analysis method (use or not of  
256 acid hydrolysis; AOAC, 2000), and/or to the level of inclusion of solubles during manufacture  
257 of the DDGS that some authors have related to EE content increase (Noll et al., 2007; Ganesan  
258 et al., 2008).

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

271 Finally, DGGS had a relative high fat content, especially in corn DDGS (from 70 in barley and  
272 wheat DDGS to 141g kg<sup>-1</sup> in corn DDGS). Fat from corn DDGS was characterized for a higher  
273 UFA/SFA ratio than barley and wheat DDGS (31 vs. 17 and 18 g g<sup>-1</sup>, respectively; P<0.05),  
274 which in addition to its higher EE content may lead to higher oxidation and rancidity potential  
275 (Cromwell et al., 2011).

#### 276 *Nutritive value of DDGS*

277 As was expected, barley DDGS was characterized by the lowest nutritive value traits of  
278 evaluated DDGS (11.9 MJ DE and 168 g DP kg<sup>-1</sup> DM), although comparable to other  
279 commonly used cereal by-products such as corn gluten feed (on av. 11.6 MJ DE, 162 g DP and  
280 406 g NDF kg<sup>-1</sup> DM; De Blas et al, 2010). The main consequence of including DDGS was the

281 increase in soluble fibre content and cellulose digestibility of the experimental diet. Soluble  
282 fibre has been frequently related with a general promotion of caecal fermentative activity  
283 (including cellulase), increasing cellulose digestibility (Falçao-Cunha et al., 2004). In addition,  
284 and in spite of the difficulties in the determination of fiber digestibility of cereals in rabbits,  
285 Villamide and De Blas (1989), with diets including up to 60 percent of cereal grain, a high  
286 dADF for barley grain has been described (0.30).

287 In spite of the higher protein and lower fibre content of the Brazilian corn (+1.7 g CP and -31 g  
288 aNDFom kg<sup>-1</sup> DM), no significant differences for the nutritive value between both corn DDGS  
289 were observed (on av. 15.3 MJ DE and 208 g DP kg<sup>-1</sup> DM). The high energy content of corn  
290 DDGS, even greater than that reported for corn grain (14.6 MJ DE kg<sup>-1</sup> DM; Villamide et al.,  
291 2010), seems to be mainly related to its high fat content (141 g EE kg<sup>-1</sup> DM), rich in MUFA  
292 (28% of total fatty acids), and highly digestible (on av. 0.93). In general, these values were  
293 slightly higher than those reported for rabbits (14.1 MJ DE and 198 g DP kg<sup>-1</sup> DM; Villamide et  
294 al., 2010), but close to those usually given for extra quality corn DDGS (15.7 MJ DE and 220 g  
295 DP kg<sup>-1</sup> DM). Literature values for DE were closer to those reported for the Brazilian corn  
296 DDGS. The inclusion of national corn DDGS led to higher digestibility of the fibre (compared  
297 to both C and Dbc diets), which could contribute to explain this higher value.

298 On the other hand, wheat DDGS might be considered as the DDGS with the highest nutritive  
299 value of those evaluated (15.7 MJ DE and 263 g DP kg<sup>-1</sup> DM), placing them close to some oil  
300 meals (such as rapeseed meal, 14.4 MJ DE and 273 g DP kg<sup>-1</sup> DM) and legume seeds (such as  
301 Australian lupin, 15.2 MJ DE and 274 g DP kg<sup>-1</sup> DM). The protein value obtained agrees with  
302 the report for the Spanish wheat DDGS (262 g PD kg<sup>-1</sup> DM; De Blas et al., 2010), however the  
303 energy value was higher than expected (13.2 MJ DE kg<sup>-1</sup> DM). This discrepancy can be  
304 partially explained by the high EE content of the evaluated wheat DDGS (76 g kg<sup>-1</sup> DM, and  
305 very digestible 0.915), which usually ranges from 32 to 67 g kg<sup>-1</sup> DM (Widyaratne and Zijlstra,  
306 2007 ; Nuez-Ortín et al., 2010), as well as by its higher dCP to that reported for wheat DDGS in  
307 rabbits (0.75 vs 0.71; De Blas et al., 2010).



308 Finally, some authors have described (Stein et al., 2006; Pahl et al., 2008) the possible  
309 negative effect of heat treatment performed during DDGS manufacture on amino acids  
310 digestibility (especially on lysine) as the main limitation for the use of DDGS in pigs. Faecal  
311 digestibility of the main amino acids in the diets including corn and wheat DDGS was similar to  
312 that observed in the control diet, even higher for some amino acids, with only the diet with  
313 barley DDGS showing a generalized decrease in amino acids availability. Pahl et al. (2008),  
314 comparing corn DDGS with great differences in heat damage, suggested that the Lys:CP ratio  
315 can be considered an indicator of heat damage, with undamaged corn DDGS being considered  
316 those with a ratio higher than 0.029 (as in the present 0.031). On the other hand, the end result  
317 of the bacterial activity in the rabbit caecum leads to a substantial change in the amino acid  
318 composition of the protein that enters the caecum, in turn this may lead to an enrichment in  
319 lysine, methionine and threonine, comparing the total faecal excretion hard and soft (Garcia et  
320 al., 2005). No information is available about the digestibility coefficients of the main amino  
321 acids for DDGS in rabbits. The literature values of apparent faecal digestibility for lysine,  
322 methionine and threonine in rabbit diets given for protein concentrates, cereals and cereal  
323 products (Garcia et al, 2005; Llorente et al., 2006, 2007) did not show any large variations from  
324 those found in this study.

325 From the results of this paper it can be concluded that, DDGS could be considered as interesting  
326 raw materials due to their high content of digestible protein and energy, comparable to other  
327 sources of protein frequently used in rabbit nutrition. On the other hand, their high fibre content  
328 (on av. 570 g of total fibre kg<sup>-1</sup> DM) makes this by-product especially interesting for rabbit  
329 nutrition compared to other monogastric species. The inclusion of corn and wheat DDGS in  
330 rabbit diets did not negatively affect the digestibility of the main amino acids, as has been  
331 observed in other species when DDGS were heat-damaged. However, their protein could be  
332 considered as relatively poor in the most limiting amino acids for rabbit diets, and a  
333 supplementation of synthetic amino acids could perhaps be required if DDGS are included at  
334 high level.

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**Table 1.** Ingredients (g/kg) of the basal diet (C) and the experimental diets (D) including the distillers dried grains with solubles (DDGS) evaluated.

	C	D
<i>DDGS evaluated</i>	-	200
<i>Basal mixture</i>	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
<i>Vitamin-mineral premix</i>	17.6	17.6
Calcium carbonate	4.6	4.6
Sodium chloride	5	5
Vitamin-micromineral mixture	5	5
Antibiotics <sup>3</sup>	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 hydroxylanisole and ethoxyquin mixture: 4 mg.

Antibiotics: Dinco-spectim (29 ppm dincomicina + 29 ppm spectinomycin), 120 ppm neomicin, Apsamix Tiamulin (50 ppm tiamulin), normally used in rabbit farms with high incidence of mucoid enteropathy (ME).

**Table 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
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 aNDFom	22	32	28	26	38
Neutral detergent fibre, aNDFom	397	392	393	365	379
Acid detergent fibre, ADFom	208	196	186	174	184
Lignin (sa)	58	48	48	44	51
Insoluble hemicelluloses, aNDFom–ADFom	189	196	208	191	195
Celluloses, ADFom–Lignin (sa)	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
<i>Amino acid composition</i>					
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
<i>Fatty acid composition</i>					
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
C18:1 $\omega$ 7	0.6	0.6	0.7	0.8	0.6
C18:1 $\omega$ 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.

**Table 3.** Chemical, amino acid and fatty acids composition of the distillers dried grains with solubles (DDGS) samples evaluated (g/kg DM) by grain source.

Grain source	DDGS				P-value
	Barley	Corn	Wheat	SEM <sup>a</sup>	
No. of samples	2	2	4		
<i>Chemical composition (g/kg DM):</i>					
Dry matter, DM	918	930	917	3.1	0.262
Ash	61 <sup>b</sup>	45 <sup>a</sup>	46 <sup>a</sup>	1.4	0.012
Ether extract	72 <sup>a</sup>	141 <sup>b</sup>	67 <sup>a</sup>	0.9	<0.001
Starch	9 <sup>a</sup>	25 <sup>b</sup>	14 <sup>ab</sup>	1.4	0.018
Crude protein, CP	262 <sup>a</sup>	305 <sup>b</sup>	353 <sup>c</sup>	2.7	<0.001
CP bound to aNDFom	86	100	76		0.267
Neutral detergent fibre, aNDFom	401 <sup>b</sup>	374 <sup>b</sup>	317 <sup>a</sup>	9.4	0.024
Acid detergent fibre, ADFom	129 <sup>b</sup>	116 <sup>ab</sup>	104 <sup>a</sup>	3.3	0.057
Lignin (sa)	9	10	19	1.9	0.123
Insoluble hemicelluloses, aNDFom–ADFom	273 <sup>b</sup>	257 <sup>ab</sup>	213 <sup>a</sup>	8.7	0.057
Celluloses, ADFom–Lignin(sa)	119 <sup>b</sup>	106 <sup>b</sup>	85 <sup>a</sup>	2.3	0.003
Neutral detergent soluble fibre	210	217	203	19.6	0.947
<i>Amino acids (g/kg of crude protein):</i>					
Alanine	45 <sup>a</sup>	75 <sup>b</sup>	39 <sup>a</sup>	1.0	<0.001
Arginine	51 <sup>b</sup>	43 <sup>a</sup>	46 <sup>a</sup>	0.7	0.022
Aspartic acid	66 <sup>b</sup>	68 <sup>b</sup>	54 <sup>a</sup>	1.0	0.002
Cysteine	16	16	20	1.5	0.489
Glutamic acid	244 <sup>b</sup>	183 <sup>a</sup>	290 <sup>c</sup>	2.9	<0.001
Glycine	47	43	45	0.8	0.255
Histidine	23 <sup>a</sup>	27 <sup>b</sup>	22 <sup>a</sup>	0.5	0.007
Isoleucine	39 <sup>b</sup>	36 <sup>ab</sup>	35 <sup>a</sup>	0.4	0.048
Leucine	74 <sup>a</sup>	114 <sup>b</sup>	66 <sup>a</sup>	2.3	<0.001
Lysine	31	31	24	1.6	0.193
Metionine	15	18	15	1.5	0.718
Phenylalanine	55 <sup>b</sup>	47 <sup>a</sup>	44 <sup>a</sup>	1.4	0.035
Proline	107 <sup>c</sup>	78 <sup>a</sup>	92 <sup>b</sup>	1.2	<0.001
Serine	50	52	50	0.4	0.173
Threonine	39 <sup>b</sup>	40 <sup>b</sup>	33 <sup>a</sup>	0.4	<0.001
Tyrosine	25 <sup>a</sup>	32 <sup>b</sup>	24 <sup>a</sup>	0.7	0.014
Valine	54 <sup>c</sup>	49 <sup>b</sup>	45 <sup>a</sup>	0.5	0.002
<i>Main fatty acids (g/kg total fatty acids):</i>					
C16:0	236 <sup>c</sup>	160 <sup>a</sup>	211 <sup>b</sup>	3.5	0.001
C18:0	22 <sup>b</sup>	22 <sup>b</sup>	16 <sup>a</sup>	1.0	0.038
C18:1 ω9	131 <sup>a</sup>	260 <sup>b</sup>	132 <sup>a</sup>	6.3	<0.001
C18:1 ω7	8 <sup>a</sup>	12 <sup>c</sup>	11 <sup>b</sup>	0.1	<0.001
C18:2	548 <sup>ab</sup>	522 <sup>a</sup>	581 <sup>b</sup>	8.4	0.065
C18:3 ω3	36 <sup>b</sup>	10 <sup>a</sup>	32 <sup>b</sup>	1.1	<0.001
SFA	267 <sup>c</sup>	188 <sup>a</sup>	232 <sup>b</sup>	4.4	0.003
MUFA	148 <sup>a</sup>	278 <sup>b</sup>	152 <sup>a</sup>	6.4	<0.001
PUFA	586 <sup>ab</sup>	534 <sup>a</sup>	615 <sup>b</sup>	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+C18:1w9+C18:1w7+C20:1+C22:1w9]; PUFA, polyunsaturated fatty acids [C18:2+C18:3w6+C18:3w3+C20:2+C22:2]. <sup>a,b,c</sup> Means not sharing the same superscript were significantly different at P<0.05.

**Table 4.** Daily feed intake, growth rate 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					SEM	P-value
	C	Dnb	Dnc	Dbc	Dnw		
No. of animals	12	12	12	12	12		
Feed intake (g/d)	141	144	133	135	139	4	0.8753
Growth rate (g/d)	53.0	54.0	50.4	52.6	55.3	1.6	0.9053
<i>Apparent digestibility coefficients<sup>2</sup>:</i>							
dDM	0.582 <sup>a</sup>	0.583 <sup>ab</sup>	0.597 <sup>bc</sup>	0.592 <sup>abc</sup>	0.600 <sup>c</sup>	0.002	0.0468
dCP	0.718	0.703	0.719	0.711	0.735	0.005	0.2262
dEE	0.879 <sup>ab</sup>	0.875 <sup>a</sup>	0.895 <sup>c</sup>	0.893 <sup>bc</sup>	0.882 <sup>abc</sup>	0.006	0.0482
dGE	0.579 <sup>a</sup>	0.582 <sup>ab</sup>	0.606 <sup>c</sup>	0.598 <sup>bc</sup>	0.609 <sup>c</sup>	0.003	0.0015
daNDFom	0.244 <sup>ab</sup>	0.263 <sup>b</sup>	0.295 <sup>c</sup>	0.234 <sup>a</sup>	0.264 <sup>b</sup>	0.005	0.0001
dADFom	0.094 <sup>a</sup>	0.134 <sup>b</sup>	0.136 <sup>b</sup>	0.083 <sup>a</sup>	0.090 <sup>a</sup>	0.006	0.0020
dHemicelluloses	0.409 <sup>bc</sup>	0.393 <sup>ab</sup>	0.437 <sup>c</sup>	0.371 <sup>a</sup>	0.428 <sup>c</sup>	0.006	0.0008
dCellulose	0.140 <sup>a</sup>	0.181 <sup>b</sup>	0.183 <sup>b</sup>	0.131 <sup>a</sup>	0.135 <sup>a</sup>	0.006	0.0017
dAlanine	0.712 <sup>b</sup>	0.686 <sup>a</sup>	0.771 <sup>c</sup>	0.765 <sup>c</sup>	0.729 <sup>b</sup>	0.010	0.0001
dArginine	0.778	0.743	0.773	0.769	0.767	0.013	0.3104
dAspartic acid	0.661	0.586	0.658	0.634	0.629	0.015	0.0029
dCysteine	0.512	0.589	0.633	0.564	0.662	0.034	0.1271
dGlutamic acid	0.809 <sup>ab</sup>	0.802 <sup>ab</sup>	0.816 <sup>b</sup>	0.795 <sup>a</sup>	0.863 <sup>c</sup>	0.007	0.0001
dGlycine	0.667	0.646	0.682	0.668	0.672	0.013	0.3551
dHistidine	0.843 <sup>b</sup>	0.814 <sup>a</sup>	0.832 <sup>ab</sup>	0.812 <sup>a</sup>	0.845 <sup>b</sup>	0.007	0.0007
dIsoleucine	0.748 <sup>bc</sup>	0.711 <sup>a</sup>	0.750 <sup>bc</sup>	0.735 <sup>ab</sup>	0.764 <sup>c</sup>	0.011	0.0092
dLeucine	0.745 <sup>b</sup>	0.714 <sup>a</sup>	0.763 <sup>b</sup>	0.753 <sup>b</sup>	0.770 <sup>b</sup>	0.009	0.0006
dLysine	0.837 <sup>c</sup>	0.778 <sup>a</sup>	0.819 <sup>bc</sup>	0.808 <sup>b</sup>	0.817 <sup>bc</sup>	0.007	0.0001
dMethionine	0.702	0.746	0.771	0.739	0.771	0.030	0.4337
dPhenylalanine	0.780	0.751	0.778	0.763	0.776	0.007	0.1262
dProline	0.771 <sup>a</sup>	0.764 <sup>a</sup>	0.767 <sup>a</sup>	0.765 <sup>a</sup>	0.812 <sup>b</sup>	0.007	0.0005
dSerine	0.718 <sup>b</sup>	0.704 <sup>a</sup>	0.760 <sup>c</sup>	0.737 <sup>b</sup>	0.756 <sup>c</sup>	0.008	0.0001
dThreonine	0.832 <sup>b</sup>	0.796 <sup>a</sup>	0.833 <sup>b</sup>	0.820 <sup>b</sup>	0.816 <sup>b</sup>	0.006	0.0005
dTyrosine	0.741 <sup>ab</sup>	0.725 <sup>a</sup>	0.773 <sup>b</sup>	0.749 <sup>ab</sup>	0.745 <sup>ab</sup>	0.009	0.0566
dValine	0.723 <sup>b</sup>	0.680 <sup>a</sup>	0.719 <sup>b</sup>	0.698 <sup>a</sup>	0.727 <sup>b</sup>	0.010	0.0072
dSulphur	0.619	0.680	0.710	0.661	0.719	0.035	0.2555

SEM: standard error of the means.

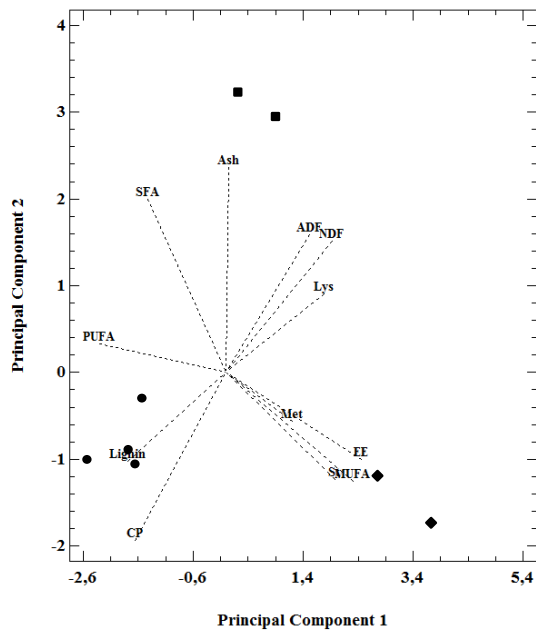
d, apparent digestibility coefficient; DM, dry matter; CP, crude protein; GE, gross energy; aNDFom, neutral detergent fibre; ADFom, Acid detergent fibre; Hemicelluloses, aNDFom-ADFom; Celluloses, ADFom-Lignin (sa).

**Table 5.** Apparent digestibility coefficients (d) of dry matter (DM), crude protein (CP), ether extract (EE) 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.

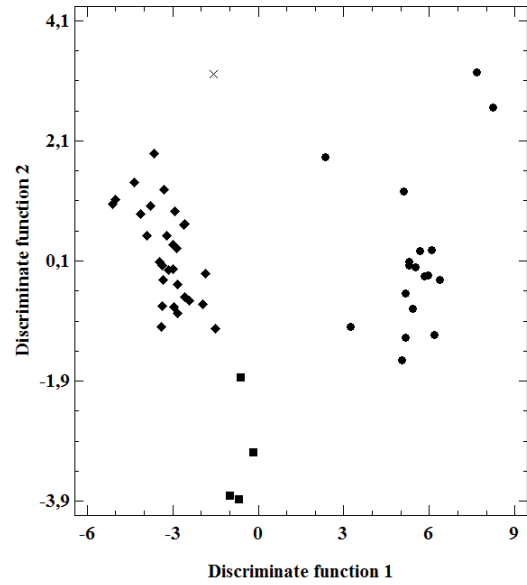
	DDGS				SEM	P-value
	National barley	National Corn	Brazilian corn	National wheat		
dDM	0.647 <sup>b</sup>	0.722 <sup>ab</sup>	0.684 <sup>ab</sup>	0.754 <sup>a</sup>	0.014	0.0547
dCP	0.635	0.656	0.704	0.748	0.016	0.0827
dEE	0.767 <sup>a</sup>	0.921 <sup>b</sup>	0.945 <sup>b</sup>	0.915 <sup>b</sup>	0.014	0.0001
dGE	0.582 <sup>c</sup>	0.718 <sup>ab</sup>	0.653 <sup>cb</sup>	0.750 <sup>a</sup>	0.014	0.0006
DP, g/kg DM	168 <sup>a</sup>	195 <sup>ab</sup>	221 <sup>b</sup>	263 <sup>c</sup>	5	0.0001
DE, MJ/kg DM	11.87 <sup>a</sup>	15.89 <sup>b</sup>	14.72 <sup>b</sup>	15.69 <sup>b</sup>	0.30	0.0001

SEM: standard error of the means.

<sup>a,b,c</sup> Means not sharing the same superscript were significantly different at P<0.05.



(a)



(b)

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521 **Figure 1.** (a) Distribution of the evaluated distillers dried grains with solubles (DDGS) by grain  
 522 source [■ barley, ◆ corn, ● wheat and × sorghum] in the first two principal components  
 523 obtained from their main chemical composition [Ash; S, starch; EE, ether extract; CP, crude  
 524 protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; Lignin; Lys, Lysine; Met,  
 525 methionine; SFA: saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA,  
 526 polyunsaturated fatty acids]. (b) Distribution of DDGS described in the literature by grain  
 527 source in the first two discriminate functions from their main available chemical composition  
 528 [dry matter; ash, CP, EE, NDF, ADF and RES (as 100–Ash–CP–EE–aNDFom–Starch)].

529 Literature DDGS sample sources: Villamide et al., 1989; Cromwell et al., 1993; NRC, 1998; Spiels et al., 2002;  
 530 Sauvant et al., 2004; Shurson et al., 2004; Distillers Grains Technology Council, 2005; Nyachoti et al., 2005; Stein et  
 531 al., 2006; Widyaratne and Zijlstra, 2006; US Grains Council, 2007; Widyaratne and Zijlstra, 2007; Babcock et al.,  
 532 2008; Emiola et al., 2009; Nuez-Ortin et al., 2009; Avelar et al., 2010; Cozannet et al., 2010; De Blas et al., 2010;  
 533 Waller, 2004; present study.

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