

# Nutrient content and digestibility of different batches of wheat distillers dried grains with solubles for laying hens

by Whiting, I.M., Rose, S.P., Mackenzie, A.M., Amerah, A.M. and Pirgozliev, V.

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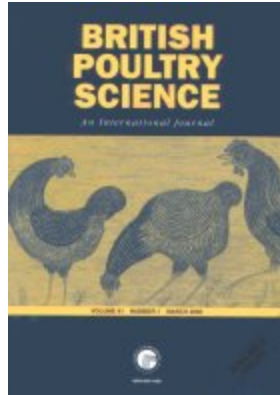
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3 **1 Nutrient content and digestibility of different batches of wheat distillers dried grains**  
4 **2 with solubles for laying hens**

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7 3 I. M. Whiting<sup>1</sup>, S. P. Rose<sup>1</sup>, A. M. Amerah<sup>2</sup>, A. M. Mackenzie<sup>1</sup>, V. R. Pirgozliev<sup>1</sup>  
8  
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10  
11 5 <sup>1</sup>*Harper Adams University, Shropshire, UK*

12  
13 6 <sup>2</sup>*Danisco Animal Nutrition, Marlborough, Wiltshire, UK*  
14  
15  
16 7

17  
18 8 *Corresponding author: Isobel Whiting*

19  
20  
21 9 *Harper Adams University*

22  
23  
24 10 [iwhiting@harper-adams.ac.uk](mailto:iwhiting@harper-adams.ac.uk)

25  
26 11 *Tel: 01952815139*  
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30  
31 13 **ABSTRACT**

32  
33  
34 14 1. Four batches of wheat distillers dried grains with solubles (DDGS) produced by a single  
35 15 production plant were used to investigate variation in digestible energy (DE) and nutrient  
36 16 digestibility for laying hens.

37  
38  
39 17 2. A total of 144 Hy-Line Brown laying hens were allocated to eight treatment groups in  
40 18 replicates of six. Experimental diets were prepared by replacing the basal feed with either 150  
41 19 g/kg or 300 g/kg of each batch of DDGS.

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43  
44 20 3. Chemical analysis of the DDGS showed variation between the different batches. Largest  
45 21 coefficients of variation were observed for starch (0.546) and total soluble non-starch  
46 22 polysaccharides (NSP; 0.276).

47  
48  
49 23 4. Digestible energy and the nutrient digestibility of each diet was measured using the ileal  
50 24 collection technique. Data were statistically analysed as a blocked 2 x 4 factorial design  
51 25 analysis of variance (ANOVA).

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53  
54 26 5. Variability between the different diets were observed for digestible energy and the  
55 27 digestibility of certain nutrients (P<0.05).

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60 Accepted for publication 11 May 2019

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3 28 6. The observed differences in energy utilisation and nutrient digestibility in laying hens  
4 29 suggested that the feeding quality of diets containing different wheat DDGS batches produced  
5 30 by a single production plant may still have large variation.  
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10  
11 32 **Key words:** Wheat DDGS, amino acids, nutrient digestibility, digestible energy, laying hens  
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## 18 19 35 INTRODUCTION

20  
21  
22 36 In the UK, wheat is the primary cereal crop used to produce ethanol. The main by-product from  
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24 37 this industry is wheat distillers dried grains with solubles (DDGS; Smith *et al.* 2006). The  
25  
26 38 conversion of starch to ethanol during processing results in DDGS containing up to three times  
27  
28 39 the concentration of some nutrients compared with the original grain (Świątkiewicz and  
29  
30 40 Koreleski, 2008). The use of DDGS in feeds for monogastrics, particularly poultry, is limited  
31  
32 41 due to its relatively high level of fibre (Nyachoti *et al.* 2005; Thacker and Widyaratne, 2007),  
33  
34 42 as poultry lack the digestive enzymes required to breakdown fibrous feed components (Bedford  
35  
36 43 and Schulze, 1998). Other issues related to the feeding quality of DDGS are mainly associated  
37  
38 44 with processing parameters. Nutrient availability of DDGS has been shown to vary  
39  
40 45 considerably between batches produced by different production sites (Belyea *et al.* 2004; Liu,  
41  
42 46 2011; Whiting *et al.* 2016; Pirgozliev *et al.* 2018). It has been suggested that some of the  
43  
44 47 variability is caused by differences in the blended proportions of wet distillers' grains (WDG)  
45  
46 48 and condensed distillers with solubles (CDS) (Belyea *et al.* 2010; Han and Liu, 2010). Maillard  
47  
48 49 reactions may occur as a result of high temperatures used during cooking and drying, with  
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50 50 lysine being particularly susceptible to heat damage (Smith *et al.* 2006). Residual starch may  
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52 51 pose a problem, as not all of the starch is converted into its constituent sugars during ethanol  
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3 52 production, and this unconverted starch will likely be in the form of indigestible resistant starch  
4  
5 53 (RS) (Sharma *et al.* 2010).  
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8  
9 54 It has been reported recently that a storage of DDGS may affect its feeding value for broilers  
10  
11 55 (Whiting *et al.* 2017). In addition to processing differences, the chemical composition of cereal  
12  
13 56 grains is variable (Pirgozliev *et al.* 2003; Azhar *et al.* 2019), thus the nutritive value of DDGS  
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15 57 produced will be inconsistent.  
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18  
19 58 The aim of the present experiment was to investigate the effect of feeding four different batches  
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21 59 of wheat DDGS produced by a single production site (Ensus Limited, UK) to laying hens when  
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23 60 incorporated at two inclusion levels.  
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## 28 29 62 **MATERIALS AND METHODS**

### 30 31 63 ***Wheat DDGS***

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33  
34 64 Four batches of wheat DDGS used in the study were produced by a single manufacturer  
35  
36 65 (ENSUS Bio refinery, Wilton, UK). Batches 1, 2 and 3 were manufactured in late 2012, early  
37  
38 66 2013 and were obtained from S.C. Feeds Ltd, Staffordshire. The fourth batch was manufactured  
39  
40 67 in January 2012 and was obtained from Target Feeds Ltd, Shropshire. All batches were stored  
41  
42 68 in bags at ambient air temperatures in a dry store until the study commenced in February 2013.  
43  
44 69 A representative sample was taken from each of the four batches and the major chemical  
45  
46 70 components were measured. Analyses were carried out in duplicate and are reported on a dry  
47  
48 71 matter basis.

### 48 49 72 ***Proximate analysis***

50  
51 73 Analyses were carried out separately on the basal feed and DDGS prior to mixing. To determine  
52  
53 74 the correct chemical composition of each experimental treatment, individual diets were  
54  
55 75 recalculated from the analysis of the basal feed and DDGS. Dry matter (DM) of the basal feed  
56  
57 76 and DDGS was determined by drying samples in a forced air oven for 48 hours at 100°C  
58  
59 77 (AOAC Official Method 934.01, 2006). Ash of the basal feed and DDGS was measured in a  
60  
78 78 muffle furnace at 500°C for 24 hours (AOAC Official Method 942.05, 2005). Crude protein

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3 79 (CP) calculated as  $N \times 6.25$  of the basal feed, DDGS and digesta was determined by the  
4 80 combustion method (AOAC Official Method 990.03, 2006) using Leco (FP-528 N; Leco Corp.,  
5 81 St. Joseph, MI) with EDTA as a standard (Sweeney, 1989). Oil (as ether extract) in the basal  
6 82 feed, DDGS and digesta was extracted with petroleum ether using a Soxtec Avanti 2050, Foss  
7 83 UK Ltd (AOAC Official Method 945.16, 2005). Gross energy (GE) of the basal feed, DDGS  
8 84 and digesta was measured using an adiabatic bomb calorimeter (Parr 6200 Instrument  
9 85 Company, Moline, IL, 61265, USA; FAO, 2003). Neutral detergent fibre (NDF) in the basal  
10 86 feed and DDGS was analysed according to Van Soest *et al.* (1991), using an FT 122 Fibertec™  
11 87 hot extraction unit (200-230V). Titanium dioxide (TiO<sub>2</sub>) in diets and digesta was determined  
12 88 using the method developed by Short *et al.* (1996). Total starch in the DDGS was determined  
13 89 by a modified version of Englyst *et al.* (2000) and non-starch polysaccharides (NSP) in the  
14 90 DDGS was determined by the method of Englyst *et al.* (1994). Amino acids (AA) in the basal  
15 91 feed, DDGS and digesta were determined by SSNIFF Spezialdiäten GmbH (Soest, Germany)  
16 92 according to the EC directives 2000/45/EC for tryptophan (OJEC, 2000), and EC/98/64 (L  
17 93 257/16) for the rest of the amino acids (OJEC, 1998). Colour scores of each DDGS sample  
18 94 were measured using an L\*a\*b\* colour space (Konica Minolta, Chroma Meter CR-400). L\*  
19 95 indicated lightness, while a\* and b\* were the chromaticity coordinates. The bulk density of  
20 96 each of the DDGS samples was measured using a chondrometer, a vessel with a known volume,  
21 97 providing constant conditions (Farm-Tec, Scunthorpe, UK).

### 98 ***Diet formulation***

99 Eight diets in total were used in this experiment. A wheat-soybean meal basal diet containing  
100 11.89 MJ/kg AME and 172.5 g/kg crude protein was prepared (Table 1). Four diets containing  
101 850 g/kg of the basal feed were mixed with 150 g/kg of each of the four DDGS batches.  
102 Similarly, the remaining four diets contained 700 g/kg of the basal feed and 300 g/kg of the  
103 respective DDGS batches. The diets did not contain any coccidiostat, antimicrobial growth  
104 promoters, prophylactic or other similar additives. All diets were fed as mash with 5 g/kg TiO<sub>2</sub>  
105 added on top as an indigestible marker. The birds were fed a single proprietary layers feed,  
106 until 22 weeks of age, when approximately 90% egg production was reached. The diets were  
107 administered for eight days with six replicate cages of birds per treatment.

108

109 Table 1 here

110

**111 *Birds and housing***

112 The study was approved by the Animal Experimental Committee of Harper Adams University.  
113 A total of 144, sixteen-week old Hy-Line Brown laying hens were obtained from a commercial  
114 supplier (Country Fresh Pullets Ltd, Oswestry, Shropshire). The birds were randomly allocated  
115 to 48 layer cages (over three tiers) in groups of three. Each cage was equipped with a separate  
116 feeder at the front and two nipple drinkers inside. Cage dimensions were 45 cm x 40 cm x 50  
117 cm and consisted of a wire mesh flooring which contained no bedding material. Temperature  
118 was maintained at 21°C and relative humidity was between 50 and 70%. The birds had *ad*  
119 *libitum* access to feed and water. Lighting was set to give 10 hours day length upon arrival and,  
120 at 20 weeks of age, lighting was increased by 30 minutes each week and 12 hours light was  
121 given each 24 hours between 23 and 24 weeks of age. On the final day of the study the birds  
122 were killed by cervical dislocation and digesta were collected from the distal ileum and pooled  
123 into one pot per cage. Digesta were immediately frozen at -20°C for 24 hours and then freeze  
124 dried. An indigestible marker technique was used to determine digestible energy (DE) and  
125 nutrient digestibility from the ileal digesta.

**126 *Statistical analysis***

127 The experiment was conducted using a randomised block design. To determine digestibility  
128 coefficients, data for this study was analysed as a blocked 2 x 4 factorial design analysis of  
129 variance (ANOVA) using Genstat 17th edition (Lawes Agricultural Trust, VSN International  
130 Ltd, Oxford, UK). The main effects analysed were related to the effects of the different DDGS  
131 batches and the two inclusion rates. In all instances, differences were reported as significant at  
132 95% confidence limits ( $P < 0.05$ ). Duncan's multiple range test was used to determine  
133 significant differences between the four DDGS samples.

134

**135 RESULTS****136 *Chemical characteristics of the studied DDGS*****137 *Proximate analysis***

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2  
3 138 The chemical compositions of the wheat DDGS samples were variable for each criterion (Table  
4 139 2). The DM content ranged from 843 to 900 g/kg with batch 4 containing ~6% less DM than  
5 140 the mean of the other DDGS samples. Gross energy ranged from 21.16 (batch 4) to 21.78 MJ/kg  
6 141 DM (batch 1) . Batch 4 contained ~10% less protein than the mean of the other DDGS samples.  
7 142 Oil concentration ranged from 41.9 (batch 2) to 61.2 g/kg DM (batch 4). The content of NDF  
8 143 ranged from 442.2 (batch 4) to 493.3 g/kg DM (batch 1). Ash content ranged from 53.1 (batch  
9 144 3) to 58.2 g/kg (batch 2). Bulk density ranged from 37.0 (batch 1) to 54.9 kg/hl (batch 4), while  
10 145 lightness from the colour scores ranged from 36.4 (batch 2) to 43.8 (batch 4). The greatest  
11 146 coefficients of variation (CV) were observed for oil (0.157) and bulk density (0.176).

147

148 Table 2 here

149

### 150 *Amino acid composition*

151 The AA composition of the studied DDGS batches are presented in Table 3. Among the  
152 indispensable AA (IAA), mean methionine (5.3 g/kg DM) and lysine (7.0 g/kg DM) were low  
153 relative to other AA. In contrast, IAA with the highest mean content were leucine (27.3 g/kg  
154 DM) and phenylalanine (16.8 g/kg DM). The greatest CV among the IAA was seen for  
155 tryptophan (0.099), while the greatest CV among the dispensable AA (DAA) was observed for  
156 glutamic acid (0.080).

157

158 Table 3 here

159

### 160 *Polysaccharide composition*

161 The polysaccharide composition of the studied DDGS batches are presented in Table 4. The  
162 distribution of constituent sugars for the studied DDGS are in order of xylose, glucose,  
163 arabinose, mannose, galactose and galaturonic acid for batches 1 and 4 and in order of xylose,  
164 glucose, arabinose, galactose, mannose and galaturonic acid for batches 2 and 3. Total NSP  
165 content of the four batches ranged from 217.5 (batch 3) to 253.7 g/kg DM (batch 4). Total  
166 soluble and insoluble NSP content ranged from 32.7 (batch 4) to 59.9 g/kg DM (batch 2) and



1  
2  
3 167 174.8 (batch 2) to 221.0 g/kg DM (batch 4), respectively. Starch content ranged from 28.0  
4  
5 168 (batch 2) to 88.1 g/kg DM (batch 4). The greatest CV's for soluble, insoluble and total NSP  
6  
7 169 was observed for galaturonic acid, while a large CV of 0.565 was observed for starch content.  
8

9 170

10  
11 171 Table 4 here

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17 173 ***Digestibility of energy and nutrients***

18  
19 174 Digestibility coefficients for energy, protein, DM and fat are shown in Table 5. Differences  
20  
21 175 were observed between the four batches of DDGS, with batch 1 having the lowest DE  
22  
23 176 (P=0.048), DM (P=0.012) and fat digestibility (P<0.001). No differences were observed  
24  
25 177 between the different batches for protein digestibility (PD). Increasing DDGS inclusion rate  
26  
27 178 from 15 to 30% improved (P<0.001) DM and fat digestibility. No interactions (P>0.05) were  
28  
29 179 observed between the DDGS batches and inclusion level.  
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31 180

32  
33 181 Table 5 here

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37  
38 183 Digestibility coefficients for both IAA and DAA are shown in Tables 6 and 7, respectively.  
39  
40 184 Batch 2 had the highest histidine digestibility (P=0.038). Differences between DDGS samples  
41  
42 185 were evident for cysteine, glutamic acid, glycine, proline and serine (P<0.05).  
43

44 186

45  
46  
47 187 Tables 6 and 7 here

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52 189 **DISCUSSION**

53  
54 190 The purpose of the experiment reported in this paper was to examine the variability between  
55  
56 191 batches of wheat DDGS produced by the same manufacturer on digestible energy and nutrient  
57  
58 192 digestibility in diets for laying hens. Different manufacturing processes at different production  
59  
60 193 plants and seasonal wheat differences may further increase the variability of DDGS samples

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3 194 that will be available to poultry feed manufacturers. However, the present comparison remains  
4  
5 195 valuable to inform the poultry industry of the variability they can expect within each harvest  
6  
7 196 year.

### 9 197 ***Chemical composition of the wheat DDGS batches***

10  
11 198 Results from the chemical analysis were generally in agreement with data published by others  
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13 199 (Thacker and Widyaratne, 2007; Vilariño *et al.* 2007; Bandegan *et al.* 2009; Oryschak *et al.*  
14  
15 200 2010; Hazzledine *et al.* 2011; Pedersen *et al.* 2014). Despite batch 4 having the lowest protein  
16  
17 201 content, it scored the highest value for lightness (43.8) compared with the other batches of  
18  
19 202 DDGS. Cozannet *et al.* (2010b) recommended colour scoring DDGS as a rapid and reliable  
20  
21 203 method for estimating AA digestibility. Although it must be advised that DDGS with L\* scores  
22  
23 204 lower than 50 may have been exposed to Maillard reactions, thus resulting in DDGS with  
24  
25 205 reduced protein quality. It should be noted that each of the studied batches of wheat DDGS had  
26  
27 206 lightness values lower than 50. The AA composition of the studied wheat DDGS were  
28  
29 207 comparable to values reported by Kluth and Rodehutsord (2010). Among the IAA the greatest  
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31 208 CV was for observed tryptophan (0.099), while the greatest CV among the DAA was observed  
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33 209 for proline (0.090). Lowest contents of IAA were observed for lysine (7.0 g/kg DM) and  
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35 210 methionine (5.3 g/kg DM), although average values were similar to those reported by the NRC  
36  
37 211 (2012).

38  
39 212 Total NSP content of the studied DDGS was similar to that reported by Widyaratne and Zijlstra  
40  
41 213 (2007) and Pedersen *et al.* (2014). Compared with the total NSP content of the different  
42  
43 214 batches of the studied DDGS, the content of certain constituent sugars and the total soluble and  
44  
45 215 insoluble fractions varied considerably. With the exception of galacturonic acid, the CV values  
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47 216 of the studied wheat DDGS were greatest for the sNSP fraction. In addition, the large range  
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49 217 observed among the different batches for starch (28.0 to 88.1 g/kg DM) are similar to findings  
50  
51 218 reported by Cozannet *et al.* (2010a). Residual starch is directly related to the fermentation  
52  
53 219 process and is particularly dependent on fermentation time, yeast and the use of enzymes. The  
54  
55 220 large variation in starch content among the different batches of DDGS may have an impact on  
56  
57 221 ethanol yield and may be of useful for the bioethanol industry to take this into account.

### 58 222 ***Digestibility of energy and nutrients***

59 223 When formulating poultry diets containing DDGS, information on energy and nutrient  
60 224 availability is important to ensure diets are balanced. When considering production costs for

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3 225 poultry feeds, determining accurate measures of available energy is crucial. The most common  
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5 226 measure of energy contained in feedstuffs for poultry is AME (Abdulla *et al.* 2016). Although  
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7 227 AME takes into account energy loss in urine, it may be confounded by loss of energy in  
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9 228 fermentation in the caeca that has little benefit to the bird. However, it may be speculated that  
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11 229 the relatively high insoluble NSP contents, and numerically low CP digestibility seen in  
12  
13 230 samples 1 and 4, indicated that, if determined, dietary N corrected AME may follow similar  
14  
15 231 variation as DE. In addition, research conducted by Pirgozliev *et al.* (2003), suggested that ME  
16  
17 232 does not always relate to the feeding value of diets. Little data are available on the digestible  
18  
19 233 energy of wheat DDGS for poultry, particularly for laying hens, and any differences may be  
20  
21 234 important in evaluating different batches of DDGS that have high dietary fibre contents. Ileal  
22  
23 235 digesta have already been used to determine AA and CP digestibility, thus, measuring DE was  
24  
25 236 fairly simple. High NSP content in cereals are associated with low available energy in poultry  
26  
27 237 feeds (Annison, 1991; Choct *et al.* 1999). A number of factors have been found to affect the  
28  
29 238 bioavailability of nutrients in DDGS. These include the type of DDGS, quality of the original  
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31 239 grain and production processes.

32  
33 240 It has been previously reported that the presence of soluble pentosans considerably reduced the  
34  
35 241 digestibility of fat (Dänicke *et al.* 1999). However, in the present study, there was no  
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37 242 relationship between fat digestibility and soluble NSP content for the four DDGS batches.  
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39 243 Findings from a broiler study by Romero *et al.* (2014) suggested that fat digestibility was less  
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41 244 affected by wheat arabinoxylans in older birds, such as those used in the present study. In  
42  
43 245 addition, the improved dietary fat and dry matter digestibility observed when increasing DDGS  
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45 246 inclusion rate from 15 to 30% may have been due to the increased fat content in the diets, which  
46  
47 247 might improve overall nutrient availability and, thus, dry matter digestibility.

48  
49 248 The results of the present study have shown that there is a large and important variation in the  
50  
51 249 digestible energy content between different batches of wheat DDGS, which may correlate to  
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53 250 insoluble NSP contents. These differences will affect the economic value of the DDGS for use  
54  
55 251 in practical laying hen feeds.

### 52 252 ***Protein and amino acid digestibility***

54  
55 253 Accurate information on the digestible protein and amino acid content of wheat DDGS is  
56  
57 254 crucial and enables the poultry feed industry to formulate biologically and economically  
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59 255 efficient diets. Precise data on digestible amino acid levels in feed ingredients for poultry  
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256 allows the feed industry to optimise the efficiency of diet preparation and minimises

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3 257 overfeeding of amino acids, thereby optimising bird performance and feed cost (Pirgozliev *et*  
4 258 *al.* 2010; Tahir and Pesti, 2012). Digestibility coefficients for lysine are low, although this was  
5 259 expected because of the high temperatures used during the manufacturing process and agreed  
6  
7 260 with studies using maize DDGS (Fontaine *et al.* 2007).  
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## 12 13 262 **CONCLUSIONS**

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16 263 Wheat DDGS is now reliably available for compounders to use in animal feeds. The economic  
17 264 value of any feedstuff depends on its price and the content of available nutrients for a particular  
18 265 strain and age of livestock. The present study provided information on digestible energy and  
19 266 nutrient digestibility of four different wheat DDGS batches when incorporated into diets for  
20 267 adult laying hens at two inclusion rates. Stochastic feed formulation techniques may now be  
21 268 employed by poultry nutritionists to account for variability in the available nutrient content  
22 269 between different batches of the same feedstuff. Stochastic feed formulation methods need to  
23 270 include data on batch variability for each of the nutrients that they consider. Differences  
24 271 between production sites will probably further increase the variability of the overall number of  
25 272 batches that are available to the industry. However, the variability estimates are valid for  
26 273 practical feed compounders that will primarily buy their DDGS batches from one production  
27 274 factory only.  
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275 *Table 1. Chemical composition and ingredients of the basal diet*

| <b>Ingredient</b>                             | <b>Amount g/kg</b> |
|---|--------------------|
| Wheat   | 687.0              |
| Hipro Soya bean meal                          | 130.0              |
| Full fat soya                                 | 70.0               |
| Soya oil                                      | 10.0               |
| Lysine  | 1.0                |
| Methionine                                    | 2.0                |
| Threonine                                     | 1.0                |
| Limestone                                     | 85.0               |
| Dicalcium phosphate                           | 10.0               |
| Salt  | 3.0                |
| Vitamin and trace element premix <sup>1</sup> | 1.0                |
| <b>Calculated composition (as fed basis)</b>  |                    |
| ME (MJ/kg)                                    | 11.89              |
| CP  | 172.5              |
| Ca  | 34.0               |
| Available P                                   | 3.1                |
| Na  | 1.7                |
| <b>Analysed values (DM basis)</b>             |                    |
| DM  | 876.9              |
| GE (MJ/kg)                                    | 13.08              |
| CP  | 138.6              |
| Fat   | 34.8               |
| NDF   | 70.1               |

276 <sup>1</sup>Vitamin and mineral premix provided (units per kg/feed): retinol, 2160 µg; cholecalciferol, 75 µg; α-tocopherol,  
 277 25 mg; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; cyanocobalamin, 0.01 mg; pyridoxine, 1.5  
 278 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80  
 279 mg; selenium, 0.3 mg; manganese, 80 mg; and zinc, 80 mg.

280

281 *Table 2. Proximate analysis, bulk density and colour measurements of the studied wheat DDGS samples*

|                                   | Batch 1 | Batch 2 | Batch 3 | Batch 4 | CV    |
|-----------------------------------|---------|---------|---------|---------|-------|
| Dry matter (g/kg)                 | 896.0   | 900.0   | 895.0   | 843.0   | 0.031 |
| Gross energy (MJ/kg DM)           | 21.78   | 21.42   | 21.68   | 21.16   | 0.013 |
| Protein (g/kg DM)                 | 353.9   | 363.0   | 363.3   | 324.8   | 0.052 |
| Oil (g/kg DM)                     | 49.8    | 41.9    | 55.0    | 61.2    | 0.157 |
| Neutral detergent fibre (g/kg DM) | 493.3   | 490.8   | 486.7   | 442.2   | 0.051 |
| Ash (g/kg)                        | 54.9    | 58.2    | 53.1    | 57.5    | 0.042 |
| Bulk density (kg/hl)              | 37.0    | 40.7    | 42.9    | 54.9    | 0.176 |
| Colour measurements               |         |         |         |         |       |
| L*                                | 38.6    | 36.4    | 36.8    | 43.8    | 0.088 |
| a*                                | 10.2    | 9.3     | 9.5     | 9.5     | 0.041 |
| b*                                | 19.7    | 17.1    | 18.1    | 21.8    | 0.107 |

282 CV = coefficient of variation

283

284 *Table 3. Amino acid composition of the studied DDGS sample (g/kg DM)*

|     | Batch 1 | Batch 2 | Batch 3 | Batch 4 | CV    |
|-----|---------|---------|---------|---------|-------|
| Ala | 16.1    | 14.7    | 17.1    | 16.9    | 0.067 |
| Arg | 13.9    | 13.2    | 13.6    | 13.2    | 0.025 |
| Asp | 18.7    | 17.9    | 19.1    | 18.7    | 0.027 |
| Cys | 6.0     | 5.9     | 6.2     | 5.9     | 0.024 |
| Glu | 91.6    | 93.1    | 93.3    | 78.5    | 0.080 |
| Gly | 14.3    | 14.2    | 14.7    | 13.4    | 0.038 |
| His | 6.9     | 6.8     | 7.3     | 7.0     | 0.031 |
| Ile | 13.4    | 13.3    | 14      | 12.9    | 0.034 |
| Leu | 27.0    | 25.0    | 29.1    | 28.0    | 0.064 |
| Lys | 6.9     | 6.8     | 7.1     | 7.0     | 0.019 |
| Met | 5.3     | 4.9     | 5.5     | 5.3     | 0.048 |
| Phe | 17.2    | 16.6    | 17.7    | 15.8    | 0.049 |
| Pro | 28.9    | 33.2    | 35.4    | 30.5    | 0.090 |
| Ser | 12.0    | 11.7    | 12.2    | 11.4    | 0.030 |
| Thr | 9.9     | 9.5     | 10.1    | 9.5     | 0.031 |
| Try | 3.7     | 4.2     | 3.3     | 3.7     | 0.099 |
| Tyr | 9.1     | 8.2     | 8.9     | 8.8     | 0.044 |
| Val | 17.1    | 16.6    | 17.8    | 16.4    | 0.037 |

285 CV = coefficient of variation

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287

288 *Table 4. Polysaccharide composition of the studied wheat DDGS samples (g/kg DM)*

|                   | Batch 1 | Batch 2 | Batch 3 | Batch 4 | CV    |
|-------------------|---------|---------|---------|---------|-------|
| sNSP              |         |         |         |         |       |
| Xylose            | 15.2    | 23.0    | 14.3    | 9.0     | 0.375 |
| Arabinose         | 11.1    | 12.9    | 11.5    | 11.2    | 0.071 |
| Mannose           | 5.7     | 4.4     | 4.0     | 4.9     | 0.154 |
| Galactose         | 2.4     | 4.1     | 3.4     | 2.5     | 0.259 |
| Glucose           | 7.2     | 10.2    | 5.4     | 1.7     | 0.580 |
| Galacturonic acid | 4.1     | 5.4     | 0.0     | 3.3     | 0.719 |
| Total sNSP        | 45.7    | 59.9    | 38.6    | 32.7    | 0.265 |
| iNSP              |         |         |         |         |       |
| Xylose            | 66.8    | 60.9    | 64.0    | 79.3    | 0.119 |
| Arabinose         | 43.2    | 39.3    | 39.8    | 46.0    | 0.075 |
| Mannose           | 6.8     | 6.1     | 6.2     | 9.3     | 0.211 |
| Galactose         | 8.6     | 6.5     | 8.2     | 8.1     | 0.118 |
| Glucose           | 63.4    | 62.0    | 60.6    | 77.1    | 0.116 |
| Galacturonic acid | 0.0     | 0.0     | 0.0     | 1.3     | -     |
| Total iNSP        | 188.8   | 174.8   | 178.8   | 221.0   | 0.110 |
| Total NSP         |         |         |         |         |       |
| Xylose            | 82.0    | 83.9    | 78.3    | 88.3    | 0.050 |
| Arabinose         | 54.3    | 52.2    | 51.3    | 57.2    | 0.049 |
| Mannose           | 12.5    | 10.5    | 10.2    | 14.2    | 0.158 |
| Galactose         | 11.0    | 10.6    | 11.6    | 10.6    | 0.043 |
| Glucose           | 70.5    | 72.2    | 66.0    | 78.7    | 0.073 |
| Galacturonic acid | 4.1     | 5.4     | 0.0     | 4.7     | 0.683 |
| Total NSP         | 234.5   | 234.7   | 217.5   | 253.7   | 0.063 |
| Starch            | 41.5    | 28.0    | 35.0    | 88.1    | 0.565 |

289 sNSP = soluble NSP

290 iNSP = insoluble NSP

291 CV = coefficient of variation

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295 Table 5. *Dietary digestible energy (DE), apparent dietary ileal digestibility coefficients for protein (PD), dry*  
 296 *matter (DMD) and fat (FD) when fed to laying hens from 22 to 23 weeks of age*

| Treatment factor                         | DE (MJ/kg DM)       | PD    | DMD                | FD                 |
|--|---------------------|-------|--------------------|--------------------|
| Digestibility coefficients               |                     |       |                    |                    |
| Batch 1                                  | 11.44 <sup>a</sup>  | 0.697 | 0.667 <sup>a</sup> | 0.656 <sup>a</sup> |
| Batch 2                                  | 12.41 <sup>b</sup>  | 0.766 | 0.777 <sup>b</sup> | 0.785 <sup>b</sup> |
| Batch 3                                  | 12.15 <sup>ab</sup> | 0.748 | 0.779 <sup>b</sup> | 0.771 <sup>b</sup> |
| Batch 4                                  | 11.52 <sup>a</sup>  | 0.729 | 0.823 <sup>b</sup> | 0.894 <sup>c</sup> |
| SEM                                      | 0.279               | 0.020 | 0.032              | 0.037              |
| 15% inclusion                            | 11.95               | 0.745 | 0.692              | 0.688              |
| 30% inclusion                            | 11.81               | 0.725 | 0.831              | 0.864              |
| SEM                                      | 0.197               | 0.014 | 0.023              | 0.026              |
| Batch 1 + 15%                            | 11.58               | 0.706 | 0.543              | 0.523              |
| Batch 2 + 15%                            | 12.17               | 0.748 | 0.704              | 0.685              |
| Batch 3 + 15%                            | 12.08               | 0.755 | 0.695              | 0.669              |
| Batch 4 + 15%                            | 11.96               | 0.772 | 0.820              | 0.876              |
| Batch 1 + 30%                            | 11.30               | 0.687 | 0.786              | 0.788              |
| Batch 2 + 30%                            | 12.65               | 0.785 | 0.850              | 0.884              |
| Batch 3 + 30%                            | 12.23               | 0.741 | 0.862              | 0.872              |
| Batch 4 + 30%                            | 11.08               | 0.686 | 0.826              | 0.915              |
| SEM                                      | 0.394               | 0.028 | 0.046              | 0.052              |
| Probabilities of statistical differences |                     |       |                    |                    |
| Batch                                    | 0.048               | NS    | 0.012              | <0.001             |
| Inclusion                                | NS                  | NS    | <0.001             | <0.001             |
| Batch*Inclusion                          | NS                  | NS    | NS                 | NS                 |

297 There is a statistically significant difference when  $P < 0.05$ ; SEM – standard error of means (5% level). Means  
 298 within a row with no common superscript differ significantly.

299

301 Table 6. *Dietary apparent ileal digestibility coefficients for indispensable amino acids when fed to laying hens*  
 302 *from 22 to 23 weeks of age*

| Treatment factor                       | Indispensable amino acids |                     |       |       |       |       |       |       |                     |       |
|--|---------------------------|---------------------|-------|-------|-------|-------|-------|-------|---------------------|-------|
|  | Arg                       | His                 | Ile   | Leu   | Lys   | Met   | Phe   | Thr   | Try                 | Val   |
| Digestibility coefficients             |                           |                     |       |       |       |       |       |       |                     |       |
| Batch 1                                | 0.657                     | 0.615 <sup>a</sup>  | 0.597 | 0.635 | 0.526 | 0.715 | 0.685 | 0.490 | 0.586               | 0.565 |
| Batch 2                                | 0.709                     | 0.679 <sup>b</sup>  | 0.664 | 0.689 | 0.604 | 0.757 | 0.733 | 0.570 | 0.693               | 0.631 |
| Batch 3                                | 0.680                     | 0.654 <sup>ab</sup> | 0.639 | 0.670 | 0.561 | 0.741 | 0.701 | 0.531 | 0.650               | 0.600 |
| Batch 4                                | 0.663                     | 0.627 <sup>ab</sup> | 0.615 | 0.646 | 0.549 | 0.724 | 0.680 | 0.501 | 0.645               | 0.575 |
| SEM                                    | 0.016                     | 0.016               | 0.018 | 0.017 | 0.024 | 0.015 | 0.014 | 0.022 | 0.020               | 0.019 |
| 15% inclusion                          | 0.688                     | 0.661               | 0.641 | 0.654 | 0.597 | 0.751 | 0.709 | 0.541 | 0.654               | 0.604 |
| 30% inclusion                          | 0.666                     | 0.626               | 0.617 | 0.666 | 0.524 | 0.717 | 0.691 | 0.505 | 0.634               | 0.581 |
| SEM                                    | 0.011                     | 0.011               | 0.013 | 0.012 | 0.017 | 0.010 | 0.010 | 0.016 | 0.014               | 0.013 |
| Batch 1 + 15%                          | 0.658                     | 0.626               | 0.606 | 0.639 | 0.552 | 0.728 | 0.690 | 0.497 | 0.594 <sup>a</sup>  | 0.572 |
| Batch 2 + 15%                          | 0.656                     | 0.604               | 0.588 | 0.631 | 0.500 | 0.702 | 0.719 | 0.483 | 0.579 <sup>ab</sup> | 0.558 |
| Batch 3 + 15%                          | 0.695                     | 0.678               | 0.646 | 0.675 | 0.605 | 0.754 | 0.710 | 0.572 | 0.658 <sup>ab</sup> | 0.621 |
| Batch 4 + 15%                          | 0.723                     | 0.680               | 0.672 | 0.702 | 0.604 | 0.760 | 0.717 | 0.569 | 0.729 <sup>b</sup>  | 0.641 |
| Batch 1 + 30%                          | 0.693                     | 0.672               | 0.646 | 0.673 | 0.602 | 0.759 | 0.681 | 0.548 | 0.653 <sup>ab</sup> | 0.608 |
| Batch 2 + 30%                          | 0.667                     | 0.636               | 0.631 | 0.668 | 0.521 | 0.722 | 0.748 | 0.514 | 0.647 <sup>b</sup>  | 0.591 |
| Batch 3 + 30%                          | 0.707                     | 0.669               | 0.654 | 0.678 | 0.628 | 0.762 | 0.692 | 0.548 | 0.653 <sup>ab</sup> | 0.616 |
| Batch 4 + 30%                          | 0.618                     | 0.585               | 0.576 | 0.614 | 0.469 | 0.685 | 0.642 | 0.454 | 0.580 <sup>ab</sup> | 0.534 |
| SEM                                    | 0.022                     | 0.023               | 0.026 | 0.023 | 0.034 | 0.010 | 0.020 | 0.031 | 0.0141              | 0.027 |
| Probability of statistical differences |                           |                     |       |       |       |       |       |       |                     |       |
| Batch                                  | NS                        | 0.038               | NS    | NS    | NS    | NS    | 0.051 | NS    | 0.006               | NS    |
| Inclusion                              | NS                        | 0.038               | NS    | NS    | 0.005 | 0.029 | NS    | NS    | NS                  | NS    |
| Batch*Inclusion                        | NS                        | NS                  | NS    | NS    | NS    | NS    | NS    | NS    | 0.011               | NS    |

303 There is a statistically significant difference when  $P < 0.05$ ; SEM – standard error of means (5% level). Means  
 304 within a row with no common superscript differ significantly.

305

306 Table 7. *Dietary apparent ileal digestibility coefficients for dispensable amino acids when fed to laying hens from*  
 307 *22 to 23 weeks of age*

| Treatment factor                       | Dispensable amino acids |        |                     |                     |                     |                     |        |        |
|--|-------------------------|--------|---------------------|---------------------|---------------------|---------------------|--------|--------|
|  | Ala                     | Asp    | Cys                 | Glu                 | Gly                 | Pro                 | Ser    | Tyr    |
| Digestibility coefficients             |                         |        |                     |                     |                     |                     |        |        |
| Batch 1                                | 0.545                   | 0.528  | 0.511 <sup>a</sup>  | 0.787 <sup>ab</sup> | 0.521 <sup>a</sup>  | 0.745 <sup>a</sup>  | 0.586  | 0.569  |
| Batch 2                                | 0.606                   | 0.601  | 0.592 <sup>b</sup>  | 0.821 <sup>b</sup>  | 0.599 <sup>b</sup>  | 0.809 <sup>b</sup>  | 0.650  | 0.631  |
| Batch 3                                | 0.587                   | 0.564  | 0.572 <sup>ab</sup> | 0.802 <sup>ab</sup> | 0.564 <sup>ab</sup> | 0.786 <sup>ab</sup> | 0.619  | 0.595  |
| Batch 4                                | 0.567                   | 0.542  | 0.535 <sup>ab</sup> | 0.778 <sup>a</sup>  | 0.534 <sup>ab</sup> | 0.752 <sup>a</sup>  | 0.590  | 0.578  |
| SEM                                    | 0.0207                  | 0.0193 | 0.0204              | 0.0092              | 0.0199              | 0.0113              | 0.0173 | 0.0199 |
| 15% inclusion                          | 0.581                   | 0.580  | 0.576               | 0.804               | 0.567               | 0.776               | 0.618  | 0.594  |
| 30% inclusion                          | 0.571                   | 0.538  | 0.529               | 0.790               | 0.542               | 0.770               | 0.604  | 0.592  |
| SEM                                    | 0.0147                  | 0.0136 | 0.0144              | 0.0065              | 0.0141              | 0.0080              | 0.0122 | 0.0141 |
| Batch 1 + 15%                          | 0.544                   | 0.540  | 0.542               | 0.791               | 0.529               | 0.751               | 0.583  | 0.554  |
| Batch 2 + 15%                          | 0.545                   | 0.516  | 0.479               | 0.783               | 0.513               | 0.738               | 0.589  | 0.583  |
| Batch 3 + 15%                          | 0.591                   | 0.600  | 0.608               | 0.813               | 0.590               | 0.803               | 0.643  | 0.610  |
| Batch 4 + 15%                          | 0.621                   | 0.603  | 0.576               | 0.829               | 0.608               | 0.815               | 0.656  | 0.651  |
| Batch 1 + 30%                          | 0.589                   | 0.584  | 0.583               | 0.806               | 0.572               | 0.787               | 0.624  | 0.598  |
| Batch 2 + 30%                          | 0.584                   | 0.543  | 0.562               | 0.798               | 0.556               | 0.785               | 0.614  | 0.591  |
| Batch 3 + 30%                          | 0.601                   | 0.596  | 0.572               | 0.806               | 0.576               | 0.762               | 0.622  | 0.614  |
| Batch 4 + 30%                          | 0.534                   | 0.489  | 0.498               | 0.750               | 0.491               | 0.743               | 0.558  | 0.541  |
| SEM                                    | 0.0293                  | 0.0136 | 0.0144              | 0.0065              | 0.0282              | 0.0080              | 0.0244 | 0.0281 |
| Probability of statistical differences |                         |        |                     |                     |                     |                     |        |        |
| Batch                                  | NS                      | 0.058  | 0.035               | 0.013               | 0.042               | <0.001              | 0.048  | NS     |
| Inclusion                              | NS                      | 0.037  | 0.026               | NS                  | NS                  | NS                  | NS     | NS     |
| Batch*Inclusion                        | NS                      | NS     | NS                  | NS                  | NS                  | NS                  | NS     | NS     |

308 There is a statistically significant difference when  $P < 0.05$ ; SEM – standard error of means (5% level). Means  
 309 within a row with no common superscript differ significantly.

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