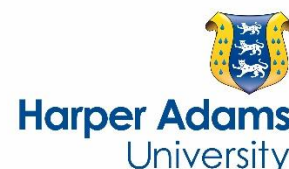


Nutrient availability of different batches of wheat distillers dried grains with solubles with and without exogenous enzymes for broiler chickens

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1 WHEAT DISTILLERS DRIED GRAINS WITH SOLUBLES FOR CHICKENS

2 **Nutrient availability of different batches of wheat distillers dried grains with solubles**
3 **with and without exogenous enzymes for broiler chickens**

4
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15

16 **ABSTRACT** Wheat distillers dried grains with solubles (DDGS) are being used increasingly
17 in the poultry feed industry but their nutritional value is variable. The aim of this experiment
18 was to examine the effect of batch to batch variation of wheat DDGS produced by the same
19 manufacturer on the growth performance, dietary N corrected apparent metabolizable energy
20 (AMEn), energy conversion ratio (ECR), total tract dry matter retention (DMR), nitrogen
21 retention (NR) and fat digestibility (FD) coefficients when fed to broilers in complete diets
22 with and without enzyme supplementation. Six UK wheat DDGS samples, produced by a
23 single manufacturer, were used in a broiler experiment. Six diets containing 150 g/kg of each
24 selected wheat DDGS sample were mixed. Each diet was then split into two batches and one
25 of them was supplemented with commercial enzyme preparation, providing 1220 units
26 xylanase and 152 units of β -glucanase /kg diet, resulting in 12 experimental diets. Each diet
27 was fed *ad libitum* to five pens of two male Ross 308 broilers from 7 to 21 d old. Enzyme
28 supplementation improved dietary AMEn, DMR, NR ($P < 0.001$) and FD ($P < 0.05$)
29 compared to non-supplemented diets. There was DDGS sample by enzyme interaction ($P <$
30 0.05) on daily weight gain and ECR. The results suggest that the variability in AMEn of
31 DDGS samples produced from a single manufacturer is greater than expected compared to
32 the variability of whole wheat samples but substantially lower than expected from wheat
33 DDGS samples from different EU manufacturers. This experiment has shown that the
34 variation in feeding value of wheat DDGS may be explained by the variability in
35 polysaccharide contents.

36 **Key Words:** wheat DDGS, batch variability, broiler, enzyme, ME

INTRODUCTION

37

38 The increase of bioethanol production resulted in more available distillers dried grains with
39 solubles (**DDGS**) for animal feed (Gamage *et al.*, 2012). Traditionally fed to ruminants, this
40 abundant and competitively priced coproduct of bioethanol production can be also used in
41 poultry diet formulations (Burton *et al.*, 2013; Opoku *et al.*, 2015). Most of the research,
42 however, was done on DDGS produced from maize, and there is a dearth of information on
43 the nutritive value of DDGS produced from wheat (Świątkiewicz *et al.*, 2013; Ivanova *et al.*,
44 2013). It is known already that compared to maize, wheat DDGS has more protein and
45 available phosphorus but also non starch polysaccharides (**NSP**) (Oryschak *et al.*, 2010;
46 Olukosi and Adebisi, 2013). As the price of cereals and protein sources in European
47 countries increases, inclusion of European produced wheat DDGS could be more routinely
48 used in broiler diets if there was more robust information on its nutrient availability and its
49 variation. Information on energy and nutrient availability of wheat DDGS, and its impact on
50 growth performance and bird health is needed.

51 The nutrient availability has been shown to vary substantially between DDGS samples
52 produced by different bioethanol plants (Bandegan *et al.*, 2009; Cozannet *et al.*, 2010). Batch
53 variability in metabolizable energy content and nutrient digestibility for layers within wheat
54 DDGS samples produced by a single production plant has been reported by Whiting *et al.*
55 (2014, 2015), although information on broilers is lacking.

56 The hypothesis to be tested in this study was that the improvement of feeding value of wheat
57 DDGS due to fiber degrading enzyme supplementation depends upon the chemical
58 composition of the DDGS. The specific objectives of the experiment were (1) to examine the
59 differences in the chemical composition of six different wheat DDGS samples produced by a
60 single manufacturer, (2) to determine the nitrogen corrected apparent metabolizable energy
61 (**AMEn**) and nutrient availability in diets containing different wheat DDGS batches, and (3)

62 to compare the growth performance of broilers fed those diets with and without enzyme
63 supplementation.

64 **MATERIALS AND METHODS**

65

66 *Experimental samples*

67

68 This report is focused on the nutritional value for broilers of six wheat DDGS samples. The
69 six wheat DDGS batches used in the study were produced by a single manufacturer (ENSUS
70 Biorefinery, Wilton, UK). The batches were manufactured in early 2013. All samples were
71 stored in bags at ambient air temperatures in a dry store. The stored DDGS samples did not
72 experience any freezing temperatures during this storage.

73

74 *Proximate analysis of samples*

75

76 Dry matter (**DM**) was determined by drying of samples in a forced draft oven at 105°C to a
77 constant weight (AOAC, 2000; method 934.01). Ash was measured in a muffle furnace at
78 500°C for 18 h (AOAC, 2000; method 942.05). Crude protein (6.25 X N) in samples was
79 determined by the combustion method (AOAC, 2000; method 990.03) using a Leco (FP-528
80 N, Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the
81 ether extraction method (AOAC, 2000; method 945.16) using a Soxtec system (Foss UK
82 Ltd.). The gross energy (**GE**) value of DDGS samples was determined in a bomb calorimeter
83 (model 6200; Parr Instrument Co., Moline, IL) with benzoic acid used as the standard. Total
84 starch (**TS**) was determined following the method of Englyst (2000). The NSP content was
85 determined by the method of Englyst (1994), whereby starch is completely dispersed and
86 then hydrolysed enzymatically. The NSP is isolated by precipitation in 80% ethanol then

87 hydrolysed by sulphuric acid and the released sugars measured by gas chromatography as
88 their alditol acetate derivatives. Titanium dioxide was determined by the method of Short *et*
89 *al.* (1996).

90 The energy conversion ratio (**ECR**) was also determined (calculation shown later). It
91 describes the relative efficiency of the use of metabolizable energy for growth, rather than
92 heat loss, implicit that a more efficient energy use towards growth is related to a lower ratio.

93

94 ***Diet preparation***

95 A basal feed containing 602.6 g/kg of wheat and 260 g/kg soybean meal, as main ingredients
96 was prepared (Table 1). Titanium dioxide was added on the top of the basal diet (6 g/kg) as
97 an indigestible marker. Six diets containing 150 g/kg of each of the six experimental wheat
98 DDGS samples were made after mixing with 850 g/kg of a basal feed. Each diet was then
99 split into two batches and one of them was supplemented with 100 g/tonne of a commercial
100 enzyme (Aextra XB, Danisco Animal Nutrition, Marlborough, UK), resulting in 12 diets in
101 total. The enzyme preparation was based on 1220 units of xylanase and 152 units of β -
102 glucanase /kg diet produced by *Trichoderma reesei*. No adjustments were made for
103 differences in dry matter between the wheat DDGS samples because only a small range of
104 differences were observed. All diets were fed as mash.

105 ***Determination of dietary metabolizable energy, nutrient digestibility and comparison of*** 106 ***broiler growth performance***

107 All procedures were approved by The Animal Experimental Committee of Harper Adams
108 University.

109 Male Ross 308 broiler chickens were obtained from a commercial hatchery. During the seven
110 day pre-experimental period, from day old to 7 days of age, the birds were reared in a single

111 floor pen and fed proprietary wheat-based diet without coccidiostats or antimicrobial growth
112 promoters, prophylactic or other similar additives. On the first day of the experiment (7 d of
113 age), the chicks were individually weighed and the heaviest and lightest birds discarded, so
114 that 120 birds were used in the experiment (average body weight 174 g, and there were no
115 significant differences between treatment groups ($P > 0.05$)). The birds were allocated to 60
116 small pens, two birds in each pen. Each of the pens has a solid floor and equipped with
117 individual feeder and drinker. Feed and water was offered *ad libitum* to birds throughout the
118 experiment. Each diet was offered to birds in 5 pens in a randomised block design (five
119 positional blocks). Information on growth and feed intake was obtained from 7 to 21 days of
120 age. Room temperature and lighting regime met commercial recommendations. For the last
121 three days of the study the solid floor of each pen was replaced with a wire mesh and excreta
122 were collected, immediately dried at 60°C and then milled. The gross energy, dry matter,
123 nitrogen, fat and titanium content of each dried excreta sample and the experimental diets
124 were determined as described for the DDGS samples.

125

126 ***Calculations***

127

128 Dietary AMEn (MJ/kg) was calculated as follows:

$$129 \quad \text{AMEn} = \text{GEd} - \text{GEex} * (\text{Tid}/\text{Tiex}) - 34.39 * \text{Nret}$$

130 where Nret is the retained nitrogen (kg) and 34.39 MJ/kg is the GE value of nitrogen.

$$131 \quad \text{Nret} = \text{Nd} - (\text{Nex} * \text{Tid})/\text{Tiex}$$

132 Dietary nitrogen retention coefficient (NR) is obtained as described below.

133 $NR = (Nd/Tid - Nex/Tiex)/(Nd/Tid)$

134 where, Nd is the nitrogen (g/kg) of the feed; Tid is the concentration of titanium dioxide in
135 the diet (g/kg); Nex is the nitrogen in the excreta (g/kg); and Tiex is the concentration of
136 titanium dioxide in the excreta (g/kg).

137

138 Dietary fat digestibility coefficient was obtained in the same way as NR.

139 Total tract dry matter retention coefficients (DMR) were calculated using the following
140 equation:

141 $DMR = (Tiex - Tid)/Tiex$

142 The ECR was determined as the AMEn ingested to achieve the weight gain over the weight
143 gain for the experimental period:

144

145 $ECR (MJ/kg) = (AME \text{ intake } (MJ))/\text{Weight gain } (kg)$

146

147 ***Statistical analysis***

148 Statistical analyses were performed using the Genstat 16 statistical software package (IACR
149 Rothamstead, Hertfordshire, England). A randomized complete block analysis of variance
150 was performed and a 6 x 2 factorial structure was used to investigate the main treatment
151 factors (six DDGS samples and the presence of enzymes) and their interaction. Differences
152 were reported as significant at $P < 0.05$ and trends were noted when the P value was near to
153 0.1.

154 Power calculations indicated that using 5 replicate pens (2 birds per pen) would be able to
155 detect a difference of 0.35 MJ/kg of dietary AMEn as statistically significant (CV% = 2.0
156 with 5% probability levels). We therefore considered that the replication was sufficient to
157 detect economically and biologically important differences.

158

159

RESULTS

160 *Dietary DDGS samples*

161 The proximate compositions of DDGS samples are summarized in Table 2. The amounts of
162 oil and protein were more variable than the GE concentration, and ranged from 41.9 to 61.2
163 g/kg DM, and 274.1 to 327.0 g/kg DM, respectively. The ash and DM contents ranged from
164 53.1 to 64.9 g/kg DM, and 843 to 900 g/kg DM, respectively.

165 The range of the total NSP content of the wheat DDGS samples was 216.8 to 253.9 g/kg DM
166 (Table 3). Xylose, glucose and arabinose were the main NSP constituent sugars in the wheat
167 DDGS samples. The mean starch content of the DDGS batches was 36.8 g/kg DM, as batch
168 C had the lowest starch content of 28.0 g/kg DM, and batch F had the highest starch content
169 of 88.1 g/kg DM, respectively.

170 *Bird performance*

171 The overall daily feed intake was 65.8 g DM or 5% lower than the expected 69.2 g DM by
172 the breeder (Aviagen Ltd., Edinburgh, UK) (Table 4). There was a DDGS batch by enzyme
173 interaction on daily weight gain ($P = 0.049$) suggesting that birds fed DDGS batch F
174 benefitted more from enzyme supplementation, compared to the rest.

175 *Dietary AMEn, ECR and nutrient availability*

176 The DMR of the diet containing DDGS batch B was lower ($P = 0.033$) compared to those of
177 diets containing DDGS batches A and D, but did not differ ($P > 0.05$) from the rest of the
178 diets. The NR of the diet containing DDGS batch B was also lower ($P = 0.021$) than diets
179 containing DDGS batches A and E, but was not different ($P > 0.05$) from the rest of the diets.

180 Enzyme supplementation improved dietary AMEn, DMR, NR ($P < 0.001$) and FD ($P < 0.05$)
181 compared to non-supplemented diets (Table 5). There was a DDGS batch by enzyme
182 interaction ($P = 0.022$) on ECR value, showing that DDGS sample F had a poorer (higher)
183 ECR ($P < 0.05$) than samples B and C when there was no enzyme addition, but there were no
184 differences ($P > 0.05$) between these samples when enzymes were added.

185 **DISCUSSION**

186 The study evaluated the effect of different wheat DDGS batches with and without enzyme
187 supplementation on dietary AMEn, nutrient availability and growth performance of broilers.
188 The DDGS batches were produced by a single production plant over a relatively short period
189 of time. The variation in chemical composition between batches (for example ranges of 52.1
190 g/kg CP, 19.3 g/kg in oil, 37.1 g/kg in total NSP, and 60.1 g/kg in starch) were either due to
191 small differences in processing conditions or differences in wheat grain used in the
192 production process. The results confirm the importance of research on batch variability of
193 wheat DDGS and the interaction with exogenous enzymes to better understand the source of
194 variation that influence its feeding quality for poultry.

195 The approximate nutrient, polysaccharide and GE contents of the experimental wheat DDGS
196 samples were similar to published reports (Bolarinwa and Adeola, 2012; Adebisi and
197 Olukosi, 2015). As expected, the studied wheat DDGS contained between two and three
198 times more NSP, fat, protein and ash compared to wheat (Pirgozliev *et al.*, 2003). Starch was

199 reduced by about 20 times (less for batch F) compared to average wheat starch contents in
200 line with fermentation process during DDGS production.

201 The growth performance was lower than the breeders recommendations (Aviagen Ltd,
202 Edinburgh, UK) but was expected for birds fed meal form feeds (Pirgozliev *et al.*, 2015a). It
203 has been widely reported that bioethanol plants have significant impact on nutritive value of
204 wheat and maize DDGS (Bandegan *et al.*, 2009; Cozannet *et al.*, 2010; Nuez Ortín and Yu,
205 2009). Batch variation in composition of maize DDGS produced by a single production plant
206 has been observed (Belyea *et al.*, 2004). Batch-to-batch variations in protein metabolic
207 characteristics and degraded protein balance in wheat DDGS from the same bioethanol
208 processing plant have been also reported (Gamage *et al.*, 2012). The chemical composition of
209 wheat, e.g. NSP and resistant starch varies, thus the polysaccharide content of DDGS
210 produced will also be variable. The main factors affecting the variability of wheat include,
211 crop nutrition, location, seasonal factors, and genetics (Pirgozliev *et al.*, 2003). Since wheat
212 for DDGS production is supplied from various sources all over the world, the observed
213 variability is not a surprise. Sharma *et al.* (2010) reported that differences in temperature
214 during the liquefaction stage may be a reason for differences in resistant starch content, thus
215 suggesting that a variation in the temperature during the process in the same plant may exist.
216 In addition, Classen *et al.* (2014) found that all stages of heat application during wheat DDGS
217 production negatively affected the content and digestibility of amino acids, thus indicating
218 the need to consider the level and control of heat application in wheat ethanol production.

219 The significant interaction between batch and enzyme addition in the WG and ECR indicates
220 that the enzyme addition particularly improved the nutritional value of batch F. This sample
221 had a high content of total and insoluble NSP and also starch. This indicates that the optimum
222 inclusion rate of exogenous fiber degrading enzymes varies between samples. Recent
223 research (Ward, 2014; Pirgozliev *et al.*, 2015b) also reported an improvement in nutrient

224 availability of high NSP content diets when supplemented with fiber degrading enzymes.
225 Although the fermentation process involving processing wheat to get ethanol could hydrolyse
226 a proportion of the NSP of the original wheat samples, the analysis of the DDGS samples
227 (Table 3) indicated that pentosans comprised 58% of the total NSP content. The NSP have a
228 structural function as the main components of plant cell walls, and the majority of NSP in
229 wheat are pentosans (Annison, 1991). The anti-nutritive properties of wheat pentosans and
230 their negative influence on growth performance of poultry have been well documented
231 (Pirgozliev *et al.*, 2015b). Although starch is considered as the main energy source in poultry
232 diets, processes such as heating can considerably alter the characteristics of starch, producing
233 starch with some degrees of structural alteration, also known as resistant starch, that is often
234 less susceptible to digestion (Englyst, 2000).

235 The determined metabolizable energy and the coefficients of retention of the diets had a
236 similar range to previous reports of diets with similar DDGS contents (Vilariño *et al.*, 2007;
237 Youssef *et al.* 2008; Cozannet *et al.*, 2010). The differences in AMEn and DMR between
238 batches did not follow a consistent pattern, although diets including DDGS batches A and D,
239 that had slightly higher GE, tended to have higher values than the rest.

240 A major objective of the study was to examine the batch to batch variability between
241 different DDGS samples from the same manufacturer. The mean AMEn of the DDGS
242 samples was 13.20 MJ/kg DM, and the % coefficient of variation between the 6 samples was
243 1.5 (a range of 0.64 MJ/kg DM). This variation is somewhat greater than expected from
244 unprocessed cereals; Pirgozliev *et al.* (2003) compared 9 wheat samples from the same
245 harvest year and found a % coefficient of variation of 0.9. Cozannet *et al.* (2010) reported a
246 range of 2.33 MJ/kg DM in 10 European wheat DDGS samples sourced from different
247 manufacturers which indicates that the variation between manufacturers may be much greater
248 compared to variation within manufacturer.

249 Supplementary enzymes improved dietary AMEn in accord with Whiting *et al.* (2015). The
250 effect of the enzymes addition on the improvement of AMEn suggests that enzymes were
251 able to convert the polysaccharide content of the DDGS. However, this did not always result
252 in improvement in growth performance in all of the DDGS samples (significant DDGS x
253 enzyme interaction). The NSP fraction has been described as the main anti-nutritive factor in
254 wheat DDGS as a feed ingredient for poultry (Cozannet *et al.*, 2010). The benefits of using
255 fiber degrading enzymes in broiler feed has been associated with reduced intestinal viscosity,
256 degradation of cell wall NSP (the pentosans comprised approximately 58% of the total NSP
257 content in the DDGS samples used in this study), and the release of encapsulated nutrients in
258 the gut (Min *et al.*, 2009; Collins *et al.*, 2012). Xylanase also increases the access of digestive
259 enzymes to substrates by disrupting the cell wall matrix (Parkkonen *et al.*, 1997), suggesting
260 that the mixture of xylanase and β - glucanase in this study may increase the access of
261 pancreatic enzymes to nutrients that may be trapped by fibers.

262 It should be noted that supplementing NSP degrading enzymes to diets containing maize
263 DDGS was not always effective in improving nutrient utilization (Min *et al.*, 2009). The
264 complexity of cereal NSP is enhanced with attached residues and branching that markedly
265 influence their physiochemical properties (Collins *et al.*, 2012). Arabinoxylans in maize are
266 heavily branched and prevent the access of exogenous enzymes, e.g. xylanase, to xylan
267 backbone thus inhibiting degradation (Appeldoorn *et al.*, 2010; Agger and Meyer, 2012).
268 Compared to maize, wheat arabinoxylans are less branched and this can partially explain the
269 effectiveness of xylanase in the reported study. Compared to maize, wheat DDGS contains
270 more mixed link β -glucans (Pritchard *et al.*, 2011; Ward, 2014), thus the presence of β -
271 glucanase may have opened more access to cleavage sites.

272 The addition of enzyme improved the AMEn of the DDGS samples by 0.30 MJ/kg DM. As
273 discussed previously there is relatively large variability between different wheat DDGS

274 samples. However, although the range in determined AMEn in the DDGS sample plus
275 enzyme was 0.51 MJ/kg DM (compared to 0.64 MJ/kg DM for the samples without enzyme)
276 there was no statistically significant evidence that enzyme addition had reduced variability
277 between the samples.

278 The results suggest that the feeding value of different wheat DDGS batches produced by a
279 single production plant may vary when fed to broilers. The DDGS batch by enzyme
280 interaction in growth performance indicated that the greatest response to enzyme
281 supplementation was in the DDGS sample with the highest insoluble polysaccharide content.
282 Enzyme supplementation improved dietary metabolizable energy and nutrient availability in
283 all diets. When formulating broiler diets the variable nutrient value of wheat DDGS should be
284 taken into account and the use of fiber degrading enzymes should be considered.

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435 **Table 1.** Ingredient composition (g/kg, as-fed) of the experimental broiler chicken balancer
 436 and experimental diets formulation

	Balancer	Experimental
Wheat	602.6	512.2
Soybean meal (48)	260.0	221.0
Wheat DDGS (sample D)	-	150.0
Soybean meal (full fat)	50.0	42.5
Vegetable oil	45.0	38.3
Monocalcium phosphate	15.0	12.8
Limestone	12.5	10.6
NaCl	2.7	2.3
Lysine	1.7	1.4
Methionine	3.5	2.9
Threonine	1.5	1.3
NaHCO ₃	1.5	1.3
Vitamin mineral premix ¹	4.0	3.4
	1000	1000
Calculated analysis (as fed)		
ME MJ/kg	13.11	12.77 ²
Crude Protein g/kg	219.4	231.0
Crude Fat g/kg	6.48	6.1
Ca g/kg	9.0	7.7
Available P g/kg	4.7	5.3
Lysine g/kg	13.0	13.8
Methionine + Cysteine g/kg	9.3	9.7
Analysed values³		
Dry matter (g/kg)	899	898
Gross energy (MJ/kg)	17.35	17.67
CP (N × 6.25) (g/kg)	226	235
Fat (g/kg)	62	59

437 This balancer was fed as a part of complete diet comprised 150 g/kg of each experimental
 438 wheat DDGS sample and 850 g/kg of the balancer. Each experimental diet met the diet
 439 specification for this strain of broiler chicken (Aviagen Ltd., Edinburgh, UK).

440 ¹The vitamin and mineral premix contained vitamins and trace elements to meet the
 441 breeder's recommendations (Aviagen Ltd., Edinburgh, UK). The premix provided (units/kg
 442 diet): retinol, 12,000 IU; cholecalciferol, 5,000 IU; α -tocopherol, 34 mg; menadione, 3 mg;
 443 thiamine, 2 mg; riboflavin, 7 mg; pyridoxine, 5 mg; cobalamin, 15 μ g; nicotinic acid, 50 mg;
 444 pantothenic acid, 15 mg; folic acid, 1 mg; biotin, 200 μ g; 80 mg Fe as iron sulfate (30%); 10
 445 mg Cu as a copper sulfate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc

446 oxide (72%); 1 mg I as calcium iodate (52%); 0.2 mg Se as sodium selenite (4.5%); and 0.5
447 mg Mo as sodium molybdate (40%).

448 ²Wheat DDGS sample D has determined AME value of 10.80 MJ/kg (data not published).

449 ³Analyses were performed in duplicate.

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452 **Table 2.** Proximate composition of the experimental wheat DDGS samples (data presented
453 on DM basis)

DDGS	Dry matter (g/kg)	Ash (g/kg)	Oil (g/kg)	Protein (g/kg)	Gross energy (MJ/kg)
A	896	54.9	49.8	317.5	21.78
B	898	64.9	42.8	326.2	20.29
C	900	58.2	41.9	327	21.42
D	892	55.1	51.3	322.4	21.77
E	895	53.1	55	325.3	21.68
F	843	57.5	61.2	274.1	21.16
ST DEV	21.9	4.17	7.33	20.53	0.572

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472 **Table 3.** Polysaccharide composition of the experimental wheat DDGS samples (g/kg DM
 473 basis)

Batch	Fraction	Ar	Xyl	Man	Gal	Gluc	Galact	NSP	Starch
A	NSPs	11.2	15.6	5.6	2.2	6.7	4.5	45.8	
	NSPn	43.5	67.0	6.7	8.9	63.6	0.0	189.7	
	NSPt	54.7	82.6	12.3	11.2	70.3	4.5	235.5	41.5
B	NSPs	13.4	24.5	6.7	5.6	14.5	5.6	70.2	
	NSPn	40.1	56.8	6.7	5.6	57.9	0.0	167.0	
	NSPt	53.5	81.3	13.4	11.1	72.4	5.6	237.2	28.5
C	NSPs	13.3	23.3	4.4	4.4	10.0	5.6	61.1	
	NSPn	38.9	61.1	6.7	6.7	62.2	0.0	175.6	
	NSPt	52.2	84.4	10.0	11.1	72.2	5.6	235.6	28.0
D	NSPs	13.5	19.1	4.5	4.5	7.8	3.4	52.7	
	NSPn	41.5	62.8	7.8	6.7	61.7	0.0	180.5	
	NSPt	54.9	81.8	11.2	11.2	70.6	3.4	233.2	31.9
E	NSPs	11.2	14.5	4.5	3.4	5.6	0.0	39.1	
	NSPn	40.2	63.7	6.7	7.8	60.3	0.0	178.8	
	NSPt	51.4	78.2	10.1	11.2	65.9	0.0	216.8	35.0
F	NSPs	10.7	9.5	4.7	2.4	1.2	3.6	32.0	
	NSPn	46.3	79.5	9.5	8.3	77.1	1.2	221.8	
	NSPt	56.9	89.0	14.2	10.7	78.3	4.7	253.9	88.1
ST DEV	s	1.18	4.55	0.72	1.08	3.13	1.45	11.18	
ST DEV	n	2.10	5.40	0.87	1.00	4.43	0.33	13.46	
ST DEV	t	1.57	2.54	1.43	0.13	2.68	1.51	6.91	15.31

474 Ar = Arabinose; Xyl = Xylose; Man = Mannose; Gal = Galactose; Glu = Glucose; Galact =
 475 Galacturonic acid ; NSPs = soluble non-starch polysaccharides; NSPn = nonsoluble non-
 476 starch polysaccharides; NSPt = total non-starch polysaccharides; ST DEV = standard
 477 deviation.

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481 **Table 4.** The effect of DDGS sample and enzyme supplementation of diets fed to broiler
 482 chickens on, daily feed intake (g DM), daily body weight gain (g), and gain to feed (g/g) ratio
 483 (data based on the feeding period from 7 to 21d age)¹

DDGS	Enzyme	FI (g DM/b)	WG (g/b/d)	G:F (g:g)
A	no	62.8	48.0 ^{ab}	0.757
B	no	64.6	48.0 ^{abc}	0.749
C	no	65.4	48.0 ^{abc}	0.741
D	no	66.9	50.0 ^{abc}	0.741
E	no	66.8	48.0 ^{abc}	0.723
F	no	65.6	46.0 ^a	0.700
A	yes	64.5	48.0 ^{abc}	0.752
B	yes	66.1	48.0 ^{abc}	0.730
C	yes	65.9	48.0 ^{ab}	0.725
D	yes	66.7	49.0 ^{abc}	0.735
E	yes	65.6	50.0 ^{bc}	0.761
F	yes	68.3	52.0 ^c	0.758
s.e.m. ²		0.90	1.10	0.0153
Main effects				
DDGS				
A		63.6 ^a	48.0	0.755
B		65.3 ^{ab}	48.0	0.740
C		65.7 ^{ab}	48.0	0.733
D		66.8 ^b	49.0	0.738
E		66.2 ^b	49.0	0.742
F		67.0 ^b	49.0	0.729
s.e.m. ²		0.64	0.80	0.0108
Enzyme				
	no	65.4	48.0	0.735
	yes	66.2	49.0	0.744
s.e.m. ²		0.37	0.50	0.0062
Probabilities				
		3.0	5.0	5.6
CV%				
DDGS		0.008	0.779	0.644
Enzyme		0.120	0.072	0.342
DDGS x Enzyme		0.312	0.049	0.080

484 FI = average daily broiler feed intake; WG = average daily broiler body weight gain; G:F =
 485 gain to feed ratio.

486 ^{a,b,c} Within DDGS or main effects mean values in a column not sharing a common superscript
 487 are significantly different.

488 ¹Each value represents the mean of five replicates.

489 ²Pooled standard error of mean.

490 **Table 5.** The effect of wheat DDGS sample and pelleting of diets fed to broiler chickens on
 491 nitrogen-corrected dietary apparent metabolisable energy (MJ/kg DM), energy conversion
 492 ratio (metabolisable energy intake:gain, MJ/kg), dry matter retention (g/g), nitrogen retention
 493 (g/g), and fat digestibility (g/g) coefficients (data based on excreta collection at the end of the
 494 study (21d age) and use of TiO₂ as indigestible marker)¹

DDGS	Enzyme	AMEn (MJ/kg DM)	ECR (MJ/kg)	DMR (g/g)	NR (g/g)	FD (g/g)
A	no	13.53	17.93 ^{abc}	0.701	0.604	0.757
B	no	12.88	17.25 ^a	0.682	0.566	0.744
C	no	13.10	17.69 ^{ab}	0.687	0.581	0.754
D	no	13.38	18.07 ^{abc}	0.694	0.584	0.785
E	no	13.24	18.37 ^{abc}	0.693	0.588	0.767
F	no	13.26	19.03 ^c	0.692	0.581	0.770
A	yes	13.72	18.33 ^{abc}	0.711	0.618	0.790
B	yes	13.21	18.18 ^{abc}	0.694	0.583	0.768
C	yes	13.65	18.91 ^{bc}	0.704	0.599	0.805
D	yes	13.68	18.67 ^{bc}	0.709	0.613	0.792
E	yes	13.53	17.81 ^{abc}	0.704	0.623	0.783
F	yes	13.39	17.72 ^{abc}	0.703	0.611	0.784
s.e.m. ²		0.120	0.393	0.0052	0.0105	0.0145
Main effects						
DDGS						
A		13.63 ^c	18.13	0.706 ^b	0.611 ^b	0.774
B		13.05 ^a	17.71	0.688 ^a	0.574 ^a	0.756
C		13.38 ^{bc}	18.30	0.696 ^{ab}	0.590 ^{ab}	0.780
D		13.53 ^{bc}	18.37	0.702 ^b	0.598 ^{ab}	0.788
E		13.38 ^{bc}	18.09	0.699 ^{ab}	0.605 ^b	0.775
F		13.33 ^b	18.37	0.697 ^{ab}	0.596 ^{ab}	0.777
s.e.m. ²		0.085	0.278	0.0037	0.0074	0.0103
Enzyme						
	no	13.23	18.06	0.691	0.584	0.763
	yes	13.53	18.27	0.704	0.608	0.787
s.e.m. ²		0.049	0.161	0.0021	0.0043	0.0059
CV%						
		2.0	4.8	1.7	3.9	4.1
Probabilities						
DDGS		<0.001	0.551	0.033	0.021	0.369
Enzyme		<0.001	0.357	<0.001	<0.001	0.006
DDGS x Enzyme		0.616	0.022	0.980	0.880	0.688

495 AMEn = nitrogen-corrected apparent metabolisable energy; ECR = energy conversion ratio;
 496 DMR = dry matter retention; NR = nitrogen retention; FD = fat digestibility.

497 ^{a,b,c} Within AMEn, DMR and NR main and DDGS values in a column not sharing a common
498 superscript are significantly different.

499 ¹Each value represents the mean of five replicates.

500 ²Pooled standard error of mean.