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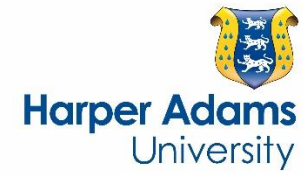

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Nutrient availability of different batches of wheat distillers dried grains with solubles with and without exogenous enzymes for broiler chickens

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

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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Experimental samples

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

Proximate analysis of samples

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

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

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

Diet preparation

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

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

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

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

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

Calculations

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

$$AMEn = GEd - GEex * (Tid/Tiex) - 34.39 * Nret$$

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

$$Nret = Nd - (Nex * Tid)/Tiex$$

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

NR = (Nd/Tid – Nex/Tiex)/(Nd/Tid)

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

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

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

DMR = (Tiex − Tid)/Tiex)

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

ECR (MJ/kg) = (AME intake (MJ))/Weight gain (kg)

Statistical analysis

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

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

RESULTS

Dietary DDGS samples

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

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

Bird performance

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

Dietary AMEn, ECR and nutrient availability

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

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

DISCUSSION

The study evaluated the effect of different wheat DDGS batches with and without enzyme supplementation on dietary AMEn, nutrient availability and growth performance of broilers. The DDGS batches were produced by a single production plant over a relatively short period of time. The variation in chemical composition between batches (for example ranges of 52.1 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 small differences in processing conditions or differences in wheat grain used in the production process. The results confirm the importance of research on batch variability of wheat DDGS and the interaction with exogenous enzymes to better understand the source of variation that influence its feeding quality for poultry.

The approximate nutrient, polysaccharide and GE contents of the experimental wheat DDGS samples were similar to published reports (Bolarinwa and Adeola, 2012; Adebiyi and Olukosi, 2015). As expected, the studied wheat DDGS contained between two and three 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 Ortın 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

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

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

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

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

The results suggest that the feeding value of different wheat DDGS batches produced by a single production plant may vary when fed to broilers. The DDGS batch by enzyme interaction in growth performance indicated that the greatest response to enzyme supplementation was in the DDGS sample with the highest insoluble polysaccharide content. Enzyme supplementation improved dietary metabolizable energy and nutrient availability in all diets. When formulating broiler diets the variable nutrient value of wheat DDGS should be 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
NaHCO3	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 values[3]		
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 [1]The 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 μg; nicotinic acid, 50 mg;
444 pantothenic acid, 15 mg; folic acid, 1 mg; biotin, 200 μg; 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 [2] Wheat DDGS sample D has determined AME value of 10.80 MJ/kg (data not published).
449 [3]Analyses were performed in duplicate.
450
451

Table 2. Proximate composition of the experimental wheat DDGS samples (data presented 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

Table 3. Polysaccharide composition of the experimental wheat DDGS samples (g/kg DM 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

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

Table 4. The effect of DDGS sample and enzyme supplementation of diets fed to broiler chickens on, daily feed intake (g DM), daily body weight gain (g), and gain to feed (g/g) ratio (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

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

[a,b,c] Within DDGS or main effects mean values in a column not sharing a common superscript are significantly different.

[1]Each value represents the mean of five replicates.

[2]Pooled standard error of mean.

Table 5. The effect of wheat DDGS sample and pelleting of diets fed to broiler chickens on nitrogen-corrected dietary apparent metabolisable energy (MJ/kg DM), energy conversion ratio (metabolisable energy intake:gain, MJ/kg), dry matter retention (g/g), nitrogen retention (g/g), and fat digestibility (g/g) coefficients (data based on excreta collection at the end of the study (21d age) and use of TiO_2 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

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

[a,b,c] Within AMEn, DMR and NR main and DDGS values in a column not sharing a common superscript are significantly different.

[1]Each value represents the mean of five replicates.

[2]Pooled standard error of mean.