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1 **Determination in broilers and turkeys of true phosphorus digestibility and retention in**
2 **wheat distillers dried grains with solubles without or with phytase supplementation**

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17 **ABSTRACT**

18 Wheat distillers dried grains with solubles (**wheat-DDGS**) is a viable source of P for poultry.
19 Two experiments were conducted to determine the true ileal P digestibility (**TPD**) and true total
20 tract P retention (**TPR**) of wheat-DDGS without or with phytase supplementation for broilers
21 and turkeys. In experiment 1 (broilers), wheat-DDGS inclusion linearly decreased ($P < 0.05$)
22 dietary ileal DM digestibility and total tract DM and P retention. The coefficient of TPD without
23 or with phytase for broilers was 0.94 or 0.96, respectively. The coefficient of TPR was 0.92 and
24 0.94 without or with phytase, respectively. In experiment 2 (turkeys), wheat-DDGS inclusion
25 linearly decreased ($P < 0.05$) dietary ileal DM digestibility and total tract DM retention. The
26 coefficient of TPD of wheat-DDGS for turkeys was 0.76 or 0.82 without or with phytase,
27 respectively. The coefficient of TPR of wheat-DDGS without or with phytase was 0.71 and 0.82,
28 respectively. Phytase had no effect ($P > 0.05$) on dietary ileal DM digestibility, total tract DM
29 retention, ileal P digestibility and total tract P retention for broilers and turkeys. Phytase had no
30 effect ($P > 0.05$) on TPD and TPR for broilers and turkeys. It was concluded that wheat-DDGS is
31 a valuable dietary source of digestible P for broilers and turkeys.

32 **Keywords:** broilers, phosphorus digestibility and retention, phytase, turkeys, wheat-DDGS

33 **1. Introduction**

34 Wheat distillers dried grains with solubles (**wheat-DDGS**) is the co-product of bioethanol
35 produced from wheat grain by the dry-grind process. It is possible to use wheat-DDGS as a
36 source of metabolisable energy and amino acids (**AA**) for broilers and turkeys (Bandegan et al.,
37 2009; Bolarinwa and Adeola, 2012), but the value of wheat-DDGS as a source of P for poultry

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38 has not been investigated. Wheat-DDGS has the potential to be a good source of digestible P for
39 poultry because substantial concentrations of phytate P are hydrolysed by the action of yeast
40 phytase during the fermentation process in bioethanol production (Liu, 2011).

41 The use of exogenous phytase in poultry diets is not new and a plethora of studies have
42 documented the efficacy of exogenous phytase in releasing phytate P and improving P
43 digestibility for poultry. Martinez-Amezcuca et al. (2004) noted that up to 25% of the total P in
44 maize-DDGS may be bound to phytate. As such, there is an opportunity to improve P
45 digestibility in wheat-DDGS for broilers and turkeys using exogenous phytase. Few studies have
46 determined the value of exogenous phytase in diets containing maize-DDGS for broilers
47 (Martinez-Amezcuca et al., 2006; Olukosi et al., 2010).

48 Broiler and turkey diets are formulated to contain optimal levels of P that best supports
49 maintenance and performance. It is essential to provide information about the digestible P
50 content of wheat-DDGS, because digestible P values of feed ingredients are a more accurate
51 measure of bird requirement compared with total P values. WPSA (2013) developed a standard
52 protocol for determining digestible P in feed ingredients for broilers and encourages using
53 digestible P as a measure of bird P requirements.

54 The objective of the current study was to determine the true ileal P digestibility (**TPD**) and
55 true total tract P retention (**TPR**) of wheat-DDGS without or with phytase supplementation in
56 broilers and turkeys.

57 **2. Materials and methods**

58 2.1. Animals and management

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59 The Scotland's Rural College Animal Experiment Committee approved all bird handling and
60 sample collection procedures.

61 On hundred and twenty-six Ross 308 male broiler chicks (Experiment 1) or 126 BUT 10 male
62 turkey poults (Experiment 2) were used for determination of TPD and TPR of wheat-DDGS.
63 Birds had *ad libitum* access to the diets and water in the entire pre- and experimental periods.
64 The birds were reared in a house with facilities to control temperature, light, and humidity. In the
65 two experiments, the birds were offered a pre-experimental diet that offers energy and nutrients
66 comparable with specific breed requirements. In each experiment, birds were allocated to one of
67 6 experimental diets in a randomised complete block design using d 14 bodyweight as blocking
68 criterion and transferred to metabolism cages on d 14. Each treatment had seven replicate cages
69 and three birds per replicate cage.

70 2.2. Diets and sample collection

71 The pre-experimental diet offered from d 1 to 14 in experiment 1 and 2 contained (as-is) 12.7
72 MJ/kg of ME, 230 g/kg of CP and 6.8 g/kg of P. The 6 experimental diets used in each
73 experiment consisted of three levels of wheat-DDGS in a maizestarch-dextrose based diet (200,
74 400 or 600 g/kg) and two levels of phytase (without or with) in a 3 × 2 factorial arrangement.
75 The phytase was added at a rate of 1000 FTU/kg. The phytase was derived from *Escherichia coli*
76 and expressed in *Schizosaccharomyces pombe*. One phytase unit was defined as the quantity of
77 enzyme required to liberate 1 µmol of inorganic P per min, at pH 5.5 from an excess of 15 µM
78 sodium phytate at 37°C.

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79 Titanium dioxide was added to the experimental diets (3 g/kg of diet) to enable determination
80 of ileal P digestibility or total tract P retention by the index method. Experimental diets were
81 offered between d 14 and 21. The ingredient and chemical compositions of the experimental
82 diets used in both experiments are shown in Table 1. Excreta were collected daily from each
83 cage for 3 d (d 18 to 20), dried and pooled within a cage. Birds were euthanized by cervical
84 dislocation on d 21 and ileal digesta were collected from the Meckel's diverticulum to
85 approximately 1 cm proximal to the ileo-cecal junction by flushing with distilled water. Ileal
86 digesta were pooled within a cage and subsampled for analyses. Samples of diets, wheat-DDGS,
87 excreta and ileal digesta were oven dried and ground to 0.5 mm particle size using a mill grinder
88 (Retsch ZM 100, F. Kurt Retsch GmbH & Co.KG, Haan, Germany) before chemical analysis.
89 Samples of diets, wheat-DDGS, ileal digesta and excreta were analyzed for P, DM and Ti.

90 2.3. Chemical analysis

91 To determine DM, samples were dried at 105 °C for 24 hours (method 930.15; AOAC, 2006)
92 in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England, UK). Ash
93 content was determined by heating wheat-DDGS samples in a muffle furnace at 500°C for 24
94 hours (Method 934.01; AOAC, 2006). Ether extract was determined using AOAC Method
95 920.39 (AOAC, 2003). Gross energy was determined in a Parr adiabatic bomb calorimeter using
96 benzoic acid as an internal standard (Model 6200, Parr Instruments, Moline, Illinois, USA).
97 Nitrogen was determined by the combustion method (method 968.06; AOAC 2006). For AA
98 analyses, samples were hydrolyzed for 24 hours in 6 N hydrochloric acid at 110°C under an
99 atmosphere of N. For Met and Cys, performic acid oxidation was carried out before acid
100 hydrolysis. The AA in the hydrolysate were determined by High Performance Liquid

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101 Chromatography after post-column derivatization [(method 982.30E (a, b, c); AOAC 2000].
102 Analysis for Ti was done as described by Short et al. (1996). Mineral concentrations in the
103 samples were determined using inductively coupled plasma spectrophotometry following the
104 procedures of Olsen and Sommers (1982). Crude fiber, NDF and ADF in the diets were
105 determined using the ANKOM's proprietary 200 Filter Bag Technique in Ankom 200 Fiber
106 Analyzer (Ankom Technology, Macedon, NY, USA). Phytate P in wheat-DDGS was determined
107 using inductively coupled plasma atomic emission spectroscopy (method 925.10; AOAC, 1990).
108 Phytase activity in diets was determined using AOAC method 2000.12 (AOAC, 2000).

109 2.4. Calculations and statistical analysis

110 Dietary ileal P digestibility or total tract P retention was calculated using the index method.
111 True ileal P digestibility or TPR was determined from the regression of P output at the ileal or
112 total tract against dietary P intake as done by Dilger and Adeola (2010). The regression model
113 was:

$$114 \quad 1. \quad PO-dmi = (TPI \times P_i) + EPL$$

115 where PO-dmi is P output (g/kg of DM intake); TPI is true P indigestibility; P_i is P intake (g/kg
116 DM) and EPL is endogenous P loss (g/kg of DM intake).

117 The coefficient of TPD or TPR was calculated from the measure of P indigestibility using the
118 following equation:

$$119 \quad 2. \quad TPD \text{ or } TPR = 1 - TPI$$

[Type text]

120 where TPD is coefficient of true ileal P digestibility, TPR is coefficient of true total tract P
121 retention and TPI is true P indigestibility, respectively.

122 Digestible phosphorus (DP) and retainable phosphorus (RP) contents in the wheat-DDGS were
123 calculated using the following equation:

124 3. $DP \text{ or } RP \left(\frac{g}{kg} \text{ DM} \right) = [(TPD \text{ or } TPR) \times DDGS - P]$

125 where TPD is coefficient of true ileal P digestibility, TPR is coefficient of true total tract P
126 retention and DDGS-P is analyzed P content (g/kg) in the wheat-DDGS.

127 Data were analyzed using the Generalized Linear Mixed Models of Genstat Statistical
128 Package (11th edition, VSN International). Statistical significance was set at $P < 0.05$ for all
129 mean comparisons. Dietary DM and P ileal digestibility and total tract retention data were
130 analyzed as a 3×2 factorial of wheat-DDGS inclusion level (200, 400 or 600 g/kg) and phytase
131 (without or with) using ANOVA procedures. Orthogonal contrasts were used to determine the
132 effects of graded wheat-DDGS intake and phytase supplementation on apparent P digestibility
133 and retention. The regression of P output against P intake was done using regression analysis
134 procedures. Improvements due to phytase supplementation were determined using ANOVA
135 procedures as the difference between the slopes of treatments not supplemented with phytase and
136 those supplemented with phytase.

137 **3. Results**

[Type text]

138 The chemical composition of the wheat-DDGS used in the current study is presented in Table
139 2. The total P, crude fibre, CP and AA contents in the wheat-DDGS were greater compared with
140 wheat.

141 Ileal digestibility and total tract retention of DM and P for broilers and turkeys offered graded
142 levels of wheat-DDGS without- or with supplemental phytase are presented in Table 3. There
143 was no wheat-DDGS \times phytase interaction ($P > 0.05$) for dietary ileal DM digestibility, total
144 tract DM retention, ileal P digestibility and total tract P retention for broilers and turkeys. In
145 broilers, increasing the inclusion level of wheat-DDGS in the diet linearly decreased ($P < 0.05$)
146 ileal DM digestibility and total tract DM retention and apparent total tract P retention but had no
147 effect ($P > 0.05$) on apparent ileal P digestibility. In turkeys, increasing the inclusion level of
148 wheat-DDGS in the diet linearly decreased ($P < 0.05$) ileal DM digestibility and total tract DM
149 retention but had no effect ($P > 0.05$) on either apparent ileal P digestibility or apparent total tract
150 P retention.

151 True P ileal digestibility and TPR in wheat-DDGS without or with supplemental phytase for
152 broilers is presented in Table 4. In broilers, the coefficient of TPD of wheat-DDGS without or
153 with supplemental phytase was 0.94 or 0.96, respectively. Corresponding coefficients of TPR
154 were 0.92 and 0.94, respectively. Phytase supplementation had no effect ($P > 0.05$) on TPD or
155 TPR in broilers. The digestible P and retainable P (DP and RP, respectively) contents in the
156 wheat-DDGS were calculated as the coefficient of TPD or TPR multiplied by the analyzed P
157 content (g/kg) in the wheat-DDGS. The DP content (g/kg) in the wheat-DDGS for broilers
158 without or with phytase was 6.0 or 6.2, respectively whereas RP content (g/kg) was 6.0 or 6.1,
159 respectively. The intercept in the regression equations in Table 4 represents endogenous P losses

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160 at the ileal and total tract. Ileal endogenous P loss (mg/kg of DMI) without or with phytase were
161 476 or 174, respectively. Endogenous P losses (mg/kg of DMI) at the total tract without or with
162 phytase were 625 or 201, respectively.

163 True P ileal digestibility and TPR in wheat-DDGS without or with supplemental phytase for
164 turkeys is presented in Table 4. In turkeys, the coefficient of TPD in wheat-DDGS without or
165 with phytase supplementation was 0.76 and 0.82, respectively. Corresponding coefficients of
166 TPR in the wheat-DDGS was 0.71 and 0.82, respectively. Phytase supplementation had no effect
167 ($P > 0.05$) on TPD or TPR in the wheat-DDGS for turkeys. Digestible P content (g/kg) in the
168 wheat-DDGS without or with phytase for turkeys was 4.9 or 5.3, respectively whereas RP (g/kg)
169 content was 4.6 or 5.3, respectively. Endogenous P losses at the ileal or total tract without or
170 with phytase are presented in Table 4. Ileal endogenous P loss (mg/kg of DMI) without or with
171 phytase were 430 or 98, respectively. Endogenous P losses (mg/kg of DMI) at the total tract
172 without or with phytase were 293 or 451, respectively.

173 **4. Discussion**

174 The objective of the current study was to determine the TPD and TPR of wheat-DDGS
175 without or with phytase supplementation for broilers and turkeys. Determination of TPD and
176 TPD in feedstuffs for broilers and turkeys is important because excessive P in poultry manure is
177 potentially harmful to the environment. The concentration of P is increased three-fold in DDGS
178 after the removal of starch in the grain during bioethanol production (Thacker and Widyaratne,
179 2007). Of greater importance is a large proportion of phytate-bound P in the grain is hydrolysed
180 by the actions of yeast phytase during fermentation, therefore increasing the concentrations of
181 non phytate P in DDGS (Liu, 2011). Because phytate-P is poorly utilised by poultry, feedstuffs

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182 containing low levels of phytate P are often desirable. However, it is unlikely that yeast phytase
183 will exert a complete hydrolyses of phytate-P during the fermentation process. Martinez-
184 Amezcua et al., 2004) observed that up to 25% of the total P in maize-DDGS was phytate-bound.
185 Therefore, there is a chance to improve P digestibility in wheat-DDGS for broilers and turkeys
186 using exogenous phytase.

187 The wheat-DDGS used in the current study contained 7.6 g/kg DM of total P which is lower
188 compared with the 12.3 g/kg DM reported by Thacker and Widyaratne (2007) or the 9.4 g/kg
189 DM noted by Nyachoti et al. (2005). Olukosi and Adebisi (2013) observed that P content in
190 eleven samples of wheat-DDGS from published data and from different sources ranged from 6.5
191 to 11.1 g/kg and concluded that P content in wheat-DDGS from different sources is markedly
192 variable. The differences in P content of wheat-DDGS are likely due to differences in the P
193 composition in the wheat used or to differences in processing techniques.

194 Increasing the inclusion level of wheat-DDGS reduced dietary DM digestibility and total tract
195 DM retention for broilers and turkeys in the current study. Increased levels of dietary fibre
196 decreases DM and nutrient digestibility in broilers (Jørgensen et al., 1996). Increasing wheat-
197 DDGS inclusion levels in a wheat-SBM based diet reduced DM and energy retention in broilers
198 (Bolarinwa and Adeola, 2012). Thacker and Widyaratne (2007) reported a reduction in apparent
199 P retention when using graded levels of wheat-DDGS in a practical wheat-SBM diet for broilers.
200 The increase in dietary fibre as wheat-DDGS replaced maize-starch in the diets may explain the
201 reduction in DM digestibility and retention observed in the current study.

202 Phytase had no effect on dietary P digestibility and retention for broilers and turkeys in the
203 current study. The efficacy of supplemental phytase to release P bound to phytate for poultry and

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204 pig have been described extensively in the literature and reviewed (Selle and Ravindran, 2007;
205 Woyengo and Nyachoti, 2011). The lack of improvement in dietary P digestibility and retention
206 as observed in the current study may be due to the characteristics of the wheat-DDGS. Liu and
207 Han (2011) assessed the concentrations of different forms of P (non phytate-P, phytate-bound P,
208 and total P) in different streams of the bioethanol production process and reported an increase in
209 maize-DDGS over maize grain of 1.8 fold in phytate-P and 10.8 fold in non-phytate P. Liu and
210 Han (2011) observed that during the fermentation process, percentage phytate-P in total P
211 decreased significantly whereas percentage non phytate-P in total P increased. These
212 observations implied that phytate underwent degradation through the actions of yeast phytase. In
213 addition, Martinez-Amezcuca et al. (2004) observed that the hydrolysis of phytate in the DDGS
214 during fermentation is often incomplete, and that heat treatment during the drying step may
215 further dissociate P from phytate in DDGS.

216 It is possible to extrapolate the TPD or TPR and basal endogenous P loss from the linear
217 relationship between undigested P and dietary P intake using the regression method. In the
218 current study, there was a strong relationship between undigested P and dietary P intake, which
219 is important when using the regression method. The regression method has been used to
220 determine TPD and TPR of feedstuffs for broilers (Dilger and Adeola, 2006) and swine
221 (Akinmusire and Adeola, 2009).

222 Mutucumarana et al. (2014) reported the coefficient of TPD in corn-DDGS to be 0.727, a
223 value that is lower compared with the 0.94 or 0.96 for the coefficient of TPD in broilers without
224 or with phytase, respectively noted in the current study. It is expected that differences in the
225 grain used, processing techniques and DDGS chemical characteristics will affect TPD in DDGS

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226 for broilers and may explain the differences in TPD noted in the current study and that of
227 Mutucumarana et al. (2004). The ileal digestible P or total tract retainable P contents in wheat-
228 DDGS were greater for broilers compared with turkeys in the current study. The difference in
229 TPD and TPR between broilers and turkeys in the current study is probably due to differences in
230 physiological maturity between the two species at 21 d of age. Uni et al. (1995; 1999) reported
231 that post hatch development of the small intestine in turkeys is slower compared with that of the
232 broiler chick. It is speculated that broilers being physiological more mature on day 28 were able
233 to utilise AA in the wheat-DDGS more efficiently compared with turkeys at the same age.

234 Endogenous P losses ranged from 98 to 625 mg/kg of DMI in the current study. Mean ileal
235 endogenous P losses in broilers were reported to be 272 mg/kg of DMI (Rutherford et al., 2002)
236 or 446 (Rutherford et al., 2004) or 418 mg/kg of DMI (Mutucumarana et al., 2014). The
237 endogenous P losses of 272 or 446 mg/kg of DMI noted by Rutherford et al. (2002; 2004) and
238 418 mg/kg of DMI noted by Mutucumarana et al. (2014) falls within the range of endogenous P
239 losses at the ileal noted in the current study for broilers. Modest differences in endogenous P
240 losses may be expected among studies due to differences in the chemical characteristics of the
241 feed ingredients or diets used.

242 Supplemental phytase had no effect on ileal or total tract endogenous P losses, TPD or TPR
243 for broilers and turkeys in the current study. The ratio of phytate P to total P in the wheat-DDGS
244 used in the current study was 0.23 and this value is similar to the average of 7 samples (0.27)
245 reported by Noblet et al. (2012). Compared with wheat, the phytate P level in the wheat-DDGS
246 used in the current study was lower than the mean of 22 wheat samples (0.15% vs. 0.25%,
247 respectively) analyzed in Noblet et al. (2012) study. At the inclusion rate of 200, 400 or 600

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248 g/kg, the diets used in the current study contained 0.3 g/kg, 0.6 g/kg or 1.0 g/kg of phytate-P,
249 respectively which may not have provided sufficient level of substrate for the supplemental
250 phytase to cause significant improvement in P digestibility. The low level of phytate bound P in
251 the wheat-DDGS used in the current study corroborates the high TPD and TPR noted for broilers
252 and turkeys and may explain the lack of phytase effect.

253 **5. Conclusions**

254 In conclusion, the results from the current study show that wheat-DDGS is an exceptional
255 source of digestible and retainable P for broilers and turkeys; thus the inclusion of wheat-DDGS
256 in the diet will reduce the use of inorganic P sources. Supplemental phytase had no effect on ileal
257 P digestibility or total tract P retention of the wheat-DDGS for broilers and turkeys most likely
258 because the wheat-DDGS contained low levels of phytate-bound P.

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263 receives support from the Scottish Government (RERAD).

264 **References**

265 Akinmusire, A. S., Adeola, O., 2009. True digestibility of phosphorus in canola and soybean
266 meals for growing pigs: Influence of microbial phytase. *J. Anim. Sci.* 87, 977-983.

[Type text]

267 AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemist,
268 Arlington, VA, USA.

269 AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemist,
270 Arlington, VA, USA.

271 AOAC, 2003. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemist,
272 Arlington, VA, USA.

273 AOAC, 2006. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemist,
274 Arlington, VA, USA.

275 Bandegan, A., Guenter, W., Hoehler, D., Crow, G. H., Nyachoti, C. M., 2009. Standardised ileal
276 amino acid digestibility in wheat distillers dried grains with solubles for broilers. *Poult.*
277 *Sci.* 88, 2592 – 2596.

278 Bolarinwa, O. A., Adeola, O., 2012. Energy value of wheat, barley and wheat distillers dried
279 grains with solubles for broiler chickens using the regression method. *Poult. Sci.* 91,
280 1928-1935.

281 Dilger, R. N., Adeola, O., 2006. Estimation of true phosphorus and endogenous phosphorus loss
282 in growing chicks fed conventional and low phytate soybean meals. *Poult. Sci.* 85, 661-
283 668.

284 Jørgensen, H., Zhao, X., Knudsen, K., Eggum, B., 1996. The influence of dietary fibre source
285 and level on the development of the gastrointestinal tract, digestibility and energy
286 metabolism in broiler chickens. *Br. J. Nutr.* 75, 379-395.

[Type text]

- 287 Liu, K., Chemical composition of distillers grains, a review. *J. Agric. Food Chem.* 59, 1508-
288 1526.
- 289 Liu, K., Han, J., 2011. Changes in mineral concentrations and phosphorus profile during dry-
290 grind processing of maize into ethanol. *Bioresour. Technol.* 102, 3110–3118.
- 291 Martinez Amezcua, C., Parsons, C. M., Noll, S. L., 2004. Content and relative bioavailability of
292 phosphorus in distillers dried grains with solubles in chicks. *Poult. Sci.* 83, 971.
- 293 Martinez-Amezcua, C., Parsons, C. M., Baker, D. H., Effect of microbial phytase and citric acid
294 on phosphorus bioavailability, apparent metabolizable energy, and amino acid
295 digestibility in distillers dried grains with solubles in chicks. *Poult. Sci.* 85, 470-475.
- 296 Mutucumarana, R. K., Ravindran, V., Ravindran, G., Cowieson, A. J., 2014. Measurement of
297 true ileal digestibility of phosphorus in some feed ingredients for broiler chickens. *J.*
298 *Anim. Sci.* 92, 5520-5529.
- 299 Noblet, J., Cozannet, P., Skiba, F., 2012. Nutritional value and utilization of wheat dried
300 distillers grain with solubles in pigs and poultry, in biofuel co-products as livestock feed -
301 Opportunities and challenges, ed, by Harinder P and Makkar S. Rome, pp. 163-174.
- 302 Nyachoti, C. M., House, J. D., Slominski, B. A., Seddon, I. R., 2005. Energy and nutrient
303 digestibilities in wheat dried distillers' grains with solubles fed to growing pigs. *J. Sci.*
304 *Food Agric.* 85, 2581-2586.

[Type text]

- 305 Olsen, S. R., Sommers, L. E., 1982. Phosphorus, in methods of soil analysis by Page, A. L.
306 Miller R. H., Keeney, D. R. 9th ed. Part II in the series Agronomy. American Society of
307 Agronomy and Soil Science Society of America Journal, Wisconsin, USA.
- 308 Olukosi O. A., Adebisi. A. O., 2013. Chemical composition and prediction of amino acid content
309 of corn- and wheat-Distillers' Dried Grains with Solubles. Anim. Feed Sci. Tech. 185,
310 182-189.
- 311 Olukosi, O. A., Cowieson, A. J., Adeola, O., 2010. Broiler responses to supplementation of
312 phytase and admixture of carbohydrases and protease in maize-soyabean meal diets
313 with or without maize Distillers' Dried Grain with Solubles. Br. Poult. Sci. 51, 434-
314 443.
- 315 Rutherford, S. M., Chung, T. K., Morel, P. C. H., Moughan, P. J., 2004. Effect of microbial
316 phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids
317 in a low-phosphorus diet for broilers. Poult. Sci. 83, 61-68.
- 318 Rutherford, S. M., Chung, T. K., Moughan, P. J., 2002. The effect of microbial phytase on ileal
319 phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43, 598-
320 606.
- 321 Selle, P. H., Ravindran, V., 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Tech.
322 135, 1-41.
- 323 Short, F. J., Gorton, P., Wiseman, J., Boorman, K. N., 1996. Determination of titanium dioxide
324 added as an inert marker in chicken digestibility studies. J. Anim. Sci. Tech. 59, 215-221.

[Type text]

325 Thacker, P. A., Widyaratne, G. P., 2007. Nutritional value of diets containing graded levels of
326 wheat distillers grains with solubles fed to broiler chicks. *J. Sci. Food Agric.* 87, 1386-
327 1390.

328 Woyengo, T. A., Nyachoti, C. M., 2011. Review: Supplementation of phytase and carbohydrases
329 to diets for poultry. *Can. J. Anim. Sci.* 91, 177-192.

330 WPSA, 2013. World's Poultry Science Association. Working Group Report. Working Group No
331 2: Nutrition of the European Federation of Branches of WPSA. Determination of
332 phosphorus availability in poultry. *World's Poult. Sci. J.* 69, 687-698.

333 Uni, Z., Noy, Y., Sklan, D., 1995. Post hatch changes in morphology and function of the small
334 intestines in heavy and light strain chicks. *Poult. Sci.* 74, 1622-1629.

335 Uni, Z., Noy, Y., Sklan, D., 1999. Post-hatch development of small intestinal function in the
336 poult. *Poult. Sci.* 78, 215-222.

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337 **Table 1**
338 Analyzed nutrient composition of wheat distillers dried grains with solubles (as-is basis)

Item	g/kg
Dry matter	858
Crude protein	326
Gross energy (MJ/kg)	18.5
Crude fibre	80.0
Ether extract	72.5
Neutral detergent fibre	389
Acid detergent fibre	223
Ash	46.0
Ca	1.60
Total P	6.50
Phytate P	1.50
K	10.6
Na	4.80
Mg	2.2
Fe	0.40
Mn	0.06
Cu	0.01
Zn	0.06
Amino acids	
Arg	11.8
His	8.30
Ile	13.7
Leu	22.6
Lys	7.70
Phe	15.8
Thr	11.5
Met	4.50
Trp	3.80
Val	16.2

[Type text]

339 **Table 2**
340 Ingredient and chemical composition of experimental diets to determine the phosphorus
341 digestibility and retention in wheat distillers dried grains with solubles for broilers and turkeys.

Item	Wheat distillers dried grains with solubles inclusion level, g/kg		
	200	400	600
Ingredients, g/kg			
Maize starch ¹	516	293.5	77
Wheat-DDGS	200	400	600
Soybean oil	18	36	48
Dextrose	100	100	100
Sucrose	130	130	130
Vitamin-mineral premix ²	2.5	2.5	2.5
Limestone	4.5	9	13.5
Common salt	4	4	4
Marker premix ³	15	15	15
Phytase premix	10	10	10
Analyzed composition ⁴			
Dry matter, g/kg	880	890	885
Phosphorus, g/kg	2.0	2.9	4.2
Calcium, g/kg	3.5	4.7	6.9
Phytase activity, FTU/kg	962	810	933

342 ¹Phytase premix replaced maize-starch at 10 g/kg.

343 ²Vitamin and mineral premix supplied per kg of diet: Vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25
344 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; nicotinic acid, 60 mg; pantothenic
345 acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese,
346 100 mg; cobalt, 1.0 mg; zinc, 82.2 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg.

347 ³Contained 1 g of titanium dioxide added to 4 g of maize-starch.

348 ⁴Values are means of duplicate analyses

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354 **Table 3**
 355 Ileal digestibility and total tract retention coefficients of dietary dry matter and phosphorus for broilers and turkeys receiving graded
 356 levels of wheat distillers dried grains with solubles with or without phytase supplementation¹.

Measurements	Broilers				Turkeys			
	Ileal DM digestibility	Total tract DM retention	Ileal P digestibility	Total tract P retention	Ileal DM digestibility	Total tract DM retention	Ileal P digestibility	Total tract P retention
Wheat-DDGS effect								
200 g/kg of wDDGS	0.79	0.79	0.63	0.60	0.75	0.75	0.45	0.19
400 g/kg of wDDGS	0.71	0.73	0.57	0.54	0.61	0.68	0.38	0.25
600 g/kg of wDDGS	0.65	0.68	0.61	0.46	0.51	0.61	0.35	0.20
S.E	0.02	0.01	0.03	0.04	0.02	0.01	0.05	0.05
P values for main effect of wDDGS levels	<0.001	<0.001	0.154	0.011	<0.001	<0.001	0.181	0.442
Phytase effect								
Without phytase	0.73	0.74	0.60	0.55	0.61	0.67	0.35	0.19
With phytase	0.70	0.73	0.61	0.52	0.64	0.69	0.43	0.24
S.E	0.01	0.08	0.03	0.04	0.02	0.01	0.04	0.04
P values for main effects of phytase	0.068	0.110	0.609	0.511	0.137	0.104	0.078	0.257
wDDGS × phytase interaction								
P values for effect of wDDGS inclusion	0.969	0.660	0.493	0.574	0.865	0.917	0.346	0.474
DDGS (linear)	<0.001	<0.001	0.392	0.003	<0.001	<0.001	0.072	0.860
DDGS (quadratic)	0.299	0.676	0.082	0.708	0.344	0.672	0.697	0.209

¹Data are means of 7 replicate pens

DM – dry matter; s.e.d - standard error of difference of mean; wDDGS – wheat distillers dried grains with solubles

[Type text]

359 **Table 4**
 360 True ileal phosphorus digestibility and true phosphorus retention of wheat distillers dried grains with solubles without or with
 361 phytase supplementation for broilers and turkeys¹.

	Regression equation ²	r ²	SE of slope ³	TPD coefficient ⁴	TPR coefficient ⁴	DP ⁵ , g/kg	RP ⁵ , g/kg
Experiment 1 - broilers							
True ileal P digestibility							
Without phytase	Y = 0.064X - 476	0.661	0.010	0.94	-	6.0	-
With phytase	Y = 0.040X + 174	0.725	0.005	0.96	-	6.2	-
True total tract P retention							
Without phytase	Y = 0.063X - 625	0.534	0.016	-	0.92	-	6.0
With phytase	Y = 0.065X - 201	0.689	0.010	-	0.94	-	6.1
Experiment 2 - turkeys							
True ileal P digestibility							
Without phytase	Y = 0.242X - 430	0.650	0.039	0.76	-	4.9	-
With phytase	Y = 0.179X - 98	0.422	0.047	0.82	-	5.3	-
True total tract P retention							
Without phytase	Y = 0.294X - 293	0.612	0.056	-	0.71	-	4.6
With phytase	Y = 0.184X + 451	0.375	0.054	-	0.82	-	5.3

362 ¹Data are means of 7 replicate pens

363 ²Ileal or excreta P output (mg/kg of DM intake) regressed against dietary P intake (mg/kg of DM). The intercept of the regression term is endogenous P
 364 loss (mg/kg of DM intake) whereas the slope is true P indigestibility.

365 ³Standard error of regression components

366 ⁴Calculated as 1 - true P indigestibility; TPD or TPR are coefficients of true ileal P digestibility or true P retention, respectively. Phytase had no effect
 367 on TPD and TPR of the wheat-DDGS.

368 ⁵DP and RP are digestible P and retainable P contents of wheat-DDGS, respectively. Calculated as coefficients of true P digestibility or retention
 369 multiplied by analyzed P content in wheat-DDGS (g/kg).