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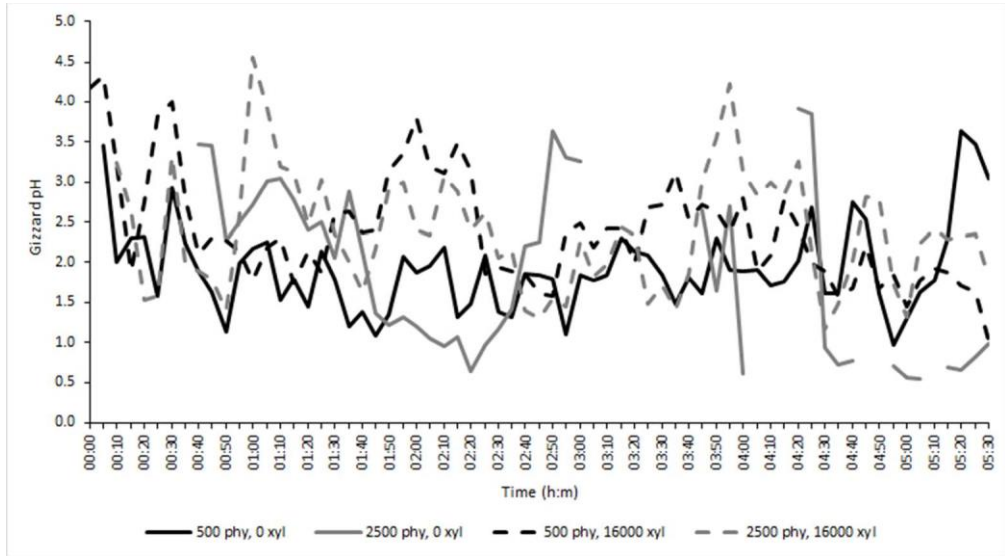


Figure 1

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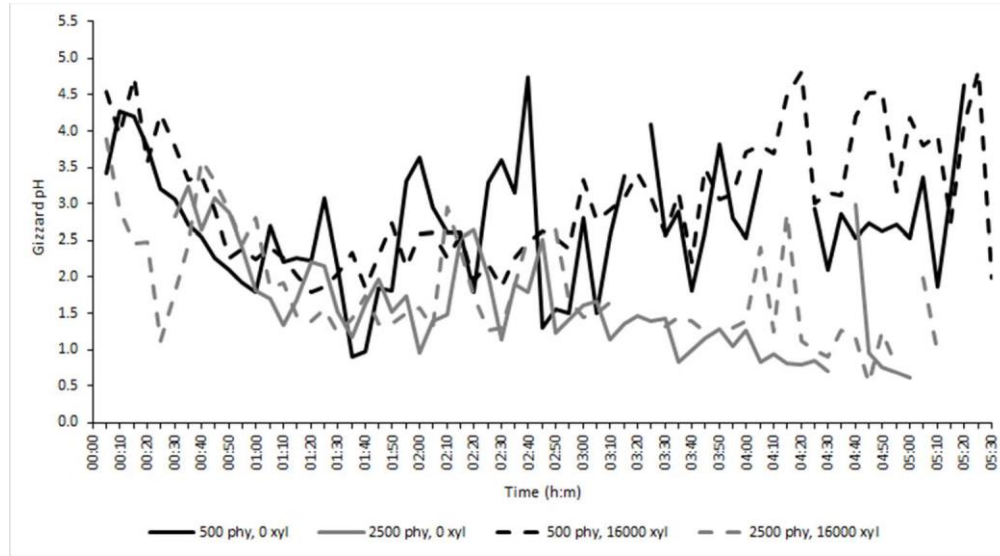


Figure 2

200x111mm (96 x 96 DPI)

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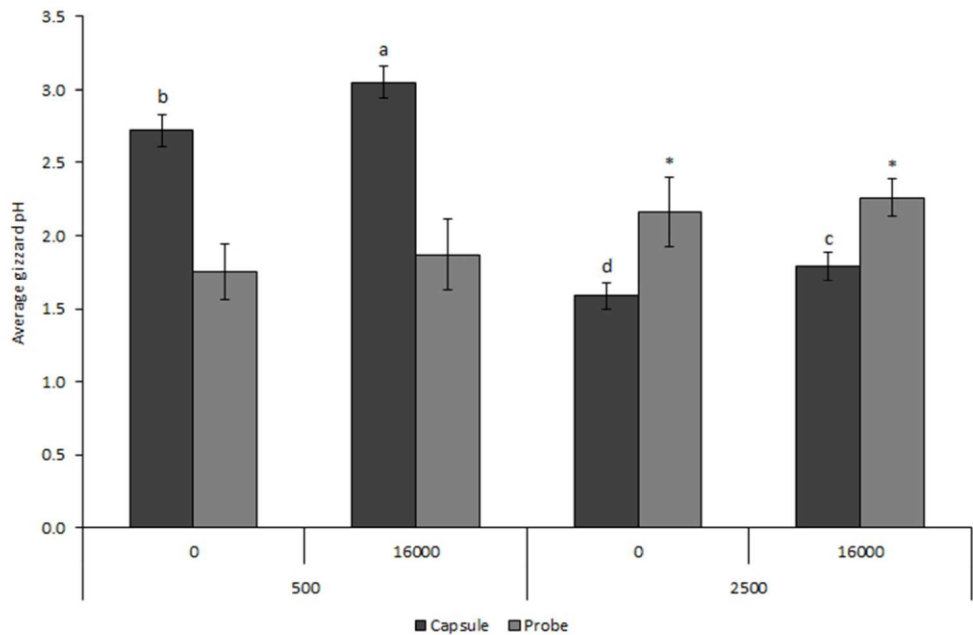


Figure 3
190x125mm (96 x 96 DPI)

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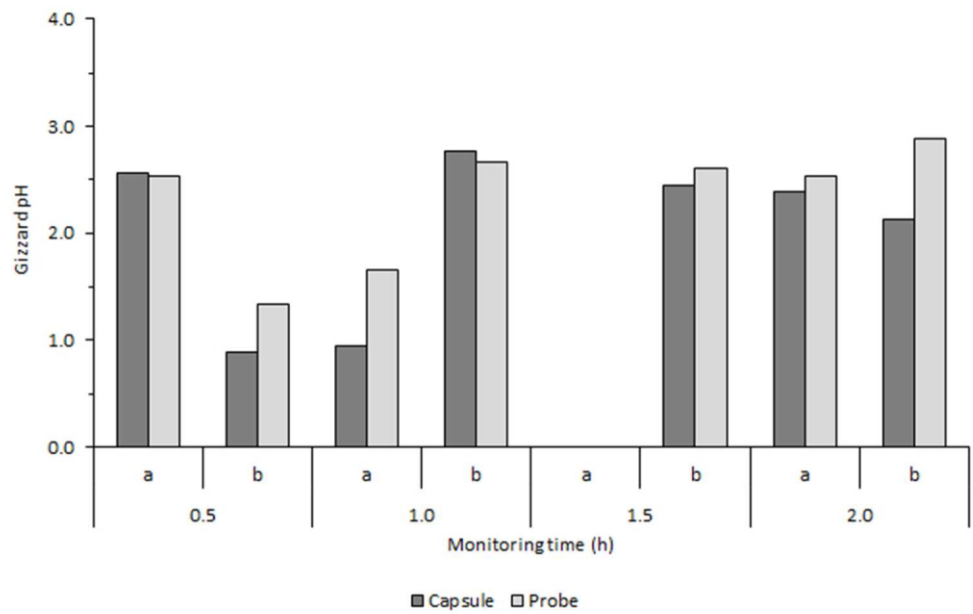


Figure 4
169x107mm (96 x 96 DPI)

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3 **Exogenous phytase and xylanase exhibit opposing effects on real-time gizzard pH in broiler**
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5 **chickens**
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Abstract

1. The current study was conducted to evaluate the influence of high phytase doses and xylanase, individually and in combination, on performance, blood inositol and real-time gastric pH in broilers fed wheat-based diets.
2. In a 42 d experiment, a total of 576 male Ross 308 broiler chicks were allocated to four dietary treatments. Treatments consisted of a 2×2 factorial arrangement, with 500 or 2500 FTU/kg phytase and 0 or 16000 BXU/kg xylanase, fed in two phases (starter 0–21; grower 21–42 d). Heidelberg pH capsules were administered to eight birds from each treatment group, pre and post diet phase change, with readings captured over a 5.5 h period.
3. At 21 and 42 d, birds fed 500 FTU/kg phytase without xylanase had on average 127g and 223 g lower weight gain than all other treatments, respectively ($P < 0.05$). At 21 d, FCR was reduced ($P < 0.01$) by 2500 FTU/kg phytase or xylanase, however, 42 d FCR was unaffected by enzyme treatment. Inositol content of plasma was twice that of the erythrocyte ($P < 0.001$), with 2500 FTU/kg phytase tending to increase ($P = 0.07$) inositol content in both blood fractions.
4. Across all treatments, capsule readings ranged from pH 0.54 to 4.84 in the gizzard of broilers. Addition of 2500 FTU/kg phytase to the grower diet reduced ($P < 0.05$) average gizzard pH from 2.89 to 1.69, whilst feeding xylanase increased ($P < 0.001$) gizzard pH from 2.04 to 2.40. In contrast, digital probe measurements showed no effect of xylanase on gizzard pH, while addition of 2500 FTU/kg phytase increased ($P = 0.05$) pH compared to 500 FTU/kg phytase with or without xylanase.
5. These findings suggested that xylanase and high phytase doses have opposite effects on real-time gastric pH, while similarly improving performance of broilers.

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3 **Keywords:** Gizzard; pH; Capsule; Phytase; Xylanase
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27 **Introduction**

28 The use of exogenous enzymes in feed is common practice in today's poultry farming. Plant
29 feedstuffs contain a variety of anti-nutritional factors (ANF), including non-starch
30 polysaccharides (NSP) and phytate, which hinder diet utilisation and encourage the use of
31 enzymes that reduce the impact of ANF. The predominant enzyme in poultry diets is phytase,
32 which is added to increase phytate hydrolysis and release phosphorus (P), thereby lowering
33 the requirement for expensive inorganic phosphorus and reducing P excretion (Nelson *et al.*,
34 1971; Ravindran *et al.*, 1995). The physiological importance of P is primarily associated to
35 bone mineralisation (Bailey *et al.*, 1986), and to a lesser extent growth performance
36 (Waldroup *et al.*, 2000; Yan *et al.*, 2001). Recent developments have led to the application of
37 higher phytase inclusion rates, referred to as superdosing (Walk *et al.*, 2013), to exploit the
38 'extra-phosphoric effects' of phytase by reducing the anti-nutritive influence of phytate on
39 protein and mineral digestion and retention. Higher phytase doses have been shown to
40 improve weight gain, FCR, meat yield, bone ash, phytate-P disappearance and inositol
41 provision in poultry (Cowieson *et al.*, 2011).

42 Arabinoxylans, the major NSP fraction in wheat, are largely indigestible and reduce nutrient
43 digestibility of the diet through increased digesta viscosity and reduced enzyme access to
44 nutrients (Choct and Annison, 1992a; Choct and Annison, 1992b). Exogenous xylanases have
45 been widely used in wheat-based diets to reduce digesta viscosity and improve nutrient
46 utilisation and growth performance of poultry (Adeola and Bedford, 2004; Choct *et al.*, 2004,
47 Gao *et al.*, 2008; Kiarie *et al.*, 2014). Reports have indicated a link between increased gizzard
48 weight and feed retention and xylanase supplementation (Masey O'Neill *et al.*, 2014; Singh *et*
49 *al.*, 2012). Svihus (2014) speculated that a greater gizzard volume and retention time may

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3 50 elevate HCl secretion and thus lower gizzard pH. However, previous reports have found no
4
5 51 effect of xylanase on gizzard pH in broiler chickens (Engberg *et al.*, 2004; Lee *et al.*, 2017c).
6
7 52 Although substrate specificity of these enzymes is different, a number of studies have
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9 53 reported synergistic responses to phytase and xylanase (Kühn *et al.*, 2013; Schramm *et al.*,
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11 54 2017; Selle *et al.*, 2003; Selle *et al.*, 2009), and hence the use of more than one enzyme is
12
13 55 becoming routine in commercial practice. When used in combination, xylanase may enhance
14
15 56 the availability of phytate within the food-matrix to phytase (Adeola and Cowieson, 2011),
16
17 57 thereby improving precaecal nutrient and mineral digestibility. By manipulating the digestive
18
19 58 process, it is possible that these enzymes can influence the digestive environment. In previous
20
21 59 studies (Lee *et al.*, 2017a; Lee *et al.*, 2018), the ability of phytase to alter gastric pH using real-
22
23 60 time pH capsule technology has been demonstrated. However, pH response to xylanase over
24
25 61 time has not yet been evaluated. Consequently, the objective of the current study was to
26
27 62 investigate the effect of high phytase inclusion rates and xylanase supplementation on growth
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29 63 performance and real-time gastric pH measurements in broiler chickens.
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35 65 **Materials and methods**

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38 66 Animal trials were presented and accepted by the Drayton Animal Health Welfare and
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40 67 Ethical Review Body and conducted according to the Animals (Scientific Procedures) Act
41
42 68 1986.

43 69 *Animal and housing*

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46
47 70 A total of 576 male Ross 308 broiler chicks were supplied from a commercial hatchery (P D
48
49 71 Hook Hatcheries Ltd, UK) in a 42-day experiment. Chicks were vaccinated against infectious
50
51 72 bronchitis at the hatchery before arriving at the experimental housing unit in two batches, one
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53 73 week apart. Birds were raised in separate rooms to allow for sufficient pH capsule monitoring
54
55 74 to be performed. On day 1, chicks were randomly allocated to one of four dietary treatments,
56

75 whereby each treatment group had eight replicate floor pens (1.5 x 1.3m) bedded on wood
 76 shavings, each containing 18 chicks. Light was provided for 23 h for 1 d.o. birds, 20 h for 2
 77 d.o. and 3 d.o. birds, and 16 h for 4-42 d.o. birds. Light intensity was provided at
 78 approximately 40 lux on d 1, reducing to a target of 20 lux over the following 10 d. The
 79 temperature of the housing unit was set to 31°C at d 1, and gradually decreased to 20°C over
 80 the rearing period. Each pen of birds was weighed on days 0, 21 and 42 of the study. Any
 81 birds withdrawn from study or died during the study were weighed manually when removed.

82 *Dietary treatments*

83 Treatments consisted of a 2 × 2 factorial arrangement, with 500 or 2500 FTU/kg phytase
 84 (modified *E. coli*-derived 6-phytase; Quantum Blue, AB Vista, Marlborough, UK) and 0 or
 85 16000 BXU/kg xylanase (family 11 xylanase derived from *Nonomurea flexuosa*; Econase
 86 XT25, AB Vista, Marlborough, UK). Treatment diets were wheat-soy based (Table 1), and
 87 formulated to meet or exceed the NRC (1994) nutritional requirements of broilers.

89 **Table 1** Composition of starter and grower broiler diets

<u>Ingredient, g/kg</u>	<u>Starter (0-21 d)</u>	<u>Grower (21-42 d)</u>
Wheat	633.0	735.7
Soybean meal 48	308.5	205.2
Soy oil	27.1	35.9
Salt	3.9	3.9
DL Methionine	1.8	0.8
Lysine HCl	2.1	2.1
Threonine	0.2	0.0
Limestone	12.8	9.7
Mono Ca Phosphorus	6.0	2.1
Premix ¹	4.0	4.0
Monteban G100	0.6	0.6
Quantum Blue ²	0.1	0.1
Nutrient composition, %		
Crude protein	21.85	17.90
ME, MJ/kg	12.45	12.97
Calcium	0.98	0.78
Phosphorus	0.71	0.59

Phytate Phosphorus	0.23	0.21
Available Phosphorus	0.46	0.37
Fat	4.12	5.04
Crude fibre	2.60	2.50
Methionine	0.50	0.34
Methionine + Cysteine	0.88	0.67
Lysine	1.28	1.00
Tryptophan	0.27	0.22
Threonine	0.80	0.62
Sodium	0.19	0.19
Chloride	0.33	0.33

¹ Starter premix- supplied per kg of diet: manganese, 100 mg; zinc, 80 mg; iron (ferrous sulphate), 20 mg; copper, 10 mg; iodine, 1.0 mg; molybdenum, 0.50 mg; selenium, 0.25 mg; retinol (vitamin A), 13.5 mg; cholecalciferol (vitamin D₃), 5 mg; tocopherol (vitamin E), 100 mg; thiamine (vitamin B₁), 3 mg; riboflavin (vitamin B₂), 10 mg; pyridoxine (vitamin B₆), 3.0 mg; cobalamin (vitamin B₁₂), 30 mg; hetra, 5.0 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; and biotin 251 mg. choline chloride, 250 mg. Grower premix- same as starter, except retinol (vitamin A), 10.0 mg.

²Quantum Blue was included at 100g/t, with an expected activity of 500FTU/kg, into all diets. Phytase matrix applied: 0.15% available phosphorus, 0.165% calcium, 0.035% sodium.

Phytase was included at 100 g/t (expected activity of 500 FTU/kg) in all diets, and assigned a matrix value of 0.15% available phosphorus, 0.165% calcium, 0.035% sodium. No matrices were used for the subsequent addition of enzymes. For treatments with 2500 FTU/kg phytase, a further 400 g/t (2000 FTU/kg) phytase was added to the basal diet. Diets were fed in two phases; starter crumb (0–21d) and grower pellet (21–42 d) and were provided *ad libitum* along with water throughout the study. Analysed nutrients in starter and grower feed are shown in Table 2.

TABLE 2 HERE

Capsule administration and data collection

Four pens per treatment were selected for capsule dosing, with eight birds per treatment (four birds from each batch, two birds per pen) being randomly selected for capsule administration

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2
3 114 on either day 19 or 20 (pre-diet phase change). The same eight birds were dosed again on
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5 115 either d 22 or 23 (post diet phase change). The Heidelberg pH Diagnostic System (fifth
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7 116 generation) from Heidelberg Medical, including a pH capsule and transceiver, was used to
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9 117 capture pH readings. Capsules were administered to birds as previously described (Lee *et al.*,
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11 118 2017a). Capsuled birds were isolated into individual pens placed within the original treatment
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13 119 pen. This allowed the transceiver to remain in close proximity to the bird, thereby optimising
14
15 120 data collection. Individual pens had separate feeders and drinkers with diets and water
16
17 121 provided *ad libitum* during the monitoring period.
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19 122 Capsule readings were collected every second, over a 5.5 h period, and aggregated into 5 min
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21 123 averages prior to analysis. Readings of pH 0, owing to lost signal between the capsule and the
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23 124 transceiver, were removed from the data set as these were not considered ‘true’ values. Data
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25 125 anomalies were removed from the data set prior to statistical analysis, as determined by
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27 126 values residing outside 3 x root mean square error (RMSE).
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29 127 Upon completion of the initial capsule readings, birds were subsequently placed back into
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31 128 their respective original treatment pen. However, following the final capsule reading at 22 or
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33 129 23 d, birds were humanely killed by electrical stunning and exsanguination. Immediately, the
34
35 130 gizzard was located and a small incision made to allow a spear tip pH probe (Oakton, USA)
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37 131 to be inserted. Concurrent to pH readings taken by the probe, capsule readings were collected
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39 132 at the same time to assess method comparability. The spear-tip probe was calibrated using the
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41 133 same pH standards (pH 1.0 and 7.0) that were used to calibrate the Heidelberg capsules to
42
43 134 maintain consistency between the two methods.

135 *Capsule Benchmarking*

136 At the end of the experiment (d 42) birds from the 500 FTU/kg phytase without xylanase
137 treatment group were used in a benchmarking assessment to confirm the accuracy of the
138 capsule readings when dosed for different periods of time. Eight birds were monitored in

1
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3 139 total, two from each group at the following time points: 0.5, 1.0, 1.5 and 2.0 h post dosing
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5 140 with the pH capsule. On completion of capsule dosing, birds were humanely euthanised and a
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7 141 spear-tip probe used to measure gizzard pH simultaneously to a capsule reading.
8

9
10 142 *Foot pad and litter scores*

11 143 External foot pad dermatitis (FPD) scores were recorded for all birds on day 21 and 42.
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13 144 Scores were assessed as follows: 1 = good condition, no lesions; 2 = mild superficial lesions
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15 145 are visible within a small area; 3 = moderate lesions, discolouration and thickening to the foot
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17 146 pad, not widespread; 4 = lesions over majority of the area, maybe inflamed; 5 = severe
18
19 147 lesions over majority of the area, may have signs of ulcers and/or scabs, haemorrhages,
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21 148 bleeding and inflammation.
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23
24 149 Litter quality, in terms of friability, was determined on day 21 and 42 for each pen.
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26
27 150 Throughout the experimental period, all pens received approximately equal quantities of
28
29 151 shavings. Scores were determined using the following criteria: 1 = fully friable - no capping
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31 152 in any area; 2 = mostly friable - very slight capping (5-40%); 3 = friable litter area reduced
32
33 153 (~50%); 4 = still small areas of friable litter - most of assessment area capped (60-75%); 5 =
34
35 154 extensive capping over all of assessment area (>80%).
36

37 155 *Blood inositol*

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39 156 Following euthanasia of capsulated birds, a terminal blood sample was collected into lithium
40
41 157 heparin vacutainers. Erythrocytes were pelleted by centrifugation at 1,500 x g for 10 min and
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43 158 an aliquot was washed by mixing with 10 volumes of phosphate-buffered saline, followed by
44
45 159 centrifugation at 1,500 x g for 10 min. Plasma samples were mixed with 2 volumes of ice-
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47 160 cold 1N-perchloric acid and held on ice for 20 min to allow precipitation of protein. Samples
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49 161 were centrifuged at 16,000 x g for 15 min at 4°C and the supernatant diluted 50-100-fold in
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51 162 18.2 MOhm.cm water. Inositol was determined by HPLC pulsed amperometry (HPLC-PAD)
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53 163 on a Dionex DX-600 HPLC System fitted with two 6-port valves. Following this, 20 ml of
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3 164 sample was injected onto a 4 mm x 50 mm CarboPac PA1 column (Dionex, UK) arranged in
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5 165 series with a 4 mm x 250 mm CarboPac MA1 column with 4 mm x 50 mm guard column of
6
7 166 the same material.

8
9 167 Initial flow rate of the 150 mM NaOH eluent was 0.4 ml/min. Once inositol had eluted from
10
11 168 the CarboPac PA1 column onto the CarboPac MA1 column, the flow through the CarboPac
12
13 169 PA1 column was switched at 1.5 min to 750 mM NaOH, at 0.4 ml min⁻¹. Eluent (150 mM
14
15 170 NaOH) from the CarboPac MA1 column was directed to an ED50 electrochemical detector
16
17 171 (Dionex) configured with a gold electrode and operating a standard Dionex carbohydrate
18
19 172 waveform. After 11.5 min, the CarboPac PA1 column was returned to the 150 mM NaOH
20
21 173 flow, in series with the MA1 column, conditioning the columns for a further 8.5 min before
22
23 174 the next injection. Inositol was eluted at approximately 10.5 min. For determination of
24
25 175 inositol concentration, peaks derived from inositol standards (0.01-0.2 nM in 20 µl) were
26
27 176 used to create a linear calibration curve ($r^2 > 0.995$) with a slope of approximately 100
28
29 177 nC.min/nmol.

30 31 32 33 178 *Statistical analysis*

34
35 179 The effect of phytase and xylanase on performance parameters and pH readings were
36
37 180 compared statistically by Least Squares ANOVA using JMP Pro 13.0 (SAS Institute Inc.,
38
39 181 Cary, NC). When considering gastric pH changes, diet phase change was included in the
40
41 182 model. When differences were significant, least square means were separated using Student's t-
42
43 183 test. Mortality, footpad and litter scores were analysed using a non-parametric Wilcoxon Test.
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45 184 Significance was accepted at $P \leq 0.05$, with trends ($P < 0.10$) discussed.
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51 186 **Results**

52
53 187 In-feed phytase activities were measured by ELISA (performed by AB Vista Lab Services)
54
55 188 and were as expected (Table 2).
56

189 *Performance*

190 The effect of phytase and xylanase dose on performance parameters in broilers is shown in
191 Table 3. At days 0-21, neither high phytase dose nor xylanase inclusion significantly
192 influenced feed intake. However, during days 21-42, feed intake was affected by a phytase
193 and xylanase interaction ($P = 0.03$), with birds fed 500 FTU/kg phytase without xylanase
194 having lower feed consumption than all the other treatments. Considering the entire
195 experimental period (d 0-42), dietary treatment had no significant effect on feed consumption
196 in birds.

197

198 TABLE 3 HERE

199

200 An interaction between phytase and xylanase ($P=0.04$) was seen for BWG from d 0 to 21,
201 whereby birds fed 500 FTU/kg phytase without xylanase gained less (127g on average) than
202 all other treatments. From d 21-42, higher doses of phytase (2500 FTU/kg) improved
203 ($P=0.04$) BWG of broilers by approximately 70g, compared to diets with 500 FTU/kg
204 phytase. Addition of xylanase, however, had no effect on BWG from d 21-42. Over the entire
205 experimental period, an interaction between phytase and xylanase ($P=0.04$) was evident, with
206 birds fed 500 FTU/kg phytase without xylanase having, on average, 223 g lower weight gain
207 than all other treatments.

208 From d 0-21, FCR was lowered ($P<0.01$) by five points with addition of 2500 FTU/kg
209 phytase and seven points with xylanase, although no interaction between these enzymes was
210 shown. However, from d 21-42 and over the entire experimental period, dietary treatment had
211 no significant effect on FCR or body weight corrected FCR.

212 Mortality was not significantly affected by treatment at any age (Table 4). However,
213 mortality was clearly higher in the starter phase than in the grower. It was noted that, in the

214 first batch of chicks, 74% of mortalities occurred within the first week. Mortality was 13.5%
 215 in the starter phase for the first batch compared to 5.1% in the second batch. Once these birds
 216 were removed, mortality was reduced during the grower phase to around 3% for both batches,
 217 which is within the expected level. Therefore, high mortality in this trial was attributed to
 218 poor chick quality in the first batch of chicks, and not dietary treatments.

219

220 **Table 4** Influence of phytase and xylanase on broiler mortality¹

221

		Mortality (%)		
Phytase (FTU/kg)	Xylanase (BXU/kg)	Days 0-21	Days 21-42	Days 0-42
500	0	5.48	3.42	8.82
2500	0	10.20	5.11	14.86
500	16000	9.58	1.44	10.94
2500	16000	12.04	1.39	13.36
	SEM	1.606	0.799	1.588
<i>P</i> -value				
Phytase		0.075	0.894	0.085
Xylanase		0.309	0.068	0.850

222 ¹Means represent the average response of 8 replicate pens (144 chicks) per treatment.

223 SEM, standard error of the mean

224

225 *Litter and FPD scores*

226 The effects of treatment on footpad and litter scores were determined at 21 and 42 d of age
 227 (Table 5). Litter scores were unaffected by treatment and were given approximate scores of 2
 228 and 3 for day 21 and 42, respectively, indicating that capping was not extensive in this trial.

229 The majority of foot pad dermatitis (FPD) scores for all treatments ranged between 1 and 2,
 230 signifying overall good-to-mild footpad conditions in birds. At day 21, FPD scores were
 231 unaffected by treatment, however, at day 42 a xylanase effect was shown (P=0.01); feeding
 232 xylanase reduced the incidence of FPD scores of 5 (severe), compared to when no xylanase
 233 was supplemented.

234 TABLE 5 HERE

235

236 *Blood inositol content*

237 Blood inositol content, measured in two separate blood fractions (erythrocyte and plasma),
 238 with results presented in Table 6. Xylanase in the diet had no effect on inositol levels, and no
 239 interaction between xylanase and phytase was shown. Addition of 2500 FTU/kg phytase
 240 tended to increase ($P = 0.056$) blood inositol level, compared to 500 FTU/kg phytase. The
 241 fraction of blood analysed had a considerable effect ($P < 0.001$) on inositol levels, with
 242 samples taken from the plasma having higher inositol content than that from erythrocytes.

243

244 **Table 6** Blood myo-inositol content in birds fed diets containing varying levels of phytase
 245 and xylanase¹

246

Blood fraction	Phytase (FTU/kg)	Xylanase (BXU/kg)	Myo-inositol (nmol/mL)
Erythrocyte	500	0	98.1
	2500	0	106.8
	500	16000	95.2
	2500	16000	136.4
Plasma	500	0	246.3
	2500	0	281.8
	500	16000	246.2
	2500	16000	294.4
RMSE			49.90
Erythrocyte			109.1
Plasma			267.2
	500		171.4
	2500		204.9
		0	183.3
		16000	193.0
<i>P</i> -value			
Phytase			0.070
Xylanase			0.584
Blood fraction			<0.001
Phytase x Xylanase			0.528

Phytase x Blood fraction	0.635
Xylanase x Blood fraction	0.842
Phytase x Xylanase x Blood fraction	0.780

247 ¹Means represent the average response of 4 birds per treatment
 248 RMSE, root mean square error
 249

250 *Gizzard pH*

251 Changes in gizzard pH over the 5.5 h period in response to supplementing phytase and
 252 xylanase to broiler starter (Figure 1) and grower (Figure 2) diets were recorded.

253

254 FIGS 1 AND 2 HERE

255

256 Capsule readings ranged from pH 0.54 to 4.84 in the gizzard of broilers across all treatments
 257 (Table 7). Following euthanasia, capsules were located in the gizzard of broilers, except for
 258 one bird in the 500 FTU/kg phytase with xylanase treatment group where the capsule was
 259 found in the crop. Data from this bird was kept in the analysis as pH readings were within the
 260 expected limits for gastric readings, and therefore it is possible that the capsule had moved
 261 out of the gizzard during euthanasia. A feed phase x phytase interaction ($P < 0.001$) was seen
 262 for gizzard capsule pH, whereby increasing phytase dose from 500 to 2500 FTU/kg had no
 263 effect on average gizzard pH (2.16 vs. 2.15) in birds fed starter diets. However, in birds fed
 264 the grower diets, increasing phytase to 2500 FTU/kg reduced gizzard pH (1.69 vs. 2.89)
 265 compared to 500 FTU/kg phytase diet. Addition of xylanase to the diet increased ($P < 0.001$)
 266 gizzard pH (2.40 vs. 2.04), irrespective of phytase dose or diet phase. There was no
 267 interaction between phytase and xylanase, indicating that these enzymes were working
 268 independently of one another.

269

270 **Table 7** Influence of diet phase, QB and XT on gizzard pH as measured using pH capsule
 271 technology¹

Phase	QB (FTU/kg)	XT (BXU/kg)	Min	Max	Average gizzard pH
Starter	500	0	0.96	3.64	1.92
	2500	0	0.54	4.09	1.92
	500	16000	1.02	4.33	2.40
	2500	16000	1.17	4.56	2.37
Grower	500	0	0.91	4.74	2.72
	2500	0	0.61	3.24	1.59
	500	16000	1.79	4.84	3.05
	2500	16000	0.54	3.89	1.79
RMSE					0.782
Starter					2.15
Grower					2.29
	500				2.52
	2500				1.92
		0			2.04
		16000			2.40
P-value					
Phase					0.060
QB					<.0001
XT					<.0001
Phase x QB					<.0001
Phase x XT					0.175
QB x XT					0.572
Phase x QB x XT					0.720

272 ¹ Means represent the average response of 8 birds per treatment

273

274 Capsule readings were compared to a standard method using a spear-tip pH probe to take
 275 gizzard pH readings following euthanasia (Figure 3). In contrast to the capsule readings, pH
 276 probe measurements showed no effect of feeding xylanase on gizzard pH (2.06 vs. 1.96),
 277 while 2500 FTU/kg phytase increased (P=0.05) gizzard pH (2.21 vs 1.81) compared to a 500
 278 FTU/kg phytase diet, irrespective of xylanase inclusion. Simultaneous to probe
 279 measurements, capsule readings were taken to allow comparisons to be made between the
 280 methods. Out of the 32 birds sacrificed, 26 of the capsules had pH readings that plateaued at

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3 281 0.50 at the time of simultaneous probe reading, indicating that the capsules had become
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5 282 unresponsive.

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7 283 FIG 3 HERE

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11 285 It would appear that the longer the monitoring period within the gizzard, the more likely the
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13 286 capsule was to become damaged, thereby prompting the 0.50 reading. This lead to a
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15 287 benchmarking experiment, that used eight 42 d birds from the 500 FTU/kg phytase without
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17 288 xylanase treatment group to dose capsules over 0.50 to 2.0 h prior to euthanasia, with pH
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19 289 recordings taken by both probe and capsule. Following euthanasia, all capsules were located
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21 290 in the gizzard of birds, except one bird dosed for 1.5 h where the capsule was located
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23 291 between the crop and gizzard. This bird gave a capsule reading of pH 2.62, however, data
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25 292 from this bird was removed from the dataset due to the capsule not being located in the
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27 293 gizzard. The range of difference between the capsule reading and the probe was -0.03 to
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29 294 +0.76, with the average difference across the eight birds being 0.30 (Figure 4). None of the
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31 295 capsules plateaued at pH 0.5, indicating that dosing up to 2 h in birds does not appear to
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33 296 cause damage to the capsules.
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39 298 FIG 4 HERE

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42 43 44 300 **Discussion**

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47 301 Research implementing higher phytase inclusion rates in poultry feed has shown enhanced
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49 302 hydrolysis of lower inositol phosphate (IP) esters created from phytate degradation, thereby
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51 303 reducing anti-nutritive effects on protein and mineral digestibility (Beeson *et al.*, 2017, Yu *et*
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53 304 *al.*, 2012). As a result, increasing phytase dose above industry standards has been shown to
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55 305 improve performance of broilers (Lee *et al.*, 2017b, Shirley and Edwards, 2003, Walk *et al.*,

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3 306 2014, Walk *et al.*, 2013). Supplementation of xylanase to wheat-based diets has shown
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5 307 improvements in broiler performance (González-Ortiz *et al.*, 2016, Wu *et al.*, 2004). This
6
7 308 response has been accredited to reductions in intestinal viscosity and enhanced AME of feed
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9 309 (Annison and Choct, 1991, Selle *et al.*, 2003, Wu *et al.*, 2004).
10
11 310 In the current study, day-old birds were 5 g lighter than expected (average weight 37g),
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13 311 although this did not appear to effect subsequent growth performance as suggested by dos
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15 312 Santos *et al.* (2010). Birds fed 2500 FTU/kg phytase, 16,000 xylanase or a combination of the
16
17 313 two, gained significantly more weight than birds fed 500 FTU/kg phytase without xylanase,
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19 314 at 21 and 42 days. A study by dos Santos *et al.* (2017) reported a significant increase in
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21 315 weight gain with 1500 FTU/kg phytase, while 16,000 xylanase showed a tendency to
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23 316 improve gain in 42 day birds, compared to feeding a standard phytase dose (500 FTU/kg)
24
25 317 alone. However, the combination of 1500 FTU/kg phytase and xylanase had no additional
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27 318 benefit on the body weight gain of broilers. A similar response was reported by Karimi *et al.*
28
29 319 (2013), suggesting that phytase and xylanase exert non-additive effects in diets based on corn
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31 320 and sorghum based of performance parameters. However, Kühn *et al.* (2013) showed that a
32
33 321 combination of 1500 FTU/kg phytase and 16,000 BXU/kg xylanase significantly increased
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35 322 weight gain in 35d wheat-fed broilers, compared to feeding these enzymes individually. This
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37 323 suggests that xylanase may give additional benefits alongside phytase in birds fed wheat-
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39 324 based diets.
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41 325 This synergy may be explained by the morphology of the wheat grain. The primary storage
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43 326 site of phytate in wheat is in the aleurone layer (O'Dell and Boland, 1972), the cell walls of
44
45 327 which are comprised essentially of b-glucans and arabinoxylans (Burton and Fincher, 2014).
46
47 328 Xylanase may increase permeability of the aleurone layer by degradation of arabinoxylan in
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49 329 the cell walls (Parkkonen *et al.*, 1997), thereby enhancing availability of phytate for
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51 330 interaction with phytase (Karimi *et al.*, 2013).
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3 331 In the current study, FCR at 21 d was significantly reduced in birds fed 2500 FTU/kg phytase
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5 332 and 16000 BXU/kg xylanase, compared to birds fed 500 FTU/kg phytase alone. However, at
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7 333 d 42, FCR was not significantly affected by higher phytase dose or xylanase. This may be
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9 334 explained by the fact that growth performance of all birds was approximately 16% ahead of
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11 335 breed standards, and FCR was around 12% lower at this age. This makes it extremely
12
13 336 challenging to observe any performance response to treatment when birds are already
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16 337 exceeding performance expectations. Even so, the combination of 16000 BXU/kg xylanase
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18 338 and 2500FTU/kg phytase gave a four point reduction in FCR (non-significant) compared to
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20 339 the 500 FTU/kg without xylanase diet. This is a considerable reduction in already well
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22 340 performing birds, and, although not statically significant, is highly commercially relevant.
23
24 341 Wet litter poses a major challenge for the poultry industry, with FPD among broilers being of
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26 342 increasing concern from both a welfare and economic standpoint. There is some evidence that
27
28 343 exogenous phytase may reduce litter quality and increase faecal moisture (Debicki-Garnier
29
30 344 and Hruby, 2003). Phytate and its lower esters have anti-nutritive effects on protein and
31
32 345 mineral digestion and absorption (Beeson *et al.*, 2017, Yu *et al.*, 2012), leading to an
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34 346 imbalance that can increase water intake and thus wet litter. Increasing phytase dose
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36 347 promotes the near-destruction of phytase and its lower esters (Walk *et al.*, 2014, Walk *et al.*,
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38 348 2013), thereby enhancing protein and mineral absorption and improving litter quality. In the
39
40 349 current study, reasonable litter quality was observed for bird age and was unaffected by
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42 350 treatment. Consequently, incidence of FPD was relatively low in birds at 21 and 42 d of age.
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44 351 Exogenous xylanase has been widely acknowledged for its ability to resolve wet litter issues,
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46 352 particularly in birds fed wheat-based diets, through soluble NSP degradation and subsequent
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48 353 reduction in digesta viscosity and faecal moisture content. In the present study, feeding
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50 354 xylanase significantly reduced the incidence of severe FPD in 42 d.o. birds. Since litter
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52 355 quality was unaffected by xylanase, other factors such as altered health status and litter
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3 356 microbial population (Kim *et al.*, 2017, Shepherd and Fairchild, 2010) may explain these
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5 357 findings, or it may be that the measures of litter quality are not currently adequate.
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7 358 Blood inositol can be a useful indicator of complete dephosphorylation of dietary phytate by
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9 359 addition of exogenous phytase to the diet. In the body, inositol is involved in a number of
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11 360 signalling pathways that support the development and growth of animals (Lee and Bedford,
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13 361 2016). Several studies have supported the benefits of inositol either by dietary
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15 362 supplementation or through high phytase inclusion rates (Cowieson *et al.*, 2015, Cowieson *et*
16
17 363 *al.*, 2013, Lee *et al.*, 2017b, Sommerfeld *et al.*, 2017, Walk *et al.*, 2014), indicating that
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19 364 inositol may play an important role in animal growth response. Previously, inositol profile
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21 365 has been determined primarily using blood plasma samples (Cowieson *et al.*, 2015,
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23 366 Sommerfeld *et al.*, 2017). However, inositol has been detected in erythrocytes of day-old and
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25 367 21 d chickens (Oshima *et al.*, 1964). In erythrocytes, myo-inositol appears to be a precursor
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27 368 for myo-inositol pentaphosphate (IP5), which interacts with haemoglobin to modulate affinity
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29 369 for oxygen (Isaacks *et al.*, 1982; Lutz, 1980). In the current study, the fraction of blood
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31 370 analysed had a considerable effect on inositol levels, with plasma inositol being more than
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33 371 twice the concentration than in erythrocytes. This is in contrast to Oshima *et al.* (1964), that
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35 372 found higher concentrations of free myo-inositol in erythrocytes than plasma. This
36
37 373 discrepancy may be the result of differences in sensitivity between the previous and more
38
39 374 current detection methods used. Nonetheless, increasing phytase dose to 2500 FTU/kg tended
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41 375 to increase inositol concentration in both blood fractions compared to the standard 500
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43 376 FTU/kg phytase inclusion rate, suggesting more complete dephosphorylation of phytate.
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45 377 Addition of xylanase to the diet had no effect on blood inositol levels, as this enzyme would
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47 378 not be expected to directly affect phytate degradation.
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49 379 It is clear from the current study and previous work (Lee *et al.*, 2017a, Lee *et al.*, 2018) that
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51 380 relatively large fluctuations in gastric pH can be detected using real-time capsule technology.
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3 381 The fact that pH is not kept at a consistent level illustrates that acid secretion is not static and
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5 382 questions the value of point-in-time measurements. Reports in both laying hens and broilers
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7 383 have shown no effect of adding xylanase to wheat- or corn-based diets on gizzard pH
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9 384 (Engberg *et al.*, 2004; Lee *et al.*, 2017c; Mirzaie *et al.*, 2012). Similarly, in the present study,
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11 385 digital pH probe measurements indicated that inclusion of xylanase into wheat-soy diets had
12
13 386 no significant influence on gizzard pH in broilers. However, in contrast, pH capsule readings
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15 387 demonstrated that inclusion of xylanase into the diet significantly increased gizzard pH from
16
17 388 2.0 to 2.4, irrespective of phytase inclusion or diet phase. Morgan *et al.* (2017) reported a pH
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19 389 2.5 optimum for xylanase degradation of wheat arabinoxylan to short-chain xylo-
20
21 390 oligosaccharides. Conditions may therefore have been optimised in the current study in terms
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23 391 of xylanase efficacy.
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26 392 Moreover, as measured by pH capsule technology, increasing phytase dose from 500 to 2500
27
28 393 FTU/kg significantly reduced gizzard pH in birds fed grower diets. A similar finding was
29
30 394 evident in a previous trial (Lee *et al.*, 2018). It has been suggested that 500 FTU/kg phytase
31
32 395 releases more Ca than P, while higher phytase doses increases P release beyond Ca, restoring
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34 396 this balance (Cowieson *et al.*, 2011). It may be this rebalancing of minerals lowers gastric pH
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36 397 with 2500 FTU/kg phytase, which accounts for the improved solubility and digestibility of
37
38 398 dietary nutrients shown with high phytase inclusion rates (Manobhavan *et al.*, 2016;
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40 399 Pirgozliev *et al.*, 2012). However, capsule results were contradictory to pH probe
41
42 400 measurements, showing an increase in gizzard pH with 2500 FTU/kg phytase compared to
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44 401 500 FTU/kg phytase. Other studies adopting point-in-time pH measurements have reported a
45
46 402 lack of effect of administering phytase doses up to 2500 FTU/kg on gastric pH (Lee *et al.*,
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48 403 2018; Nourmohammadi *et al.*, 2011; Radcliffe *et al.*, 1998) while, application of much higher
49
50 404 phytase inclusion rates of 5000 FTU/kg has been shown to increase gizzard pH in broilers
51
52 405 (Walk *et al.*, 2012). Therefore, this may suggest that much higher enzyme doses are required
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3 406 to enable detection of a noticeable response to treatment using current methods. Even so, the
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5 407 direction of response, particularly for phytase, is conflicting between capsule and probe
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7 408 methods.

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9 409 There are clear differences between the two methods used in this study to record gizzard pH,
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11 410 which may explain these opposing conclusions. For example, *in-situ* and *ex-situ* pH probe
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13 411 readings are taken at one point-in-time, once the animal has been sacrificed. Conversely, *in*
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15 412 *vivo* pH capsules take readings every second for several hours in the live animal, thereby
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17 413 providing a more representative outlook on real-time acid secretions in response to treated
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19 414 feed. It may be this ability to detect fluctuations in gastric pH that allows treatment responses
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21 415 to be realised, which would otherwise be missed using standard point-in-time methods.
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24 416 However, a limitation to the capsule technology is that only a restricted number of birds can
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26 417 be capsuled at the same time, due to the number of detection devices available. In order to
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28 418 determine the comparability between these two methods and the effect of euthanasia on
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30 419 gastric pH, capsule readings were taken simultaneous to probe measurements. However, the
31
32 420 majority of capsules appeared to plateau at pH 0.50 at the point of probe measurement,
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34 421 suggesting potential damage to the capsule. In light of this, a benchmarking experiment was
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36 422 undertaken to confirm the accuracy of the capsule readings when dosed for different periods
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38 423 of time. The average pH difference between probe and the capsule readings was 0.30, with a
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40 424 range of -0.03 to +0.76. This suggested that digital probe measurements read higher than the
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42 425 capsule. This may be due to the positioning of the H⁺ ion sensor within the food bolus when
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44 426 measurements are taken. The orientation of the capsule cannot be controlled, however, taking
45
46 427 into account the size of the capsule (2cm in length) compared to the size of the gizzard, it
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48 428 could be assumed that the H⁺ ion sensor would be located in the outer region of the food
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50 429 bolus, where exposure to gastric acid secretions is high. In contrast, the probe was inserted
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52 430 directly into the centre of the food bolus, the region less exposed to gastric secretions.
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3 431 Therefore, the method of choice may be dependent on the research question, as to whether a
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5 432 change in acid secretion is to be determined or the pH of the food bolus. Since none of the
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7 433 capsules plateaued at pH 0.5, this would suggest that dosing up to 2.0 h did not cause damage
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9 434 to the capsules, as indicated after a 5.5 h dosing period. However, capsule readings obtained
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11 435 over 5.5 h in the live bird did not suggest capsule damage, and therefore it is possible that this
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13 436 damage only becomes apparent once the bird has been killed. Further investigation is
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15 437 required for intermediate dosing periods to confirm the potential maximum period for capsule
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17 438 administration.
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21 22 440 **Conclusions**

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25 441 The current study demonstrated that body weight gain and FCR of broilers can be improved
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27 442 by addition of higher phytase doses and xylanase in wheat-based diets. Increasing phytase
28
29 443 dose had the tendency to increase inositol in the blood, suggesting more complete phytase
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31 444 degradation with higher phytase inclusion rates. Addition of xylanase and higher phytase
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33 445 dose appeared to have opposite effects on real-time gastric pH, as measured by capsule
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35 446 technology. Supplementation of xylanase increased gizzard pH, while feeding high phytase in
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37 447 the grower diet led to a reduction in gizzard pH. However, these findings were not supported
38
39 448 by probe measurements, indicating inconsistencies between the methods. The fact that
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41 449 xylanase and high phytase doses had opposing effects on real-time gastric pH, while giving
42
43 450 similar performance responses, indicated that gastric conditions were not solely accountable
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45 451 for animal performance.
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47 452

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6
7 456 Vista.

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11 **References**

12 458

13 459
14
15 460 ADEOLA, O. & BEDFORD, M.R. (2004) Exogenous dietary xylanase ameliorates viscosity-
16 461 induced anti-nutritional effect in wheat based diets for White Pekin Ducks (*Anas*
17 462 *Platyrinchos domesticus*). *British Journal of Nutrition*, **92**: 87-94.

18
19 463 ADEOLA, O. & COWIESON, A.J. (2011) BOARD-INVITED REVIEW: opportunities and
20 464 challenges in using exogenous enzymes to improve nonruminant animal production. *Journal*
21 465 *of Animal Science*, **89**: 3189-3218.

22
23 466 ANNISON, G. & CHOCT, M. (1991) Anti-nutritive activities of cereal non-starch
24 467 polysaccharides in broiler diets and strategies minimizing their effects. *World's Poultry*
25 468 *Science Journal*, **47**: 232-242.

26
27 469 BAILEY, C., LINTON, S., BRISTER, R. & CREGER, C. (1986) Effects of graded levels of
28 470 dietary phosphorus on bone mineralization in the very young poult. *Poultry Science*, **65**:
29 471 1018-1020.

30
31 472 BEESON, L.A., WALK, C.L., BEDFORD, M.R. & OLUKOSI, O.A. (2017) Hydrolysis of
32 473 phytate to its lower esters can influence the growth performance and nutrient utilization of
33 474 broilers with regular or super doses of phytase. *Poultry Science*, **96**: 2243-2253.

34
35 475 BURTON, R.A. & FINCHER, G.B. (2014) Evolution and development of cell walls in cereal
36 476 grains. *Frontiers in Plant Science*, **5**: 456.

37
38 477 CHOCT, M. & ANNISON, G. (1992a) Anti-nutritive effect of wheat pentosans in broiler
39 478 chickens: roles of viscosity and gut microflora. *British Poultry Science*, **33**: 821-834.

40
41 479 CHOCT, M. & ANNISON, G. (1992b) The inhibition of nutrient digestion by wheat
42 480 pentosans. *British Journal of Nutrition*, **67**: 123-132.

43
44 481 CHOCT, M., KOCHER, A., WATERS, D.L., PETTERSSON, D. & ROSS, G. (2004) A
45 482 comparison of three xylanases on the nutritive value of two wheats for broiler chickens.
46 483 *British Journal of Nutrition*, **92**: 53-61.

47
48 484 COWIESON, A.J., WILCOCK, P. & BEDFORD, M.R. (2011) Super-dosing effects of
49 485 phytase in poultry and other monogastrics. *World's Poultry Science Journal*, **67**: 225-236.

50
51 486 COWIESON, A.J., AURELI, R., GUGGENBUHL, P. & FRU-NJI, F. (2015) Possible
52 487 involvement of myo-inositol in the physiological response of broilers to high doses of
53 488 microbial phytase. *Animal Production Science*, **55**: 710-719.

- 1
2
3 489 COWIESON, A.J., PTAK, A., MACKOWIAK, P., SASSEK, M., PRUSZYNSKA-
4 490 OSZMALEK, E., ZYLA, K., SWIATKIEWICZ, S., KACZMAREK, S. & D, J. (2013) The
5 491 effect of microbial phytase and myo-inositol on performance and blood biochemistry of
6 492 broiler chickens fed wheat/corn-based diets. *Poultry Science*, **92**: 2124-2134.
- 8 493 DEBICKI-GARNIER, A.M. & HRUBY, M. (2003) The effect of phytase and betaine on
9 494 broiler performance and excreta characteristics. Proceedings of the 14th European
10 495 Symposium on Poultry Nutrition, Lillehammer, Norway,
- 12 496 DOS SANTOS, T.T., MASEY O'NEILL, H.V., GONZÁLEZ-ORTIZ, G., CAMACHO-
13 497 FERNÁNDEZ, D. & LÓPEZ-COELLO, C. (2017) Xylanase, protease and superdosing
14 498 phytase interactions in broiler performance, carcass yield and digesta transit time. *Animal*
15 499 *Nutrition*, **3**: 121-126.
- 17 500 DOS SANTOS, T.T., CORZO, A., KIDD, M.T., MCDANIEL, C.D., TORRES FILHO, R.A.
18 501 & ARAÚJO, L.F. (2010) Influence of in ovo inoculation with various nutrients and egg size
19 502 on broiler performance. *The Journal of Applied Poultry Research*, **19**: 1-12.
- 21 503 ENGBERG, R.M., HEDEMANN, M.S., STEENFELDT, S. & JENSEN, B.B. (2004)
22 504 Influence of whole wheat and xylanase on broiler performance and microbial composition
23 505 and activity in the digestive tract. *Poultry Science*, **83**: 925-938.
- 25 506 GAO, F., JIANG, Y., ZHOU, G.H. & HAN, Z.K. (2008) The effects of xylanase
26 507 supplementation on performance, characteristics of the gastrointestinal tract, blood
27 508 parameters and gut microflora in broilers fed on wheat-based diets. *Animal Feed Science and*
28 509 *Technology*, **142**: 173-184.
- 30 510 GONZÁLEZ-ORTIZ, G., OLUKOSI, O. & BEDFORD, M.R. (2016) Evaluation of the effect
31 511 of different wheats and xylanase supplementation on performance, nutrient and energy
32 512 utilisation in broiler chicks. *Animal Nutrition*, **2**: 173-179.
- 34 513 ISAACKS, R.E., KIM, C.Y., JOHNSON, A.E., JR., GOLDMAN, P.H. & HARKNESS, D.R.
35 514 (1982) Studies on avian erythrocyte metabolism. XII. The synthesis and degradation of
36 515 inositol pentakis (dihydrogen phosphate). *Poultry Science*, **61**: 2271-2281.
- 38 516 KARIMI, A., COTO, C., MUSSINI, F., GOODGAME, S., LU, C., YUAN, J., BEDFORD,
39 517 M.R. & WALDROUP, P.W. (2013) Interactions between phytase and xylanase enzymes in
40 518 male broiler chicks fed phosphorus-deficient diets from 1 to 18 days of age. *Poultry Science*,
41 519 **92**: 1818-1823.
- 43 520 KIARIE, E., ROMERO, L.F. & RAVINDRAN, V. (2014) Growth performance, nutrient
44 521 utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or
45 522 with supplemental xylanase. *Poultry Science*, **93**: 1186-1196.
- 47 523 KIM, J.H., HAN, G.P., SHIN, J.E. & KIL, D.Y. (2017) Effect of dietary calcium
48 524 concentrations in phytase-containing diets on growth performance, bone mineralization, litter
49 525 quality, and footpad dermatitis score in broiler chickens. *Animal Feed Science and*
50 526 *Technology*, **229**: 13-18.
- 52 527 KÜHN, I., BEDFORD, M.R. & MÄNNER, K. (2013) Effect of phytase and xylanase on
53 528 growth and bone mineralisation in broilers fed wheat based diets, *19th European Symposium*
54 529 *on Poultry Nutrition*, (Potsdam, Germany, World's Poultry Science Association).

- 1
2
3 530 LEE, S.A. & BEDFORD, M.R. (2016) Inositol- An Effective Growth Promotor? *World's*
4 531 *Poultry Science Journal*, **72**: 743-760.
- 5
6 532 LEE, S.A., DUNNE, J., MOTTRAM, T. & BEDFORD, M.R. (2017a) Effect of diet phase
7 533 change, dietary Ca and P level and phytase on bird performance and real-time gizzard pH
8 534 measurements. *British Poultry Science*, **58**: 290-297.
- 9
10 535 LEE, S.A., NAGALAKSHMI, D., RAJU, M.V.L.N., RAMA RAO, S.V. & BEDFORD,
11 536 M.R. (2017b) Effect of phytase superdosing, myo-inositol and available phosphorus
12 537 concentrations on performance and bone mineralisation in broilers. *Animal Nutrition*, **3**: 247-
13 538 251.
- 14
15 539 LEE, S.A., WISEMAN, J., MASEY O'NEILL, H.V., SCHOLEY, D.V., BURTON, E.J. &
16 540 HILL, S.E. (2017c) Understanding the direct and indirect mechanisms of xylanase action on
17 541 starch digestion in broilers. *Journal of World's Poultry Research*, **7**: 35-47.
- 18
19 542 LEE, S.A., DUNNE, J., FEBERY, E., WILCOCK, P., MOTTRAM, T. & BEDFORD, M.R.
20 543 (2018) Superdosing phytase reduces real-time gastric pH in broilers and weaned piglets.
21 544 *British Poultry Science*, **In Press**.
- 22
23 545 LUTZ, P.L. (1980) On the oxygen affinity of bird blood. *American Zoologist*, **20**: 187-198.
- 24
25 546 MANOBHAVAN, M., ELANGOVAN, A.V., SRIDHAR, M., SHET, D., AJITH, S., PAL,
26 547 D.T. & GOWDA, N.K. (2016) Effect of super dosing of phytase on growth performance,
27 548 ileal digestibility and bone characteristics in broilers fed corn-soya-based diets. *Journal of*
28 549 *Animal Physiology and Animal Nutrition (Berl)*, **100**: 93-100.
- 29
30 550 MASEY O'NEILL, H.V., SINGH, M. & COWIESON, A.J. (2014) Effects of exogenous
31 551 xylanase on performance, nutrient digestibility, volatile fatty acid production and digestive
32 552 tract thermal profiles of broilers fed on wheat- or maize-based diet. *British Poultry Science*,
33 553 **55**: 351-359.
- 34
35 554 MIRZAIE, S., ZAGHARI, M., AMINZADEH, S., SHIVAZAD, M. & MATEOS, G.G.
36 555 (2012) Effects of wheat inclusion and xylanase supplementation of the diet on productive
37 556 performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens.
38 557 *Poultry Science*, **91**: 413-425.
- 39
40 558 MORGAN, N.K., WALLACE, A., BEDFORD, M.R. & CHOCT, M. (2017) Efficiency of
41 559 xylanases from families 10 and 11 in production of xylo-oligosaccharides from wheat
42 560 arabinoxylans. *Carbohydrate Polymers*, **167**: 290-296.
- 43
44 561 NELSON, T.S., SHIEH, T.R., WODZINSKI, R.J. & WARE, J.H. (1971) Effect of
45 562 supplemental phytase on the utilization of phytate phosphorus by chicks. *Journal of*
46 563 *Nutrition*, **101**: 1289-1294.
- 47
48 564 NOURMOHAMMADI, R., HOSSEINI, S.M., SARAEE, H. & ARAB, A. (2011) Plasma
49 565 thyroid hormone concentrations and pH values of some GI-tract segments of broilers fed on
50 566 different dietary citric acid and microbial phytase levels. *American Journal of Animal and*
51 567 *Veterinary Sciences*, **6**: 1-6.
- 52
53 568 NRC (1994) Nutrient Requirements of Poultry, (Washington, DC, National Academic Press).
- 54
55
56
57
58
59
60

- 1
2
3 569 O'DELL, B.L. & BOLAND, A. (1972) Distribution of phytate and nutritionally important
4 570 elements among the morphological components of cereal grains. *Journal of Agricultural &*
5 571 *Food Chemistry*, **20**: 718-721.
- 6
7 572 OSHIMA, M., TG., T. & WILLIAMS, A. (1964) Variations in the concentration of phytic
8 573 acid in the blood of the domestic fowl. *Biochemical Journal*, **92**: 42-46.
- 9
10 574 PARKKONEN, T., TERVILÄ-WILO, A., HOPEAKOSKI-NURMINEN, M., MORGAN,
11 575 A., POUTANEN, K. & AUTIO, K. (1997) Changes in wheat micro structure following in
12 576 vitro digestion. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, **47**: 43-
13 577 47.
- 14
15 578 PIRGOZLIEV, V., BEDFORD, M., ODUGUWA, O., ACAMOVIC, T. & ALLYMEHR, M.
16 579 (2012) The effect of supplementary bacterial phytase on dietary metabolisable energy,
17 580 nutrient retention and endogenous losses in precision fed broiler chickens. *Journal of Animal*
18 581 *Physiology and Animal Nutrition*, **96**: 52-57.
- 19
20 582 RADCLIFFE, J.S., ZHANG, Z. & KORNEGAY, E.T. (1998) The Effects of Microbial
21 583 Phytase, Citric Acid, and Their Interaction in Corn-Soybean Meal-Based Diet for Weanling
22 584 Pigs. *Journal of Animal Science*, **76**: 1880-1886.
- 23
24 585 RAVINDRAN, V., BRYDEN, W.L. & KORNEGAY, E.T. (1995) Phytates: Occurrence,
25 586 Bioavailability and Implications in Poultry Nutrition. *Poultry and Avian Biology Reviews*, **6**:
26 587 125-143.
- 27
28 588 SCHRAMM, V.G., DURAU, J.F., BARRILLI, L.N.E., SORBARA, J.O.B., COWIESON,
29 589 A.J., FÉLIX, A.P. & MAIORKA, A. (2017) Interaction between xylanase and phytase on the
30 590 digestibility of corn and a corn/soy diet for broiler chickens. *Poultry Science*, **96**: 1204-1211.
- 31
32 591 SELLE, P.H., RAVINDRAN, V. & PARTRIDGE, G.G. (2009) Beneficial effects of xylanase
33 592 and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral
34 593 retention and growth performance in wheat-based broiler diets. *Animal Feed Science and*
35 594 *Technology*, **153**: 303-313.
- 36
37 595 SELLE, P.H., RAVINDRAN, V., RAVINDRAN, G., PITTOLO, P.H. & BRYDEN, W.L.
38 596 (2003) Influence of Phytase and Xylanase Supplementation on Growth Performance and
39 597 Nutrient Utilisation of Broilers Offered Wheat-based Diets. *Asian-Australasian Journal of*
40
41 598 *Animal Science*, **16**: 394-402.
- 42
43 599 SHEPHERD, E.M. & FAIRCHILD, B.D. (2010) Footpad dermatitis in poultry. *Poultry*
44 600 *Science*, **89**: 2043-2051.
- 45
46 601 SHIRLEY, R.B. & EDWARDS, H.M., JR. (2003) Graded levels of phytase past industry
47 602 standards improves broiler performance. *Poultry Science*, **82**: 671-680.
- 48
49 603 SINGH, A., O'NEILL, H.V.M., GHOSH, T.K., BEDFORD, M.R. & HALDAR, S. (2012)
50 604 Effects of xylanase supplementation on performance, total volatile fatty acids and selected
51 605 bacterial population in caeca, metabolic indices and peptide YY concentrations in serum of
52 606 broiler chickens fed energy restricted maize–soybean based diets. *Animal Feed Science and*
53 607 *Technology*, **177**: 194-203.
- 54
55
56
57
58
59
60

- 1
2
3 608 SOMMERFELD, V., KUNZEL, S., SCHOLLENBERGER, M., KUHN, I. &
4 609 RODEHUTSCORD, M. (2017) Influence of phytase or myo-inositol supplements on
5 610 performance and phytate degradation products in the crop, ileum, and blood of broiler
6 611 chickens. *Poultry Science*, **97**: 920-929.
- 7
8 612 SVIHUS, B. (2014) Function of the digestive system. *The Journal of Applied Poultry*
9 613 *Research*, **23**: 306-314.
- 10
11 614 WALDROUP, P.W., KERSEY, J.H., SALEH, E.A., FRITTS, C.A., YAN, F., STILBORN,
12 615 H.L., CRUM, R.C., JR. & RABOY, V. (2000) Nonphytate phosphorus requirement and
13 616 phosphorus excretion of broiler chicks fed diets composed of normal or high available
14 617 phosphate corn with and without microbial phytase. *Poultry Science*, **79**: 1451-1459.
- 15
16 618 WALK, C.L., BEDFORD, M.R. & MCELROY, A.P. (2012) Influence of limestone and
17 619 phytase on broiler performance, gastrointestinal pH, and apparent ileal nutrient digestibility.
18 620 *Poultry Science*, **91**: 1371-1378.
- 19
20 621 WALK, C.L., SANTOS, T.T. & BEDFORD, M.R. (2014) Influence of superdoses of a novel
21 622 microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in
22 623 young broilers. *Poultry Science*, **93**: 1172-1177.
- 23
24 624 WALK, C.L., BEDFORD, M.R., SANTOS, T.S., PAIVA, D., BRADLEY, J.R.,
25 625 WLADECKI, H., HONAKER, C. & MCELROY, A.P. (2013) Extra-phosphoric effects of
26 626 superdoses of a novel microbial phytase. *Poultry Science*, **92**: 719-725.
- 27
28 627 WU, Y.B., RAVINDRAN, V., THOMAS, D.G., BIRTLES, M.J. & HENDRICKS, W.H.
29 628 (2004) Influence of method of whole wheat inclusion and xylanase supplementation on the
30 629 performance, apparent metabolisable energy, digestive tract measurements and gut
31 630 morphology of broilers. *British Poultry Science*, **45**: 385-394.
- 32
33
34 631 YAN, F., KERSEY, J.H. & WALDROUP, P.W. (2001) Phosphorus Requirements of Broiler
35 632 Chicks Three to Six Weeks of Age as Influenced by Phytase Supplementation. *Poultry*
36 633 *Science*, **80**: 455-459.
- 37
38 634 YU, S., COWIESON, A., GILBERT, C., PLUMSTEAD, P. & DALSGAARD, S. (2012)
39 635 Interactions of phytate and myo-inositol phosphate esters (IP1-5) including IP5 isomers with
40 636 dietary protein and iron and inhibition of pepsin. *Journal of Animal Science*, **90**: 1824-1832.
- 41
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3 Figure 1. Effect of phytase and xylanase on gizzard pH in broilers fed starter diets. Phytase
4 (phy) was supplemented at 500 or 2500 FTU/kg, and xylanase (xyl) at 0 or 16000 BXU/kg.
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6 Data points represent means of 8 birds per treatment.
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10 Figure 2. Effect of phytase (phy) and xylanase (xyl) on gizzard pH in broilers fed grower
11 diets. Phytase (phy) was supplemented at 500 or 2500 FTU/kg, and xylanase (xyl) at 0 or
12 16000 BXU/kg. Data points represent means of 8 birds per treatment.
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18 Figure 3. Comparison of different methods, capsule or probe, on gizzard pH measurements in
19 broilers. Broilers were fed diets supplemented with phytase at 500 or 2500 FTU/kg and
20 xylanase at 0 or 16000 BXU/kg. Different letters denote significant difference for a specific
21 method at $P < 0.05$, with trends ($P < 0.10$) indicated by an asterisk. Error bars indicate \pm
22 standard error of the mean. Capsule and probe data show means of 8 birds per treatment.
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28 Figure 4. Benchmarking assessment comparing capsule and probe pH measurements taken
29 0.5, 1.0, 1.5 and 2.0 h post capsule application. Replicate birds per time point are indicated by
30 letters 'a' and 'b'. Data from one replicate bird following 1.5 h capsule dosing is missing due
31 to the capsule being located between the crop and gizzard.
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Table 2 Expected and analysed diet composition for broilers

Phase	Phytase (FTU/kg)		Xylanase (BXU/kg)		Calcium (%)		Phosphorus (%)		Crude protein (%)		ME (MJ/kg)	
	Target	Analysed	Target	Analysed	Target	Analysed	Target	Analysed	Target	Analysed	Target	Analysed
Starter	500	722	0	-	0.98	0.99	0.71	0.61	21.85	20.3	12.45	11.9
	2500	2390	0	-	0.98	1.20	0.71	0.65	21.85	21.8	12.45	11.8
	500	868	16000	12300	0.98	1.22	0.71	0.63	21.85	22.3	12.45	11.7
	2500	2260	16000	11800	0.98	0.79	0.71	0.57	21.85	22.4	12.45	11.9
Grower	500	493	0	-	0.78	0.63	0.59	0.4	17.90	18.7	12.97	12.7
	2500	2500	0	-	0.78	0.66	0.59	0.44	17.90	18.6	12.97	12.4
	500	677	16000	14100	0.78	0.61	0.59	0.43	17.90	19.2	12.97	12.8
	2500	2670	16000	14100	0.78	0.67	0.59	0.43	17.90	19.3	12.97	12.5

Table 3 Effect of phytase and xylanase on broiler performance¹

Phytase (FTU/kg)	Xylanase (BXU/kg)	Initial body weight (g)	Feed intake (kg)			Weight gain (kg)			FCR			^{bwc} FC R
			Days 0-21	Days 21-42	Days 0-42	Days 0-21	Days 21-42	Days 0-42	Days 0-21	Days 21-42	Days 0-42	Days 0-42
500	0	36.6	1.28	3.79 ^b	4.94	0.99 ^b	2.25	3.24 ^b	1.29	1.69	1.52	1.52
2500	0	36.4	1.35	4.04 ^a	5.24	1.10 ^a	2.37	3.47 ^a	1.23	1.71	1.51	1.51
500	16000	36.5	1.34	3.99 ^a	5.19	1.12 ^a	2.32	3.43 ^a	1.20	1.72	1.51	1.51
2500	16000	36.5	1.33	4.01 ^a	5.18	1.13 ^a	2.36	3.49 ^a	1.18	1.70	1.48	1.48
	RMSE	0.00	0.098	0.135	0.280	0.063	0.100	0.115	0.033	0.059	0.060	0.060
500		3.7	1.31	3.89	5.06	1.05	2.29	3.34	1.25	1.70	1.52	1.52
2500		3.6	1.34	4.02	5.21	1.12	2.36	3.48	1.20	1.70	1.50	1.50
	0	3.6	1.31	3.91	5.09	1.04	2.31	3.35	1.26	1.70	1.52	1.52
	16000	3.6	1.34	4.00	5.18	1.12	2.34	3.46	1.19	1.71	1.50	1.50
<i>P</i> -value												
Phytase		0.621	0.322	0.012	0.149	0.006	0.041	0.002	0.001	0.987	0.384	0.383
Xylanase		0.981	0.540	0.089	0.364	0.001	0.415	0.011	1	0.489	0.293	0.292
Phytase x Xylanase		0.836	0.286	0.025	0.124	0.044	0.287	0.044	0.063	0.317	0.724	0.725

Means of 8 replicate pens per treatment; main effects given as least square means
^{a,b,c} Data in a column not sharing a common superscript letter significantly differ at *P*<0.05.
 RMSE, root mean square error; FCR, feed conversion ratio (intake:gain) corrected for mortality and withdrawn birds; ^{bwc}FCR, FCR corrected for body weight

Table 5 Effect of phytase and xylanase on broiler litter and footpad dermatitis scores at 21 and 42 days¹

Phytase (FTU/kg)	Xylanase (BXU/kg)	Footpad score										Litter score	
		Day 21					Day 42					Day 21	Day 42
		Score 1	Score 2	Score 3	Score 4	Score 5	Score 1	Score 2	Score 3	Score 4	Score 5		
500	0	71	25	4	0	0	4	46	33	14	4	2.0	3.1
2500	0	70	19	7	2	1	10	43	28	13	5	2.1	3.3
500	16000	63	29	4	3	0	13	46	28	12	1	2.0	3.1
2500	16000	63	31	4	2	1	20	54	20	4	1	2.0	3.0
<i>P</i> -value													
Phytase		0.705	0.663	0.447	0.342	0.151	0.143	0.860	0.110	0.186	0.735	0.317	1.000
Xylanase		0.264	0.437	0.983	0.922	1.000	0.053	0.338	0.238	0.110	0.009	0.317	0.651

¹Means represent the average response of 8 replicate pens (144 chicks) per treatment.