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Influence of a microbial phytase on the performance and the utilisation of energy, crude protein and fatty acids of young broilers fed on phosphorus-adequate maize- and wheat-based diets

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Abstract   1. The effects of microbial phytase on the performance and nutrient utilisation in broilers fed on phosphorus-adequate starter diets were examined in this study. The effect of phytase on the apparent ileal digestibility of fatty acids was of particular interest. Two grain types (maize and wheat) and two inclusion concentrations of a phytase enzyme from *Escherichia coli* expressed in *Schizosaccaromyces pombe* (0 or 500 phytase units (FTU)/kg of feed) were evaluated in a 2×2 factorial arrangement of treatments.
2. Supplemental phytase improved the weight gain and feed per gain, but had no effect on the feed intake of young broilers receiving phosphorus-adequate diets.
3. Phytase supplementation improved the apparent ileal digestibility of nitrogen and phosphorus in both diet types. Phytase supplementation tended to improve the apparent ileal digestible energy in wheat-based diets, but had no effect on the apparent metabolisable energy in both diet types.
4. Supplementation of phytase increased the apparent ileal digestibility of fat, with similar effects for the different fatty acids measured. Increments on ileal fat digestibility due to phytase were not dependent on the type of diet.
5. Dietary supplementation of microbial phytase enhanced not only the digestibility of phosphorus, but also that of nitrogen and fat, exhibiting increased ileal digestibility for all fatty acids in P-adequate maize- and wheat-based diets.

INTRODUCTION

The ability of microbial phytase to improve the bioavailability of phytate-bound phosphorus (P) and to sustain the performance and skeletal health of poultry fed on low-P diets is now clearly established. There is also growing evidence that phytate negatively influences the utilisation of nutrients other than P, including amino acids, and the metabolisability of energy (Camden *et al.,* 2001; Rutherfurd *et al.,* 2002; Selle and Ravindran, 2007; Cowieson *et al.,* 2009). This so-called "extra-phosphoric" effect, however, is not widely considered in feed formulations because responses to microbial phytase supplementation on nutrients other than P in birds receiving low-P diets have been varying and the underlying mechanisms have not been fully understood (Selle and Ravindran, 2007). The addition of microbial phytase to P-adequate diets may provide evidence for the influence of the enzyme on bird performance and the utilisation of energy-yielding nutrients, independent of its effect on P availability.

Although the influence of microbial phytase on the utilisation of energy and amino acids in poultry has been examined in a number of studies, corresponding information on fat and fatty acids is limited. Nonetheless, increments in fat digestibility might be an important mechanism determining the effects of phytase on energy digestibility in chickens. Leeson (1993)

hypothesised that calcium (Ca) complexes with fatty acids to form insoluble soaps in the gut lumen, thereby lowering fat digestibility. There is also evidence that phytate interacts with lipids in maize (Cosgrove, 1966). Other reports have also suggested interactions between Ca, fat and phytate in the diet. For instance, Matika *et al.* (1990) found that dietary tallow reduced phytate P utilisation in young chicks and increased the percentage of fat excreted as soap fatty acids.

The aim of the present study was to determine the effects of phytase supplementation in P-adequate diets on the performance and nutrient utilisation, with emphasis on fat/fatty acids, of young broilers.

MATERIALS AND METHODS

Microbial phytase

The enzyme product, an *E. coli* phytase expressed in *Schizosaccaromyces pombe* (Phyzyme® XP, Danisco Animal Nutrition – DuPont Industrial Biosciences, Marlborough, UK), was tested for its ability to impact nutrient utilisation in P-adequate diets. The product contained a minimum phytase activity of 5000 FTU/g and the addition of 100 g enzyme/tonne provided a minimum of 500 FTU/kg diet. One unit of phytase (FTU) is defined as the quantity of enzyme which releases 1 μmol of inorganic P/min from 0.15 mM sodium phytate at pH 5.5 at 37°C. The enzyme was sprayed into a wheat carrier, the final product activity concentration measured, and this premix was applied in dry form to the relevant experimental diets.

Diets

The experiment had a completely randomised design with a 2 × 2 factorial arrangement of treatments evaluating two grain types (maize and wheat) and two inclusion concentrations of microbial phytase (0 or 500 FTU/kg diet). Maize- and wheat-based diets were formulated to meet the Ross 308 recommendations (Ross, 2007; Table 1) for major nutrients, including Ca (10 g/kg) and available P (4.5 g/kg) for broiler starters. The Ca: total P ratio was maintained at 1.4:1. Titanium dioxide (3 g/kg) was added to all diets as an indigestible dietary marker. Each formulated diet was then divided into two equal batches and microbial phytase was added to one batch of each basal diet.

Birds and housing

Experimental procedures were conducted in accordance with the Massey University Animal

Table 1. *Composition and calculated analysis (g/kg as fed) of the basal diets*

Ingredient	Maize-based diet	Wheat-based diet
Maize	496.4	–
Wheat	–	546.2
Soya bean meal, 48%	265.7	226.5
Wheat bran	40.0	20.0
Distillers' dried grains with solubles (DDGS)	70.0	70.0
Maize gluten meal, 60%	50.0	50.0
Animal fat– soya bean oil blend	29.4	38.5
Lysine.HCl	4.4	5.1
DL-methionine	2.9	2.8
L-threonine	1.4	1.7
Titanium dioxide	3.0	3.0
Sodium chloride	3.4	3.2
Limestone	12.4	13.1
Dicalcium phosphate	16.7	15.8
Trace mineral–vitamin premix[1]	3.1	3.1
Calculated analysis		
Metabolisable energy, MJ/kg	12.64	12.64
Crude protein	230	230
Lysine	13.9	13.8
Methionine	6.8	6.5
Methionine + cysteine	10.9	10.8
Threonine	10.0	9.7
Digestible lysine	12.0	12.0
Digestible methionine	6.5	6.1
Digestible methionine + cysteine	8.9	8.9
Digestible threonine	7.8	7.8
Calcium	10.0	10.0
Available phosphorus	4.5	4.5
Phytate P	2.7	2.5

[1]Supplied per kg of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 mg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 mg; Zn, 60 mg.

Ethics Committee guidelines. Day-old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and allocated to 24 cages (8 chicks/cage) in electrically heated battery brooders so that the average bird weight per cage was similar. Each of the 4 dietary treatments was randomly assigned to 6 cages. The birds were transferred to grower cages on d 12. The battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per d. The temperature was maintained at 31°C on d 1 and was gradually reduced to 22°C by 21 d of age. The experimental diets, in mash form, were offered *ad libitum* from d 1 to d 21 and water was freely available throughout the trial. Body weights and feed intake were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Feed per body-weight gain values were corrected for the body weight of any bird that died during the course of the experiment.

Ileal digestibility determination

On d 21, four birds were randomly selected from each replicate cage, killed by intravenous injection of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand) and digesta were collected from the lower half of the ileum, as described by Ravindran *et al.* (2005). Digesta from birds within a cage were pooled, lyophilised, ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at –4°C until laboratory analysis. Representative samples of diets and digesta were analysed for dry matter, titanium, gross energy (GE), nitrogen (N), fat, fatty acids, Ca and P.

The apparent metabolisable energy (AME) determination

Feed intake and total excreta output of each cage were measured from d 17 to d 20 post hatch. Excreta from each cage were pooled, mixed in a blender and sub-sampled. Each sub-sample was lyophilised, ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at –4°C pending analysis. Representative samples of diets and excreta were analysed for dry matter, GE, N, fat, Ca and P.

Chemical analysis

Dry matter and fat contents were determined using standard procedures (method 930.15 and 991.36, respectively; AOAC, 2005). Nitrogen content was determined by combustion (method 968.06; AOAC, 2005) using a CNS-200 carbon, N and sulphur auto analyser (LECO® Corporation, St. Joseph, MI, USA). Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. For the measurement of Ca and P, samples were wet digested with a mixture of nitric and perchloric acids, and the concentration of Ca and P were determined at specific wavelength for each of these elements (Ca, 393.3 and P, 185.9 nm) by inductively coupled plasma-optical emission spectroscopy with a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA; Fetcher *et al.*, 1998). Titanium contents were determined in a UV spectrophotometer using the method of Short *et al.* (1996).

To measure the fatty acid composition, the procedure reported by Sukhija and Palmquist (1988) was used. The samples were subjected to solvent extraction, purification and esterification followed by gas chromatographic analysis. To measure the proportion of fatty acids in the excreta that were present as soap, the procedure reported by Atteh and Leeson (1984) was used. In brief, the samples were subjected to two stages of petroleum ether extraction. The first extraction removed neutral fat and free fatty acids which were not present as soap. Thimbles containing the residue of the first extraction were placed in 25% hydrochloric acid for about 2 h at room temperature to liberate the fatty acids that were presente as soap. The samples were then freeze-dried and the process of ether extraction was repeated. The second ether extract was considered to represent the fatty acids that were present as soap.

Calculations

The AME values were calculated using the following formula with appropriate corrections made for differences in DM content.

$$\begin{aligned} \text{AME (MJ/kg diet)} \\ = ((\text{Feed intake} \times \text{GEdiet}) \\ - (\text{Excreta output} \times \text{GEexcreta}))/\text{Feed intake} \end{aligned}$$

The N-corrected AME (AME_n) values were calculated by correcting for N equilibrium (zero retention) by using a factor of 36.52 kJ/g N retained in the body (Hill and Anderson, 1958). The apparent ileal nutrient digestibility was calculated, using titanium dioxide as indigestible marker, as shown below:

$$\begin{aligned} \text{Apparent ileal nutrient digestibility} \\ = ((\text{Nt/Ti})_d - (\text{Nt/Ti})_i)/(\text{Nt/Ti})_d, \end{aligned}$$

where $(\text{Nt/Ti})_d$ = ratio of nutrient to titanium in diet, and $(\text{Nt/Ti})_i$ = ratio of nutrient to titanium in ileal digesta.

The apparent ileal digestible energy (AIDE) was calculated by multiplying the diet GE content by the apparent ileal energy digestibility coefficient.

Data analysis

Cage was considered as experimental unit for performance and digestibility data. Data were analysed as a two-way factorial arrangement of treatments using the General Linear Models procedure of SAS (2004). Differences were considered to be significant at $P < 0.05$.

RESULTS

The analysed P, Ca and other nutrients in experimental diets were in close agreement with the calculated values and confirmed the adequacy of P in both maize- and wheat-based diets (Table 2).

Table 2. *Determined analysis (g/kg DM) of the basal diets*

Ingredient	Maize-based diet	Wheat-based diet
Dry matter	887	878
Gross energy, MJ/kg	19.4	19.4
Crude protein (N x6.25)	256	273
Calcium	11.3	11.4
Total phosphorus	8.0	8.2
Fat	73.7	73.6
Myristic acid (C14:0)	0.235	0.277
Palmitic acid (C16:0)	11.6	13.0
Palmitoleic acid (C16:1)	1.18	1.45
Stearic acid (C18:0)	3.51	3.82
Oleic acid (C18:1)	19.5	18.5
Linoleic acid (C18:2)	33.1	31.5
Linolenic acid (C18:3)	2.60	3.12
Arachidic acid (C20:0)	0.238	0.170
Total saturated fatty acids	15.6	17.3
Total unsaturated fatty acids	56.3	54.6

Performance

Mortality during the experiment was negligible. Only two out of the 192 birds died and the deaths were not related to any specific treatment.

The main effect of grain type tended to be significant ($P = 0.08$) for weight gain, with higher weight gain for maize-based diets (Table 3). The addition of microbial phytase improved the weight gain ($P < 0.01$) of birds compared to those receiving diets without phytase. Dietary treatments had no effect ($P > 0.05$) on the feed intake. The main effect of grain type was significant ($P < 0.001$) for feed per gain, with lower feed per gain value for maize-based diets. The addition of microbial phytase lowered ($P < 0.001$) feed per gain of birds compared to those receiving diets without

Table 3. *Influence of grain type and microbial phytase on weight gain (g/bird), feed intake (g/bird) and feed per gain (g feed/g gain) in broiler starters, 0–21 d post hatch[1]*

	Phytase	Weight gain	Feed intake	Feed per gain
Maize	–	826	1121	1.357
	+	869	1130	1.306
Wheat	–	793	1110	1.407
	+	847	1172	1.383
SEM[2]		14.7	21.4	0.0084
Main effects				
Grain type				
Maize		848	1126	1.331
Wheat		820	1141	1.395
Phytase				
–		810	1116	1.382
+		858	1151	1.345
Probabilities, $P \leq$				
Grain type		0.08	NS	***
Phytase		**	NS	***
Grain type × Phytase		NS	NS	NS

NS, not significant; **$P < 0.01$; ***$P < 0.001$.
[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[2]Pooled standard error of mean.

phytase. No interaction ($P > 0.05$) was observed between grain type and phytase for any of the measured performance variables.

The apparent ileal nutrient digestibility

Birds fed on maize-based diets had higher ($P < 0.05–0.001$) ileal digestibility coefficients of N, Ca, P and GE, and AIDE than those receiving wheat-based diets (Table 4). The addition of microbial phytase increased ($P < 0.05$) the ileal digestibility of N and P, but had no effect

Table 4. *Influence of grain type and microbial phytase on apparent ileal digestibility coefficients of nitrogen (N), calcium (Ca), phosphorus (P) and gross energy (GE), and apparent ileal digestible energy (AIDE;MJ/kg DM) in broiler starters[1]*

	Phytase	Ileal digestibility coefficient N	Ca	P	GE	AIDE
Maize	–	0.832	0.449	0.522	0.758	14.74
	+	0.841	0.466	0.611	0.759	14.75
Wheat	–	0.771	0.360	0.453	0.665	12.91
	+	0.805	0.366	0.497	0.696	13.53
SEM[2]		0.0079	0.0378	0.0242	0.0087	0.1691
Main effects						
Grain type						
Maize		0.836	0.458	0.566	0.758	14.74
Wheat		0.788	0.363	0.475	0.680	13.22
Phytase						
–		0.801	0.405	0.487	0.711	13.83
+		0.823	0.416	0.554	0.727	14.14
Probabilities, $P \leq$						
Grain type		***	*	**	***	***
Phytase		*	NS	*	0.08	0.08
Grain type × Phytase		NS	NS	NS	0.09	0.09

NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[1]Each value represents the mean of 6 replicates.
[2]Pooled standard error of mean.

Table 5. *Influence of grain type and microbial phytase on the apparent ileal digestibility coefficients of fatty acids, saturated fatty acids (SFA), unsaturated fatty acids (UFA) and fat in broiler starters*[1]

	Phytase	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	SFA	UFA	Fat
Maize	−	0.868	0.822	0.923	0.779	0.845	0.843	0.897	0.660	0.823	0.834	0.831
	+	0.889	0.832	0.940	0.805	0.851	0.861	0.915	0.707	0.842	0.855	0.852
Wheat	−	0.777	0.734	0.872	0.721	0.775	0.762	0.810	0.504	0.744	0.745	0.756
	+	0.840	0.772	0.898	0.781	0.816	0.801	0.845	0.590	0.798	0.790	0.801
SEM[2]		0.0152	0.0120	0.0085	0.0162	0.0091	0.0104	0.0087	0.0276	0.0114	0.0093	0.0073
Main effects												
Grain type												
Maize		0.878	0.827	0.931	0.792	0.848	0.852	0.906	0.684	0.832	0.844	0.842
Wheat		0.808	0.753	0.885	0.751	0.795	0.782	0.827	0.548	0.771	0.767	0.778
Phytase												
−		0.822	0.778	0.897	0.750	0.810	0.803	0.853	0.582	0.784	0.789	0.794
+		0.864	0.802	0.919	0.793	0.833	0.831	0.880	0.649	0.820	0.822	0.827
Probabilities, *P* ≤												
Grain type		***	***	***	*	***	***	***	***	***	***	***
Phytase		*	0.06	*	*	*	*	**	*	**	**	***
Grain type × Phytase		NS	NS	NS	NS	0.06	NS	NS	NS	NS	NS	NS

NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[1]Each value represents the mean of 6 replicates.
[2]Pooled standard error of mean.

($P > 0.05$) on that of Ca. No grain type × phytase interaction ($P > 0.05$) was observed for these parameters. However, for GE and AIDE, grain type × phytase interaction tended ($P = 0.09$) to be significant. These variables were not affected by phytase addition in maize-based diets, but tended to increase with phytase supplementation in wheat-based diets.

The apparent ileal digestibility coefficient of fat ($P < 0.001$) and all fatty acids ($P < 0.05$–0.001) was higher in birds fed on maize-based diets than those fed on wheat-based diets (Table 5). Phytase supplementation increased ($P ≤ 0.06$–0.001) the ileal digestibility of fat and all fatty acids. The addition of phytase improved the digestibility of fat by 4.2% and that of myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidic acids by 5.1, 3.1, 2.5, 5.7, 2.8, 3.5, 3.2 and 11.5%, respectively. No grain type × phytase interaction was observed for the apparent ileal digestibility of fatty acids ($P = 0.18$–0.61) and fat ($P = 0.11$). Phytase supplementation increased ($P < 0.01$) the ileal digestibility of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) by 4.6 and 4.2%, respectively, compared to unsupplemented diets. Grain type × phytase interaction ($P > 0.05$) was not observed for the apparent ileal digestibility of SFA and UFA, indicating that the effect of microbial phytase supplementation on these parameters were similar, irrespective of the different grain type.

Total tract nutrient retention

Birds fed on maize-based diets had higher ($P < 0.001$) AME_n and total tract retention of fat, N

and Ca ($P < 0.05$) compared to those fed on wheat-based diets (Table 6). The addition of phytase had no effect ($P > 0.05$) on the total tract retention of fat and AME_n. However, the main effect of phytase for N and Ca retention tended ($P = 0.08$) to be significant. Birds fed on diets with added microbial phytase showed higher N and Ca retention compared to those receiving diets with no phytase supplementation. The main effects of grain type and phytase and also the interaction between grain type and phytase were not significant ($P > 0.05$) for P retention and excreta soap concentration.

DISCUSSION

Birds fed on maize-based diets tended to grow faster and were more efficient in utilising feed than those fed on wheat-based diets. Supplementation of wheat-based diets with exogenous xylanase was intentionally avoided in the present study and, hence, this was an expected result. This approach was taken to overcome any confounding effects of combining phytase and xylanase.

In the present study, irrespective of the grain type, phytase addition to P-adequate diets improved the weight gain and feed efficiency of young broilers. Growth responses to phytase supplementation in P-inadequate diets are mediated mainly through increased feed intake (Selle and Ravindran, 2007). In this study, the effect of phytase on feed per gain was numerically more pronounced in maize-based diets compared to wheat-based diets (3.9 vs. 1.7%). The lower response in birds fed on wheat-based diets was mainly due to the numerically higher feed intake compared to those fed on maize-based diets (5.2 vs. 0.80%).

Table 6. *Influence of grain type and microbial phytase on the nitrogen-corrected apparent metabolisable energy (AME$_n$; MJ/kg dry matter), excreta soap concentration (%) and total tract retention coefficients of fat, nitrogen (N), calcium (Ca) and phosphorus (P) in broiler starters[1]*

				Retention			
	Phytase	AME$_n$	Excreta soap[2]	Fat	N	Ca	P
Maize	–	13.48	47.0	0.829	0.597	0.435	0.512
	+	13.48	42.8	0.822	0.613	0.454	0.544
Wheat	–	12.76	44.0	0.785	0.525	0.353	0.498
	+	12.79	44.7	0.789	0.545	0.414	0.519
SEM[3]		0.073	3.97	0.0094	0.0095	0.0220	0.0310
Main effects							
Grain type							
Maize		13.48	44.9	0.825	0.605	0.444	0.528
Wheat		12.78	44.3	0.787	0.535	0.383	0.509
Phytase							
–		13.12	45.5	0.807	0.561	0.394	0.505
+		13.14	43.7	0.805	0.579	0.434	0.531
Probabilities, $P \leq$							
Grain type		***	NS	***	***	*	NS
Phytase		NS	NS	NS	0.08	0.08	NS
Grain type × Phytase		NS	NS	NS	NS	NS	NS

NS, not significant; *$P < 0.05$; ***$P < 0.001$.
[1]Each value represents the mean of 6 replicates.
[2]Excreta fat as soap as proportion of total fat.
[3]Pooled standard error of mean.

These data suggest that observed performance responses were due to changes in digestibility of protein and fat/fatty acids and possibly increased the nutrient intake especially in wheat-based diets.

Supplementation of P-adequate diets with phytase improved ileal P digestibility (13.8%), which is interpreted as a phytase-induced release of phytate-bound P. The ability of phytase to improve P availability by hydrolysing phytate-bound P in poultry diets is well-documented (Simons *et al.*, 1990). In maize-based diets, the ileal P digestibility coefficient (0.522) and analysed P (8.0 g/kg) in the unsupplemented diet gives a digested P of 4.18 g/kg; the difference, undigested P at 3.82 g/kg, would be available for hydrolysis by the added microbial phytase. Given that the addition of 500 FTU/kg diet reduced undigested P from 3.82 to 3.11 g/kg, it follows that the added microbial phytase in P-adequate maize-based diets degraded at least 18.5% of the undigested P. The corresponding value for wheat-based diets was 8.9%. The efficiency of hydrolysis of inositol phosphate esters is dependent on several factors including dietary P concentrations, phytase activity, form and location of phytate in feedstuffs, and conditions in the gastrointestinal tract (Adeola and Sands, 2003).

The addition of microbial phytase in P-adequate diets also caused significant improvements in apparent ileal digestibility of N (2.7%). The positive effect of microbial phytase on the digestibility of N is consistent with a number of previous reports (Rutherfurd *et al.*, 2002; Cowieson *et al.*, 2004). The possible mechanisms underlying the protein/amino acid responses have been discussed in detail by Selle *et al.* (2012). These responses may be attributed, in part, to the capacity of phytase to release phytate-bound nutrients by hydrolysing phytic acid. Recent studies suggest that phytate increases endogenous protein losses (Cowieson and Ravindran, 2007; Cowieson *et al.*, 2009) and can cause hyper secretion of mucin and depression of enzyme activities in the digestive tract of birds (Cowieson *et al.*, 2004; Liu *et al.*, 2008). Amelioration of these negative effects may also have contributed to the observed improvements in apparent N digestibility.

The lack of significant diet type × phytase interaction for all parameters indicates that the efficacy of microbial phytase was similar in both diet types. These results are in contrast with previous published data. Selle *et al.* (2000) stated that the configuration of maize protein limits the access of phytase to basic amino acid residues and, therefore, the initiation of protein–phytate complex formation. Ravindran *et al.* (1999) and Rutherfurd *et al.* (2002) also reported that phytase increased the ileal digestibility of essential amino acids in wheat to a considerably greater extent than in maize. It has been suggested by Ravindran *et al.* (1999) that microbial phytase may be acting on wheat in a manner similar to that of exogenous xylanase, by disrupting the cell wall matrix and enhancing contact between digestive enzymes and cell contents. However, in the current study, the AIDE and ileal GE digestibility data showed wheat-based diets tended to be more responsive to phytase supplementation than

maize-based diets. Improvements in the energy utilisation of wheat-based diets with added phytase are consistent with previous reports (Ravindran et al., 1999, 2000; Wu et al., 2003).

In the present study, inclusion of microbial phytase had no effect on the AME_n and total tract retention of fat and P (Table 6) despite having positive effects on the ileal digestibility of fat, P and energy. These results are difficult to explain since total tract measurements over a 4-d period are more likely to be representative of the whole body situation than spot measurements of ileal digestibility at point of death. These findings, however, may be partly related to the fact that the classical excreta-based AME_n measurements do not reflect the actual responses in absorbed energy because of the modifying effects of caecal micro-organisms on energy and nutrient utilisation and the contribution of microbial mass to energy and nutrient output in the excreta. The variable and modifying effects of hindgut microflora on protein digestion have been previously demonstrated (Ravindran et al., 1999).

The apparent ileal digestibility of SFA and UFA was positively affected by microbial phytase supplementation (4.6 and 4.2%, respectively). Accordingly, supplemented phytase improved the apparent ileal digestibility of fat by 4.2% when measured as ether extract. Linoleic and oleic acids were the major UFA in the experimental diets, whereas palmitic acid was the major SFA (Table 2). The digestibility of these three fatty acids was improved by approximately 3% by added microbial phytase. The significant improvement observed in ileal digestibility of fat with phytase is consistent with the findings of Camden et al. (2001) and Liu et al. (2010) who reported that phytase increased the digestibility of fat in broilers by 3.4 and 5.1%, respectively. However, this is the first report revealing the effects of phytase on the digestibility of fatty acids. One or more of the following events may explain the observed improvements in the digestibility of fat/fatty acids by phytase supplementation.

(i) Phytase, by hydrolysing phytate, may reduce the formation of metallic soaps (Ca-fat) in the gut lumen (Ravindran et al., 2000). It is possible that the high molar ratio of Ca to phytate in P-adequate diets leads to the formation of insoluble Ca–phytate complexes which have the capacity to further complex with fatty acids in the gut lumen (Cosgrove, 1966) to form insoluble soaps, thereby lowering fat digestibility. However, this was not confirmed in the present experiment, as no effect of phytase was observed on excreta soap percentage at the total tract level.

(ii) Supplementation of phytase may overcome the adverse effect of phytate on the activity of lipase, phospholipases A1 and A2 (Erlanson, 1975; Knuckles, 1988) and hyper secretion of bile. Bile is a rich source of phospholipids and there is suggestive evidence that bile output is increased in the presence of phytate (Cowieson et al., 2009).

(iii) As high bile salt concentration has inhibitory effects on co-lipase for binding sites on the substrate (Borgstrom, 1975), exogenous phytase may reduce this negative effect by preventing hyper secretion of bile. Co-lipase is essential to initiate the action of pancreatic lipase on triglyceride emulsions. Co-lipase and bile salts are competitive inhibitors for binding sites on the substrate and, at very high bile salt concentrations, co-lipase is displaced from the surface by bile salts, with a resultant inhibition of pancreatic lipase activity (Borgstrom, 1975).

(iv) Similar to a glycosidase, phytase may have the ability to disrupt the cell wall matrix, allowing better access of endogenous enzymes and secretions such as lipase and bile salts to the lipid fraction (Juanpere et al., 2005).

The lack of interactions between phytase supplementation and grain type in the apparent ileal digestibility of fatty acids indicated that there were no major differences in the direction and magnitude of the effects of phytase between grain types. Although the mechanisms of the increments of ileal fat digestibility for each grain type cannot be identified from the current data, they can certainly vary due to differences in the location and accessibility of phytate and fat in each grain, as well as factors such as the concentration of water-soluble non-starch polysaccharides which are higher in wheat than in maize and are known to negatively affect fat digestibility (Dänicke et al., 1999).

The present results illustrated that phytate present in whole grain and oil seeds may act as a moderate anti-nutritive factor which can impair the availability of protein and fatty acids, and eventually depress the growth performance and feed efficiency of young broilers even in P-adequate diets. These adverse effects can be overcome by supplemental phytase. Improved performance with supplemental phytase was generally associated with improved nutrient digestibility.

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