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Buttiauxella phytase maintains growth performance in broilers fed diets with reduced nutrients under a commercial setting

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Abstract

The effect of down specifying nutrients in diets supplemented with *Buttiauxella* spp. phytase was studied in a commercial trial. Three treatments were tested with five replicate groups, each containing 700, one-day-old straight run Ross 308 broilers. Birds were fed pelleted diets from days 0-42 in four phases: starter (days 0-10); grower (days 11-21); finisher 1 (days 22-35) and finisher 2 (days 36-42). A nutritionally adequate, unsupplemented, positive control (PC) diet based on wheat, corn and soybean meal was compared against two down specified, negative control (NC) diets containing *Buttiauxella* phytase supplemented at 500 or 1000 FTU/kg. The reduction level was 0.134 and 0.159% unit for digestible phosphorus, 0.164 and 0.189% unit for calcium, 0.03 and 0.04% unit for sodium, 0.283 and 0.309 MJ/kg for nitrogen corrected apparent metabolizable energy in all phases and variable digestible amino acids in different phases, respectively for the diets containing the phytase at 500 and 1000 FTU/kg. An unsupplemented NC diet was not included, as it would have caused welfare and health issues. Weight gain and mortality-corrected feed conversion ratio for birds receiving phytase at either inclusion levels were equivalent to the PC group. Feed intake was increased by 500 FTU/kg phytase (P<0.05) during 0-21 d vs PC. Including 1000 FTU/kg phytase reduced water intake vs PC at 0-42 d and water-to-feed intake ratios, after the starter phase (P<0.05). Carcass yield in birds supplemented with either phytase level was not different from PC. Tibia ash was unaffected by treatment. Estimated feed costs (inclusive of phytase) were lower in supplemented than un-supplemented (PC) diets, by 10.0 to 13.7 €/ton diet. The trial demonstrated that reducing nutrient specifications of diets supplemented with *Buttiauxella* phytase maintained growth performance, lowered feed costs, with production benefits maximised at inclusion levels of 1000 FTU/kg.

Keywords: amino acids; broilers; growth performance; nutrient matrix; phytase

1. Introduction

It is well known that up to 70-80% of the total phosphorus (P) content of plant-based poultry feed ingredients is in the form of phytate (myo-inositol hexaphosphate), and that phytate-bound P is poorly available to poultry and other monogastric animals (Humer *et al.*, 2015; Selle and Ravindran, 2007). Phytate can bind minerals (particularly Ca) and other trace minerals, such as Zn, Cu and Fe, proteins and amino acids (AA) in specific regions of the gastrointestinal tract of poultry, inhibiting the digestion and utilisation of these nutrients (Selle and Ravindran, 2007; Selle *et al.*, 2000, 2009, 2012). Further, it has been suggested that phytate can impair the activity of endogenous protease enzymes, leading to increased HCl secretion in the stomach and increased sodium bicarbonate secretion in the small intestine. This can compromise Na^+ dependent transport systems and the activity of the sodium-potassium pump in cells, reducing the absorption of nutrients such as glucose and AA (Cowieson *et al.* 2004; Ravindran *et al.* 2006; Selle *et al.*, 2012). These multiple antinutritive effects of phytate are undesirable to feed manufacturers and producers.

Applying an exogenous microbial phytase can markedly improve phytate degradation, P availability and utilisation. Phytase supplementation in poultry diets has become widespread practice. New phytases with enhanced efficacy are continuously being developed and the science underpinning the optimal formulation of least-cost diets containing phytase is developing rapidly. A current *Buttiauxella* phytase, that has demonstrably higher activity at lower pH levels than other phytases (Christensen *et al.*, 2017; Menezes-Blackburn *et al.*, 2015), has recently been shown to achieve 70-90% ileal phytate degradation at an incorporation level of ~1000 FTU/kg in corn-based diets (Amerah *et al.*, 2014; Bello *et al.*, 2019; Li *et al.*, 2016). Its effects on P and Ca digestibility have been well studied and multiple digestibility and efficacy trials have enabled the development of robust matrix values that define the precise amounts by which these nutrients can be down specified in the diet due to the expected contribution (the nutrient-release capability or improved nutrient availability) of the phytase (Kim *et al.*, 2018; Li *et al.*, 2018; Liu *et al.*, 2014; Truong *et al.*, 2015). Down specification of dietary nutrients with no loss of growth performance can offer additional value to producers due to feed cost savings.

Phytase contributions for increased P and Ca availability are generally well accepted by poultry nutritionists for products with good supporting data. However, there is currently less acceptance of matrix values for digestible AA, energy and sodium (Na), despite increasing evidence reporting 'extra-phosphoric' effects of new generation phytases on these nutrients. Phytate binds directly to AA in the low pH environment of the upper gastrointestinal tract, reducing amino acid absorption and increasing endogenous losses (Cowieson *et al.*, 2004; Selle *et al.*, 2000). A measurable improvement in amino acid digestibility with phytase can be expected, but evidence suggests that effects are not the same for all phytases. The *Buttiauxella* phytase was recently shown to produce a greater improvement in ileal digestibility of total AA than an *E. coli* phytase at equivalent dose-levels, and the dose-response curves of the two phytases differed (Dersjant-Li and Kwakernaak, 2019). Across multiple studies it has been estimated that the *Buttiauxella* phytase improves ileal digestibility of AA by between 3.7 to 15% when dosed at 1000 FTU/kg in corn-based diets, (Amerah *et al.*, 2014; Dersjant-Li and Kwakernaak, 2019; Kiarie *et al.* 2015; Li *et al.*, 2015; Truong *et al.*, 2015). These studies showed that the degree of improvement was directly related to phytate degradation, and there may be concurrent improvements in ileal digestibility of Na (+36 to +96% vs NC) (Dersjant-Li and Kwakernaak, 2019; Truong *et al.*, 2015), and metabolizable energy (ME) (Dersjant-Li and Kwakernaak, 2019). The modelling of data from seven ileal digestibility trials in broilers fed diets supplemented with *Buttiauxella* phytase at 129-2,346 FTU/kg has enabled the determination of matrix values for digestible AA, Na and ME (Plumstead *et al.*, 2013). However, although fully replicated research trials have been conducted, *in vivo* validation of these values in large-scale, less controlled commercial trials is lacking.

The aim of this study was to test whether the application of a full nutrient matrix, comprising down specifications of P, Ca, important digestible AA, ME and Na, to diets supplemented with a commercially available *Buttiauxella* phytase at two dose-levels, could maintain growth performance, slaughter yields, bone ash and mineral content in broilers reared under commercial conditions. Data was used to estimate the potential impact of the full nutrient matrix application on total feed costs and production benefits.

2. Materials and methods

Birds and housing

All animal care procedures were approved by the Institutional Animal Ethics Committee (Schothorst Feed Research, Lelystad, the Netherlands). The experiment was conducted in a commercial setting that avoided unnecessary discomfort of the animals and conformed to European Union Guidelines on animal treatment, management, housing husbandry and slaughtering conditions (EC, 2007). Hence, an unsupplemented negative control (NC) diet could not be used.

A total of 10,500 Ross 308 one-day-old broilers (mixed male and female) were obtained from a commercial hatchery and assigned to three dietary treatments across a house divided into 15 large replicate penned areas within commercial broiler houses, each containing 700 birds and with five pen replicates per treatment, in a completely randomised block design. The pens had a surface area of 47.5 m^2 resulting in an initial stocking density of 14.7 birds/m^2 rising to 40.2 kg/m^2 at point-of-slaughter. Wood shavings were used as litter. Ambient temperature was maintained initially at 34.5 °C decreasing to 19 °C at 42 days-old, under a light-dark cycle of 24L:0D for the first 24 h followed by 22L:2D thereafter. From d 3 onwards the light-dark cycle was 8:4:10:2. Birds were vaccinated against Newcastle disease (Avinew Neo, Boehringer Ingelheim, Ingelheim an Rhein, Germany) on d 7 and infectious bursal disease on day 21 (Poulvac Bursine -2, Zoetis, Kalamazo, MI, USA). The trial lasted until 42 d. Birds were given *ad libitum* access to water and pelleted diets during the trial.

Dietary treatments

The three dietary treatments included a positive control (PC) diet and two experimental diets each comprising a nutrient-reduced NC supplemented with a commercial *Buttiauxella* 6-phytase expressed in *Trichoderma reesei* (Axtra®PHY, DuPont Nutrition and Biosciences, Leiden, the Netherlands), at 500 or 1000 FTU/kg (NC1 500 FTU/kg feed

or NC2 1000 FTU/kg feed). The PC diet was a wheat, corn and soybean meal-based diet, formulated in four feeding phases providing adequate nutrients for broilers (Aviagen, 2014). The diet provided 11.7, 11.9, 12.2 and 12.6 MJ/kg ME and 11.5, 11.0, 10.0 and 9.5 g/kg digestible Lys in starter (days 0-10), grower (days 11-21) finisher 1 (days 22-35) and finisher 2 (days 36-42) phases, respectively. The detailed composition of the diets is given in Table 1. Phased NC diets were formulated with nutrient reductions in P, Ca, energy, essential AA and Na. The nutrient reduction for NC1 and NC2 diets were calculated according to the manufacturer's recommendations (DuPont Nutrition & Biosciences) for *Buttiauxella* phytase supplementation at 500 FTU/kg or 1000 FTU/kg based on dietary estimated phytate levels and the age of broilers. The targeted reduction level was 0.134 and 0.159% unit for digestible P, 0.164 and 0.189% unit for Ca, 0.03 and 0.04% unit for Na, 0.283 and 0.309 MJ/kg for nitrogen corrected apparent metabolizable energy (AMEn), respectively for the diets containing the phytase at 500 and 1000 FTU/kg in all phases. The digestible AA reduction differed based on the feeding phases and dose levels (Table 2). The nutrient reduction was achieved by replacement of calcium carbonate, monocalcium phosphate, sodium carbonate, poultry fat and synthetic AA Lys, Met, Thr and Val, with variable diamol content (a flow agent used in feed), so that the composition of the other dietary components remained the same. The NC diets were not administered unsupplemented, as this would have been unethical in the large-scale setting of this commercial trial, as it would have induced welfare issues in the birds. The phytase supplemented diets were fed as complete diets for the starter phase. The grower and finisher phases were produced by mixing 80% of the basal diet with 20% of a complement test diet formulated with the necessary nutrient down-specifications and containing the test phytase at a level designed to result in the targeted activity in the final diets. The diets were produced in a feed mill specialised in the production of experimental diets (ABZ, Leusden (for starter and complement diets) and Nijkerk (for basal diets), the Netherlands). All diets were fed as pellets; the starter diet was fed as a small pellet (2.3 mm) and the grower and finisher diets were fed as a standard pellet (3.0 mm).

Sampling and measurements

Body weight (BW) was estimated on arrival at the experimental farm (d 0) from 180 birds selected at random. At day 10 and 21, BW was monitored per pen by automatic weighing scale. At d 42, all birds were weighed per pen manually since automatic weighing plateaus are not accurate for measuring BW of late-grower and finisher stage birds. Feed intake was recorded at the end of each period on a per pen basis and used to calculate feed conversion ratio (FCR), corrected for mortality. On d 42, 20 birds per pen (10 males, 10 females) were individually weighed and eviscerated. Carcass weight, wing, leg, breast filet and abdominal fat pad weights were recorded. In addition, the left tibia bones from four birds per pen (two males and two females) were extracted and pooled for the determination of de-fatted tibia ash content. Tibias were stripped of adjacent tissues and dried overnight initially at 40 °C and subsequently at 70 °C. Fat was extracted using a Soxhlet apparatus (Thermo Fisher Scientific, Roskilde, Denmark) and 100% petroleum ether, according to modified methods of Watson *et al.* (2006). Fat-extracted-tibias were dried for 4 h at 103 °C and ashed in a muffle furnace for 24 h at 700 °C to determine bone ash content.

Chemical analysis

Representative samples of all treatment diets were analysed for dry matter, crude protein, crude fat, starch, crude fibre, Ca, P, phytate-P and phytase. Nutritional analyses were performed by Schothorst Feed Research (Lelystad, the Netherlands).

Samples were analysed in duplicate for all analyses. Nutrients were analysed according to the following methods: moisture, NEN-ISO 6496 (1999); crude protein, NEN-EN-ISO 16634 (2008); crude fat, NEN-ISO 6492 (1999); crude fibre, NEN-ISO 6865 (2001); starch, NEN-ISO 15914 (2005); phosphorus, NEN-ISO 6491 (1999); calcium, NEN-EN-ISO 6869 (2001) and; ash, NEN-ISO 5984 (2003). Phytate phosphorus (inositol hexa-phosphate) concentrations in diets and phytase activities in the diets were determined by DuPont Laboratories (Brabrand, Denmark), using the methods described by Yu *et al.* (2012). One phytase unit (FTU) was defined as the amount of enzyme that released 1 μmol of inorganic orthophosphate from a sodium phytate substrate per min. at pH 5.5 and 37 °C (AOAC, 2000).

Calculations

FCR was calculated based on mortality body weight corrected body weight gain (BWG) and feed intake from days 0-10, 11-21, 21-42 and days 0-42. Mortality body weight was estimated as 80% of the average bodyweight (measured by the weighing plateau) in the pen on the day when the bird was removed, using the following equation:

$$\sum_{i=1}^{n} (0.8 \times (\text{number of mortality})_i \times (\text{average weight of living broilers})_j,$$

in which i was the day of mortality.

Statistical analyses

Data obtained for each measurement were analysed for outliers. Outliers were defined as observations whose residual values exceeded the standard error of the residuals by more than 2.5 times. Where outliers were identified

Table 1. Ingredient and calculated nutrients composition (g/kg, as fed basis) of the positive control and basal (NC) diets and calculated feed costs.

	Starter (days 0-10)			Grower 1 (days 11-21)			Finisher 1 (days 22-35)			Finisher 2 (days 36-42)		
	PC	NC 1	NC 2	PC	NC 1	NC 2	PC	NC 1	NC 2	PC	NC 1	NC 2
Ingredient (g/kg)												
Wheat	375	375	375	353	354	355	400	400	400	400	400	400
Corn	200	200	200	250	250	250	229	229	230	234	235	236
Soybean meal	297	297	297	263	263	263	207	208	208	209	209	209
Rapeseed meal	33.5	33.5	33.5	50.0	50.0	50.0	75.0	75.0	75.0	66.2	66.2	66.2
Poultry fat	40.5	33.1	32.6	42.4	34.7	34.0	51.0	43.3	42.6	60.0	52.3	51.6
Soybean oil	2.10	2.10	2.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salt	3.10	3.10	3.10	3.20	3.30	3.10	3.20	3.30	3.20	3.20	3.20	3.20
Premix Maxiban[2]	5.0	5.0	5.0	5.0	5.0	5.0						
Premix broiler starter[3]	5.0	5.0	5.0									
Premix Narasin[4]							5.0	5.0	5.0			
Premix broiler standard[5]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Monocalcium phosphate	11.8	4.90	3.60	8.30	1.30	0.00	7.10	0.10	0.00	6.00	0.00	0.00
Limestone	13.9	12.7	12.6	11.7	10.5	10.4	10.6	9.40	9.30	9.80	8.70	8.70
Sodium bicarbonate	1.50	0.40	0.00	1.50	0.30	0.10	1.50	0.30	0.00	1.50	0.40	0.00
Lysine	2.50	2.30	2.00	2.70	2.40	2.30	2.60	2.40	2.20	2.10	1.90	1.70
DL-methionine	2.60	2.40	2.30	2.40	2.20	2.10	2.00	1.80	1.70	1.60	1.50	1.40
Threonine	1.00	0.90	0.70	1.00	0.80	0.70	0.90	0.70	0.60	0.60	0.50	0.40
Valine	0.70	0.50	0.40	0.70	0.60	0.40	0.60	0.40	0.30	0.30	0.10	0.00
Diamol	0.00	17.3	20.2	0.10	17.0	19.4	0.00	16.6	17.7	0.00	15.6	16.7
Phytase, FTU/kg		500	1000		500	1000		500	1000		500	1000
Calculated nutrients (g/kg)												
Crude protein	216	216	215	207	206	206	191	191	191	188	188	187
ME (MJ/kg)	11.72	11.42	11.41	11.92	11.64	11.61	12.24	11.96	11.93	12.55	12.27	12.24
Calcium	9.3	7.6	7.4	7.5	5.9	5.6	6.9	5.2	5.2	6.3	4.9	4.9
Total phosphorus	6.4	4.9	4.6	5.6	4.1	3.8	5.3	3.8	3.7	5.0	3.7	3.7
Ret. phosphorus	3.7	2.4	2.1	3.0	1.7	1.4	2.8	1.4	1.4	2.6	1.5	1.5
Av. phosphorus	4.1	2.5	2.2	3.3	1.7	1.4	3.0	1.4	1.4	2.7	1.4	1.4
Phytate phosphorus	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Non-phytate phosphorus	4.0	2.4	2.1	3.2	1.6	1.3	2.9	1.3	1.3	2.6	1.2	1.2
Lysine	12.9	12.7	12.6	12.4	12.2	12.1	11.2	11.1	10.9	10.8	10.6	10.5
Digestible lysine	11.5	11.3	11.1	11.0	10.8	10.7	10.0	9.8	9.7	9.5	9.4	9.2
Methionine	5.8	5.6	5.5	5.4	5.3	5.2	4.9	4.7	4.6	4.5	4.4	4.2
Digestible methionine	5.4	5.3	5.1	5.1	4.9	4.8	4.6	4.4	4.3	4.2	4.0	3.9
Methionine + cysteine	9.4	9.2	9.1	9.0	8.8	8.7	8.3	8.1	8.0	7.9	7.7	7.6
Digestible methionine + cysteine	8.4	8.2	8.1	8.0	7.8	7.7	7.3	7.2	7.0	6.9	6.8	6.6
Analysed nutrients (g/kg)												
Dry matter	902	902	900	877	877	876	888	888	888	894	892	891
Crude protein	209	-	-	204	-	-	194	-	-	190	-	-
Crude fat	89	83	83	66	-	-	75	-	-	83	-	-
Starch	357	-	-	367	-	-	383	-	-	382	-	-
Crude fibre	29	-	-	33	-	-	33	-	-	33	-	-
Calcium	9.3	7.7	7.4	7.7	6.1	5.8	7.5	5.8	5.7	7.8	6.3	6.3
Phosphorus	6.4	5.0	4.7	5.8	4.2	3.9	5.6	4.0	3.9	5.2	3.8	3.8
Phytate-P	2.3			2.2			2.4			2.3		
Analysed phytase (FTU/kg)	242	770	974	143	406	984	170	520	846	285	460	935
Feed costs (€/100 kg diet)[6]	28.74	27.74	27.52	25.75	24.65	24.38	24.44	23.35	23.16	23.70	22.68	22.52

[1] NC diets were not given as stand-alone diets (for ethical reasons) but supplemented with phytase according to treatment. NC1 was supplemented with 500 FTU/kg *Buttiauxella* spp. phytase and NC2 was supplemented with 1000 FTU/kg *Buttiauxella* spp. phytase. Phytase was dosed based on analysed product activity by an independent laboratory (LUFA, Nord West, Oldenburg, Germany).

[2] Supplies per kg diet 50 mg Narasin/ Nicarbazine (51772) Maxiban.

[3] Supplies per kg diet: vitamin A, 5,000 IU; vitamin D 25-hydroxycholeciferol 1000 IU; vitamin E, 40 mg; vitamin K3, 0.5 mg; vitamin B1, 1.5 mg; vitamin B2, 1.5 mg; pantothenic acid, 2.5 mg; niacin, 25 mg; biotin, 225 mcg; vitamin B12, 5 mcg; folic acid, 0.5 mg; vitamin B6, 1 mg; choline chloride 125 mg; Fe, 10 mg (as $FeSO_4.H_2O$); total Cu, 0.04 mg; Zn, 15 mg (as Zn chelate from glycine); Mn, 10 mg (as MnO); total I, 0.5 mg; total Se, 0.05; anti-oxidant, 25 mg (E310/E321/E324).

[4] Containing 14,000 mg narasin /kg product, providing 70 mg narasin per kg diet.

[5] Supplies per kg diet: vitamin A, 10,000 IU; vitamin D3, 3,333 IU; vitamin E, 50 mg; vitamin K3, 2.5 mg; vitamin B1, 2.5 mg; vitamin B2, 7.5 mg; pantothenic acid, 15 mg; niacin, 50 mg; biotin, 250 mcg; vitamin B12, 25 mcg; folic acid, 1.5 mg; vitamin B6, 5 mg; choline chloride 500 mg; Fe, 50 mg (as $FeSO_4.H_2O$); Cu, 12.5 mg (as $CuSO_4.5H_2O$); Zn, 70 mg (as $ZnSO_4.H_2O$); Mn, 75 mg (as MnO); total I, 2 mg (as KI); Se, 0.25 (as Na_2SeO_3); anti-oxidant, 4.17 mg butylhydroxytolueen (E310); 4.17 mg propyl gallaat (E310) and 4.17 mg ethoxyquin (E324).

[6] Calculated based on market prices in 2014 including phytase cost.

Table 2. Nutrient reduction (g/kg or MJ/kg actual reduction)in the *Buttiauxella* phytase-supplemented diets.[1,2]

Nutrient reduction versus positive control (g/kg)	500 FTU/kg phytase				1000 FTU/kg phytase			
	Starter	Grower	Finisher 1	Finisher 2	Starter	Grower	Finisher 1	Finisher 2
Calcium	1.64	1.64	1.64	1.43*	1.89	1.89	1.69*	1.43*
Digestible phosphorus	1.34	1.34	1.34	1.15*	1.59	1.59	1.35*	1.15*
AMEn (MJ/kg)[3]	0.283	0.283	0.283	0.283	0.309	0.309	0.309	0.309
Digestible lysine	0.206	0.171	0.156	0.156	0.379	0.316	0.288	0.288
Digestible methionine + cysteine	0.174	0.145	0.143	0.143	0.321	0.267	0.262	0.262
Digestible valine	0.208	0.173	0.167	0.167	0.382	0.318	0.307	0.272*
Digestible threonine	0.169	0.141	0.132	0.132	0.314	0.261	0.244	0.244
Sodium	0.302	0.302	0.302	0.302	0.41	0.41	0.41	0.41

[1] Starter: days 0-10; grower: days 11-21; finisher 1: days 22-35; finisher 2: days 36-42.

[2] * indicates where values were lower than the target matrix values (i.e. reduction achieved was less than target, e.g. the target for digestible phosphorus is 1.34 and 1.59 g/kg for finisher phases, at 500 and 1000 FTU/kg respectively). This occurred where it was not possible to achieve the target down-specification of the nutrient, due to not enough inorganic P to replace or not possible to reduce the digestible AA levels. In the former case, Ca reduction was calculated as the actual digestible P reduction × 1.25, to maintain Ca: P balance.

[3] Nitrogen corrected apparent metabolizable energy.

among growth performance and tibia ash data, the entire growth performance record of data relating to that pen was excluded from the analysis. Where identified for slaughter/carcass yield data, outliers were designated as missing values. Data were analysed based on pen as the experimental unit and analysed using GenStat® for Windows (version 17; VSNI, Hemel Hempstead, UK). Growth performance data were analysed by ANOVA with dietary treatment as a fixed effect and block as a random effect. The following model was used:

$$Y_{ij} = \mu + Block_i + Diet_j + e_{ij},$$

in which: Y_{ij} = dependent variable; μ = overall mean; $Block_i$ = block effect (i=1…5); $Diet_j$ = effect of dietary treatment, (j=1…3), and; e_{ij} = residual error. Differences between treatments were identified based on Least Significant Difference (LSD) values.

Carcass yield and tibia ash data were analysed as a 2×3 factorial arrangement with two genders and three dietary treatments. Differences between treatments were identified based on LSD values. Differences were considered significant at $P<0.05$ and $P<0.1$ was considered a strong trend.

3. Results

Enzyme recoveries, analysed nutrients and down specifications

Phytase was dosed based on the analysed activity of the product. The analysed phytase activity in the diets ranged from 73 to 106% of targeted activity across treatments (Table 1). Analysed values of crude protein in the PC were within 10% of calculated values across all dietary phases. Analysed values of P and Ca were within 10% of calculated values for all phases and treatments except for Ca in the finisher 2 diet, where values were 24 to 28% higher than calculated. Analysed phytate-P values in the PC diets were between 7% (finisher 1) and 15% (grower) lower than calculated.

The target down specifications in the phytase supplemented diets were achieved for all nutrients in the starter and grower diets (Table 2). In finisher diets supplemented with 1000 FTU/kg phytase, it was not possible to formulate diets with the full digestible P and valine matrix values, due to there being insufficient monocalcium phosphate to replace, or it not being possible to further reduce digestible AA levels. Therefore, in these diets the analysed digestible P reductions (vs PC) were slightly below the target values (digestible P reduction in finisher 1 vs PC: -1.59% (target), -1.35% (achieved); finisher 2 -1.59% (target), -1.15% (achieved)). In order to maintain the Ca:P balance in these diets the Ca down specification was adjusted to equate to 1.25 times the digestible P down specification. This resulted in the Ca reductions in the finisher diets being slightly below the full matrix value intended (Ca reduction in finisher 1 vs PC: -1.89% (target), 1.69% (achieved); finisher 2: 1.89% (target), 1.43% (achieved) (Table 2).

Growth performance

Average BWG and FCR of the birds receiving phytase supplementation at 500 FTU/kg and 1000 FTU/kg in the NC diets were equivalent to those of the respective PC. This was evident for all growth phases individually and overall ($P>0.05$; Table 3). Phytase at 500 FTU/kg increased feed intake vs PC during grower (d 11-21) and starter-grower (d 0-21) phases ($P<0.05$) and tended to increase overall feed intake (d 0-42; $P=0.10$). Phytase at 1000 FTU/kg reduced water intake vs PC over the whole trial (d 0-42; $P<0.05$) and reduced water to feed intake ratio during grower 1 (d 11-21), finisher (d 22-42) and whole trial (d 0-42) phases ($P<0.05$). Mortality was not influenced by treatments ($P>0.05$). For

Table 3. Effect of full matrix application of a *Buttiauxella* phytase, at two dose-levels, on growth performance of broilers from 0-42 days of age.[1,2]

	PC	NC + *Buttiauxella* phytase		LSD	*P*-value
		500 FTU/kg	1000 FTU/kg		
Starter (days 0-10)					
BWG (g/bird)[3]	225	227	225	5.3	0.627
Feed intake (g/bird)	253	252	257	12.8	0.669
FCRc (g/g)[4]	1.123	1.110	1.142	0.0600	0.481
Water intake (ml)	545	558	518	35.9	0.089
Water/feed intake	2.157	2.214	2.026	0.1980	0.142
Mortality (%)	1.00	1.70	1.60	0.927	0.226
Grower (days 11-21)					
BWG (g/bird)	638	662	651	24.8	0.144
Feed intake (g/bird)	880[a]	914[b]	896[ab]	22.2	0.025
FCRc (g/g)	1.380	1.381	1.377	0.0409	0.983
Water intake (ml)	1,589[ab]	1,643[b]	1,519[a]	79.4	0.021
Water/feed intake	1.805[b]	1.798[b]	1.695[a]	0.0609	0.005
Mortality (%)	0.45	0.87	0.69	0.617	0.332
Starter-grower (days 0-21)					
BWG (g/bird)	863	889	875	27.7	0.165
Feed intake (g/bird	1,133[a]	1,166[b]	1,153[ab]	23.9	0.040
FCRc (g/g)	1.313	1.311	1.317	0.0223	0.826
Water intake (ml)	2,134	2,200	2,062	111.8	0.061
Water/feed intake	1.883[b]	1.888[b]	1.798[a]	0.0725	0.039
Mortality (%)	1.46	2.61	2.26	1.316	0.180
Finisher (days 22-42)					
BWG (g/bird)	1,803	1,817	1,811	78.9	0.919
Feed intake (g/bird)	3,328	3,405	3,407	95.0	0.154
FCRc (g/g)	1.846	1.875	1.881	0.050	0.278
Water intake (ml)	6,042	6,057	5,851	257.9	0.179
Water/feed intake	1.815[b]	1.779[ab]	1.724[a]	0.0671	0.040
Mortality (%)	1.63	1.87	1.57	0.867	0.718
Overall (days 0-42)					
BWG (g/bird)	2,666	2,706	2,687	83.4	0.571
Feed intake (g/bird)	4,461	4,571	4,559	111.2	0.100
FCRc (g/g)	1.673	1.689	1.697	0.028	0.184
Water intake (ml)	8,176[b]	8,258[b]	7,824[a]	350.9	0.047
Water/feed intake	1.833[b]	1.807[b]	1.716[a]	0.0767	0.019
Mortality (%)	3.09	4.48	3.83	1.971	0.320
Feed cost (€/kg BW)	0.414	0.400	0.398		

[1] Phytase was added to a NC diet containing nutrient down specifications as described in Table 2, where full matrix means nutrient reduction is not only P and Ca but also including of other nutrients (e.g. digestible amino acids, nitrogen corrected apparent metabolizable energy, and Na).
[2] Superscript letters in the same row with no common superscripts are significantly different ($P<0.05$).
[3] Body weight gain.
[4] Feed conversion ratio corrected for mortality.

the whole trial period, the mortality was within a range of 3.1-4.5%, which is typical and acceptable under commercial conditions.

Bone ash and mineral content

Phytase added to the basal diets with nutrient reductions had no negative effect on tibia ash content, or Ca content at slaughter age (d 42; Table 4). There was no effect of gender on tibia ash or Ca content, and no interaction between phytase treatment and gender, but tibia P content tended to be lower in males than females ($P=0.06$).

Slaughter/carcass yield

Among birds selected for slaughter yield assessments on d 42, there were no interactions between phytase treatment and gender, but live weights, carcass weights and post-slaughter leg weights (relative to carcass) were greater in males than females ($P<0.001$ in all cases) and abdominal fat pads (relative to carcass) were heavier in females than males ($P<0.001$; Table 5). Phytase supplementation affected live weight at slaughter ($P<0.01$) and carcass weight ($P<0.05$; Table 5), which were increased in birds fed diets supplemented with 500 FTU/kg phytase compared with the PC. These parameters were numerically increased in

Table 4. Effect of full matrix application of *Buttiauxella* phytase[1], at two dose-levels, on average tibia ash and mineral content (g/kg fat free dry matter) of 42-day old broilers; results of a factorial arrangement analysis (3×2 factorial arrangement with 2 levels of gender[2] and 3 levels of dietary treatment).

Treatment	Gender	Ash	Ca	P
PC	male	516.6	186.9	97.6
NC1 + phytase at 500 FTU/kg	male	511.3	188.2	96.5
NC2 + phytase at 1000 FTU/kg	male	508.8	187.7	95.8
PC	female	516.0	187.2	97.9
NC1 + phytase at 500 FTU/kg	female	516.0	187.1	97.8
NC2 + phytase at 1000 FTU/kg	female	512.6	187.9	97.0
LSD, treatment × gender interaction		8.45	6.04	1.64
P-value, treatment × gender interaction		0.624	0.923	0.580
PC		516.3	187.0	97.8
NC1 + phytase at 500 FTU/kg		513.7	187.6	97.2
NC2 + phytase at 1000 FTU/kg		510.7	187.8	96.4
LSD, treatment		5.97	4.27	1.16
P-value, treatment		0.173	0.921	0.082
	male	512.2	187.6	96.7
	female	514.9	187.4	97.6
	LSD, gender	4.88	3.49	0.95
	P-value, gender	0.269	0.910	0.061

[1] Phytase was added to a NC diet containing nutrient down specifications as described in Table 2, where full matrix means nutrient reduction is not only P and Ca but also including of other nutrients (e.g. digestible amino acids, nitrogen corrected apparent metabolizable energy, Na).
[2] The left tibia bones were collected from 4 birds per pen (2 males and 2 females).

Table 5. Effect of full matrix application of *Buttiauxella* phytase[1], at two dose-levels, on average slaughter yields of 42-day old broilers; results of a factorial arrangement analysis (3×2 factorial arrangement with 2 levels of gender[2] and 3 levels of dietary treatment).[3]

Treatment	Gender	Live BW (g)	Carcass weight (g)	Carcass (% of live weight)	Weight, relative to carcass (%)			
					Wings	Legs	Breast fillet	Abdominal fat pad
PC	male	3,133	2,088	66.63	10.27	30.93	31.99	0.65
NC1 + phytase at 500 FTU/kg	male	3,227	2,149	66.56	10.32	31.04	31.90	0.59
NC2 + phytase at 1000 FTU/kg	male	3,204	2,149	67.04	10.37	31.47	31.67	0.55
PC	female	2,603	1,744	66.64	10.36	30.35	31.78	0.73
NC1 + phytase at 500 FTU/kg	female	2,733	1,822	66.64	10.27	30.34	32.38	0.71
NC2 + phytase at 1000 FTU/kg	female	2,630	1,767	67.18	10.33	30.78	32.16	0.64
LSD, treatment × gender interaction		91.2	65.2	0.656	0.214	0.545	0.628	0.101
P-value, treatment × gender interaction		0.455	0.458	0.960	0.550	0.939	0.198	0.828
PC		2,877^{a}	1,923^{a}	66.64	10.31	30.65^{a}	31.89	0.69^{b}
NC1 + phytase at 500 FTU/kg		2,989^{b}	1,992^{b}	66.60	10.29	30.70^{a}	32.13	0.65^{ab}
NC2 + phytase at 1000 FTU/kg		2,928^{ab}	1,966^{ab}	67.11	10.35	31.14^{b}	31.91	0.59^{a}
LSD, treatment		63.9	45.6	0.460	0.149	0.378	0.436	0.070
P-value, treatment		0.003	0.012	0.051	0.736	0.018	0.462	0.031
	male	3,188^{b}	2,129^{b}	66.75	10.32	31.16^{b}	31.85	0.60^{a}
	female	2,656^{a}	1,778^{a}	66.83	10.32	30.50^{a}	32.11	0.69^{b}
	LSD, gender	51.7	36.9	0.372	0.121	0.306	0.353	0.057
	P-value, gender	<0.001	<0.001	0.718	0.957	<0.001	0.159	<0.001

[1] Phytase was added to a NC diet containing nutrient down specifications as described in Table 2, where full matrix means nutrient reduction is P and Ca and included other nutrients (e.g. digestible amino acids, nitrogen corrected apparent metabolizable energy, Na).
[2] The carcass yield was measured using 20 birds per pen (10 males, 10 females).
[3] Superscript letters in a column without a common superscript are significantly different (P<0.05).

birds supplemented with 1000 FTU/kg phytase. In addition, carcass weight, as a percentage of live weight, tended to be increased among broilers supplemented with 1000 FTU/kg phytase vs PC (P<0.10). There were effects of phytase treatment on post-slaughter drumstick weights (P<0.05) and abdominal fat pad (P<0.05). Drumstick weights were increased in birds supplemented with 1000 FTU/kg phytase, vs PC, but abdominal fat pad weights were reduced in this group vs PC. Again, there were no interactions between gender and phytase treatment for these parameters.

Feed costs

Calculated total feed costs (€/ton diet) for all diets, based on market ingredient prices at the time of the trial (2014) and including the costs of phytase, are presented in Table 1. Across all phases, feed costs were consistently lower for the 1000 FTU/kg phytase-supplemented nutrient-reduced diets and were 11.8 to 13.7 €/ton diet lower than the respective PC, depending on dietary phase.

4. Discussion

Applying the correct nutrient matrix values for phytase will enable more accurate and cost-effective diet formulations. However, it requires a robust understanding of the effects of phytase on nutrient release and of how this is affected by substrate levels, dietary composition, phytase dose and bird biology, in commercially relevant settings. The ultimate aim is to supply just the right level and balance of nutrients in the diet so that, when combined with the nutrient-release capability of the specific phytase incorporated at the specified dose-level, the requirements for maintenance and growth are optimally met whilst feed costs are minimised.

In matrix validation it is important that achieved nutrient levels in the PC diets match the accepted requirements for growth and maintenance of the strain and that those in the nutrient-reduced phytase-supplemented diets match the intended down-specifications and were below requirements. The calculated and analysed nutrient levels of the test diets in this trial suggested this was so, as nutrient levels in the PC diets were broadly in line with published breeder specifications for the Netherlands (Aviagen, 2014) which was in line with industry standard and lower than the general recommendations. In addition, there was good adherence between achieved and targeted nutrient reductions, with only moderate (10-20%) under achievements in Ca, digestible P and digestible Val down-specifications in the finisher diets, for reasons which have already been discussed in the materials and method section. It was not possible to verify the nutritional inadequacy of the NC diets in terms of growth performance, as it would not have been ethical to administer the NC diets as stand-alone diets in such a large-scale setting. However, several smaller-scale studies have previously shown that moderate and comparable reductions in energy, minerals (Ca, P) and/or AA in similar corn-soybean meal-based diets, reduced broiler performance (BWG and FCR) compared with a PC diet (Amerah *et al.*, 2014; Dersjant-Li *et al.*, 2018; Liu *et al.*, 2015). In particular, the study by Liu *et al.* (2015) demonstrated significant reductions in BWG from 2.721 kg/bird in the PC to 2.525 kg/bird (-7.20%) in the NC diet over a 40 d feeding period, and increased FCR from 1.551 in the PC to 1.605 (+3.48%) in the NC diets. The reductions in P, Ca, Na, energy and certain essential digestible AA, based on the contribution of 1000 FTU/kg of the same phytase, showed that the nutrient reduction was slightly lower in magnitude than those implemented in the present study due to different feed composition, e.g. corn soybean meal based diets used in the study of Liu *et al.*, 2015.

The absence of an effect of treatment on performance measures during any feeding phase or for the overall trial period suggested that the tested nutrient matrix values applied with the respective dose levels of the phytase were appropriate for maintaining performance at a level equivalent to a nutritionally adequate diet. The absence of a difference in tibia ash, P and Ca content across treatments supported this conclusion. In addition, the current study showed that across three treatments (as no significant differences were observed among treatments) body weights of birds exhibited close adherence to breeder performance objectives (Figure 1).

The FCR showed similar response as BW, for example, BW at 42 d: 2.75 vs 2.77 kg; FCR for 0-42 d: 1.66 vs 1.72 for phytase at 500 FTU/kg vs the Aviagen objectives for mixed sex Ross 308 broilers. As this is a matrix validation study, close adherence to breeder performance objectives indicated the performance are comparable to the results from the practical commercial production settings.

Several previous broiler studies using the same *Buttiauxella* phytase reported increased ileal amino acid digestibility (+3.7 to 15% vs NC) when dosed at 500 to 2,000 FTU/kg (Amerah *et al.*, 2014; Dersjant-Li and Kwakernaak, 2019). Improved sodium digestibility (+36 to 96%) and/or digestibility or retention of AMEn (+1.95 to 4%) has additionally been reported (Dersjant-Li and Kwakernaak, 2019; Liu *et al.*, 2014; Truong *et al.*, 2015). In addition, a smaller-scale study with the same phytase at the same dose-levels in nutrient-reduced diets reported improved BWG and FCR equivalent to a PC, which was accompanied by dose-dependent improvements in ME, N digestibility

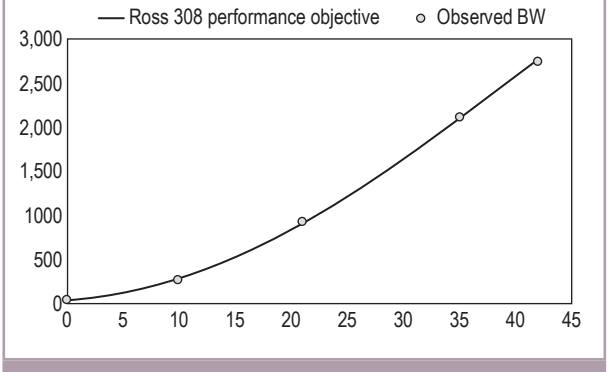

Figure 1. Comparison of body weight at different ages across three treatments to breeder's performance objective (Aviagen, 2014).

and retention (Liu *et al.*, 2015). Based on these reports and the results of the current study, it can be expected that the action of the (appropriately dosed) phytase in degrading phytate improved the availability of P, Ca, digestible AA and energy, which adequately compensated for the nutrient reductions in the NC diets.

Previous studies have reported an effect of phytase in reducing digesta retention time, leading to increased feed intake (Liu *et al.*, 2014, 2015; Selle and Ravindran 2007, 2008). Results of the present study supported this, as feed intake was increased by phytase inclusion at 500 FTU/kg during the grower phase, but, interestingly, feed intake was not increased by 1000 FTU/kg. A similar pattern was observed in the studies by Liu *et al.* (2014, 2015). In contrast, water intake (and water-to-feed intake ratio) was reduced in response to phytase supplementation at 1000 FTU/kg during the grower and finisher phases and for the overall trial period. According to current published data, this effect has not previously been reported and may result in reduced excreta moisture and improved litter quality. It has previously been shown that increasing dietary Na levels increases water consumption in young broilers (Viera *et al.*, 2003), so it may be reasonable to consider that the observed reductions in water intake among birds fed the diets with greatest nutrient reduction could have been due to decreased Na in feed. Studies on *Buttiauxella* and other microbial phytases have previously demonstrated clear improvements in ileal Na digestibility with phytase supplementation (Dersjant-Li and Kwakernaak, 2019; Ravindran *et al.*, 2008; Selle *et al.*, 2009; Truong *et al.*, 2015), as well as direct correlations between increased Na digestibility and increased amino acid/protein digestibility (Dersjant-Li and Kwakernaak, 2019; Truong *et al.*, 2015). The Na reductions applied as part of the nutrient matrix with 1000 FTU/kg phytase (a reduction from 1.8 to 1.4 g/kg) would appear to have been appropriate for maintaining growth performance and for achieving a favourable intestinal sodium balance whilst reducing the water intake needs of the birds during later growth phases.

Carcass weights and cut up parts at slaughter are additional production measures of importance to producers, which can provide further insight into the adequacy of energy and amino acid requirements provided by the diet. It was notable that, among the birds sampled at 42 d for these measurements, the total live and carcass weights at slaughter appeared to be higher among birds fed the nutrient-reduced phytase-supplemented diets than from birds fed the PC diet, which was unexpected. In part, because the increased weights (vs PC) were only significant in the lower, not the higher, phytase-dose diets, but more because a prior comparison of bodyweight gains across treatments (which included the entire sample of birds) revealed no differences during any individual feeding phase or overall from 0-42 d. It may be possible that the way in which birds were selected at d 42 for slaughter and carcass yield measurements was influential. Even though this was intended to be random, it could have been biased towards larger, heavier birds, which were easier to catch, as the mean BWG over 0-42 d was not significantly different among three treatments, although the BWG was 40 g greater in birds fed diets containing 500 FTU/kg phytase compared to PC.

The birds receiving the 500 FTU/kg phytase treatment exhibited increased leg weights vs PC, which may have contributed to increased carcass weights. Given that tibia ash was unaffected by treatment among the sub-sample of birds at day 42, the increased leg weights of these birds were more likely to have been due to increased muscle rather than bone mass. Scheideler and Ferket (2000) reported such an effect of microbial phytase supplementation on broiler leg quarter weights that was not attributable to bone density and was presumed to be due to increased muscle protein deposition. In addition, the abdominal fat percentage must be interpreted with caution given the above comments about sampling. The lower percentage of abdominal fat among broilers fed the 1000 FTU/kg phytase-supplemented nutrient-reduced diets could suggest an improved AA and energy balance which may reduce fat deposition. However, there was no discernible effect on FCR which might otherwise have supported this hypothesis.

Based on market prices in 2014, the feed cost analysis indicated that applying the tested matrix values to the experimental diets resulted in a reduction in the total costs of the diets (including phytase cost across all four phases) by 10.5 and 12.6 €/ton at 500 and 1000 FTU/kg respectively, compared with the PC diet. On a feed costs per kg weight gain basis, the cost reductions equated to 1.4 euro cents/kg BWG when phytase was dosed at 500 FTU/kg and 1.6 euro cents/kg BW gain at 1000 FTU/kg. Whilst experimental diets were not the same as commercially formulated diets, this provided an indication of potential cost savings when the diets were formulated using a feed optimisation program with the application of the tested matrix values. It is worth considering that, in practice, the addition of diamol to the diets as a replacement for the reduced nutrients (which was done in order to maintain an equivalent overall composition across dietary treatments and so enable comparison) would not be necessary. This means that the feed cost reductions of applying the tested matrix values are potentially greater than those estimated here. In this study, due to the commercial setting, nutrient digestibility was not measured, however, it may be speculated that the AA down-specification in the phytase supplemented diets would reduce the nitrogen output and improve sustainability of poultry production.

5. Conclusions

The use of dose-dependent digestible AA, ME and Na matrix values in addition to a mineral (P and Ca) down specifications in *Buttiauxella* phytase-supplemented diets maintained broiler tibia ash, growth performance, slaughter and carcass yields equivalent to those of a nutritionally adequate diet when tested in a commercial setting. Application of the full matrix down specifications resulted in feed cost savings, which were greatest with a phytase dose of 1000 FTU/kg. This data suggested it is economically prudent to take account of the ME, digestible AA and Na contribution of *Buttiauxella* phytase in broiler feed formulation to maximise production benefits.

Conflict of interest

Y. Dersjant-Li and L. Marchal are employees of DuPont Nutrition and Biosciences, which manufactures the *Buttiauxella* phytase used in the trial.

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