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The Effects of Phytase and Xylanase Supplementation on Performance and Egg Quality in Laying Hens

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ABSTRACT

1. The aim of this study was to evaluate the effects of phytase and xylanase and their interaction on laying hen performance, egg quality, phosphorus digestibility, phytate breakdown, volatile fatty acid (VFA) production and peptide YY concentration.

2. Two hundred and forty hens were allocated to cages at 22 weeks of age based on a 3 x 2 arrangement with phytase (0, 300 or 1500 FTU/kg) and xylanase (0 vs. 12 000 BXU/kg) as factors.

3. Phytase increased hen-day production ($P < 0.05$), daily egg mass ($P < 0.05$), and phosphorus digestibility with increasing levels of phytase ($P < 0.001$). Phytase fed at 1500 FTU/kg reduced IP6 and IP5 and increased myo-inositol concentration in gizzard digesta ($P < 0.05$). Phytase fed at 300 FTU/kg reduced IP6 in ileal digesta ($P < 0.05$); however IP6 and IP5 were further reduced and myo-inositol increased when phytase was added at 1500 FTU/ kg ($P < 0.05$).

4. Xylanase improved feed efficiency when phytase was fed at 300 FTU/kg ($P < 0.05$). In the absence of phytase, xylanase reduced DM and Ca digestibilities ($P < 0.05$).

5. Neither phytase nor xylanase had an effect on peptide YY or caecal VFA concentrations.

KEYWORDS

Digestibility; Egg Production; Laying hens; Phosphorus; Phytase; Xylanase

Introduction

Exogenous enzymes are added to monogastric animal diets in order to increase nutrient availability from feed, improving feed efficiency and reducing feed costs. Non-starch polysaccharides (NSP) such as arabinoxylans (the major form of NSP in wheat-barley based diets) possess anti-nutritional properties (Lazaro et al. 2003; Mathlouthi et al. 2002) and high levels of intake increase digesta viscosity and reduce nutrient digestibility through encapsulation (Kim et al. 2005; Zhang et al. 2014). Wheat is also a poor source of available phosphorus (P) due to high levels of phytate (Hídvégi and Lásztity 2003). Phytate (myo-inositol hexaphopshate, IP6) P is poorly available for digestion and absorption by monogastric animals. Phytate is also an anti-nutrient which chelates with cations, forming insoluble phytate complexes, reducing nutrient utilisation (Vohra et al. 1965; Selle et al. 2009). The addition of enzymes such as phytase and xylanase to wheat based diets can help to increase nutrient availability.

Exogenous phytases are now routinely added to low P diets in order to degrade dietary phytate improving phytate P bioavailability and reducing P excretion. The standard inclusion level of exogenous phytase added to most pig and poultry diets is 500 FTU/kg with the exception of layer diets were the standard inclusion level of exogenous phytase is 300 FTU/kg (Dersjant-Li et al. 2015). Recently there has been increased interest in including phytase at levels higher than standard inclusion (super-dosing) due to associated improvements in performance beyond that of an animal fed a P adequate control diet (Santos et al. 2014; Manobhavan et al. 2016; Cowieson et al. 2011). This increase in performance is thought to be due to greater phytate degradation, increasing myo-inositol release and reducing the anti-nutritional effects of phytate (Dersjant-Li et al. 2015; Adeola and Cowieson 2011).

Xylanase is a NSP degrading enzyme which works by breaking down long-chain arabinoxylans into small-chain xylo-oligomers, releasing nutrients for digestion by the animal and by reducing

digesta viscosity associated with high arabinoxylan intake (Masey O'Neill et al. 2014; Zhang et al. 2014). It has also been suggested the addition of xylanase generates oligosaccharide production that is fermented and increases volatile fatty acid (VFA) production thereby stimulating the release of peptide YY (Singh et al. 2012; Keenan et al. 2012). Peptide YY is a hormone produced primarily by endocrine cells in the ileum and colon and is a mediator of the ileal brake mechanism, inhibiting gastric emptying and increasing gastric digestion of nutrients (Allen et al. 1984) with a consequential improvement in performance. Courtin et al., (2008) found that xylanase results in the production of oligosaccharides which have been shown to have a prebiotic effect in chickens (De Maesschalck et al. 2015) and increase VFA production (Goodlad et al. 1987).

The use of phytase and xylanase in pig and broiler diets has received a lot of attention; however there is limited data available with regards to layer diets. The addition of both phytase and xylanase may produce complimentary effects as the increased retention time resulting from the release of peptide YY with xylanase inclusion should allow phytase to be more effective and increase the level of breakdown of phytate. Silversides et al. (2006) studied the interaction between phytase and xylanase in wheat based diets fed to laying hens. They found that enzyme addition had limited effects on production performance when added to P-deficient diets; however phytase increased egg weight in the presence of supplementary xylanase, but had no effect when added alone, suggesting an interaction between the two enzymes.

The objectives of this study were to evaluate the effects of phytase and xylanase and their interaction on laying hen performance, egg quality, P digestibility, phytate breakdown, VFA production and blood concentration of peptide YY.

Materials and methods

Animals

This study was conducted at the University of Leeds, Spen farm and received ethical approval from the University of Leeds Animal Welfare and Ethical Review Committee. A total of 240 Bovans Brown hens were used starting the experiment at 22 weeks of age (1.8 ± 0.02 kg). Hens remained on trial for 24 weeks. Hens were allocated to 60 enriched colony cages (63 x 73 cm) with 4 birds per cage creating 10 replicates per treatment. The health status of the birds was checked daily. The temperature was maintained at 21℃ with controlled ventilation.The lighting regime was set so that hens had 16 hours of light and 8 hours of dark per day.

Experimental design

This trial design was a 3 x 2 arrangement with phytase (0, 300 or 1500 FTU/kg Quantum Blue) and xylanase (0 vs. 12 000 BXU/kg Econase XT) as factors. The xylanase was beta 1-4, endo-xylanase (Econase® XT, AB Vista, Marlborough, Wiltshire, UK). The phytase was an E.coli 6-phytase (AB Vista, Marlborough, Wiltshire, UK). Diets were wheat/barley based and formulated to meet or exceed the nutrient requirements of laying hens according to Bovans Brown Nutrition Management Guide except for P, Ca and Na (300 FTU phytase matrix applied). Hens had ad libitum access to feed and water at all times. The second phase diets fed from 34 to 46 weeks of age included titanium dioxide (TiO_2) at 4 g/kg as a marker to determine nutrient utilisation. The diet composition is shown in Tables 1 and 2. Feed was analysed for gross energy, crude protein, lysine, crude fibre, ether extract, phosphorus and calcium (Sciantec Analytical Services Ltd. Stockbridge Technology Centre, Selby, UK). Diets were also analysed for phytase activity by an ELISA method, using Quantiplate Kits for Quantum Blue as supplied by Envirologix (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK) and

xylanase activity by ELISA method using Quantiplate Kits for Econase XT (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK).

Measurements

Hen body weight was recorded at the beginning and on the final day of the trial. Weekly feed intake and egg production per cage were recorded. Egg quality was determined at the end of the trial using eggs collected per cage over 3 consecutive days. Egg quality variables included whole egg weight, shell weight, shell thickness, shell strength, yolk weight, and yolk colour. The height and the weight of the albumen was also measured in order to calculate haugh units. Each egg was individually weighed and shell strength was determined using an Egg Force Reader (Orka Food Technology Ltd, Ramat HaSharon, Israel). Albumen height was measured using a Baxlo micrometer (Baxlo Precision, Barcelona, Spain). Yolk colour was measured using a yolk colour fan chart (DSM YolkFan^{TM}). Egg shells and egg yolks were individually weighed. Egg shells were dried at 100 ℃ for 24 hours and shell thickness was measured at three poi nts on the midline.

At the end of the trial (46 weeks of age) 2 birds per cage were killed by a schedule I method (cervical dislocation and exsanguination). Blood was collected from the carotid artery into heparinised tubes. The blood was centrifuged at 2 000 x g, 4 ℃ for 15 minutes. Plasma samples were stored at -20 ℃ prior to analysis of peptide YY concentration using a commercial Chicken Peptide YY ELISA kit (CUSABIO, Wuhan, Hubei Province, China, CSB-EL019128CH). Immediately after blood collection the digestive tract of each hen was removed and digesta samples were collected from the proventriculus/gizzard, ileum (from the meckel's diverticulum to the ileo-caecal junction) and the caeca. Contents from the proventriculus and the gizzard for each bird were removed and mixed and the pH of the digesta were measured using a FC202D pH electrode for measurements in semi-solids (HI99161, Hanna Instruments, Woonsocket, RI).

The digesta sample from the ileum was mixed and the pH was measured as above. The contents from both the caeca were mixed and the pH was measured as above. The digesta samples were then frozen at -80 ℃ for subsequent analysis.

Feed and digesta samples were freeze-dried and finely ground before undergoing wet ash sample preparation (method 975.03 AOAC 2012) for analysis of TiO_2 (Short et al. 1996). Briefly, samples were ashed for 13 hours at 580℃ and then dissolved in 7.4M sulphuric acid. The contents were then filtered (Whatman 541) using distilled water. Hydrogen peroxide was then added and the colour intensiry measured at 405nm. Ileal digestibility values for dry matter (DM), calcium (Ca), magnesium (Mg), P, and potassium (K) were calculated using TiO_2 as a marker. Ileal digesta samples were ashed at 550 ℃ for 16 hours and digested in 5M HCl. Dry m atter analysis of samples was performed after oven drying the samples at 102 ℃ for 24 hours. Minerals were determined by means of inductively coupled plasma optical emission spectroscopy (ICP–OES) (Thermo Scientific iCAP 7400). Quantification of IP_{3-6} in the proventriculus/gizzard and ileal digesta was performed using HPIC with post column derivitization and UV detection at 290 nm. HPLC with pulsed amperometric detection was used to determine concentrations of myo-inositol.

Caecal contents were analysed for VFA concentrations (acetate, propionate, butyrate) using gas chromatography (Varian 3400). Caproic acid was used as the internal standard and helium was used as the carrier gas. The Schutte (1992) method was used for analysis with some modifications. For analysis 2 g of the caecal contents was centrifuged at 15 000 x g for 10 minutes and 0.5 ml of supernatant acidified with 50 µl phosphoric acid (850 ml/l). Following this 0.3 ml of the internal standard was added. Distilled water was then added to obtain a final volume of 1 ml and 0.5 ml of this sample was transferred to an ultra-filtrate Eppendorf (Amicon 0.5ml 10K; MerckMillipore, Darmstadt, Germany) and centrifuged at 14000 x g for 20 minutes and the final solution was injected into the column of the gas-liquid chromatograph.

Statistical analysis

Data was analysed for main effects and their interaction over the 24 week period by ANOVA according to a general linear model procedure (IBM SPSS version 20). The model included the main effects of phytase and xylanase and their interaction. Differences were classed as significant if $P < 0.05$. Tukey's post-hoc test was used to separate treatment means where significant differences were detected. Performance data were analysed using cage as the experimental unit. The individual bird was the experimental unit for blood, digestibility, and VFA analysis.

Results

Diet analysis is shown in tables 1 and 2. Analysed values of the experimental diets were similar to calculated values except for calcium which was higher than expected. The addition of phytase and/or xylanase had no effect on overall weight gain. The addition of phytase increased hen-day production ($P < 0.05$) irrespective of phytase level. The addition of phytase also increased daily egg mass ($P < 0.05$) and again this improvement was observed at both phytase levels with no additional benefit at the super-dose level. A phytase x xylanse interaction was observed for FCR ($P < 0.05$) with xylanse improving feed efficiency when phytase was fed at 300 FTU/kg (Table 3). Neither the addition of phytase or xylanse had an effect on egg quality parameters at the end of the trial (Table 4). The addition of xylanse tended ($P = 0.064$) to reduce Ileal pH at 24 weeks (7.45 vs. 7.28). Plasma peptide YY concentration was not affected by phytase (107.2, 102.0 and 105.1 ± 5.12 pg/ml for 0, 300 and 1500 FTU/kg phytase respectively) or xylanase (102.6 and 106.9 ± 4.35 pg/ml for 0 and 12 000 BXU/kg xylanase respectively) supplementation.

Phytase addition increased P digestibility at 300 FTU/kg and further increased digestibility at 1500 FTU/kg phytase ($P < 0.001$; Table 5). A phytase x xylanase interaction was observed for DM and Ca digestibility. The addition of xylanase in the absence of phytase reduced DM and Ca digestibility ($P < 0.05$). Neither phytase nor xylanase had an effect on K digestibility. Phytase addition tended to increase Mg digestibility ($P = 0.061$).

Concentrations of $IP_{(3-6)}$ and myo-inositol in gizzard and Ileal digesta are shown in Table 6. Phytase at 1500 FTU/kg significantly reduced IP6 and IP5 ($P < 0.05$; $P < 0.05$) and increased myo-inositol concentrations ($P < 0.05$) in gizzard digesta. In ileal digesta, phytase reduced IP6 at both 300 FTU/kg ($P < 0.001$) and 1500 FTU/kg ($P < 0.001$), with levels at 1500 FTU/kg phytase being significantly lower than at 300 FTU/kg phytase. Phytase at 1500 FTU/kg also reduced IP5 ($P < 0.05$) and increased myo-inositol concentrations ($P < 0.001$) in ileal digesta. Neither phytase nor xylanase had an effect on VFA concentrations in caecal contents at the end of the trial (Table 7).

Discussion

The addition of 300 or 1500 FTU/kg phytase increased hen-day production and daily egg mass; however there was no difference between the two levels. This suggests that 300 FTU/kg phytase was enough to meet the P requirements for optimal production of these hens. This is in accordance with EFSA (2013) that found the addition of phytase to laying hen diets increased laying rate and egg mass when compared to birds fed a negative control diet, however performance between birds fed 150 FTU/kg and 1200 FTU/kg phytase were similar. A phytase x xylanase interaction was observed for FCR. The improvement in FCR with xylanase addition at 300 FTU/kg phytase may be due to an increase in substrate availability for degradation by phytase as xylanase has been shown in vitro to increase aleurone cell wall permeability,

increasing the availability of phytate for degradation (Parkkonen et al. 1997) rather than an increase in retention time as plasma peptide YY concentration was not affected by xylanase in the current study. An improvement in FCR with xylanase addition was not seen when phytase was added at 1500 FTU/kg phytase. Super-dose levels of phytase are receiving more and more attention as high levels of phytase liberate more available P, energy and amino acids (Cowieson, Wilcock, and Bedford 2011), increase mineral bioavailability (Kies et al. 2006), reduce phytate, thus reducing its anti-nutritional effect and increase myo-inositol (Lee and Bedford 2016; Woyengo, Weihrauch, and Nyachoti 2012) which has been shown to have numerous beneficial effects (Zyla et al. 2004; Cowieson et al. 2013). Feed conversion ratio was low and similar for super-dose levels of phytase irrespective of xylanase level suggesting that the super-dose level improved feed efficiency, thus there was no further scope for improvement with the addition of xylanase. Work by Lee et al. (2017) found that 1500 FTU/ kg phytase reduced FCR by 4 points when added to medium and high P broiler diets and suggested that FCR is the most sensitive parameter to super-dosing.

Neither the addition of phytase or xylanase had an effect on egg quality parameters which was surprising as previous studies have found that the addition of phytase improves egg quality with regards to egg shell strength and thickness due to an increase in P and Ca availability (Lim et al. 2003; Englmaierová et al. 2017). The lack of response in the current study may be due to the higher analysed Ca than intended providing the bird with sufficient calcium for eggshell formation and thus improving Ca availability would have no further benefit. The addition of xylanase tended to reduce Ileal pH. Changes in pH may be due to modification of the intestinal microflora as a result of enzyme supplementation. Sheng et al. (2013) found that the addition of xylanase reduced ileal pH in broiler chickens. They measured Lactobacillus concentrations in rectal digesta which was increased with the addition of xylanase suggesting the lower pH level was due to increased microflora proliferation and fermentation. An increase in gut fermentation

is thought to release peptide YY, slowing gut transit time and enhancing gastric digestion of nutrients (Keenan et al. 2012), however plasma peptide YY concentration was not affected by xylanase supplementation in this study. Masey O'Neill et al. (2014) investigated the effects of xylanase addition to broiler diets on performance, nutrient digestibility and caecal VFA content. They found that xylanase supplementation at 6 000 and 32 000 BXU/ kg improved FCR and nutrient digestibility. They also found that the addition of xylanase increased caecal VFA content suggesting the products from enzyme hydrolysis have a prebiotic role. However VFA production was not influenced by the addition of xylanase in the current experiment which may suggest that the fermentation of fibre was already optimised in the gut and thus there was no benefit in adding xylanase.

The addition of 300 FTU/kg phytase to layer hen diets increased P digestibility and this was further improved with the addition of 1500 FTU/ kg phytase increasing P availability contributing to an increase in hen-day production and daily egg mass. However performance was improved with the addition of phytase to a similar level for both 300 and 1500 FTU/kg phytase suggesting, as mentioned above, addition of 300 FTU/kg phytase was enough to meet hen P requirements for performance. A phytase x xylanase interaction was observed for DM and Ca digestibility as the addition of xylanase to diets with 0 FTU/ kg phytase reduced DM and Ca digestibility. Xylanase is a NSP degrading enzyme which breaks down long-chain arabinoxylans into small-chain xylo-oligomers and thus should reduce digesta viscosity and increase nutrient digestion (Masey O'Neill, Smith, and Bedford 2014; Zhang et al. 2014). However it has been suggested that xylanase has a quadratic response on digesta viscosity as the addition of high levels of NSP enzymes can actually increase digesta viscosity (Passos et al. 2015; Yi et al. 2013). This increase in viscosity is thought to be due to the enzyme breaking down insoluble NSP into soluble NSP increasing the water holding capacity of the digesta (Yi et al. 2013; Passos et al. 2015; Choct et al. 2004). The addition of xylanase had no effect on digestibility when phytase

was added at 300 or 1500 FTU/ kg. This result is difficult to explain; however phytase has been shown to have an effect on digesta viscosity in broiler chickens. Wu et al. (2004) found that when xylanase (1000 units/kg) alone and xylanase (1000 units/kg) and phytase (500 U/ kg) together were added to wheat—soy based diets, digesta viscosity was reduced in all sections of the intestine. When phytase was added alone, digesta viscosity was reduced in the duodenum and ileum, but not in the jejunum. In contrast Juanpere et al. (2005) found that the addition of 500 U/ kg phytase to maize based diets increased digesta viscosity in broilers.

Information on IP6 degradation in the proventriculus/gizzard is relatively scarce compared to the ileum. The IP6 data in the current study shows that the super-dose level of phytase had an additional benefit on phytate hydrolysis, reducing IP6 in the proventriculus/gizzard and ileum below that of the levels found when 300 FTU/ kg phytase was added to the diet; however IP6 concentrations remained relatively high. The addition of 1500 FTU/kg phytase to layer diets reduced IP6 concentration in the proventriculus/gizzard by 67% when compared to 0 FTU/kg which is in contrast to Walk et al. (2014) who found that IP6 concentration was 98% lower with the inclusion of 500 FTU/kg phytase to broiler diets deficient in aP (0.15%) and Ca (0.16%).They were unable to detectable IP6 levels with the inclusion of 1,000 or 1,500 FTU/kg. Beeson et al. (2017) found IP6 concentration was reduced by 85% when 1500 FTU/kg was added to a broiler diet deficient in aP (0.15%) and Ca (0.16%). In the current study, the 1500 FTU/kg level of phytase did not significantly improve performance above the standard inclusion level (300 FTU/kg). The fact that the concentrations of IP6 in the gizzard and the ileum at 1500 FTU/kg were still relatively high suggests that some of the benefits of "super-dosing" due to near complete phytate destruction may have been missed in this study due to either insufficient phytase or diet interference with the activity of the phytase. The considerably higher analysed Ca than intended may have reduced the efficacy of phytase as high Ca:P ratios have been shown to compromise IP6 hydrolysis due to formation of Ca-phytate complexes (Selle et al.

2009; Amerah et al. 2014) highlighting the importance of measuring IP6 data with regards to how successful the phytase was in the role of super-dosing.

The addition of 1500 FTU/ kg phytase to layer diets increased myo-inositol in both the proventriculus/gizzard and the ileum. Studies in broilers have shown that the addition of myo-inositol to diets increases bird performance (Zyla et al. 2004; Cowieson et al. 2013; Farhadi et al. 2017). Farhadi et al. (2017) found that the addition of myo-inositol to P deficient broiler diets improved weight gain and feed efficiency so that performance was similar to birds fed a positive control diet. Cowieson et al. (2013) found that the addition of myo-inositol to broiler diets improved feed efficiency during the finisher phase but they found that the addition of myo-inositol during the starter phase had a negative effect on feed efficiency. The increase in myo-inositol with the addition of 1500 FTU/kg phytase suggest that super-dose levels of phytase and related improvements in performance such as the improvement in FCR with or without xylanase are associated with phytate destruction (Walk et al. 2014).

Conclusion

In conclusion, phytase consistently improved egg production, improved P digestibility and at super-dose levels increased the breakdown of phytate in laying hens fed wheat/barley based diets. Xylanase alone had a limited effect on laying hen performance, but did reduce DM and Ca digestibility when added to diets without phytase. There was evidence of interactions between phytase and xylanase with xylanase improving FCR when conventional levels (300 FTU/kg phytase) of phytase were used; however this was not associated with an increase peptide YY production.

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