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*Article*

# Phytase and Xylanase Supplementation Improves Phosphorus Digestibility in Laying Hens

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**Simple Summary:** Phosphorus plays an important role in laying hen nutrition and productivity. It is usually added to poultry diets in an inorganic form, that is easily digestible by birds. However, inorganic phosphate is a finite resource and excess amounts can be released with the excreta into the environment, which can lead to water pollution. Poultry diets are largely plant based and contain phosphorus in the form of phytate that is not easily digestible by birds. By providing enzymes in the diets of laying hens, this plant-based phosphorus can be made available, reducing the need to include inorganic phosphorus in the diet. In this study we provided older laying hens with a mixed-enzyme product, containing phytase and xylanase, and demonstrated improved retention and ileal digestibility of phosphorus and gross energy as well as reduced excretion of phosphorus into the environment. These results indicate that the use of this mixed-enzyme product can result in a more efficient utilization of phosphorus as well as a reduced risk to the environment.

**Abstract:** Phosphorus is required by laying hens for a variety of biological functions. It is usually provided in excess, in a readily digestible inorganic form in diet formulations. However, this does not represent an efficient use of a finite resource and can result in water pollution. Plant bound phosphorus in phytate, is common in commercial poultry diets, and represents an alternate source of phosphorus, however poultry lacks the enzyme to digest it. By adding enzymes to their diets, the availability of this phytate-bound phosphorus can be increased allowing for the reduced inclusion of inorganic P in the diet formulation. Therefore, this study was conducted to examine the effect of a natural enzyme complex that contains phytase and xylanase in layer diets. Novogen Brown Classic layers (n=314) were fed one of two diets: (T1) control diet or (T2) control diet plus a natural mixed-enzyme product with both, phytase and xylanase. Experimental diets were provided for 12 days after which the excreta from each pen were collected for an additional three days to determine total retention and ileal digestibility of phosphorus and gross energy. At the end of the trial the birds were euthanized, and their ileal contents were collected. Productivity parameters were also recorded over the course of the study. The amount of phosphorus detected in the excreta of the control group was significantly higher than the amount detected in the enzyme supplemented group ($P<0.05$). Both phosphorus retention and phosphorus ileal digestibility were significantly higher in the enzyme supplemented birds ($P<0.001$). Gross energy digestibility was significantly higher in the enzyme supplemented birds for both the total tract and ileal samples ($P<0.001$). There were only numerical improvements, not significant, in the layer productivity parameters. This study showed that the enzyme supplement can improve total retention and ileal digestibility of phosphorus and gross energy in older layers.

**Keywords:** layer hen; xylanase; phytase; phosphorus retention; ileal digestibility; gross energy digestibility

## 1. Introduction

Phosphorus (P) is required by laying hens due to its role in bone formation and maintenance, energy utilization and cell structure [1]. Jungle fowl (*Gallus gallus*), the wild relatives of the

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domesticated chicken (*Gallus domesticus*), obtain digestible P from their scavenging lifestyle, which includes the consumption of a variety of insects [2], while domesticated chickens are generally fed plant-based diets lacking in readily digestible P. Approximately 30-40% of the P present in plants is digestible by birds with the remaining source of dietary P provided in the form of Phytate [1]. Phytate is poorly digested by birds with only 10% of P from phytate present in wheat and corn being digested [1]. In addition to being poorly digestible itself, phytate can also reduce the digestibility of other minerals, amino acids, and energy [3]. To achieve the required dietary amounts of digestible P, additional inorganic P is often included in the diet formulation of broilers and layers in excess. This excess P in poultry manure can cause surface water eutrophication so steps should be taken to minimize this impact [4].

Chickens are unable to digest the majority of dietary phytate due to their low endogenous production of phytases [5]. As a result, exogenous enzymes are often added to poultry diets, with phytase being the most widely used [6]. Dietary phytase can increase the availability of phytate-bound P while also decreasing the antinutritional effects of phytate [6]. Phytases function by hydrolyzing phytate into myo-inositol and phosphate groups, releasing a usable, inorganic form of P [5]. Improvements to P digestibility and utilization have been demonstrated in layers supplemented with exogenous phytases [7–11]. Dietary phytase has also been shown to improve productivity in terms of increased hen-day production and daily egg mass [7].

Xylanase is another enzyme that is commonly included in poultry diets to improve nutrient uptake from cereals. The primary cereal grain due to its nutrient composition in layer least cost feed formulations is wheat. However, wheat contains non-starch polysaccharides (NSPs) at relatively high concentrations and poultry lack the endogenous enzyme xylanase to digest NSPs. The addition of xylanase is thought to provide productivity benefits by hydrolyzing NSPs, reducing the encapsulation effects of plant cell walls making nutrients encapsulated available to the bird and decreasing digesta viscosity leading to improved enzyme activity, digestion and absorption of nutrients [12,13].

The combination of phytase and xylanase has been well explored in broilers, with both enzymes thought to have complimentary effects with xylanase increasing retention time, affording the phytase additional time to breakdown phytate [7]. In layers, Silversides [14] demonstrated the benefits of both phytase and xylanase in terms of an increased egg weight, with no improvements observed when the enzymes were provided individually. Taylor et al., [7], found that the combination of phytase (300 FTU/kg) and xylanase improved Feed Conversion Ratio (FCR) attributing the synergistic effect to an increased cell wall permeability rather than an increased retention time. Interestingly, no interaction was observed when xylanase was provided alongside phytase (1500 FTU/kg) with the authors attributing this to the effect of the xylanase being overwhelmed by the positive effects of the larger dose of phytase [7].

A number of authors have demonstrated that the benefits of enzyme supplementation diminish with the age of the bird. In a meta-analysis investigating the effects of phytase on layers, Bougouin [11] identified age at the start of trial as one of three independent effects (the other two being length of trial and dietary Ca content) that impact phosphorus retention, with the beneficial effects of phytase reduced in older birds. The diminishing benefit of enzymes with age was also reported by Olukosi et al., [15] who provided broilers with a cocktail of enzymes including xylanase and phytase and found the greatest benefit of supplementary enzymes was found in the youngest birds. In the meta-analysis by Bougoiun [11], the average age of a laying hen at the start of the feeding trials was 35 weeks (with a minimum age of 20-week and a maximum of 56). In this study, we investigated the effects of a mixed-enzyme product containing phytase and xylanase on older laying hens (95 weeks at trial start) in terms of phosphorus retention, ileal phosphorus digestibility and productivity.

## 2. Materials and Methods

The study protocol and animal care were in accordance with European Union Directive 2010/63/EU covering the protection of animals used for experimental or other scientific purposes. The live animal trial was conducted at Feedtest, (Wettin-Löbejün, Germany). The study procedures were

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approved by Kilfovet's Animal Welfare Body (21-06) and State Administration Office (Landesverwaltungsamt Halle, Sachsen-Anhalt, Germany) and permission to conduct a feeding study using a non-registered feed additive was granted prior to start of the study (AZ 203.2.1/76.21). The study was conducted to investigate the effect of supplementing layer hen diets with a mixed-enzyme product containing phytase and xylanase (Allzyme® Spectrum, Alltech, Inc; 3031 Catnip Hill Road, Nicholasville, KY). The effect of the feed additive in terms of total retention and ileal digestibility of both phosphorus and gross energy, as well as the additive's effect on various productivity parameters, was evaluated by means of a negatively controlled, blinded, randomized, GCP-like study.

A total of 314 Novogen Brown Classic layers (95wk of age, approximately 14wks in to second lay) were evenly distributed and randomly allocated to 1 of 2 dietary treatments with 20 replicates. They were housed in 4 $m^2$ pens with 7 or 8 hens per pen. Pens were equipped with 8 nipple drinkers, a round feeder and a three-seating laying box, suitable for up to 18 hens. The barn temperature was maintained between 14 and 16 °C and the lighting program was 10 hours dark, 14 hours light, in accordance with German animal welfare legislation. Feed and water were always available ad libitum during the study. Initial bedding consisted of wood shavings, with excreta patches removed and new material added as required. Prior to the start of the study, the birds were checked for general health and were only included if considered healthy.

Experimental diets were prepared by Research Diet Services (Broekweg, Hoge Maat 10, 3961 NC Wijk bij Duurstede, Netherlands) according to commonly used tabulated nutritional values of feedstuffs and raw material specifications. The feed was initially prepared as one batch which was then divided into two sub-batches which were supplemented with either 150g/tonne of wheat (T1) or 150g/tonne of Allzyme® Spectrum (T2) (ASC) (Alltech Inc., Kentucky, KY, USA). ASC is composed of Allzyme SSF from Aspergillus niger NCIMB 30289 (with the primary activity of phytase (1000 SPU/g) fortified with endo-1,4-b-xylanase (7500 EPU/g) produced by Trichoderma citrinoviride Bisset (IMI SD135). One solid-state fermentation phytase unit (SPU) is defined as the amount of enzyme required to liberate 1 µmol of inorganic phosphate per minute at pH 5.5 and 50 °C [16]. EPU is defined as the amount of enzyme which releases 0.0083 µmol of reducing sugars (xylose equivalent) per minute from oat spelt xylan at pH 4.7 and 50 °C [17]. The ingredient composition of each diet is summarized in Table 1. The diets were provided to the hens as mash. Subsamples of each diet were collected immediately after preparation and sent for analysis of enzyme activity (Alimetrics, Finland) and for crude nutrient analysis (SGS Germany GmbH; dry matter, crude ash, crude protein, ether extract, crude fibre, sugar, starch; estimation of $AME_N$) as well as gross energy, phosphorus and titanium. The analytical composition of the experimental diets is shown in Table 2.

**Table 1.** Ingredient composition of the experimental diets.

| Ingredient | T1 Control diet (%) | T2 Treatment diet (%) |
|---|---|---|
| Wheat | 44.28 | 44.27 |
| Corn | 20.00 | 20.00 |
| Soyabean meal | 13.51 | 13.51 |
| Sunflower HIPRO | 7.00 | 7.00 |
| Oil-Soya | 2.27 | 2.27 |
| Fine limestone | 2.00 | 2.00 |
| Coarse limestone | 8.24 | 8.24 |
| TIMAB MCP | 0.37 | 0.37 |
| Salt | 0.24 | 0.24 |
| Sodium Bicarbonate | 0.15 | 0.15 |
| Lysine HCL | 0.21 | 0.21 |
| DL Methionine | 0.18 | 0.18 |
| L Threonine | 0.06 | 0.06 |
| *Vit/TE premix | 1.00 | 1.00 |
| Titanium oxide | 0.50 | 0.50 |

| | | |
|---|---|---|
| Allzyme® Spectrum | 0.00 | 0.01 |

*The content of vitamins and trace minerals per kg of feed: vit. A: 10,000 I.U.; vit. D3: 2000 I.U.; vit. E: 25 mg; vit. K: 1.5 mg; vit. B1: 1.0 mg; vit. B2: 3.5 mg; D-pantothenic acid: 12 mg; vit. B6: 1.0 mg; vit. B12: 15 µg/; Niacin: 30 mg; Folic acid: 0.8 mg; Biotin: 100 µg; choline chloride: 350 mg; Mn: 54 mg; Fe: 50 mg; Cu: 10 mg; Zn: 60 mg; I: 0.7 mg; Se: 100 µg; anti-oxidant mixture 125mg.

**Table 2.** Analytical composition of the experimental diets.

| Diet component | T1 Control diet | T2 Treatment diet |
|---|---|---|
| Dry matter (%) | 91.0 | 90.9 |
| Ash (%) | 13.6 | 14.0 |
| Crude protein (%) | 16.1 | 15.7 |
| Crude fiber (%) | 3.0 | 3.1 |
| Crude fat (%) | 4.2 | 4.4 |
| Sugar (%) | 2.4 | 2.8 |
| Starch (%) | 41.6 | 40.4 |
| Metabolizable energy MJ/kg | 11.2 | 11.0 |
| Phosphorus (mg/kg) | 4500 | 4500 |
| Titanium oxide (mg/kg) | 2850 | 2460 |
| 3-Phytase (U/kg) | 335 | 744 |
| Xylanase (U/kg) | 33 | 673 |

Performance observations for this study included body weight, feed intake, number and weight of eggs, feed conversion ratio and mortality. Eggs were classified as either marketable or non-marketable (cracked, broken or soft-shelled). After receiving the experimental diets for 12 days, the excreta were collected to determine total tract digestibility. This was done over three consecutive days in which pens were equipped with heavy plastic sheets to allow for excreta collection. The plastic sheets were in the pens for three hours per day to allow for the continuous collection of the excreta, then pooled by pen. Samples were stored at -18 °C between days. Following the three-day excreta collection period, the hens continued to be fed the experimental diets for one more day. The hens were then slaughtered and the terminal third of the section from Meckl's diverticulum to 2 cm before the ileo-caeco-colonic junction was flushed with deionized water and pooled per pen. The feed, excreta and digesta samples were analyzed by SGS Analytics (Germany, GmbH) for phosphorus, gross energy (Berthelot's calorimeter) and the undigestible titanium marker. Digestibility was calculated using the following formula:

$$\boldsymbol{Digestibility_{Nutrient}} = \mathbf{1} - \frac{\boldsymbol{Titanium_{Diet}} \times \boldsymbol{Nutrient_{Excreta}}}{\boldsymbol{Titanium_{Excreta}} \times \boldsymbol{Nutrient_{Diet}}} \times 100$$

where: $Titanium_{diet}$ and $Titanium_{excreta}$ are the concentration (mg/kg) detected in the analysed samples and $Nutrient_{diet}$ and $Nutrient_{excreta}$ are the concentrations (mg/kg) of Phosphorus or gross energy detected in the analysed samples.

The results were analyzed statistically with pen considered the unit of study. Grubb's test was first conducted to identify potential outliers. One pen showed extreme and implausible results for digestibility values and was removed from the analysis of digestibility but kept for performance. Differences between the control and treatment group were determined using Minitab's general linear model procedure (v18, State College, PA, USA).

## 3. Results

### *3.1. Bird Health and Performance*

Mortality was low, with only one bird dying over the course of the study. At slaughter, two birds from the control group and three birds from the treatment group were found to have internal abnormalities and were excluded from the pooled precaecal digesta analysis. The productivity parameters observed over the 15-day supplementation period are shown in Table 3. No significant

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differences were observed between the control and treatment groups. However, the majority of the measured productivity parameters were higher in the supplemented group, including total and marketable eggs and laying rates. The feed conversion ratios were also numerically lower in the treatment group suggesting a higher feed efficiency than the control group.

**Table 3.** The effect of 15-day dietary supplementation of layer diets at a level of 0% or 0.01% with a phytase and xylanase feed additive on layer hen productivity parameters.

| Productivity Parameter | Control | Treatment | Standard Error | *p* value |
|---|---|---|---|---|
| Marketable eggs (n) | 90.36 | 95.80 | 2.85 | 0.184 |
| Total eggs (n) | 92.50 | 97.40 | 2.93 | 0.244 |
| Marketable egg mass (g) | 5955 | 6339 | 193 | 0.164 |
| Total egg mass (g) | 3121 | 3449 | 200 | 0.254 |
| Feed consumption (g) | 13283 | 13710 | 296 | 0.314 |
| Mean body weight (g) | 1908.8 | 1898.4 | 17.6 | 0.680 |
| Marketable laying rate (%) | 77.41 | 81.30 | 2.41 | 0.261 |
| Total laying rate (%) | 79.25 | 82.65 | 2.47 | 0.336 |
| Individual marketable egg weight (g/egg) | 65.90 | 66.21 | 0.578 | 0.708 |
| Daily marketable egg weight (g/day) | 50.98 | 53.82 | 1.61 | 0.221 |
| Daily total egg weight (g/day) | 52.43 | 54.74 | 1.69 | 0.338 |
| Daily feed consumption (g/day) | 113.72 | 116.41 | 2.47 | 0.448 |
| Marketable feed conversion ratio (kg/kg) | 2.27 | 2.17 | 0.06 | 0.251 |
| Total feed conversion ratio (kg/kg) | 2.21 | 2.14 | 0.06 | 0.387 |

*3.2. Phosphorus in the Excreta*

The amount of phosphorus detected in the excreta of the control group was significantly higher than the amount detected in the enzyme supplemented group (Figure 1).

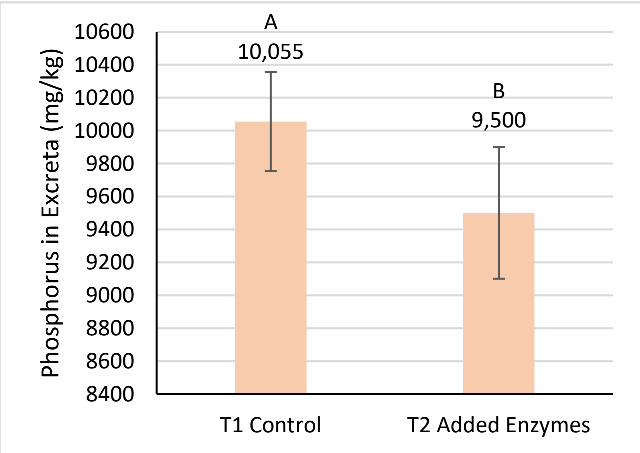


**Figure 1.** The amount (mg/kg) of phosphorus (± 95% C.I.) detected in the excreta collected of the control (T1) or enzyme supplemented (T2) laying hens. Columns marked with different letters differed significantly (p=0.042).

*3.3. Total retention and ileal digestibility of phosphorus*

Both phosphorus retention and phosphorus ileal digestibility were significantly higher in the enzyme supplemented birds (Figure 2).

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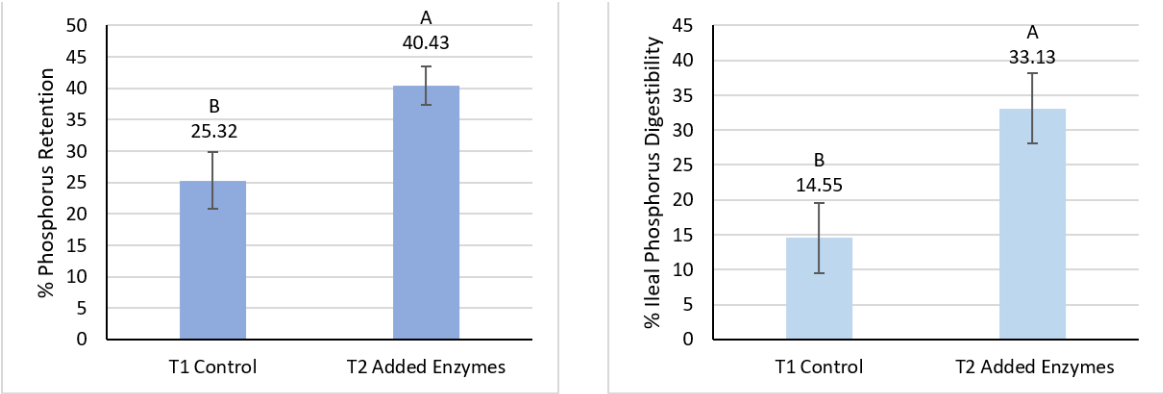
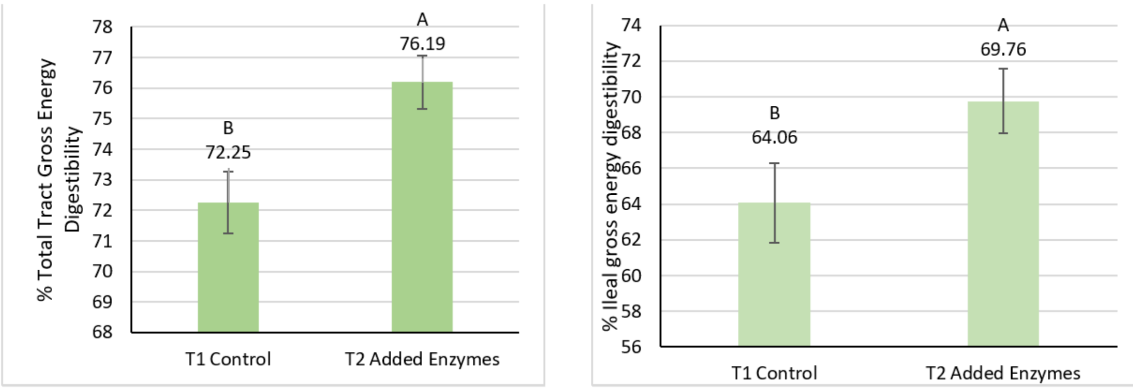



(**A**) (**B**)

**Figure 2.** Mean percent (± 95% C.I.) phosphorus retained (A) and ileal phosphorus digestibility (B) values of laying hens provided a control diet (T1) or a diet supplemented with phytase and xylanase (T2). Columns marked with different letters differ significantly (p<0.001).

### *3.4. Total retention and ileal digestibility of Gross Energy*

Gross energy digestibility was significantly higher in the enzyme supplemented birds for both the total tract (Figure 3A) and ileal samples (Figure 3B).




(**A**) (**B**)

**Figure 3.** Mean percent (± 95% C.I.) gross energy retained (A) and ileal gross energy digestibility (B) values of laying hens provided a control diet (T1) or a diet supplemented with phytase and xylanase (T2). Columns marked with different letters differed significantly (p<0.001).

## 4. Discussion

It is common practice in poultry diets to add several single enzymes in to the diet to reduce the effects of antinutrient factors and improve digestibility, it would therefore be beneficial to reduce complexity within the feed mill by using a product with multi-enzyme activity to work together within the diet. The results of this study demonstrate that supplementing older layer hen diets with an additive containing both phytase and xylanase can significantly (P<0.001) improve total retention and ileal digestibility of both phosphorus and gross energy. This indicates that the enzyme supplement used in this study was able to release the bound P to phytate and make it available to the bird to absorb and utilize. The benefits of this enzyme combination in terms of improving phosphate digestibility has been reported by other authors in broilers [15,18,19] and turkeys [18,20]. Similar improvements to P retention and digestibility are less often reported in layers. Taylor et al., [7] found no effect of the combination of the two enzymes on ileal digestibility, while diets containing phytase alone improved P digestibility with increasing phytase concentration. This is in contrast to our findings in which both P retention and ileal digestibility were both significantly higher when compared to the control group. When broilers were fed the same enzyme preparation as in the current

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study, similar effects on ileal digestibility were observed [19]. The enzyme supplementation resulted in significantly greater digestibility values when compared to a negative control diet when provided as part of either a wheat-soybean meal based diet as well as part of a corn-soybean meal based diet [19]. In both studies the enzyme supplemented diets were deficient in P and performed as well as a positive control diet, containing sufficient levels of P. This improved utilization of nutrients provides the opportunity to formulate diets in a more efficient way. The effect of phytase alone on P retention and ileal digestibility in laying hens is well established in younger birds [9–11,21]. The results of the current study fall within the range of values reported for mean P retention in a meta-analysis investigating the effect of phytases on laying hens [11], albeit closer to the lower end of the range, which may reflect the effect of the advanced age of the birds used in this study.

In the current study, gross energy digestibility was significantly ($P<0.001$) improved in both the total tract and ileal samples. This indicates that the enzyme supplement used in this study was able to improve nutrient digestibility and absorption, which may be through the xylanase reducing the viscosity of the digesta and releasing the encapsulated nutrients within the plant cell wall. Bauer [19], found similar improvements when providing the same enzyme preparation to broilers fed corn or wheat-based diets. Wu [22] also demonstrated improved gross energy digestibility when the combination of xylanase and phytase was provided to broilers, while each enzyme alone did not result in a significant increase. In contrast, more variable results have also been reported by other authors when laying hens were fed phytase and xylanase, with increasing levels of xylanase supplementation not necessarily leading to improved apparent metabolizable energy [14]. Additionally, phytase alone was found by some authors to have no beneficial effect on metabolizable energy in layers fed reduced phosphorus diets [10,23]. The observed positive effect on gross energy digestibility may not only be attributable to the release of encapsulated nutrients and reduced digesta viscosity from the addition of xylanase to the diet but also may be due to the production of xylooligosaccharides (XOSs) [24–26]. Arabinoxylans in wheat are broken down by xylanases in the diet producing XOS [24]. XOS has been reported to increase short chain fatty acid producing bacteria, the products of which can be used as a source of energy [27].

There was no significant effect of diet on the productivity parameters measured in this study. However, this is not surprising based on the short duration of the trial. Despite its short duration, the productivity parameters, including total and marketable eggs and laying rates, were generally numerically higher in the enzyme-supplemented group, suggesting that production differences may have been achievable over a longer period. Zhai et al., [9] reported the findings of one short-term (7d) and two long-term(each 12wks) trials investigating the efficacy of phytase in laying hens and showed that ileal digestibility was demonstrable in the short-term study, while long-term studies were required to show differences in egg productivity parameters, which is in agreement with our findings. In addition, the duration of the study was found to be one of three significant independent effects identified by Bougain et al., [11] in their meta-analysis of layers and broilers provided with dietary phytases. Future work on this enzyme preparation could take place over a longer duration to identify whether the productivity parameters can also be increased. Some authors have reported that the benefits of phytase diminish over time, and with birds of this advanced age, it would be interesting to see how long this supplementation remains beneficial.

To address the diminishing effects of enzymes in older birds, some authors have suggested increasing the enzyme dose provided based on the age of the animals [23]. In a meta-analysis investigating the effects of phytases on broilers and layers, the average dose provided to laying hens was found to be 371 FTU/kg. In this study an intermediate dose of 750 IU/kg of phytase was provided and found to be effective in these comparatively old birds. For laying hens at 25 weeks of age a dose of 500 FTU/kg was found to improve P digestibility and retention, but by 31 weeks a dose of 1000FTU/kg of phytase was required to show significant improvements to these digestibility parameters. Other authors have provided phytase doses as high as 5000 FTU/kg while also providing lower levels of inorganic P, and have showed that these doses are well tolerated while increasing P availability and reducing the need for dietary inorganic P [28].

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Inorganic phosphorus, that is easily metabolized by poultry, is a finite resource, with many different sectors of the agriculture industry requiring its use. Current reserves could be depleted within 100 years [29]. Despite the finite nature of inorganic P, it is still currently often used in excess in animal diets, translating to excess phosphorus in the excreta which can then be applied to the land as fertilizer and therefore is an environmental concern often associated with excess phosphorus in soils and potential pollution of water resources. In their analysis of the inorganic phosphorus cycle in the food chain, Cordel et al., [29] identified minimizing phosphorus losses from farms and food commodity chains as important strategies to simultaneously address this impending phosphate scarcity and water pollution. Using phytases and other enzymes to make phytate-bound phosphorus more biologically available to livestock provides an opportunity to use alternate phosphorus sources. It also allowed for reduced inorganic phosphorus to be added to the diets and less phosphorus excreted by the birds into the environment. In this study, the enzyme-treated group had improved P retention and ileal digestibility. They also excreted significantly less P in the excreta than the control group, indicating that the addition of the enzyme supplement in this study can help reduce the environmental impact of laying hens. Again, these findings are similar to other authors [30] and this strategy represents an opportunity to use phytases to reduce the negative impact of excess dietary inorganic phosphorus that can contribute to groundwater pollution [4,29,31].

## 5. Conclusions

The results of this 15-day study demonstrate that the combined phytase and xylanase supplementation of older laying hens fed a wheat-based diet can help ameliorate the negative effects of the NSP and phytate present in layer diets by improving total phosphorus retention and phosphorus ileal digestibility, along with improved gross energy digestibility, while productivity was not affected over the short term. These results indicate that this mixed-enzyme product can be used in older layers to add greater flexibility when formulating diets and reduce excess phosphorus release into the environment. Enzymes are an essential tool for the poultry feed industry, their importance in making poultry production more sustainable, both environmentally and economically is well established, by reducing waste and increasing the addition of co-products. Therefore, further studies are needed to examine the effect of this mixed-enzyme product on amino acid digestibility in layers to see the potential uplift in the available amino acids from the diet and see to what extent the addition of this enzyme supplement can reduce waste and increase the addition of co-products.

**Author Contributions:** Conceptualization, K.B., J.T-P., and C.A.M.; methodology, K.B., D.C., and H.W.; validation, K.B. and H.W.; formal analysis, K.B., D.C., and J.D.K..; investigation, K.B., D.C.; resources, K.B., and D.C.; data curation, K.B., D.C.; writing—original draft preparation, J.D.K.; writing—review and editing, All authors; supervision, K.B., J. T-P. and C.A.M.; project administration, K.B.; funding acquisition, C.A.M. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Data is available from the authors on request.

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**Conflicts of Interest**: The authors H.W., C.A.M., J.D.K. and J. T-P. are employees of Alltech which produces and markets Allzyme® Spectrum, the commercial product used in this study.

## References


1. *Nutrient Requirements of Poultry*; National Academies Press: Washington, D.C., 1994; Vol. 1994; ISBN 978-0-309-04892-7.
2. Collias, N.E.; Collias, E.C. A Field Study of the Red Jungle Fowl in North-Central India. *Condor* **1967**, *69*, 360–386, doi:10.2307/1366199.
3. Woyengo, T.A.; Nyachoti, C.M. Review: Anti-Nutritional Effects of Phytic Acid in Diets for Pigs and Poultry - Current Knowledge and Directions for Future Research. *Can. J. Anim. Sci.* **2013**, *93*, 9–21, doi:10.4141/CJAS2012-017.
4. Abbasi, F.; Fakhur-un-Nisa, T.; Liu, J.; Luo, X.; Abbasi, I.H.R. Low Digestibility of Phytate Phosphorus, Their Impacts on the Environment, and Phytase Opportunity in the Poultry Industry. *Environ. Sci. Pollut. Res.* **2019**, *26*, 9469–9479, doi:10.1007/s11356-018-4000-0.
5. Nelson, T.S. Thy Hydrolysis of Phytate Phosphorus by Chicks and Laying Hens. *Poult. Sci.* **1976**, *55*, 2262–2264, doi:10.3382/ps.0552262.
6. Naghshbandi, M.P.; Moghimi, H. Stabilization of Phytase on Multi-Walled Carbon Nanotubes via Covalent Immobilization. In *Methods in Enzymology*; Academic Press, 2020; Vol. 630, pp. 431–451 ISBN 9780128201435.
7. Taylor, A.E.; Bedford, M.R.; Pace, S.C.; Miller, H.M. The Effects of Phytase and Xylanase Supplementation on Performance and Egg Quality in Laying Hens. *Br. Poult. Sci.* **2018**, *59*, 554–561, doi:10.1080/00071668.2018.1483575.
8. Gautier, A.E.; Walk, C.L.; Dilger, R.N. Effects of a High Level of Phytase on Broiler Performance, Bone Ash, Phosphorus Utilization, and Phytate Dephosphorylation to Inositol. *Poult. Sci.* **2018**, *97*, 211–218, doi:10.3382/ps/pex291.
9. Zhai, H.X.; Wang, J.P.; Zhang, Q.; Aureli, R.; Tschambser, A.; Faruk, M.U. Evaluation of the Efficacy of a Novel Phytase in Short-Term Digestibility and Long-Term Egg Production Studies with Laying Hens. *Poult. Sci.* **2022**, *101*, 101894, doi:10.1016/j.psj.2022.101894.
10. Liu, N.; Liu, G.H.; Li, F.D.; Sands, J.S.; Zhang, S.; Zheng, A.J.; Ru, Y.J. Efficacy of Phytases on Egg Production and Nutrient Digestibility in Layers Fed Reduced Phosphorus Diets. *Poult. Sci.* **2007**, *86*, 2337–2342, doi:10.3382/ps.2007-00079.
11. Bougouin, A.; Appuhamy, J.A.D.R.N.; Kebreab, E.; Dijkstra, J.; Kwakkel, R.P.; France, J. Effects of Phytase Supplementation on Phosphorus Retention in Broilers and Layers: A Meta-Analysis. *Poult. Sci.* **2014**, *93*, 1981–1992, doi:10.3382/ps.2013-03820.
12. O'Neill, H.V.M.; Smith, J.A.; Bedford, M.R. Multicarbohydrase Enzymes for Non-Ruminants. *Asian-Australasian J. Anim. Sci.* **2014**, *27*, 290–301, doi:10.5713/ajas.2013.13261.
13. Aftab, U.; Bedford, M.R. The Use of NSP Enzymes in Poultry Nutrition: Myths and Realities. *Worlds. Poult. Sci. J.* **2018**, *74*, 277–286, doi:10.1017/S0043933918000272.
14. Silversides, F.G.; Scott, T.A.; Korver, D.R.; Afsharmanesh, M.; Hruby, M. A Study on the Interaction of Xylanase and Phytase Enzymes in Wheat-Based Diets Fed to Commercial White and Brown Egg Laying Hens. *Poult. Sci.* **2006**, *85*, 297–305, doi:10.1093/ps/85.2.297.
15. Olukosi, O.A.; Cowieson, A.J.; Adeola, O. Age-Related Influence of a Cocktail of Xylanase, Amylase, and Protease or Phytase Individually or in Combination in Broilers. *Poult. Sci.* **2007**, *86*, 77–86, doi:10.1093/ps/86.1.77.
16. McKinney, K.; Combs, J.; Becker, P.; Humphries, A.; Filer, K.; Vriesekoop, F. Optimization of Phytase Production from *Escherichia coli* by Altering Solid-State Fermentation Conditions. *Fermentation* **2015**, *1*, 13–23. https://doi.org/10.3390/fermentation1010013.
17. Kouzounis, D.; Hageman, J.A.; Soares, N.; Michiels, J.; Schols, H.A. Impact of Xylanase and Glucanase on Oligosaccharide Formation, Carbohydrate Fermentation Patterns, and Nutrient Utilization in the Gastrointestinal Tract of Broilers. *Animals* **2021**, *11*, 1285–1285. https://doi.org/10.3390/ani11051285.
18. Olukosi, O.A.; González-Ortiz, G.; Whitfield, H.; Bedford, M.R. Comparative Aspects of Phytase and Xylanase Effects on Performance, Mineral Digestibility, and Ileal Phytate Degradation in Broilers and Turkeys. *Poult. Sci.* **2020**, *99*, 1528–1539, doi:10.1016/j.psj.2019.11.018.
19. Bauer, M. The Effects of Exogenous Enzyme Supplementation on the Performance, Bone Quality, and Nutrient Utilization of Broiler Chickens, University of Kentucky, 2023.
20. Ingelmann, C.J.; Witzig, M.; Möhring, J.; Schollenberger, M.; Kühn, I.; Rodehutscord, M. Effect of Supplemental Phytase and Xylanase in Wheat-Based Diets on Prececal Phosphorus Digestibility and Phytate Degradation in Young Turkeys. *Poult. Sci.* **2018**, *97*, 2011–2020, doi:10.3382/ps/pey030.
21. Pongmanee, K.; Kühn, I.; Korver, D.R. Effects of Phytase Supplementation on Eggshell and Bone Quality, and Phosphorus and Calcium Digestibility in Laying Hens from 25 to 37 Wk of Age. *Poult. Sci.* **2020**, *99*, 2595–2607, doi:10.1016/j.psj.2019.12.051.

 doi:10.20944/preprints202401.1236.v1




22. Wu, Y.B.; Ravindran, V.; Thomas, D.G.; Birtles, M.J.; Hendriks, W.H. Influence of Phytase and Xylanase, Individually or in Combination, on Performance, Apparent Metabolisable Energy, Digestive Tract Measurements and Gut Morphology in Broilers Fed Wheat-Based Diets Containing Adequate Level of Phosphorus. *Br. Poult. Sci.* **2004**, *45*, 76–84, doi:10.1080/00071660410001668897.
23. Javadi, M.; Pascual, J.J.; Cambra-López, M.; Macías-Vidal, J.; Donadeu, A.; Dupuy, J.; Carpintero, L.; Ferrer, P.; Cerisuelo, A. Effect of Dietary Mineral Content and Phytase Dose on Nutrient Utilization, Performance, Egg Traits and Bone Mineralization in Laying Hens from 22 to 31 Weeks of Age. *Animals* **2021**, *11*, 1495, doi:10.3390/ani11061495.
24. Ribeiro, T.; Cardoso, V.; Ferreira, L.; Lordelo, M.; Coelho, E.; Moreira, A.; Domingues, M.; Coimbra, M.; Bedford, M.R.; Fontes, C. Xylo-oligosaccharides display a prebiotic activity when used to supplement wheat or corn-based diets for broilers. *Poult. Sci.* **2018**, *97*, 4330–4341. https://doi.org/10.3382/ps/pey336.
25. Bautil, A.; Verspreet, J.; Buyse, J.; Goos, P.; Bedford, M.R.; Courtin, C.M. Arabinoxylan-oligosaccharides kick-start arabinoxylan digestion in the aging broiler. *Poult. Sci.* **2020**, *99*, 2555–2565. https://doi.org/10.1016/j.psj.2019.12.041.
26. Morgan, N.K.; Gomes, G.A.; Kim, J.C. Comparing the efficacy of stimbiotic and a combination of xylanase and beta-glucanase, in broilers fed wheat-barley based diets with high or low AME. *Poult. Sci.* **2021**, *100*, 101383. https://doi.org/10.1016/j.psj.2021.101383
27. De Maesschalck, C.; Eeckhaut, V.; Maertens, L.; De Lange, L.; Marchal, L.; Nezer, C.; De Baere, S.; Croubels, S.; Daube, G.; Dewulf, J.; et al. Effects of Xylo-Oligosaccharides on Broiler Chicken Performance and Microbiota. *Appl. Environ. Microbiol.* **2015**, *81*, 5880–5888. https://doi.org/10.1128/aem.01616-15.
28. Saleh, A.A.; Elsawee, M.; Soliman, M.M.; Elkon, R.Y.N.; Alzawqari, M.H.; Shukry, M.; Abdel-Moneim, A.M.E.; Eltahan, H. Effect of Bacterial or Fungal Phytase Supplementation on the Performance, Egg Quality, Plasma Biochemical Parameters, and Reproductive Morphology of Laying Hens. *Animals* **2021**, *11*, 1–11, doi:10.3390/ani11020540.
29. Cordell, D.; Drangert, J.O.; White, S. The Story of Phosphorus: Global Food Security and Food for Thought. *Glob. Environ. Chang.* **2009**, *19*, 292–305, doi:10.1016/j.gloenvcha.2008.10.009.
30. Meyer, E.; Parsons, C. The Efficacy of a Phytase Enzyme Fed to Hy-Line W-36 Laying Hens from 32 to 62 Weeks of Age. *J. Appl. Poult. Res.* **2011**, *20*, 136–142, doi:10.3382/japr.2010-00212.
31. Lei, X.G.; Stahl, C.H. Biotechnological Development of Effective Phytases for Mineral Nutrition and Environmental Protection. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 474–481, doi:10.1007/s002530100795.