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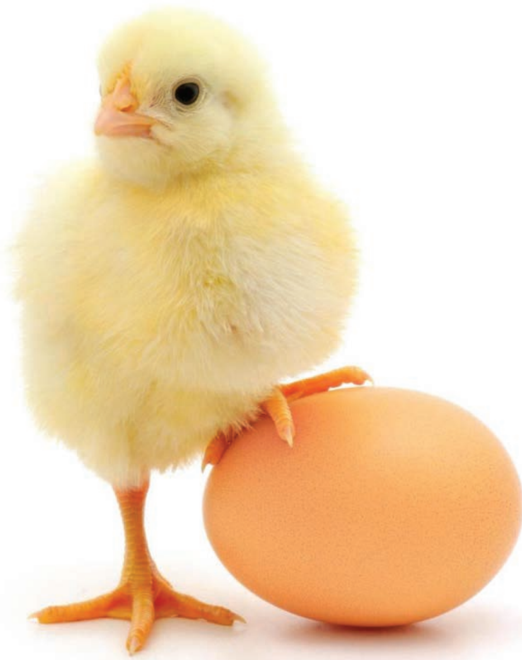

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Research Article
Effectiveness of an Enzyme Cocktail to Mitigate Negative Effects of a Nutrient Deficient Diet on Broiler Performance

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Abstract
Background and Objective: A study was conducted to evaluate the efficacy of utilizing an enzyme cocktail consisting of phytase, xylanase, amylase and protease in broilers. **Materials and Methods:** Day-of-hatch Ross 308 male broiler chicks were randomly allocated to one of three dietary treatments: Positive control (PC, nutrient adequate), Negative control (NC, PC diet reduced by ME, dig AA, Ca, aP and Na) or NC plus enzyme cocktail (phytase, xylanase, amylase and protease); NCEC. All birds were weighed on day 10, 21 and 35 to obtain performance parameters: Body weight (BW), feed consumption (FC) and feed conversion ratio (FCR). On d21, tibias were collected from 2 birds per pen, for the determination of ash content and breaking strength. **Results:** On d35, birds fed NCEC and PC had larger BW ($p<0.05$). Similarly, through day 35, the NCEC had lower ($p<0.05$) FCR than the NC however both were still higher ($p<0.05$) than the PC. Compared to the NC diets, birds fed NCEC or PC had improved tibia ash and breaking strength ($p<0.05$) on day 21. **Conclusion:** (Enzyme cocktail improved performance and bone mineralization of birds fed suboptimal ME diets)It can be concluded that the enzyme cocktail used in this current study can improve performance and bone mineralization of birds fed a diet that is formulated to be suboptimal in terms of ME, AA, Ca, aP and Na. Furthermore, the use of such an enzyme cocktail could enable the formulation of lower-cost diets and contributes to the profitability of the production of poultry products.

Key words: Amylase, bone, growth performance, phytase, protease, xylanase

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Monogastrics such as poultry lack the necessary endogenous enzymes necessary to properly hydrolyze phytate, leaving much of the organic phosphorus within grains and oilseeds unavailable to the birds[1,2]. Increases in feed cost become unavoidable due to large amounts of inorganic phosphorus added to diets to account for the phosphorus (P) requirement by the bird[3].

Microbial phytase is the most commonly used exogenous enzyme in the feed for monogastric animals. Phytase hydrolyzes phytic acid and its salt phytate (myo-inositol hexakisphosphate, IP6), resulting in the formation of inositol pentakis-, tetrakis-, tris-, bis- and monophosphate (IP5-IP1) in a stepwise manner and five inorganic phosphates (Pi)[4,5]. Inclusion of phytase in the diet not only makes the phytate-bound phosphorus available to the bird but also reduces the anti-nutritional effect of phytate, thus improving the digestibility of phosphorus (P), calcium (Ca), amino acids (AA) and the utilization of energy[6,7]. This in turn allows for reductions in the use of expensive inorganic phosphorus and increases the utilization of phytate as an available phosphorus (aP) source[3]. Reduced aP in broiler diets have detrimental effects on performance and mineral utilization. Broilers fed nutrient deficient diets, specifically aP deficient diets, have reduced body weight, feed conversion, nutrient utilization and bone mineralization[2,3,8,9]. The inclusion of phytase in reduced aP diets has been observed to improve body weight, feed conversion, nutrient utilization and bone mineralization in broilers[2,3,8,9].

Commercially available phytases are derived from various bacteria (e.g. *Escherichia coli* but *tiauxella* sp.) or fungi and have different catalytic and biochemical properties[10-12]. Development of novel phytases showing increased activity at low pH and exhibiting high stability to pepsin may offer efficient degradation of phytate earlier in the gastrointestinal tract[13-15]. *Buttiauxella* sp. phytase has been developed with improved thermostability, pepsin resistance and increased activity towards phytate and phytate-protein complex at low pH in comparison to *Escherichia coli* and fungal phytases[5]. *Buttiauxella* sp. phytase possesses a high phytate-degrading efficacy, rapidly decreasing dietary phytate concentration in the gastrointestinal tract of broilers by 60-90%[16,17]. *Buttiauxella* sp. phytase improved growth performance and nutrient utilization when fed to poultry and swine[18,19].

Although, phytase inclusion has been shown to ameliorate the effects of low aP diets, the question remains if there are further benefits provided by phytase inclusion in conjunction with xylanase, amylase and protease in broilers. It has been observed that the use of an enzyme cocktail that containing xylanase, amylase and protease activities improved nutrient digestibility in poultry[20,21]. Olukosi *et al.*[9] and Gracia *et al.*[22] have similarly reported improved performance when carbohydrases are used. The positive effect of these enzymes are suggested to be due to the enhancement of nutrient digestibility and digestion of soluble and insoluble non-starch polysaccharides (NSP)[9]. The association that occurs between phytase and carbohydrases has been categorized as additive, or synergistic[23,24]. In the absence of carbohydrases, the cell wall of feedstuffs high in NSP shields nutrients, including phytate P, making it inaccessible to phytase, thus, reducing the efficiency of the enzyme[9]. Similarly, insufficiency of phytase will prevent carbohydrases from liberating other nutrients that may be bound with the phytate molecule[9]. Also, the limitation imposed by a nutrient may attenuate the response that another enzyme may produce[23]. For example, when ME is limiting, the presence of phytase may fail to improve performance, even though P is liberated from phytate[9]. In contrast, limitation imposed by insufficient P in the absence of phytase may limit the response to additional ME made available by the use of carbohydrases[9].

The objective of the current experiment was to evaluate the ability to ameliorate broiler performance in response to diets with reduced ME, dig AA, Ca, P and Na by utilizing an enzyme cocktail which included phytase, xylanase, amylase and protease.

MATERIALS AND METHODS

This study was approved by the animal care and use committee, Texas A&M University (TAMU) Institutional Animal Care and Use Committee (AUP No. 2018-0181) and were consistent with the Guide for the Care and Use of Agricultural Animals in Research and Teaching guidelines[25].

Experimental design: A total of 600 Cobb 500 male broiler chicks were used in this study. Birds were equally housed at 20 birds per pen, with ten replicate pens per treatment, for a total of 30 pens (1.22×1.83 m). Birds were randomly assigned to each pen, however initial body weights (BW) were equalized. Each pen was lined with new pine shavings as bedding material and equipped with one bell feeder and nipple drinking system. Birds were allowed ad libitum access to feed and water. Birds were housed in an environmentally controlled tunnel ventilated broiler house.

Experimental diets: Pens were blocked within and treatments were assigned at random to one of seven dietary treatments. The experimental diets included a nutrient adequate positive control (PC), negative control (NC) diet which was the PC diet reduced by 156 kcal/kg ME, 0.05-0.06% dig AA, 0.2% Ca, 0.19% aP and 0.04% Na and NC supplemented with phytase (1,000 FTU/kg), xylanase (2,000 U/kg), amylase (200 U/kg) and protease (4,000 U/kg), NCEC. Birds were fed a three-phase diet consisting of a starter (day 0-10, crumble), grower (day 11-21, pellet) and finisher (day 22-35, pellet).

Growth performance data and carcass yield: Mortalities were collected, recorded and weighed daily. All birds and feed were weighed on day 10, 21 and 35 (at the end of each dietary phase) for the determination of body weight (BW), feed consumption (FC) and calculation of feed conversion ratio (FCR).

Bone ash and breaking strength: On day 21, 2 birds, respectively, were randomly selected per pen, euthanized by cervical dislocation and their left and right tibias collected and pooled for the determination of tibia ash and breaking strength. The left tibia was used for determination of tibia ash. The right tibia was used for determination of breaking strength. Tibia ash was determined by removing fibula, muscle and connective tissue and the bones were dried at 100°C for 12 hrs before defatting in diethyl ether for 8 hrs and air drying. De-fatted tibias were dried again at 100°C for 12 hrs and then ashed in ceramic crucibles at 600°C for 24 hrs. Breaking strength was measured by the 3-point bending test using TA XT Plus100 Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY, USA).

Statistical analysis: Data are reported by pen as the experimental unit. Data were analyzed by analysis of variance (ANOVA) using Minitab Software (Mintab 21.4.1) to investigate the effect of treatments in a randomized design. Means separation was achieved using Tukey's Honest Significant Difference test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Growth performance data and carcass yield: The effect of dietary treatment on feed consumption, body weight and feed conversion ratio is presented by phase (Table 1) and cumulatively (Table 2). Reductions in nutrients negatively influenced daily feed consumption. Cumulatively through day 10, no differences in FC were observed ($p>0.05$). From day 11-21, birds fed NCEC increased FC ($p<0.05$) compared to NC and PC diets. Therefore day 0-21 FC overall was increased ($p<0.05$) in the NCEC compared to both NC and PC diets. From day 21-35, no differences ($p>0.05$) were observed in FC. Cumulatively through d35, birds fed NCEC, had increased FC ($p<0.05$) compared to both NC and PC diets.

Exogenous enzyme supplementation increased BW ($p<0.01$) throughout the experiment compared to the NC diets. On day 10, 21 and 35, NCEC improved BW ($p<0.05$) similar to that of the PC and both PC and NCEC were heavier ($p<0.05$) than the NC.

Cumulatively through day 10, no differences in FCR were observed between dietary treatments ($p<0.05$). From day 11-21 and from day 22-35, birds fed NCEC had improved FCR ($p<0.05$) compared to NC and were similar to the PC. Overall on day 35, the NCEC had lower ($p<0.05$) FCR than the NC however both were still higher ($p<0.05$) than the PC.

Table 1: Effect of an enzyme cocktail on growth performance in broilers, by phase[1]

Days	FC (g/bird/day)	FCR (g/g)	BW (kg)	Mortality (%)
Starter (day 0-10)				
PC	31.470	1.215	0.294^{a}	3.600
NC	31.290	1.258	0.282^{b}	2.500
NCEC	31.600	1.227	0.299^{a}	3.400
Pooled SEM	0.300	0.007	0.001	0.400
p-value	0.110	0.100	0.000	0.920
Grower (day 11-21)				
PC	89.730^{b}	1.325^{b}	1.027^{a}	4.500
NC	82.180^{c}	1.468^{a}	0.916^{b}	2.600
NCEC	96.240^{a}	1.3/2^{b}	1.063^{a}	4.100
Pooled SEM	0.720	0.006	0.008	0.500
p-value	0.000	0.000	0.000	0.710
Finisher (day 22-35)				
PC	162.140	1.671^{c}	2.379^{a}	5.800
NC	166.500	1.848^{a}	2.169^{b}	2.500
NCEC	169.850	1.773^{b}	2.400^{a}	5.000
Pooled SEM	0.899	0.007	0.015	0.483
p-value	0.004	0.000	0.000	0.620

[1]All performance data is corrected for mortality, $^{a-c}$Means within column with different superscripts differ at $p<0.05$

Table 2: Effect an enzyme cocktail on growth performance in broilers[1]

Days	FC (g/bird/day)	FCR (g/g)
0-21		
PC	59.9810[b]	1.310[c]
NC	57.320[c]	1.390[ab]
NCEC	63.650[a]	1.390[bc]
Pooled SEM	0.415	0.005
p-value	0.000	0.000
0-35		
PC	93.250[b]	1.510[c]
NC	93.570[b]	1.650[a]
NCEC	97.590[a]	1.570[b]
Pooled SEM	0.521	0.005
p-value	0.004	0.000

[1]All performance data is corrected for mortality, [a-c]Means within column with different superscripts differ at p<0.05

Table 3: Effect of an enzyme cocktail on tibia ash content and breaking strength in broilers

	Day 21 Tibia Ash (%)	Day 21 breaking strength (kg)
PC	54.87[a]	38.08[a]
NC	46.77[b]	22.25[b]
NCEC	54.41[a]	38.63[a]
Pooled SEM	0.33	0.995
p-value	0.000	0.000

[a,b]Means within column with different superscripts differ at p<0.05

Tibia ash and breaking strength: The effect of dietary treatment on tibia ash and breaking strength is presented in Table 3. Compared to PC, birds fed the NC diets exhibited reduced tibia ash (p<0.05) at day 21. Compared to the NC diets, birds fed NCEC had improved tibia ash (p<0.05) sampled on day 21. Compared to the PC, birds fed the NC diets exhibited reduced bone breaking strength (p>0.05) at day 21. Compared to the NC diets, birds fed NCEC improved tibia breaking strength (p<0.05) sampled on day 21.

DISCUSSION

Plant feedstuffs may present anti-nutritional factors that impair diet utilization. For example, non-starch polysaccharides are poorly digestible and may act as barriers, preventing the access of enzymes to the nutrients[26]. In this study, the enzyme cocktail was used in corn, wheat, soybean meal, rapeseed meal, wheat bran and soy hulls based diets that were marginally deficient in ME, AA, Ca, aP and Na. Phytase acts on the phosphate groups associated with the inositol ring of phytate and thus releases P. It is expected that the use of phytase would result in improved performance if P is the limiting nutrient for growth. The use of the enzyme cocktail is expected to act on resistant starch and improve the accessibility and solubility of nutrients, thus improving ME and protein digestibility. Therefore, it is expected the enzyme cocktail would improve performance if ME is limiting. The objective of the current experiment was to evaluate the production benefits of applying substrate-based matrices for combined xylanase, amylase and protease with phytase in broilers.

Cumulative feed consumption in the current experiment was increased with the inclusion of the enzyme cocktail. The inclusion of the enzyme cocktail increased FC numerically when compared with its negative control. Similar results were reported by Pirgozliev *et al*.[27] with an average increase in feed intake of 11.7% in birds fed diets supplemented with phytase compared to un-supplemented diets. A study conducted by Wu *et al*.[28] reported an average increase in feed intake of 1101 g/bird with no enzyme supplementation to 1220 g/bird with xylanase supplementation and 1254 g/bird with both xylanase and phytase supplementation. Cowieson and Adeola[23] reported an average increase in feed intake of 6.2% in birds fed diets supplemented with similar enzyme cocktail. Average body weight in the current experiment was increased with the inclusion of enzyme cocktail as well. The inclusion of the enzyme cocktail increased BW when compared with its negative control. Birds fed the enzyme cocktail performed similarly to PC and increased BW when compared with the NC fed birds. Similar results were reported by Pieniazek *et al*.[3] with increases in BW being observed in birds supplemented with phytase. A study conducted by Williams *et al*.[29] reported increased BW in birds supplemented with xylanase in reduced-energy diets. Wu *et al*.[28] reported an increase in weight gain of 16.5% in birds supplemented with xylanase and 17.5% in birds supplemented with xylanase and phytase compared to un-supplemented diets. Cowieson and Adeola[23] reported combined inclusion of a similar enzyme cocktail to the un-supplemented diet improved body weight gain by 14.0%. Olukosi and Adeola[9] reported the addition of an enzyme cocktail without phytase alone did not affect BW, when phytase alone or in combination with the other enzymes were added, there was an increase in BW above the un-supplemented diet. These results are consistent with the findings of Dalolio *et al*.[30], who reported the supplementation of enzyme blends to diets with reduced mineral and energy levels promoted similar feed intake and weight gain compared to birds fed a nutrient adequate diet. The results of the present experiment demonstrate that birds benefit from dietary phytase supplementation in combination with other enzymes.

In the current experiment, the inclusion of the enzyme cocktail improved FCR to levels of the PC diet. Similar results were reported by Pirgozliev *et al*.[31] with improvements in feed conversion efficiency by an average of 9.4% with phytase supplementation compared to the low P diets. Improved performance was also observed by Esmaeilipour *et al.*[32] and Williams *et al.*[29] when xylanase was supplemented in the diet. Wu *et al.*[28] reported the feed:gain ratios of birds fed diets supplemented with xylanase or phytase were lowered by 4.9 and 2.9%, respectively, compared to un-supplemented diets. Wu *et al.*[28] also reported the combination of xylanase and phytase resulted in further reductions in feed:gain, 5.4%, respectively. A study conducted by Cowieson and Adeola[23] reported supplementation of an enzyme cocktail linearly improved FCR when compared to the un-supplemented diet. Olukosi *et al.*[9] reported the combination of enzymes in a cocktail improved feed efficiency above the un-supplemented diet.

Increases in tibia ash percentage are a good indicator of increased bone mineralization due to the increased availability of P, Ca, Zn and Cu from the phytate-mineral complex by the action of phytase[33]. In the present experiment, tibia bone ash percent were increased with the inclusion of the enzyme cocktail. Similar observations were reported by Shirley and Edwards[34] with increases in bone ash percent from 26-41% with the addition of phytase supplementation in low P diets. Olukosi and Adeola[35] reported supplementation of phytase alone or combination with xylanase increased tibia bone ash content compared to un-supplemented diet. A study conducted by Francesch and Geraert[36] found that reducing dietary nutrient levels resulted in reduced bone ash, calcium and phosphorus contents in comparison with a nutrient adequate diet. In addition, Francesch and Geraert[36] reported improved bone mineralization by supplementing the nutrient-deficient with a xylanase, glucanase and phytase blend. In the current experiment, day 21 tibia ash percent and breaking strength were improved with the inclusion of the enzyme cocktail when compared with its negative control. These improvements in tibia ash percent and breaking strength suggest that mineral availability was increased with the supplementation of the enzyme cocktail.

CONCLUSION

It can be concluded that enzyme cocktail of phytase, xylanase, amylase and protease can improve performance and bone mineralization, of birds fed a diet that is formulated to be suboptimal in terms of ME, AA, Ca, aP and Na. Furthermore, the use of such an enzyme cocktail enables the formulation of lower-cost diets and contributes to the profitability of the production of poultry products.

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