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Nutritional value of field bean-containing diets for broilers without and with phytase, xylanase and protease enzymes, alone or in a combination

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ABSTRACT

The effects of exogenous phytase (PHY), xylanase (XYL) and protease (PRO) alone and in a combination, when supplemented to diets based on three field bean cultivar samples with different chemical composition, on dietary metabolisable energy (ME), nutrient digestibility, gastrointestinal tract (GIT) development, feed intake (FI), weigh gain (WG) and feed conversion ratio (FCR) were studied. Diets based on beans with lower phytic acid and fibre content had higher ME, fat ($P<0.05$) and dry matter retention ($P<0.001$) coefficients, which coupled with greater feed efficiency, e.g. FCR ($P<0.001$). Dietary PHY alone reduced FCR ($P<0.001$) and XYL alone improved dietary ME ($P<0.05$). An interaction ($P<0.05$) between enzymes regarding FI and WG occurs, but PHY seem to be the main contributor to improved performance. There was an interaction ($P<0.05$) between been cultivars and enzymes regarding nitrogen retention (NR) as PHY produced greater coefficient when supplemented to low phytate diet. The overall weight of the total GIT was not changed by the enzymes or bean cultivars ($P>0.05$). The changes observed in different GIT segment is unlikely to have biological significance.

Key words: field beans; broilers; xylanase; phytase; protease; metabolisable energy; growth performance.

Introduction

The constantly increasing price of soybean meal (SBM) and the recent ban on the use of ingredients of animal origin in some parts of the world, grain legumes, including field beans (*Vicia faba L. var. minor*), are considered possible alternative protein sources because of the similarity of their amino acid profiles to SBM (Nalle et al., 2010). Due to their adaptation to the climate, large amounts of field beans can be produced in many parts of Europe (Crépon et al. 2010). However, field beans also contain anti-nutritional factors such as oligosaccharides, non-starch polysaccharides (NSP), tannins and glucosides, which can explain why the poultry industry has been reluctant to use field beans in diet formulations (David et al. 2024). Field beans also contain phytate, which is present in both testa and cotyledons of the beans (Rubio et al. 1992). To alleviate the negative impact of anti-nutritional factors in field beans, various practices have been suggested, including genetic selection, mechanical processing, heat treatments, and exogenous fibre degrading enzyme supplementation (Woyengo and Nyachoti 2012; Abdulla et al., 2016b). Although designed to work on specific substrate, the exogenous enzymes can also work in additive and synergistic manner (Ravindran et al., 1999). It can be expected that dietary supplementation with PHY and/or XYL can improve broiler performance in diets low in Ca, P, energy and amino acid (Wu et al. 2004). The mode of action of dietary protease (PRO) is not well understood, thus supplementation with protease (PRO) needs further investigation (Walk et al., 2018; Watts et al. 2020). However, the effects of enzyme combinations can also be subadditive (Wu et al., 2004) and even antagonistic (Saleh et al., 2004). Recent research showed that broilers fed field beans-based diets responded to tannase (Abdulla et al., 2016a, 2016b) or a mixture of tannase, pectinase and XYL (Abdulla et al., 2017), but there is a lack of information on the impact of feeding value for broilers of bean-based diets supplemented with PHY, XYL and PRO, alone or in a combination.

Therefore, the objective of this experiment was to determine the effect of supplemental PHY, XYL and PRO alone and in a combination on broiler performance, dietary metabolisable energy (ME) and nutrient digestibility when fed bean containing nutritionally sufficient diets.

Materials and methods

The experiment was completed at the National Institute of Poultry Husbandry (NIPH) and approved by the Research Ethics Committee of Harper Adams University, UK (Project number PG26-201404). The manuscript has been prepared in compliance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

Field bean cultivar samples

Based on previous research (Abdulla et al., 2021a, b; Pirgozliev et al., 2023), field beans from cultivars Divine, Clipper and Buzz were selected for this study. Cultivar Divine had greater apparent metabolizable energy (AME) and crude protein (CP) and low NSP and phytate contents (Table 1). The opposite, cultivar Buzz had low AME and CP, high phytate and medium NSP contents. The concentration of AME, CP and phytate were medium, but NSP was high in Clipper field beans.

Diet formulation

Three diets containing 300 g/kg of one of the three freshly hammer-milled (passing a 4 mm screen) experimental field bean samples (from cultivars Buzz, Clipper and Divine) were mixed (Table 2). Each diet was then split into eight equal batches, seven of which were supplemented with commercial PHY, XYL or PRO both singularly and in combinations. One sample from each of the eight batches was not supplemented, resulting in 24 dietary treatments in total. All enzymes were provided by Danisco Animal Nutrition (DuPont

Industrial Biosciences, Marlborough, UK). Danisco® Xylanase (EC 3.2.1.8) is a fungal endo-1,4-ß-xylanase biosynthesised from *Trichoderma reesei*. Phyzyme® XP (EC 3.1.3.26) is a bacterial 6-phytase, produced from a species of *Escherichia coli* and is expressed in a *Saccharomyces pombe*. Axtra® PRO (EC 3.4.21.62) is a subtilisin protease, expressed in *Bacillus subtilis*. Enzymes were added on top of the diets, in powder form, providing an activity of 1000, 2000 and 4000 units/kg diet (U/kg) for PHY, XYL and PRO, respectively. These activities were suggested as optimal by the producer. No adjustments were made for differences in dry matter between the field bean samples because only a small range of differences were observed and deemed insignificant. All diets were supplied with 5 g/kg of titanium dioxide (TiO_2) as an indigestible marker on the top and were fed as mash.

Husbandry and sample collection

Male Ross 308 broiler chickens were obtained from a commercial hatchery at one-day old and were placed in a single floor pen and fed on a proprietary broiler starter feed until 6 days of age. On the first day of the experimental period (at 7 days of age), the chicks were individually weighed and randomly placed in one of the experimental pens. Two birds were placed in each pen (0.16 m^2 solid floor area) within a controlled environment. Each diet was fed at random to 9 pens from 7 to 21 days of age, resulting in 216 pens, over three time periods. The rearing conditions were similar to those of previous experiments (Abdulla et al. 2016a, b; 2017). Access to feed and water was *ad libitum* throughout the experimental period.

During the last four days of the experiment, the solid floor of each pen was replaced with a wire mesh. Excreta were collected every day, stored in a freezer until last day collection, then oven dried at 60^{o} C and milled after leaving at room temperature for three nights and weighing. At the end of the study (at 21 days of age), the two birds in each pen were killed by cervical dislocation, weighed, digesta from the distal part of the ileum of the broiler chickens of each pen were collected, pooled and freeze dried, and then milled to pass through a 0.8 mm sieve.

The empty weights of the gastrointestinal tract (GIT) segments including, proventriculus and gizzard (PG), pancreas, jejunum (situated between the duodenal loop and Meckel's diverticulum) and ileum (situated between the Meckel's diverticulum and ileo-caecal junction) from of the heavier bird in each pen were taken and the weight of each of the segments was expressed as absolute and relative to BW (Abdulla et al. 2016a, b).

Laboratory analysis and calculations

The gross energy (GE), dry matter (DM), nitrogen (N) and crude fat (CF), as ether extract, of each dried excreta sample and the experimental diets, as well as crude protein in the digesta were determined, as explained previously (Pirgozliev et al., 2014). Titanium dioxide concentration in the diet, digesta and excreta was determined using the method described by Peddie et al. (1982). The AME and N-corrected AME (AMEn) contents of the diets were determined according to the method of Hill and Anderson (1958). The coefficients of fat (FR), dry matter (DMR) and nitrogen (NR) retention coefficients (based on excreta analysis) and ileal nitrogen (ND) digestibility (based on digesta analysis) of diets were determined as described elsewhere (Whiting et al., 2017; Watts et al., 2021). The mean daily feed intake (FI g/b/d), weight gain (WG), and feed conversion ratio (FCR) were also measured over 14 days (from 7 to 21 days of age) of the experiment.

Statistical analyses

Statistical analyses were performed using the Genstat statistical software package (Genstat 23rd release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The data was compared statistically by analysis of variance (ANOVA) using a 3 x 2 x 2 x 2 factorial arrangement of treatments. The main effects analysed were related to the impacts of field bean cultivars with and without the exogenous enzymes, single or in combinations. Means were separated employing Tukey's test. In all instances, differences were reported as significant at $P \leq 0.05$.

Results

Effect of the experimental diets on growth performance variables

Results on growth performance are presented in Table 3, and data on observed interactions is presented in Tables 3 A and 3 B. Field bean cultivar did not affect feed intake (FI), and there was PHY x XYL x PRO interaction ($P<0.05$) (Table 3 A). Phytase supplementation increased FI ($P=001$) by 4.4%, although exogenous XYL reduced ($P<0.05$) FI by 2.6%. Dietary PRO did not have an impact on FI ($P>0.05$). There was also PHY x XYL x PRO interaction ($P<0.05$) for weight gain (WG) (Table 3 B), as PHY supplementation increased WG ($P<0.01$) by 6.5 % but the impact of XYL and PRO was numerical only ($P>0.05$). Diets based on cultivar Divine produced lower feed conversion ratio (FCR), i.e. high feed efficiency, ($P<0.001$) compared to Buzz and Clipper-based diets. Phytase supplementation reduced FCR ($P=0.05$) by 1.98%. Dietary XYL and PRO did not change ($P>0.05$) FCR.

Energy and nutrient availability of the experimental diets

Results on dietary energy and nutrient utilisation are presented in Table 4, and interactions in tables 4 A and 4 B. Diets based on Divine bean cultivar had higher ME ($P<0.05$) and DMR ($P<0.001$) coefficient than those based on Buzz or Clipper, and the values did not differ ($P>0.05$) between Buzz or Clipper based diets. Feeding PHY decreased ($P<0.001$) FR by 4.3%, but did not affect the rest of the variables. Dietary XYL increased AME ($P=0.002$), AMEn and DMR ($P<0.001$) by 0.18 MJ/kg DM and 1%, respectively. There was a bean cultivar x PHY x XYL interaction ($P<0.05$) in NR, showing that diet based on cultivar Divine responded positively to PHY alone, but NR was reduced when combining PHY and XYL (Table 4 A). Dietary PHY increased ND ($P<0.05$), but PRO supplementation did not bring changes (Table 4 B).

Effect of the experimental diets on digestive tract development of the birds

The overall weight of the total GIT, jejunum and ileum as % of total body weight was not changed ($P>0.05$) by field bean cultivar or enzyme supplementation (Table 5). Feeding PHY reduced ($P<0.05$) the weight of the duodenum by 7.7%. There was a field bean cultivar x PHY interaction ($P<0.05$) on the development of the pancreas, showing an increase in the pancreas of birds fed Divine and PHY (Table 5 A). Further interaction ($P<0.05$) on the PG development was observed between beans, PHY and PRO (Table 5 B). Birds fed Divine based diets had greater PG weight ($P>0.05$) when supplemented with PHY and PRO compared to Divine based enzyme free or PHY only supplemented diets although no changes in PG weight were observed for the other field beans.

Discussion

The overall composition of the experimental diets was within the expected range. Therefore, the design allowed an examination of the effects of different field bean cultivars inclusion to the diet or the supplementation of the three enzymes individually or in combination.

Effect of the field bean cultivars

Non-starch polysaccharides and phytic acid in field beans can form strong complexes with proteins, starch, and minerals (Selle and Ravindran, 2007). Thus, the observed lower FCR, greater FR, DMR and AME in birds fed Divine (the bean sample with the lowest phytate and

NSP content) over those fed Buzz and Clipper-containing diets is not a surprise. Also, in a previous study (Abdulla et al., 2021a), slightly better broiler performance, ME and nutrient retention was found with Divine compared to those of the other two bean cultivars. However, the greater differences in the results in the current experiment than those of the same cultivar samples in the previous study (Abdulla et al., 2021a) may be due to using a higher proportion of beans in this study (300 g/kg diet) than the previous one (200 g/kg diet). The reported results are in line with the observed improved production performance, ME and nutrient digestibility in broilers fed low anti-nutrients compared to a high anti-nutrient diets (Kalmendal et al*.,* 2011). Pirgozliev et al. (2023) also explained changes in dietary ME and feed efficiency of birds fed field bean containing diets with differences in the carbohydrate fraction of dietary beans. In contrast, Nalle (2010) did not find significant differences in broilers' performance when fed four different bean cultivars with an NSP range like that reported in this study. However, the relatively low inclusion rate of 200 g/kg beans may be the reason for the results reported by Nalle (2010).

The inclusion of different field bean cultivars did not alter the development of the GIT segments, although there were few interactions with the exogenous enzymes regarding some of the GIT segments. Nalle (2010) also did not find difference in the relative empty weights of most of the GIT segments for 21day old male Ross 308 birds, fed diets containing 200 g/kg of different field bean cultivars. The bean with PHY interaction produced enlarged pancreas in Divine fed birds coupled with the improved NR. The secretion of pancreatic enzymes might be affected by the concentration of enzymes and substrates or products of their hydrolysis in the lumen of the small intestine following a negative feedback mechanism (Kubena et al., 1983). Mahagna et al. (1995) reported that secretion of pancreatic amylase and proteases was reduced when chicks were fed diets supplemented with the same enzymes. If the efficiency of digestion is consistently suboptimal, whether due to ingredient quality, microbial interaction of anti-nutritive factors, the GIT responds by increasing both size (surface area) and digestive enzyme output (Abdulla et al. 2016a, 2017). The relationship between phytic acid and protein digestibility is well documented (Selle and

Ravindran, 2007; Pirgozliev et al., 2009). A reduction in phytate concentration due to exogenous PHY supplementation should enable better utilisation of CP, amino acids and other nutrients in young birds. Although phytate degradation was not determined in this study, it may be speculated that the release of nutrients in the lumen of the small intestine, due to PHY supplementation, would lead to a release of more enzymes from the pancreas, thus explaining the enlargement of the pancreas in relation to PHY.

The differences in phytate and NSP contents in bean samples may be the reason for the observed interactions regarding PG development, e.g. cultivar Divine contained less anti-nutrients and responded differently to the exogenous enzymes. However, compared to other reports on GIT development (Wu et al., 2004; Nalle, 2010; Oso et al., 2017), the observed differences in this study are relatively small and may not represent changes in the functions of organs.

Effect of the enzymes

Effects of dietary enzyme combination on broiler growth performance and nutrient utilisation are frequently inconsistent. Although some authors reported antagonism between different enzymes (Saleh et al., 2004), while others found the effects of enzyme combinations to be subadditive (Wu et al., 2004), additive (Romero et al., 2014; Olukosi et al., 2015), and synergistic (Ravindran et al., 1999). Differences in the type of enzymes tested, as well as in the experimental design, specifically the dietary composition and the use of enzyme complexes rather than monocomponent enzyme preparations, can partially explain the conflicting and highly variable results reported (Walk et al., 2018). When several enzymes are tested together the effect of the enzyme preparation cannot always be attributed to the addition of a specific enzyme.

The positive effect of PHY on protein digestibility and broiler growth in a low P diet is expected (Selle and Ravindran, 2007; Pirgozliev and Bedford, 2013). Diets fed in this study were formulated to contain less available P, i.e. 3.9 g/kg average *vs* 4.4 g/kg recommended, which may be the reason for the observed superior feed efficiency and ND in birds fed PHY only. Phytate is also an irritant that increases the endogenous losses from broiler's gastrointestinal tract (Pirgozliev et al., 2012), thus hydrolysing dietary phytates will further increase the benefits of supplementary PHY. However, dietary P was not determined in this study, thus firm conclusion is incorrect.

The benefits of using fibre-degrading enzymes in broiler feed has been associated with reduced intestinal viscosity, degradation of cell wall NSP and the release of encapsulated nutrients in the gut (Šimić et al., 2023; Whiting et al., 2023;). The experimental field beans, representing 30% of the diets, on average contained 21% of NSP, in addition to the over 40% wheat in the diets, thus explaining the benefits observed in increasing ME and DMR, in line with a reduction in pancreas enlargement in this study when XYL was supplemented in the diets.

Although, the birds cannot produce XYL and sufficient amounts of PHY in their GIT, the wide range of endogenous PRO synthesised and released in the GIT is generally considered to be sufficient to optimise feed protein utilisation (Le Heurou-Luron et al., 1993). This may be the reason for the lack of significant effects following supplementation of PRO singularly on the measured parameters in the current study. However, nitrogen and amino acid digestibility reported for poultry indicate that valuable amounts of protein pass through the GIT without being completely digested (Lemme et al., 2004). This undigested protein represents an opportunity for the use of supplemental exogenous PRO in broiler feeds to improve protein digestibility (Watts et al. 2020). Improvements in nutrient digestibility and performance of broilers have been reported following supplementation of exogenous PRO alone and in a combination with other exogenous enzymes, such as PHY and XYL (Olukosi et al., 2015; Romero et al., 2014).

Results from *in vitro* studies have shown that phytate limits nutrient availability in field beans and PHY alone or in combination with carbohydrase (cellulase) additively increases their relative nutrient availability (Luo et al., 2010). Xylanase increases the access of proteolytic enzymes to substrates, e.g. phytate and protein, in the aleurone layer of wheat (Parkkonen et al., 1997), suggesting that XYL may increase the access of the enzymes to substrates by disrupting the cell wall matrix. Frolich (1990) stated that phytic acid is not the component that is solely responsible for the decreased nutrient availability in diets, as dietary fibre itself also might be of importance. Thus, PHY might also assist the hydrolytic functions of XYL by dephosphorylation of phytate. The ability of exogenous PRO to increase the solubilisation and fermentation of hemicellulose by removing structural proteins in the cell wall, allowing faster access of ruminal microbes to digestible substrates has been demonstrated in ruminant models (Colombatto and Beauchemin, 2009). All this supports the observed overall improvements in FI and WG of birds fed a combination of XYL, PHY and PRO, although PHY was the main contributor.

Incorporating locally produced protein sources in diets may reduce reliance on soybean meal associated with land use change, contributing towards sustainability for broiler industry. Thus, further research on feeding value of field beans for poultry is important. The use of exogenous enzymes may further help optimising dietary formulations.

Conclusion

Supplementation of PHY alone improved feed efficiency, and XYL alone improved the ME of field bean-containing diets for broiler chickens. The use of a combination of PHY, XYL and PRO improved overall feed intake and weight gain of birds. The observed interactions between the enzymes and field bean cultivar samples show that studies involving various enzyme activities considering substrates in beans/ diets are warranted.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 1. Chemical composition and N-corrected apparent metabolizable energy (AMEn) content of the of the experimental field beans (dry mater basis)

Ingredient	Buzz	Clipper	Divine
Dry matter (g/kg)	845	854	866
Crude protein (N x 6.25) (g/kg)	276	285	300
Ether extract (g/kg)	10.7	9.4	9.2
Phytate (mg/g)	20.8	16.6	13.4
Total non-starch polysaccharide (g/kg)	190	250	180
Soluble non-starch polysaccharide (g/kg)	51	73	46
Insoluble non-starch polysaccharide (g/kg)	139	177	134
Total starch (g/kg)	452	397	434
AMEn (MJ/kg)	8.20	9.16	9.96

Adapted from Abdulla et al., 2021a

Table 2. Ingredient composition (g/kg, as-fed) of the experimental diet formulations

Ingredient	Buzz	Clipper	Divine
Wheat	404.2	404.2	404.2
Buzz bean	300	-	-
Clipper bean	-	300	-
Divine bean	-	-	300
Soybean meal (Crude protein = 48%)	27.0	27.0	27.0
Full fat soybean meal	127.5	127.5	127.5
Maize gluten meal	35.0	35.0	35.0
Soy oil	65.0	65.0	65.0
Lysine HCL	2.3	2.3	2.3
DL Methionine	5.8	5.8	5.8
L Threonine	2.4	2.4	2.4
Monocalcium phosphate	10.0	10.0	10.0
Limestone	14.0	14.0	14.0
Sodium chloride	2.8	2.8	2.8
Vitamin/mineral premix[1]	4.0	4.0	4.0
Total	1000	1000	1000
Calculated composition			
ME (MJ/kg)	12.77	13.06	13.33
Crude protein (g/kg)	221	224	229
Lysine (g/kg)	12.8	12.1	13.0
Methionine + cysteine (g/kg)	8.5	8.6	8.5
Calcium (g/kg)	8.2	8.3	8.2
Phosphorus available (g/kg)	4.0	3.9	3.8

Analysed values (as-fed)			
Dry matter (g/kg)	883	881	883
Gross energy (MJ/kg)	17.60	17.67	17.69
Crude protein (g)	189	194	199
Fat (g)	95	95	95

Each experimental diet met or exceeded the diet specification for this strain of broiler chicken (Aviagen Ltd., Edinburgh, UK). [1]Vitamin and mineral premix provided per kg diet: 50 mg nicotinic acid, 34 mg α-tocopherol, 15 mg pantothenic acid, 7 mg riboflavin, 5 mg pyridoxine, 3.6 mg retinol, 3 mg menadione, 2 mg thiamine, 1 mg folic acid, 200 μg biotin, 125 μg cholecalciferol, 15 μg cobalamin, 100 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 1 mg iodine, 0.5 mg cobalt, 0.5 mg molybdenum and 0.2 mg selenium.

Table 3. Effect of experimental diets on growth performance of broilers

Treatment factor	FI (DM g/b/d)	WG (g/b/d)	FCR (g:g)
Bean cultivar			
Buzz	52.7	40.3	1.308^{b}
Clipper	53.2	40.6	1.315^{b}
Divine	53.2	42.0	1.269^{a}
SEM	0.60	0.49	0.0077
Enzyme			
PHY			
-	51.9	39.7	1.310
+	54.2	42.3	1.284
XYL			
-	53.7	41.3	1.304
+	52.3	40.6	1.291
PRO			
-	52.8	40.9	1.294
+	53.2	41.0	1.300
SEM (df = 181)	0.49	0.40	0.0063
P values			
B	0.784	0.040	<0.001
PHY	0.001	<0.001	0.005
XYL	0.046	0.257	0.149
PRO	0.606	0.946	0.483
Interactions			
B x PHY	0.803	0.636	0.620
B x XYL	0.983	0.755	0.289

B x PRO	0.888	0.868	0.233
PHY x XYL	0.014	0.006	0.400
PHY x PRO	0.406	0.827	0.240
XYL x PRO	0.578	0.782	0.616
PHY x XYL x PRO	0.025[†]	0.017[‡]	0.706
B x PHY x XYL	0.235	0.235	0.921
B x PHY x PRO	0.442	0.406	0.880
B x XYL x PRO	0.363	0.427	0.422
B x PHY x XYL x PRO	0.135	0.091	0.680

B, bean cultivar; PHY, phytase; XYL, xylanase; PRO, protease; FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; Each mean represents values from nine replicate pens of two birds each; Bird performance was determined from 7 to 21 days of age; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$; [a,b]Values within a column with different superscripts differ significantly; [†]PHY x XYL x PRO interaction in FI is presented in Table 3 A; [‡]PHY x XYL x PRO interaction in WG is presented in Table 3 B.

[†]**Table 3 A.** Interactions on feed intake of broiler chickens (SEM = 0.99)

	XYL	no		yes	
PHY	PRO	no	yes	no	yes
no		52.9^{bc}	53.9^{bc}	51.0^{ab}	49.6^{a}
yes		54.5^{c}	53.5^{bc}	52.9^{bc}	55.8^{c}

PHY, phytase; XYL, xylanase; PRO, protease; ^{a,b,c}Values within a table with different superscripts differ significantly.

[‡]**Table 3 B.** Interactions on weight gain of broiler chickens (SEM = 0.79)

	XYL	no		yes	
PHY	PRO	no	yes	no	yes
no		40.2^{ab}	41.3^{bc}	39.2^{ab}	37.9^{a}
yes		42.5^{bc}	41.1^{b}	41.9^{bc}	43.6^{c}

PHY, phytase; XYL, xylanase; PRO, protease; ^{a,b,c}Values within a table with different superscripts differ significantly.

Table 4. Energy and nutrient availability of the experimental diets for broilers

Treatment factor	AME (MJ/kg DM)	AMEn (MJ/kg DM)	FR	NR	DMR	ND
Bean cultivar						
Buzz	14.41^{b}	13.60	0.755^{a}	0.673	0.684^{a}	0.719
Clipper	14.41^{b}	13.58	0.768^{a}	0.674	0.683^{a}	0.718
Divine	14.56^{a}	13.70	0.791^{b}	0.685	0.693^{b}	0.719
SEM	0.048	0.046	0.0074	0.0029	0.0020	0.0036
Enzyme						
PHY						
-	14.50	13.68	0.788	0.675	0.687	0.714
+	14.41	13.58	0.754	0.680	0.686	0.724
XYL						
-	14.37	13.54	0.765	0.679	0.683	0.719
+	14.55	13.72	0.777	0.676	0.690	0.719
PRO						
-	14.46	13.63	0.775	0.677	0.686	0.718
+	14.45	13.62	0.768	0.678	0.687	0.720
SEM (df = 181)	0.039	0.038	0.0061	0.0024	0.0016	0.0030
P values						
B	0.042	0.158	0.003	0.006	<0.001	0.920
PHY	0.101	0.070	<0.001	0.130	0.517	0.021
XYL	0.002	<0.001	0.154	0.427	<0.001	0.981
PRO	0.897	0.880	0.417	0.839	0.818	0.581
Interactions						
B x PHY	0.292	0.307	0.550	0.269	0.249	0.074
B x XYL	0.802	0.773	0.500	0.743	0.477	0.365

B x PRO	0.338	0.306	0.053	0.369	0.614	0.301
PHY x XYL	0.172	0.183	0.582	0.308	0.110	0.210
PHY x PRO	0.624	0.697	0.666	0.134	0.391	0.033[‡]
XYL x PRO	0.982	0.980	0.766	0.556	0.962	0.085
PHY x XYL x PRO	0.910	0.973	0.839	0.288	0.635	0.453
B x PHY x XYL	0.620	0.722	0.583	0.011[†]	0.739	0.760
B x PHY x PRO	0.597	0.563	0.715	0.916	0.568	0.059
B x XYL x PRO	0.471	0.523	0.538	0.239	0.434	0.575
B x PHY x XYL x PRO	0.648	0.609	0.576	0.752	0.583	0.841

B, bean cultivar; PHY, phytase; XYL, xylanase; PRO, protease; AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; NR, coefficient of nitrogen retention; FR, coefficient of fat retention; DMR, coefficient of dry matter retention; ND, coefficient of ileal nitrogen digestibility; Each value represents the mean of nine replicate pens of two birds each; Dietary AME, AMEn, CPR, FD, DMR, CPD and DMD variables were determined between 17 and 21 days of age; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$; [a,b]Values within a column with different superscripts differ significantly; [†]B x PHY x XYL interaction in NR is presented in Table 4 A; [‡]PHY x PRO interaction in ND is presented in Table 4 B.

†**Table 4 A.** Interactions on nitrogen retention (on excreta) coefficients (SEM = 0.0059)

	PHY	no		yes	
Bean cultivar	XYL	no	yes	no	yes
Buzz		0.675^{ab}	0.673^{ab}	0.670^{ab}	0.673^{ab}
Clipper		0.676^{ab}	0.665^{a}	0.679^{ab}	0.678^{ab}
Divine		0.672^{ab}	0.688^{bc}	0.701^{c}	0.680^{ab}

PHY, phytase; XYL, xylanase; ^{a,b,c}Values within a table with different superscripts differ significantly.

‡**Table 4 B.** Interactions on nitrogen digestibility (on digesta) coefficients (SEM = 0.0042)

PHY	PRO	no	yes
no		0.708^{a}	0.719^{ab}
yes		0.727^{b}	0.720^{ab}

PHY, phytase; PRO, protease; ^{a,b}Values within a table with different superscripts differ significantly.

Table 5. Effect of experimental diets on the gastrointestinal tract development of broiler chickens (data presented as a percentage of the body weight)

Treatment factor	Total GIT	Pancreas	PG	SI	Duodenum	Jejunum	Ileum
Bean cultivar							
Buzz	8.5	0.4	3.2	4.9	1.2	2.2	1.4
Clipper	8.4	0.4	3.2	4.8	1.2	2.2	1.4
Divine	8.3	0.4	3.1	4.7	1.2	2.2	1.3
SEM	0.08	0.01	0.04	0.07	0.02	0.04	0.02
Enzyme							
PHY							
-	8.4	0.4	3.1	4.9	1.3	2.2	1.4
+	8.3	0.4	3.2	4.7	1.2	2.2	1.4
XYL							
-	8.4	0.5	3.1	4.8	1.2	2.2	1.4
+	8.4	0.4	3.2	4.8	1.2	2.2	1.4
PRO							
-	8.3	0.4	3.1	4.7	1.2	2.2	1.3
+	8.5	0.4	3.2	4.8	1.2	2.2	1.4
SEM (df = 181)	0.07	0.01	0.03	0.06	0.02	0.03	0.02
P values							
B	0.126	0.776	0.236	0.231	0.184	0.460	0.424
PHY	0.256	0.553	0.105	0.041	0.010	0.086	0.477
XYL	0.773	0.005	0.022	0.635	0.826	0.486	0.977
PRO	0.133	0.735	0.085	0.418	0.528	0.806	0.156
Interactions							
B x PHY	0.829	0.043[†]	0.978	0.907	0.272	0.605	0.803

B x XYL	0.754	0.118	0.854	0.689	0.893	0.727	0.595
B x PRO	0.212	0.292	0.055	0.813	0.755	0.903	0.078
PHY x XYL	0.089	0.207	0.295	0.203	1.000	0.065	0.494
PHY x PRO	0.259	0.957	0.586	0.115	0.054	0.454	0.108
XYL x PRO	0.430	0.597	0.412	0.670	0.366	0.997	0.682
PHY x XYL x PRO	0.393	0.509	0.184	0.117	0.925	0.073	0.076
B x PHY x XYL	0.450	0.617	0.919	0.400	0.355	0.425	0.237
B x PHY x PRO	0.067	0.095	0.034[‡]	0.426	0.613	0.682	0.307
B x XYL x PRO	0.153	0.939	0.086	0.410	0.102	0.379	0.274
B x PHY x XYL x PRO	0.906	0.262	0.181	0.543	0.260	0.852	0.683

B, bean cultivar; PHY, phytase; XYL, xylanase; PRO, protease; GIT, gastrointestinal tract (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG, proventriculus and gizzard; SI, small intestine (including duodenum, jejunum and ileum); Each value represents the mean of nine replicate pens; Gastrointestinal tract development was determined at 21 days of age using the heavier bird in each pen; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P\leq0.05$; ; [†]B x PHY interaction in pancreas size as a proportion of body weight is presented in Table 6 A; [‡]B x PHY x PRO interaction in PG is presented in Table 6 B.

†**Table 6 A.** Interactions on the pancreas size (data presented as a percentage of the body weight) (SEM = 0.01)

PHY	no	yes
Bean cultivar		
Buzz	0.4^{a}	0.4^{a}
Clipper	0.4^{a}	0.4^{a}
Divine	0.4^{a}	0.5^{b}

PHY, phytase; ^{a,b}Values within a table with different superscripts differ significantly.

‡**Table 6 B.** Interactions on proventriculus and gizzard size (data presented as a percentage of the body weight) (SEM = 0.07)

	PHY	no		yes	
Bean cultivar	PRO	no	yes	no	yes
Buzz		3.1^{ab}	3.2^{ab}	3.3^{b}	3.2^{ab}
Clipper		3.2^{ab}	3.1^{ab}	3.2^{ab}	3.3^{b}
Divine		3.0^{a}	3.2^{ab}	3.0^{a}	3.3^{b}

PHY, phytase; PRO, protease; ^{a,b}Values within a table with different superscripts differ significantly.