Feeding value of field beans (*Vicia faba* L. var. *minor*) with and without enzyme containing tannase, pectinase and xylanase activities for broilers

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Feeding value for chicks of field beans (*Vicia faba* L. var. *minor*) with and without enzyme containing tannase, pectinase and xylanase activities

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ABSTRACT

Effects of field beans with various tannin content (T) and exogenous enzyme containing tannase, pectinase and xylanase activities on N-corrected dietary apparent metabolisable energy (AMEn), coefficients of dry matter (DMR) and nitrogen (NR) retention, fat digestibility (FD), gastrointestinal tract (GIT) development, jejunal villus morphometry, ileal digesta viscosity and sialic acid (SA) were examined. Birds' growth performance and energy conversion ratio (ECR) were also measured. Birds were fed one of eight mash diets. A control diet was prepared that had major ingredients of 400.0 g/kg wheat and 127.0 g/kg soybean meal (SBM), and contained 221 g/kg CP and 12.83 MJ/kg metabolisable energy in agreement with breeder's recommendation. To reduce nutrient density the control diet also contained 119.1 g/kg washed sand. Another three diets containing 300 g/kg of each of three experimental field bean cultivar samples in replacement for soybean meal and sand were also mixed in order to have metabolisable energy and CP in a range similar to the control diet. Each diet was fed to nine pens with two Ross 308 male broilers following randomisation.

Diets high in T had low (P<0.001) N-corrected apparent metabolisable energy (AMEn), ECR, DMR and NR. Feeding field beans increased (P<0.001) the weights of the pancreas and the proventriculus and gizzard (PG) of the birds. Supplementing diets with the enzyme mixture containing tannase, pectinase and xylanase activities improved (P<0.001) feed conversion efficiency, AMEn and all nutrient utilisation coefficient despite the T in diets. The enzyme mixture reduced ileal digesta viscosity (P<0.001) and the weight of the pancreas, the total GIT and the PG (P<0.05) of the birds. It can be concluded that the feeding value of field beans with different T contents may vary when fed to broilers. The enzyme mixture supplementation improved feeding value of diets for broilers. The beneficial effect of the addition of enzyme mixture containing tannase, pectinase and xylanase activities to poultry diets seems to be mediated through reduced ileal digesta viscosity and improved nutrient availability.

KEYWORDS

Field bean; tannase enzyme; broiler; ME; digestibility

1. Introduction

Continuous increase in the demand for soybean meal has led to raising its price, particularly after prohibition of animal protein inclusion in poultry diet by European Union (O'Neill et al., 2012). Soybean is an imported feed ingredient which affects the stability of its price and availability in market (Ravindran et al., 2010; O'Neill et al., 2012). Moreover, large amount of the available soybean meal in the market is produced from genetically modified crops which worries consumers and is not convenience for organic production (Vicenti et al., 2009). These factors have inspired nutritionists to do research on locally grown legumes aiming their optimum and potential seize as an alternative to soybean meal in poultry diet (Crepon, 2006;

Ravindran et al., 2010). Grain legumes are considered reasonable candidates to soybean meal replacement because of the similarity of their amino acid profiles to those of soybean meal (Wiryawan and Dingle, 1999). The field beans (*Vicia faba*), unlike the soybean, yields quite satisfactorily in the cooler and shorter growing season of' the upper North Temperature Zone. Due to favourable climate conditions, field beans can be produced at a high amount and in wide area in Europe (Crépon et al., 2010).

Field beans are not regularly used in poultry diet formulations because of the presence of antinutritional factors including soluble non-starch polysaccharides (NSP) and tannins (Longstaff and McNab, 1991a,b). Although the beneficial effect of feeding fibre-degrading enzymes to legume-containing diets has been studied (Castanon and Marquardt, 1989; Cowieson et al., 2003), there is a lack of information on the effect of multi enzyme preparation on feeding value of field beans for broilers.

Tannase or tannin acyl-hydrolase (E.C. 3.1.1.20) catalyzes the hydrolysis of ester bonds present in gallotannins, complex tannins and gallic acid esters (Aguilar et al., 2007). Commercially available tannase products generally have other enzyme activities, primarily amylase, pectinase and galactosidase (Boadi and Neufeld, 2001). The application of these tannase-containing enzymes is in food and beverages processes. Little is known of its potential use in poultry feed. Chamorro et al. (2015) found no effect of exogenous tannase on growth performance in chickens fed diet rich in polyphenols, although Abdulla et al. (2016a) showed that dietary tannase can improve feeding value of field beans containing diet for broilers. Although some research has been done (Abdulla et al., 2016b), more information is needed on the effect of multi enzyme preparation (also containing tannin degrading enzymes), on diets with different field bean samples (with different tannin contents) and in comparison with other low-tannin diets.

The main objective of this experiment, therefore, was to determine the effect of supplementary multi enzyme preparation, containing tannase, xylanase, amylase, pectinase and galactosidase activities on dietary metabolisable energy, nutrient utilisation, ileal digesta viscosity, ileal villus morphometry and gastrointestinal tract development when feeding diets containing field beans with different tannin contents to chickens. The overall feed intake, weight gain and feed conversion efficiency of the birds were also measured.

2. Materials and methods

2.1. *Experimental samples*

This report is focused on the nutritional value for broilers of three UK grown field bean samples. The three field bean samples used in the study were produced during 2013 harvest year. All samples were stored in tote bags at ambient air temperatures in a dry store. The stored field bean samples did not experience any freezing temperatures during this period.

2.2. *Proximate analysis of samples*

Dry matter (DM) was determined by drying of samples in a forced draft oven at 105°C to a constant weight (AOAC, 2000; method 934.01). Crude protein (CP; 6.25 X N) in samples was determined by dry combustion method (AOAC, 2000; method 990.03) using a Leco (FP-528 N, Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC, 2000; method 945.16) using a Soxtec system (Foss UK Ltd.). The gross energy (GE) value of the field bean samples was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL) with benzoic acid used as the standard. Total starch (TS) was determined following the method of Englyst et al. (2000). The non-starch polysaccharides (NSP) content was determined by the method of Englyst et al. (1994), whereby starch is completely dispersed and then hydrolysed enzymatically. The NSP

is isolated by precipitation in 80% ethanol then hydrolysed by sulphuric acid and the released sugars measured by gas chromatography as their alditol acetate derivatives. The total phenol, total tannin in the control diet, as well as representative samples of studied field bean cultivars, all as tannic acid equivalent, were determined by applying the procedure suggested by Makkar et al. (1993). Whereas condensed tannins, as leucocynidin equivalent, for the same samples were determined by using the assay described by Porter et al. (1985)

2.3. *Diet preparation*

Birds were fed one of eight mash diets. A control diet was prepared that had major ingredients of 400.0 g/kg wheat and 127.0 g/kg soybean meal (SBM), and contained 221 g/kg CP and 12.83 MJ/kg metabolisable energy in agreement with breeder's recommendation (Aviagen Ltd., Edinburgh, UK) (Table 1). To reduce nutrient density, the control diet also contained 119.1 g/kg washed sand. Another three diets containing 300 g/kg of each of three experimental field bean cultivar samples in replacement for soybean meal and sand were also mixed in order to have AME and CP in a range similar to the control diet (Table 1). Each diet was then split into two batches and one of them was supplemented with an enzyme mixture (Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland) resulting in eight diets in total. The determined enzyme activities of the enzyme mixture were; tannase or tannin acyl-hydrolase (E.C. 3.1.1.20) 3400 units / kg diet (following the method of Bajpai and Patil (1996) at pH 5.5; determined by Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland), pectinase (EC 3.2.1.15) 6220 units/kg diet (ESC Standard Analytical Method SAM027 at pH 4.5 and 40°C; determined by Enzyme Services & Consultancy, Ystrad Mynach, UK); xylanase (EC 3.2.1.8) 6100 units/kg diet (ESC Standard Analytical Method SAM036 at pH 5.3 and 50°C, using 1.2% BSA in the extraction; determined by Enzyme Services & Consultancy, Ystrad Mynach, UK), and there were some additional

amylase and α-galactosidase activities. The enzyme mixture preparation was synthesised by *Aspergillus niger*. The enzyme was in a liquid form and the reported enzyme activities were obtained after spraying 17ml/kg on the top of diets. The dry matter content of non-supplemented diets was adjusted by spraying of 17ml water per kg of diet. After spraying the diets were thoroughly mixed in a horizontal mixer.

Diets were free of coccidiostat, antimicrobial growth promoters, prophylactic and other similar additives.

2.4. *Determination of dietary metabolisable energy, nutrient utilisation, mucin losses and comparison of broiler growth performance*

All procedures were approved by The Animal Experimental Committee of Harper Adams University.

Male Ross 308 broiler chickens were obtained from a commercial hatchery. During the pre-study period, from day old to 13 days of age, the birds were reared in a single floor pen and fed proprietary wheat-based diet without coccidiostats or antimicrobial growth promoters, prophylactic or other similar additives. At the beginning of the study, at 14 days of age, 144 chicks were allocated to 72 small pens with 0.160 m^2 solid floors area, two birds in each pen. Room temperature and lighting program followed breeder's recommendations (Aviagen Ltd., Edinburgh, UK). Feed and water was offered *ad libitum* to birds throughout the experiment. Each diet was offered to birds in 9 pens in a randomised block design. Information on growth performances was obtained from 14 to 21d age. Excreta were collected quantitatively for the last four days of the study from 17 to 21d age and feed intake was also recorded. The gross energy, dry matter, nitrogen, and fat of each dried excreta sample and the experimental diets were determined as described for the field bean samples. The AMEn of the diets was calculated as described by Hill and Anderson (1958). The energy conversion ratio (ECR) was

151 determined as the AMEn ingested to achieve the weight gain over the weight gain for the
152 experimental period. The coefficients of total tract fat digestibility (FD), dry matter (DMR)
153 and nitrogen retention (NR) were determined as the difference between intake and excretion
154 (retention) of the nutrient divided by their respective intake.
155 The energy conversion ratio (ECR) was also determined as the AMEn ingested to achieve the
156 weight gain over the weight gain for the experimental period (Whiting et al., 2016). It
157 describes the relative efficiency of the use of metabolisable energy for growth, rather than
158 heat loss, implicit that a more efficient energy use towards growth is related to a lower ratio.
159 The mucin secretions in excreta were measured using the concentration of the sialic (SA) as a
160 marker, following the periodate-resorcinol method (Jourdian et al., 1971). The method
161 involves conversion of free and glycosidically bound SA to chromogenic substances by
162 treatment with periodic acid followed by resorcinol. The colour of the samples was stabilised
163 by 2-methyl-propan-2-ol, and after centrifugation the absorbance of the supernatant was
164 determined spectrophotometrically at 630 nm (Spectronic 301; Milton Roy Company,
165 Ivyland, PA). This procedure detects total, free, and glycosidically bound N-acetyl
166 neuraminic (sialic) acid. The endogenous mucin losses in excreta are presented in results and
167 tables as SA. The total SA excretion was obtained by multiplying the SA concentration by the
168 amount of dry excreta collected.
169
170 **2.5. *Digesta viscosity***
171 On the last day of the study, at 21 days of age, the two birds in each pen were weighed and
172 killed by cervical dislocation. The ileal digesta from both birds in each pen were collected and
173 pooled, then centrifuged (10 000g for 2 min). The viscosity of the supernatant (in centipoise
174 (cP) units) was measured using a rotating cone and cup viscometer (model DV – II + LV,
175 Brookfield Engineering Laboratories, USA) as described by Bedford and Classen (1992).

176 **2.6. *Gastrointestinal tract development and ileal villus morphometry***

177 The relative empty weights of GIT segments including proventriculus, gizzard, small intestine
178 and pancreas of each bird were also determined as previously described (Amerah and
179 Ravindran, 2008; Pirgozliev et al., 2016). After that, approximately 5 cm of the middle part of
180 the jejunum, between the point of bile duct entry and Meckel's diverticulum, of one of the
181 birds was sampled and stored for 2 wk in 10% formalin-buffered saline. The samples then
182 were embedded in paraffin wax, sectioned at approximately 5 µm, and 3 gut segments were
183 fixed in each slide. Morphometric measurements were determined on 20 intact well-oriented
184 villus–crypt units for each slide (microscope Microtec, TEC Microscopes LTD, Axbridge,
185 UK; CCD camera Infinity 2, Lumen*era* Corporation, Ottawa, Canada; Image analysis
186 software, Infinity Analyse – Infinity 2-2 for Windows version 6.5.2, Lumen*era* Corporation,
187 Ottawa, Canada). The height and the width of the villus, and the crypt depth were determined
188 as previously described (Viveros et al., 2011). The distance between the bottom of the crypt
189 and the outside of the intestine was measured and described as muscle thickness.

190

191 **2.7. *Statistical procedures***

192 Statistical analyses were performed using the Genstat statistical software package (Genstat
193 15th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The studied
194 variables were compared statistically by a two way ANOVA using a 2 × 4 factorial
195 arrangement of treatments. The main effects were the enzyme supplementation and the four
196 diet formulations (three bean samples and one control diet) giving a total of eight dietary
197 treatments. The differences between the treatments means of the four diet formulations were
198 separated using Duncan's multiple range tests. In addition, an orthogonal comparison contrast
199 test was performed to compare the control diet with the mean of three field bean diets and the

200 interaction with exogenous enzymes. In all instances, differences were reported as significant
201 at P ≤0.05. Tendencies towards significance (P ≤0.1) were also reported.

202 **3. Results**

203 The field bean compositions are summarised in Table 2. The amount of CP was more variable
204 than the ether extract content, and ranged from 244.6 to 304.5 g/kg DM, respectively. The
205 total phenols and tannins, as tannic acid equivalent, and condensed tannins, as leucocyanidins,
206 varied between 6.9 to 10.9, 6.1 to 8.3, and 4.5 to 7.3 g/kg DM for Maris Bead and Sultan,
207 respectively.
208 The mean total NSP content of the field bean samples was 179.6 g/kg DM, comprising 42.5
209 g/kg DM of soluble and 137.1 g/kg DM of insoluble NSP, respectively (Table 3). Glucose,
210 galacturonic acid, and arabinose were the main NSP constituent sugars in the field bean
211 samples. The sample of cultivar Sultan had not only the highest tannin content but also
212 contained more soluble NSP compared to the rest of the studied bean samples. The mean
213 starch content of the field bean samples was 444.7 g/kg DM, as Wizard cultivar sample had
214 the lowest (424.0 g/kg DM), and Sultan cultivar sample the highest (467.0 g/kg DM) starch
215 content.
216 There were no mortalities, and the overall weight of the birds was 0.867 kg (data not in
217 tables), and in agreement with breeder's recommendation (Aviagen Ltd, Edinburgh, UK)
218 (Table 4). Birds fed control diet had higher (P <0.001) daily FI and WG, compare to the birds
219 fed the rest of the diets. Diet containing cultivar Sultan had low FCE compared to the rest of
220 the diets (P<0.001). Feeding the enzyme mixture tended (P=0.090) to reduce daily FI, and
221 improved dietary FCE by 3.5% (P<0.001) compared to non-supplemented diets. Orthogonal
222 comparison contrast test showed that birds fed bean containing diets had higher (P<0.001) FI
223 and WG compared to the control, but no significant difference was detected for FCE. There
224 were no significant differences in diet formulation x enzymes interactions.

225 The results on dietary available energy and nutrient utilisation are summarised in Table 5.
226 Sultan containing diets had relatively low metabolisable energy and nutrient utilisation
227 coefficients compared to the rest of the field beans containing diets (P<0.001). Feeding the
228 enzyme mixture improved dietary AMEn by 0.56 MJ/kg DM (4.1%) (P<0.001). Enzyme
229 supplementation also improved (P<0.001) GE metabolisability, DMR, NR and FD by 3.8%,
230 3.6%, 2.5% and 9.0%, respectively. The means of the three field bean diets for AMEn, ECR,
231 and DMD were higher (P<0.001) and NR lower than the control diet. The enzyme mixture
232 improved FD (P<0.001), although did not affect AMEn:GE ratio. However, the AMEn:GE
233 ratio was changed by dietary formulation (P<0.001), as diet based on Sultan had higher,
234 although the control diet has lower ratio, compared to the rest. There were no significant
235 differences in diet formulation x enzymes interactions.
236 Feeding the experimental diets did not significantly influence the relative weight of the small
237 intestine of the bird (Table 6). Dietary inclusion of beans increased the weight of the PG
238 compared to control fed birds. Birds fed the control diet had smaller (P<0.05) pancreas
239 compared to the rest. Overall, enzyme mixture supplementation reduced (P<0.05) the weights
240 of the GIT, PG and the pancreas, but did not influence significantly the small intestine.
241 Contrast test showed that compared to the control, birds fed bean containing diets had
242 increased pancreas (P<0.001), proventriculus and gizzard (P<0.001), and total GIT (P<0.05),
243 although none of the treatments changed weight of the small intestine. There was no
244 significant diet formulation x enzymes interactions.
245 Dietary enzyme mixture reduced (P<0.001) viscosity by 46.5% (Table 7). Feeding diets
246 containing Sultan (high in T), reduced (P=0.005) the concentration of SA in excreta compared
247 to the rest of the diets. Feeding enzyme however, did not influence SA concentration, but
248 reduced total SA secretion by 9.4% (P<0.001). Compared to the mean of the bean diets
249 feeding the control diet increased (P<0.05) ileal digesta viscosity (8.31 vs 6.78 cP), and total

SA (P<0.001) (329 vs 257). No significant diet formulation x enzymes interactions were observed.

The results on jejunal histomorphological parameters are presented on Table 8. Feeding the control diet increased the muscle thickness of the jejunum compared to feeding Maris Beads and Sultan (P=0.038). Tannase supplementation tended (P=0.061) to reduce the muscle thickness of the wall of the jejunum. Villus high and width were not affected (P>0.05) by the diets and enzyme supplementation. Orthogonal comparison contrast test showed that birds fed bean containing diets had decreased (P<0.001) jejunal crypt depth compared to the control fed birds (216 vs 240 nm) and no diet formulation x enzymes interactions were observed (P>0.05).

4. Discussion

The purpose of the experiment reported in this paper was to determine whether tannase-containing enzyme could be used to improve available energy and nutrient utilisation in field bean containing diets when fed to growing broiler chicks. It was important to evaluate exogenous tannase efficiency using different field bean cultivar samples because of the large variation in the agronomic production and chemical composition of beans available to the animal feed industry.

The sample of bean cultivar Sultan had higher tannin and soluble NSP contents, followed by Wizard and Maris Bead samples. Tannins are hydro soluble and high molecular weight polyphenolic compounds. Tannins have the ability to precipitate macromolecules (such as proteins, cellulose, starch, etc.) and minerals by forming strong complexes (Lekha and Lonsane, 1997). However, compared to Maris Beads and Wizard, Sultan also had a lower metabolisable energy, DMD and a higher ECR most probably due its higher tannins and soluble NSP content. In addition, Sultan had a lower CP content. The lower metabolisable energy and CP content of these diets may have directly affected growth performance.

Reduced mucin losses (measured as SA) in birds fed cultivar Sultan, may be associated with a reduced number of GIT microflora (Pirgozliev et al., 2008). Redondo et al. (2014) also reported reduced bacterial number in excreta when birds were fed tannin containing diets. However, the birds in this study were not under specific microbial challenge, so gut health benefits from dietary tannin contents were not expected.

Tannins can form complexes with proteins and bind to enzymes, thus tannins may stimulate pancreatic secretion in a manner analogous to that of proteinase inhibitors from legume seeds (Griffiths, 1980), suggesting an explanation on the increased pancreas size in birds fed field bean containing diets compared to the control fed birds in this study. This is in agreement with previous reports that also found an increased pancreas in broilers fed high-tannin diets (Kubena et al., 1983; Ahmed et al., 1991; Abdulla et al., 2015). Thus suggesting that the increase in pancreas weight of birds fed field beans might have been related to higher dietary tannin contents.

The multi enzyme preparation used in this study had not only tannase, but also xylanase, amylase, pectinase and galactosidase activities. The novel aspect of this experiment was to study the effect of the tannase in diets that varied in tannin contents. The control diet was formulated to contain no tannins so no effect of tannase was expected. However, the bean based diets had different tannins contents thus different responses to tannase were expected. A previous report demonstrated that tannase was effective in improving the nutrient availability and performance of broilers fed a diet containing a high tannin field bean sample (Abdulla et al., 2016a, b). No enzyme by diet interaction was observed in the present study and the feeding value of all diets was improved with the same magnitude. Therefore, the potential for tannase alone to improve feeding value of diets was not dependent upon the tannin content and the other enzyme activities, most likely xylanase, may have been more important.

299 The most noticeable response to dietary multi enzyme preparation was in reducing digesta
300 viscosity by 46.5%. High digesta viscosity is usually associated with high content of dietary
301 water-soluble NSP (Choct and Annison, 1992). These NSP have a significant capacity to
302 attract and hold water and could directly interact with water molecules to form a large
303 network or mesh-like structure, thereby increasing the viscosity of digesta. Pectinase, tannase
304 and xylanase are known to have the ability to degrade NSP in plants (Zyla et al., 2000;
305 García-Conesa et al., 2001), thus explaining the observed reduction in ileal digesta viscosity.
306 The detrimental impact of high intestinal viscosity on dietary nutrient digestibility and
307 absorption is well documented (Choct and Annison, 1992). The viscous properties have
308 adverse effects on the diffusion and convective transport of pancreatic enzymes, substrates
309 and the end products of the digestion process (Johnson et al., 1984; Isaksson et al., 1982). An
310 increase in intestinal viscosity associated with enhanced bacterial fermentation can also
311 depress fat digestion (Danicke et al. 1999).
312 The enzyme mixture supplementation improved feed efficiency by 3.5%, an increase that is
313 similar to those reported by Abdulla et al. (2016a, b) in 21 d-old broilers fed field beans
314 containing diet supplemented with a similar enzyme preparation.
315 The weight of pancreas as a percentage of BW decreased with the enzyme mixture
316 supplementation by 7.1%, a decrease that is similar to the 6.4% found by Abdulla et al.
317 (2016a) for broilers of similar age when fed a similar enzyme preparation. Feeding 1000 units
318 of xylanase/kg diet also reduced the weight of the pancreas by 10% (Wu et al., 2004). In
319 addition, Gracia et al. (2003) found a reduced relative weight of pancreas by 17% after adding
320 1720 units of α-amylase/kg diet. This indicates that secretion of pancreatic enzymes might be
321 affected by the concentration of enzymes and substrates or products of their hydrolysis in the
322 lumen of the small intestine following a feedback mechanism (Kubena et al., 1983). Tannins
323 are able to bind to enzymes, reducing their bioavailability (Singh, 1984), thus the destruction

of tannins by tannase may reduce the secretion of pancreatic enzymes. Mahagna et al. (1995) also reported that secretion of pancreatic amylase and proteases was reduced when chicks were fed diets supplemented with amylase and protease. The combination of fiber degrading enzymes used in this study may also improve the availability of substrates trapped by fibers via disrupting the cell wall matrix (Parkkonen et al., 1997) further reducing the needs of pancreatic enzymes.

The weight of the GIT as a percentage of BW decreased with the studied enzyme mixture supplementation by 4.6%, which is similar to the 4.5% and slightly lower that the 6.3% found by Gracia et al. (2003) and Wu et al. (2004), respectively, when feeding α-amylase or a mixture of phytase and xylanase to broilers. The weight of the PG was particularly affected and decreased by 7.1%, a decrease that is similar to the 6.1% reported by Abdulla et al. (2016a) when fed the same enzyme to broilers of similar age. Wu et al. (2004) also reported a reduced weight of the PG by 7.4% when feeding a mixture of phytase and xylanase to broilers. A similar trend was observed by Gracia et al. (2003) after feeding α-amylase to broilers at similar age. The reduction in GIT in birds given enzyme mixture containing diets paralleled the reduction in digesta viscosity and intestinal muscle thickness and the improvement in metabolisable energy, nutrient utilisation and feed efficiency in agreement with Abdulla et al. (2016a). In general, if the efficiency of digestion is consistently suboptimal, whether due to ingredient quality, microbial interaction of anti-nutritive factors, the GIT responds by increasing in both size (surface area) and digestive enzyme output (Bedford, 2006). Birds fed multi enzyme mixture also secreted less mucin thus supporting the view that the reduction in GIT in this experiment might have been related to enhanced efficiency of digestion.

Jejunal morphometry is not always the key factor associated with better function and production in poultry (Wu et al., 2004; Pirgozliev et al., 2010), thus the lack of correlation with productive performance is not surprising.

5. Conclusions

The results from this study show that a commercial enzyme preparation containing tannase, pectinase and xylanase activities proved to be a highly effective in improving dietary available energy, nutrient utilisation, and feed efficiency when fed to chickens. The results also showed that the feeding value of field beans with different tannin contents may vary when fed to broilers although there were no interactions with the enzyme preparation used in the study.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 1. Ingredient composition (g/kg, as-fed) of the experimental broiler chicken balancer and control diet formulations

	Control	Maris Bead	Sultan	Wizard
Wheat	400.0	404.2	404.2	404.2
Bean (Maris Bead)	-	300.0	-	-
Bean (Sultan)	-	-	300.0	-
Bean (Wizard)	-	-	-	300.0
SBM (CP=48%)	190.4	27.0	27.0	27.0
Full fat soya meal	127.0	127.5	127.5	127.5
Maize gluten meal	35.0	35.0	35.0	35.0
Washed sand	119.1	-	-	-
Soya oil	82.5	65.0	65.0	65.0
Lysine	6.0	2.3	2.3	2.3
Methionine	6.8	5.8	5.8	5.8
Threonine	2.4	2.4	2.4	2.4
Monocalcium phosphate	10.0	10.0	10.0	10.0
Limestone	14.0	14.0	14.0	14.0
Salt	2.8	2.8	2.8	2.8
Vitamin/mineral premix	4.0	4.0	4.0	4.0
Total	1000	1000	1000	1000
Calculated values				
ME (MJ/kg)	12.83	13.12	12.65	13.15
Crude protein (g/kg)	221	217	201	216
Ether extract (g/kg)	113	97	97	97
Ca (g/kg)	7.9	8.1	8.2	8.2
Av P (g/kg)	4.4	4.4	4.4	4.4
Total lysine (g/kg)	15.1	12.4	11.8	12.7
Total methionine + cysteine (g/kg)	13.5	8.6	8.4	8.6
Analysed values (as-fed)				
DM (g/kg)	855	877	876	876
GE (MJ/kg)	16.21	17.57	17.52	17.60
CP (g/kg)	197	198	183	197
Ether extract (g/kg)	112	95	95	95
Total phenols[a]	1.312	2.770	3.791	3.084
Tannins[a]	0.452	1.991	2.550	2.159
Condensed tannins[b]	0.00	1.17	1.86	1.53

* Vitamin and mineral premix provided (units · kg–1 feed): µg: retinol 2160, cholecalciferol 75; mg: alpha-tocopherol 25, menadione 1.5, riboflavin 5, pantotenic acid 8, cyanocobalamin 0.01, pyridoxine 1.5, thiamine 1.5, folic acid 0.5, niacin 30, biotin 0.06, I 0.8, Cu 10, Fe 80, Se 0.3, Mn 80, Zn 80. Diets were not supplemented with coccidiostat. The vitamin and mineral premix was supplied by Target Feeds Ltd, Whitchurch, UK.

[a] As tannic acid equivalent

[b] As leucocyanidin equivalent

532 Table 2. Chemical composition of the experimental field bean cultivar samples (DM basis)

	Field beans		
	Maris Bead	Sultan	Wizard
Dry matter (g/kg)	858	856	855
Ether extract (g/kg)	10.5	11.7	10.5
Crude protein (g/kg)	304.5	244.6	299.7
Gross energy (MJ/kg)	18.41	18.27	18.59
Total phenols (g/kg)[a]	6.9	10.9	8.1
Tannins (g/kg)[a]	6.1	8.3	6.8
Condensed tannins (g/kg)[b]	4.5	7.3	6.0

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534 [a] As tannic acid equivalent

535 [b] As leucocyanidin equivalent

536 Note: All data are the results of a chemical analysis conducted in triplicate.

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Table 3. Carbohydrate contents (g/kg DM) of the studied field bean cultivars

Bean cultivar	Fraction	NSP constituent sugars										
		rha	fuc	ara	xyl	man	gal	glu	GlcA	GalA	Total	Total starch
Maris Bead	Soluble sugar	0.9	0.7	7.6	2.8	1.4	4.9	1.5	0.0	10.1	30.0	
	Insoluble sugar	0.2	0.2	12.5	11.4	4.2	3.3	80.9	0.0	12.7	125.5	
	Total sugar	1.1	0.9	20.1	14.3	5.6	8.2	82.3	0.0	22.8	155.4	443
Sultan	Soluble sugar	1.0	0.4	9.7	3.7	2.1	5.4	15.4	0.0	17.1	54.8	
	Insoluble sugar	0.0	0.5	11.4	8.2	4.6	3.1	96.1	0.0	11.6	135.4	
	Total sugar	1.0	0.9	21.0	11.9	6.6	8.5	111.5	0.0	28.7	190.1	467
Wizard	Soluble sugar	0.8	0.5	11.1	3.6	2.0	5.6	4.9	0.0	14.2	42.8	
	Insoluble sugar	0.3	0.4	11.8	15.8	5.0	3.2	101.8	0.0	12.1	150.4	
	Total sugar	1.2	0.9	23.0	19.5	6.9	8.8	106.7	0.0	26.3	193.2	424

Note: All data are the results of a chemical analysis conducted in duplicate.

rha = rhamnose; fuc = fucose; ara = arabinose; xyl = xylose; man = mannose; gal = galactose, glu = glucose; GlcA = glucuronic acid; GalA = galacturonic acid; Total-NSPs = total non-starch polysaccharides.

562 Table 4. Daily feed intake (FI), daily weight gain (WG) and feed conversion efficiency (FCE) ratio of broiler
563 chickens fed the experimental diets.

Treatment factor	FI (g DM/b/d)	WG (g/b/d)	FCE (g:g)
Diet formulation			
Bean (Maris Bead)	75.8[a]	62.9[b]	0.829[b]
Bean (Wizard)	75.7[a]	61.4[ab]	0.811[b]
Bean (Sultan)	76.6[a]	58.5[a]	0.764[a]
Control (no beans)	82.9[b]	67.3[c]	0.812[b]
SEM	1.11	1.11	0.0065
Enzymes			
-	78. 8	62.3	0.790
+	76.7	62.8	0.818
SEM	0.79	0.78	0.0046
p-Value			
Diet formulation	<0.001	<0.001	<0.001
Enzymes	0.069	0.649	<0.001
Diet x Enzymes interactions*	0.921	0.890	0.293

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565 Notes: SEM, Standard error of the mean; p-Value, Comparison of the mean of dietary sources.
566 Each mean represents values from 9 replicate pens of 2 chicks each; Bird performance was determined from 13
567 to 21 d age; There is statistically significant difference between treatments when $P \leq 0.05$.
568 [a,b,c]Values within a column with different superscripts differ significantly at $P \leq 0.05$.
569 * As there were no significant (P>0.05) diet formulation x enzymes interactions only the main treatment factor
570 effects are presented in the table.
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585 Table 5. Dietary available energy and nutrient retention coefficients

Treatment factor	AMEn (MJ/kg DM)	ECR	Retention coefficients GE	DM	NR	FD
Diet formulation						
Bean (Maris Bead)	14.10^{c}	17.01^{b}	0.699^{bc}	0.669^{c}	0.649^{b}	0.744
Bean (Wizard)	14.16^{c}	17.46^{b}	0.705^{c}	0.677^{c}	0.657^{bc}	0.757
Bean (Sultan)	13.74^{b}	18.00^{c}	0.680^{a}	0.644^{b}	0.634^{a}	0.718
Control (no beans)	13.12^{a}	16.21^{a}	0.688^{ab}	0.612^{a}	0.660^{c}	0.750
SEM	0.089	0.176	0.0045	0.0035	0.0031	0.0148
Enzymes						
-	13.50	17.11	0.680	0.639	0.642	0.710
+	14.06	17.23	0.706	0.662	0.658	0.774
SEM	0.063	0.124	0.0032	0.0025	0.0022	0.0105
p-Value						
Diet formulation	<0.001	<0.001	0.001	<0.001	<0.001	0.278
Enzymes	<0.001	0.501	<0.001	<0.001	<0.001	<0.001
Diet x Enzymes interactions *	0.917	0.331	0.771	0.739	0.645	0.949

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587 Notes: AMEn, N-corrected apparent metabolisable energy; GE, gross energy; ECR, energy conversion ratio;
588 DM, Coefficient of total tract dry matter retention; NR, Coefficient of total tract nitrogen retention; FD,
589 Coefficient of total tract fat digestibility; SEM, Standard error of the mean; p-Value, Comparison of the mean of
590 dietary sources.
591 Each mean represents values from 9 replicate pens of 2 chicks each; Dietary DMR, NR and FD were determined
592 between 17 and 21 d age; There is statistically significant difference between treatments when $P \leq 0.05$.
593 ^{a,b,c}Values within a column with different superscripts differ significantly at $P \leq 0.05$.
594 * As there were no significant (P>0.05) diet formulation x enzymes interactions only the main treatment factor
595 effects are presented in the table.
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607 Table 6. Gastrointestinal tract development responses to the experimental diets

Treatment factor	Total GIT (%)	PG (%)	Pancreas (%)	SI (%)
Diet formulation				
Bean (Maris Bead)	7.75[ab]	2.75[a]	0.42[a]	4.59
Bean (Wizard)	7.65[ab]	2.55[b]	0.42[a]	4.68
Bean (Sultan)	7.96[b]	2.76[a]	0.44[a]	4.76
Control (no beans)	7.41[a]	2.33[c]	0.36[b]	4.72
SEM	0.126	0.058	0.01174	0.1070
Enzymes				
-	7.87	2.69	0.42	4.76
+	7.51	2.50	0.39	4.62
SEM	0.089	0.041	0.0083	0.076
p-Value				
Diet formulation	0.025	<0.001	<0.001	0.713
Enzymes	0.007	0.002	0.018	0.192
Diet x Enzymes interactions *	0.764	0.194	0.612	0.617

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609 Notes: GIT (%), Gastrointestinal tract as a proportion of the body weight; PG (%), Proventriculus and gizzard as
610 a proportion of the body weight; SI (%), Small intestine as a proportion of the body weight; SEM, Standard error
611 of the mean; p-Value, Comparison of the mean of dietary sources;
612 Each mean represents values from 9 replicate pens; Gastrointestinal tract development were determined at 21 d
613 old using heavier bird in each pen; There is statistically significant difference between treatments when $P \leq 0.05$.
614 [a,b,c]Values within a column with different superscripts differ significantly at $P \leq 0.05$.
615 * As there were no significant ($P > 0.05$) diet formulation x enzymes interactions only the main treatment factor
616 effects are presented in the table.
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627 Table 7. Ileal digesta viscosity and sialic acid secretion responses to the experimental diets

Treatment factor	cPa	SA (µg/g DM)	Total SA (µg)
Diet formulation			
Bean (Maris Bead)	7.12	1.01[b]	256[a]
Bean (Wizard)	6.82	1.03[b]	255[a]
Bean (Sultan)	6.40	0.94[a]	259[a]
Control (no beans)	8.31	1.03[b]	329[b]
SEM	0.548	0.020	7.8
Enzymes			
-	9.33	1.01	288
+	4.99	1.00	261
SEM	0.387	0.014	5.5
p-Value			
Diet formulation	0.096	0.005	<0.001
Enzymes	<0.001	0.628	<0.001
Diet x Enzymes interactions *	0.940	0.193	0.293

628

629 Notes: cPa, Dynamic ileal digesta viscosity; SA (µg/g DM), Sialic acid concentration in excreta; Total SA (µg),
630 Total sialic acid excretion; SEM, Standard error of the mean; p-Value, Comparison of the mean of dietary
631 sources

632 Each mean represents values from 9 replicate pens; Viscosity of the supernatant (in centipoise (cPa) units) was
633 determined at 21 d old; There is statistically significant difference between treatments when $P \leq 0.05$. [a,b]Values
634 within a column with different superscripts differ significantly at $P \leq 0.05$.

635 * As there were no significant (P>0.05) diet formulation x enzymes interactions only the main treatment factor
636 effects are presented in the table.

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646 Table 8. Jejunum histomorphological variables (μm) responses to the experimental diets

Treatment factor	Muscle thickness	Crypt depth	Villus high	Villus width
Diet formulation				
Bean (Maris Bead)	181[a]	217	1045	185
Bean (Wizard)	197[ab]	210	978	170
Bean (Sultan)	180[a]	222	999	196
Control (no beans)	201[b]	240	1025	185
SEM	6.3	8.3	33.8	10.7
Enzymes				
-	196	222	1015	180
+	184	222	1008	188
SEM	4.4	5.9	23.9	7.5
p-Value				
Diet formulation	0.038	0.072	0.532	0.403
Enzymes	0.061	0.979	0.856	0.435
Diet x Enzymes interactions *	0.525	0.573	0.447	0.765

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648 Notes: SEM, Standard error of the mean; p-Value, Comparison of the mean of dietary sources.

649 Each mean represents values from 9 replicate pens and was determined at 21 d old;

650 There is statistically significant difference between treatments when $P \leq 0.05$.

651 [a,b]Values within a column with different superscripts differ significantly at $P \leq 0.05$.

652 * As there were no significant (P>0.05) diet formulation x enzymes interactions only the main treatment factor

653 effects are presented in the table.