Exogenous tannase improves feeding value of a diet containing field beans (Vicia faba) when fed to broilers

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Exogenous tannase improves feeding value of diet containing field beans (*Vicia faba)* when fed to broilers

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Abstract 1. A total of 72 male Ross 308 broilers were used in a study to investigate the effect of dietary tannase on apparent metabolisable energy (AME), coefficients of dry matter (DMR) and nitrogen (NR) retention and fat digestibility (FD) of a diet containing 300g/kg field beans (*Vicia faba*). Growth performance variables and gastrointestinal tract development were also measured.

2. Two treatments were used in this study: control (C) and C + 3400 tannase units (TU) per kg feed. Diets were formulated to be nutritionally adequate with the exception that the AME was lower than recommended (12.65 MJ/kg vs 12.97 MJ/kg, respectively).

3. Inclusion of tannase increased AME by 0.4 MJ/kg DM ($P<0.05$). Tannase supplementation improved dietary DMR ($P<0.05$), NR (<0.001) and FD ($P<0.05$) by 2.8, 3.2 and 6.5%, respectively.

4. Birds fed tannase had 4.4% reduction in feed intake and 2.6% improvement in gain to feed ratio ($P<0.05$). Compared to control diet, birds fed tannase had reduced relative to body weight (%BW) proventriculus and gizzard and pancreas weights, 3.29 *vs* 3.09% and 0.47 *vs* 0.44%, respectively.

5. The mechanisms of action of the studied enzyme require further elucidation.

Global demand for dietary protein has recently led to an unstable increase in the supply and price of soybean. In addition, the use of soybeans has led to consumer resistance because much of it comes from genetically modified crops unsuitable for use in organic farming (Vicenti et al., 2009). These circumstances have stimulated research on alternative protein sources, especially high-protein legumes, free of genetic modifications that can satisfy the protein requirements (Ravindran et al., 2010; Laudadio et al., 2011). The content of antinutrients in field beans (*Vicia faba*), primarily polysaccharides and tannins, are the main reason reduced nutrient digestibility and growth performance of broilers fed field bean based diets (Longstaff and McNab, 1991). Although the beneficial effect of feeding fibre degrading enzymes to legume containing diets is known (Castanon and Marquardt, 1989; Cowieson et al., 2004) there is lack of information on the effect of tannin degrading enzymes on feeding value of field beans for broilers.

The main objective of this experiment, therefore, was to determine the effect of supplementary tannase, an enzyme that hydrolyses tannins, on dietary metabolisable energy, nutrient utilisation and gastrointestinal tract development. The overall feed intake, weight gain and feed conversion efficiency of the birds were also measured.

MATERIALS AND METHODS

Materials and methods

Field bean sample

A UK grown field bean sample of cultivar Sultan from 2013 harvest year was used in a broiler feeding experiment. This field bean cultivar was selected for the experiment because of its relatively high content of hydrolysable tannins and low metabolisable energy (Abdulla et al., 2015). Before the animal feeding experiment, the sample was hammer-milled using a 4

mm screen and then mixed in a horizontal mixer with the other feed ingredients. Freshly milled sample in duplicate was used for analyses and in the feeding study to avoid spoilage.

The gross energy (GE) of the bean samples was determined using a bomb calorimeter (Parr Instrument Company, Moline, IL). Nitrogen was determined by the combustion method (AOAC, 2000) using a LECO (FP-528 N, LECO Corp., St. Joseph, MI). The crude protein (CP) values were obtained as N x 6.25. Oil (as ether extract) in the bean sample was extracted with diethyl ether by the ether extraction method (AOAC, 2000) using a Soxtec system (Foss UK Ltd.). The contents of non-starch polysaccharides (NSP), hydrolysable tannins (HT) and trypsin inhibitors (TI) in the experimental field bean sample were determined by the methods of Englyst et al. (1994), Makkar et al. (1993) and Smith et al. (1980), respectively.

Diet preparation

A control diet (C) containing 300 g/kg field bean sample was prepared (Table 1). The diet was then split into two batches and one of them was supplemented with 3400 units/kg (TU) of propriety tannase (Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland) resulting in diet CT. The enzyme had also 6220 units/kg of pectinase and less than 200 units/kg of phytase activity. The enzyme preparation was based on tannase produced by *Aspergillus niger*. The enzyme was in a liquid form and 17ml/kg was sprayed on the top of diet CT. The dry matter content of diet C was adjusted by spraying of 17ml water per kg of diet. After spraying the diets were thoroughly mixed in a horizontal mixer.

Birds, husbandry and sample collection

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

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 d of age. On the first day of the experimental period (at 7 d of age), the chicks were individually weighed and assigned to one of the experimental pens. Two birds were placed in each pen (0.4m X 0.4m solid floor area) within a controlled environment room. Each diet was fed at random to 16 pens from 7 to 21d age. Room temperature and lighting program followed commercial recommendations (Aviagen Ltd., Edinburgh, UK). Access to the mash form feed and the water was *ad libitum*.

During the last four days of the experiment, from 17 to 21 d age, the solid floor of each pen was replaced with a wire mesh. All excreta were collected daily and refrigerated. On the last collection the samples from each pen were pooled, the total amount was immediately dried at 60◦C and then milled. Representative samples of dry and milled excreta were taken for analyses. Feed intakes were also measured for the same period.

On the last day of the study, at 21d age, the two birds in each pen were weighed and killed by cervical dislocation. The empty weights of gastrointestinal tract (GIT) segments, including proventriculus and gizzard (PG), pancreas and small intestine, of each bird were determined, according to the procedures described by Amerah and Ravindran (2008). The weights of the segments were presented as relative to BW (% BW).

Metabolisable energy and nutrient utilisation determination

Excreta were oven-dried in forced draft oven at 60°C to constant weight, weighed, and milled to pass through a 0.75 mm mesh. The gross energy, nitrogen and oil in feed and excreta were determined as for the field bean sample. The dietary N-corrected apparent metabolisable energy (AMEn) was calculated as described by Hill and Anderson (1958). The coefficients of total tract fat dry matter (DMR) and nitrogen retention (NR), and fat digestibility (FD) were

determined as the difference between the respective nutrient intake and nutrient excreted divided by the intake.

STATISTICAL ANALYSES

Statistical analyses were performed using the Genstat statistical software package (Genstat 15th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The metabolisable energy content of the experimental diets, broiler growth performance, and nutrient utilisation were compared statistically by ANOVA. In all instances, differences were reported as significant at $P < 0.05$.

RESULTS AND DISCUSSION

The determined chemical composition of the Sultan cultivar field bean sample contained 856g/kg dry matter, 18.27 MJ/kg GE, 245 g/kg CP, 12 g/kg oil, 190 g/kg total NSP (135g non-soluble and 55 g soluble), 12.3 mg/g HT and 2.3 mg/g TI, respectively (results are presented on dry matter basis). All birds were healthy throughout the study period and there was no mortality. There was no effect of treatment on final body weight and weight gain of the birds (Table 2). Birds fed diet TC had reduced feed intake (FI) but improved feed conversion efficiency (FCE) ($P<0.05$) compared to the control fed birds.

Exogenous tannase supplementation improved dietary AMEn by 0.4 MJ/kg compared to the control ($P<0.05$) (Table 3). Similarly, tannase supplementation resulted in improved DMR, NR and FD ($P<0.05$).

Birds fed tannase had reduced the relative proventriculus and gizzard weight, and also reduced the relative pancreas weights compared to birds fed the control diet ($P<0.05$) (Table 4). There was no effect ($P>0.05$) of dietary treatment on the relative weight of the small intestine.

The study evaluated the efficacy of supplementary tannase enzyme on growth performance, energy and nutrient utilisation and GIT development when field bean diet was fed to broilers. The data demonstrate that young broilers are sensitive to dietary supplementation with exogenous tannase. There are no previous published studies of dietary tannase supplementation in broiler feeds. However, there are published reports on the negative impact of high dietary tannin on the studied variables (Jansman, 1993; Brufau et al., 1998; O'Neill et al., 2012).

The diets were relatively high in HT content from the Sultan field bean cultivar inclusion (approximately 3.5 g/kg diet). The growth of the birds did not differ between diets and was in the expected range for broilers reared in similar environment and fed mash diets (Karadas et al., 2014; Pirgozliev et al., 2015a, 2015b). In agreement with improved AMEn and nutrient utilisation, birds fed tannase supplemented diet had an improved FCE. This is in line with Kubena et al. (1983) who reported reduced feed efficiency when high tannin diets were fed to poultry.

Tannins are able to form complexes with proteins, so they can also bind to enzymes, which have implications for their biological activity. It has been reported that high-tannin inclusion reduces the activities of all digestive enzymes in various *in vitro* and *in vivo* assays (Griffiths, 1979; Griffiths & Moseley, 1980; Singh, 1984). This supports the increased nutrient utilisation coefficients in the recent report suggesting that exogenous tannase was able to hydrolise at least part of the dietary tannins and alleviate their negative impact observed in other studies. This is in line with the observed improved AME, N and amino acid digestibility of broilers when fed diets low in tannin compared to high tannin diets (Nyachoti et al., 1996; Brufau et al., 1998; O'Neill et al., 2012).

Kubena et al. (1983) and Ahmed et al. (1991) also found an increased pancreas in broilers fed high-tannin diets. Similar to trypsin inhibitors, tannins are also able to 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 reduced pancreas size in birds fed tannase in this study.

Kubena et al. (1983) found that the weights of PG of birds fed high tannin feed (15 g tannic acid per kg diet) was lower compared to the control fed birds. This is the opposite of our findings that reducing dietary tannin (via supplementing diets with tannase) reduced the relative PG weights of birds. In the present study the diets contained about 3.5 g/kg hydrolysable tannins (measured as tannic acid), although Kubena et al. (1983) had 15 g tannic acid per kg diet.

In conclusion supplementation of field bean-based diets with tannase enzyme improved feed efficiency, dietary metabolisable energy and nutrient utilisation. Although the beneficial effects associated with tannase treatment were in line with pancreatic size reduction, it is possible that the pectinase activity in the tannase preparation may have also influenced the responses of the birds. The mechanisms require further elucidation.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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Table 1. *Diet formulation (g/kg 'as-fed') of the diets*

Ingredient	
Wheat	404.2
Soybean meal (480 g/kg CP)	27.0
Full-fat soybean	127.5
Maize gluten meal	35.0
Field bean	300.0
Soya oil	65.0
Lysine HCl	2.3
DL Methionine	5.8
L Threonine	2.4
Monocalcium phosphate	10
Limestone	14.0
Salt	2.8
Vitamin-trace mineral premix*	4.0
	1000
Calculated nutrient composition	
ME (MJ/kg)	12.65
Protein (g/kg)	212
Lysine (g/kg)	12.4
Met + Cys (g/kg)	9.4
Ca (g/kg)	8.2
P non-phytate (g/kg)	4.0
Determined nutrient composition	
Gross energy (MJ/kg DM)	18.34
Protein (g/kg)	171
Fat (g/kg)	132
Dry matter (g/kg)	885

* Vitamin and trace-mineral premix provided per kg diet: 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

Table 2. *The effect of experimental diets on growth performance of broilers*

	FI (g DM/b/d)	WG (g/b/d)	FCE (g:g)
Control	59.0	53.5	0.906
Tannase	56.4	52.4	0.930
SEM (df=31)	0.74	0.50	0.0070
P	0.025	0.165	0.031

Each mean represents values from 16 replicate pens of 2 chicks each; Bird performance was determined from 7 to 21 d age; There is statistically significant difference between treatments when $P \leq 0.05$.

Table 3. *The effect of experimental diets on apparent metabolisable energy (AME), dry matter (DMR), and nitrogen (NR) retention, and fat digestibility (FD)*

	AMEn (MJ/kg DM)	DMR	NR	FD
Control	13.20	0.641	0.634	0.744
Tannase	13.60	0.659	0.654	0.792
SEM (df=31)	0.079	0.0039	0.0033	0.0107
P	0.003	0.006	<0.001	0.007

Each mean represents values from 16 replicate pens of 2 chicks each; Dietary AME, DMR, NR and FD were determined between 17 and 21 d age; There is statistically significant difference between treatments when $P \leq 0.05$.

Table 4. *The effect of experimental diets on broilers gastrointestinal tract development (data presented as relative to BW (%BW))*

	Total GIT (% BW)	P&G (% BW)	SI (% BW)	Pancreas (% BW)
Control	9.39	3.29	5.64	0.47
Tannase	9.26	3.09	5.73	0.44
SEM (df=31)	0.102	0.048	0.104	0.008
P	0.363	0.012	0.565	0.042

Each mean represents values from 16 replicate pens; Gastrointestinal tract development were determined at 21 d old using the bigger birds in each pen; There is statistically significant difference between treatments when $P \leq 0.05$. GIT – gastrointestinal tract; P&G – proventriculus and gizzard; SI – small intestine.

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