Nutritional value of raw and micronized field beans (*Vicia faba* L. var. *minor*) with and without enzyme supplementation containing tannase for growing chickens


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Nutritional value of raw and micronized field beans (*Vicia faba* L. var. *minor*) with and without enzyme supplementation containing tannase for growing chickens

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ABSTRACT

An experiment examined the effects of two field bean cultivar samples with different tannin contents, the effect of heat treatment (micronizing), and the effect of dietary enzyme containing tannase, pectinase and xylanase activities on N-corrected dietary apparent metabolisable energy (AMEn), coefficients of total tract dry matter (DMD) and ether extract digestibility (EDD), nitrogen retention (NR), tannin degradability, gastrointestinal tract (GIT) development, and endogenous mucin losses excretion in broiler chickens. A control diet was prepared that contained 221 g/kg crude protein and 12.83 MJ/kg metabolizable energy. Four additional diets containing 300 g/kg of each of two untreated or micronized experimental field bean cultivar samples were also mixed. Each diet was then split into two batches and one of them was supplemented with 3400 units/kg of proprietary tannase enzyme resulting in ten diets in total. Each diet was fed to seven pens that contained two randomly selected male broilers. Birds fed the high tannin bean sample had a lower weight gain (P<0.001), and a
lower determined metabolisable energy (P<0.05), and DMD (P<0.001) but a higher tannin
degradability (P<0.001). Compared to the control diet, feeding field beans increased
(P<0.001) the weights of the proventriculus and gizzard of the birds, and also increased
endogenous mucin losses (P<0.05). Supplementing diets with tannase-containing enzyme
improved dietary AMEn (P<0.001), DMD (P<0.001), NR (P<0.001) and DEE (P<0.05), but
did not change (P>0.05) tannin digestibility. Heat treatment of the beans reduced the
degradability of condensed tannins and increased endogenous mucin losses (P<0.05). This
experiment has shown that there are differences in the feeding value of different field bean
samples and these are not improved by heat treatment. Enzyme supplementation improved
the feeding value of all diets regardless of the bean samples or heat treatment (no treatment
factor interactions, P>0.05). Further research is warranted to study the effectiveness of
tannase supplementation in poultry diet formulations by dose response trials with purified
tannase preparations.

Field bean; tannase; heat treatment; broiler chicken; ME; digestibility
1. Introduction

Grain legumes, including field beans (*Vicia faba* L. var. *minor*), are considered possible alternative protein sources to soybean meal because of the similarity of their amino acid profiles (Wiryawan and Dingle, 1999; Gatta et al. 2013). Large amount of field beans can be produced in many parts of Europe because of their adaptation to the climate in addition to their cultivar diversity that allows them to be cultivated in winter and spring (Crépon et al. 2010; Duc et al. 1999). The poultry industry has been reluctant to use field beans in diet formulations due to the presence of antinutritional factors including oligosaccharides, soluble non-starch polysaccharides (NSP) and tannins (Longstaff and McNab, 1991a,b). Field beans also contain some pyrimidine glucosides (vicine and covicine) that reduce egg size in laying hens (Mateos and Puchal, 1981). However, the antinutritional influence of vicine and covicine in broilers is not consistent (Grosjean et al., 2000; Metayer et al., 2004; Vilarino et al., 2009). In order to alleviate the negative impact of antinutritional factors in field beans, different practices with various successes have been suggested, including genetic selection, mechanical processing, heat treatments, and exogenous fibre degrading enzyme supplementation (Van der Pole et al. 1991; Cowieson et al. 2003; Woyengo and Nyachoti, 2012).

Recent research in our laboratory (Abdulla et al. 2016a,b) found that exogenous tannase can also improve feeding value of field beans in diets for broilers. However, there is a lack of knowledge on the interaction with bean cultivar sample, and whether the bean sample has been heat treated.

The main objective of this experiment, therefore, was to determine the effect of heat treatment (micronizing) and exogenous tannase on dietary metabolisable energy, nutrient utilisation, and gastrointestinal tract development when feeding diets containing two different
field bean cultivar samples 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 two UK grown field bean samples that were fed either as raw or as micronized to broiler chickens. The two field bean samples used in the study were Maris Bead (Spring cultivar) and Sultan (Winter cultivar). Both cultivar samples were produced in the UK during 2013 harvest year, and were stored in porous synthetic bags at ambient air temperatures in a dark, dry store. The samples were chosen because of their different tannin contents, although there were differences in their proximate composition. The stored field bean samples did not experience any freezing temperatures during this period. The bean samples were milled through a 4 mm screen. Each sample was then split on two and half of it was micronized (130°C, 90 sec, 2 microns wave length; Heraeus Noblelight GmbH, Germany).

2.2. Diet preparation

Birds were fed one of ten mash diets. A control diet was prepared that had major ingredients of 404.2 g/kg wheat and 127.5 g/kg soybean meal (SBM), and contained 221 g/kg CP and 12.83 MJ/kg metabolizable 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 four diets containing 300 g/kg of each of two untreated or micronized 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 (Table 1).

Each diet was then split into two batches and one of them was supplemented with the proprietary tannase (Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland) resulting in ten diets in total. The determined enzyme activities of the proprietary tannase were; tannase 3400 units / kg, pectinase 6220 units/kg; xylanase 6100 units/kg, and there were some additional amylase and alpha-galactosidase activities. The enzyme preparation was based on tannase produced by *Aspergillus niger* in a submerged fermentation methodology. The enzyme was in a liquid form and 17ml/kg was sprayed on the top of diets. The dry matter content of non-supplemented diets was adjusted by spraying of 17ml water per kg of diet. Additional water was added to diets containing micronized beans to adjust for the water loss during heat treatment. The diets were thoroughly mixed in a horizontal mixer.

### 2.3. Animal husbandry, determination of dietary metabolisable energy, nutrient utilisation, tannin degradability, endogenous mucin losses and comparison of broiler growth performance

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

One hundred and forty male Ross 308 broiler chickens in total were obtained from a commercial hatchery. During the pre-study period, from day old to 6 days of age, the birds were reared in a single floor pen and fed a proprietary wheat-based diet without coccidiostats or antimicrobial growth promoters, or other similar additives. At the beginning of the study, at 7 days of age, 140 chicks were allocated to 70 small pens with 0.160 m² solid floors area,
two birds in each pen. Feed and water was offered ad libitum to birds throughout the experimental period. Each diet was offered to birds in 7 pens in a randomised block design. Information on growth and feed intake was obtained from 7 to 16 days of age. The temperature was kept at 29°C at 7d age and was gradually reduced to 22°C at the end of the 10 d feeding period (16 days of age). The light regimen was 18 h light and 6 h dark. At 12 days of age, the solid floor of each pen was replaced with a wire mesh and excreta samples were collected for four consecutive days from each pen, immediately dried at 60°C and then milled for further analyses. The feed intake for the same period was also measured. The gross energy, dry matter, nitrogen, and fat of each dried excreta sample and the experimental diets were determined as described in Chapter 2.5. The AMEn of the diets was calculated as described by Hill and Anderson (1958). The coefficients of total tract ether extract (DEE) and dry matter (DMD) digestibility, and nitrogen retention (NR) were determined as the difference between intake and excretion of the nutrient divided by its respective intake. The degradation in the GIT of tannins was described as tannin degradability (TD), when tannins were presented as tannic acid equivalent, and as condensed tannin degradability (CTD), when tannins were presented as leucocyanidin equivalent. The endogenous mucin losses in excreta were measured using the concentration of the sialic acid (SA) as a marker, following the periodate-resorcinol method (Jourdian et al. 1971).

2.4. Gastrointestinal tract development

At the end of the experiment, at 16 day of age, all birds were killed by cervical dislocation and weighed. The empty and relative weights of GIT segments from proventriculus to caeca of the heavier bird in each pen were also determined according to the procedure used by Amerah and Ravindran (2008).

2.5. Proximate analysis of samples
Dry matter (DM) was determined by drying samples in a forced draft oven at 105°C to a constant weight. Crude protein (6.25 X N) in samples was determined by dry combustion method (AOAC, 2000) 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), using a Soxtec system (Foss UK Ltd.). The gross energy (GE) value of the samples was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL), and benzoic acid was used as the standard. Total starch (TS) was determined following the method of Englyst et al. (2000). The non-starch polysaccharides (NSPs) 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, non-tannin phenol, total tannin (all as tannic acid equivalent) in the representative samples of excreta, as well as freshly milled raw and micronized studied field bean cultivars, the control diet and other feed ingredients were determined by applying the procedure used by Makkar et al. (1993). The condensed tannins in the same samples were determined as leucocyanidin equivalent as described by Porter et al. (1985).

2.6. Statistical analysis

The experiment was arranged as a randomised block analysis of variance with 10 treatments each with 7 replicates. The treatments were arranged 2 x 2 x 2 factorial with a further two specific orthogonal contrasts for the control diets. The 2 x 2 x 2 factorial arrangement had field bean cultivar (Maris Bead or Sultan), enzyme (with and without tannase) and micronizing (with and without). The first specific orthogonal contrasts was Control 1 (no enzyme) vs Control 2 (with enzyme), and the second contrast was mean of all bean diets vs
mean of the two control diets. In all instances, differences were reported as significant at P ≤0.05. Tendencies towards significance (0.05<P ≤0.1) were also reported.

3. Results

Overall, with the exception of total starch content, Maris Bead contained higher nutrient and lower anti-nutrient comparing to Sultan field bean cultivar, and the crude protein content (CP) was more variable than the oil and GE. Crude protein varied from 244.6 (Sultan) to 304.5 (Maris Bead) g/kg DM. The total phenols and tannins, as tannic acid equivalent, and condensed tannins, as leucocyanidins, differ from 6.9 to 10.9, 6.1 to 8.3, and 4.5 to 7.3 g/kg DM for Maris Bead and Sultan (Table 2). Micronizing slightly reduced the tannin contents of the beans. The carbohydrate content of the field bean samples has been illustrated in table 3, as Sultan contained more carbohydrates than Maris Bead. The total starch concentration, as g/kg DM, was 443 and 467, the total NSPs 155.4 and 190.1 including 30.0 and 54.4 soluble and 125.5 and 135.4 insoluble sugars in Maris Bead and Sultan, respectively. Glucose, galacturonic acid, arabinose, xylose, galactose and mannose were the main NSP constituent sugars in the field bean samples.

The birds fed field bean diets had a lower daily feed intake (P<0.001), and weight gains (P<0.001) than the birds fed the control diets (Table 4). Bean based diets had lower NR (P<0.001), and DEE (P=0.009), but a higher determined AMEn (P<0.001) compared to the control diet.

Changes in DMD followed the same directions as metabolisable energy (table 4).

Tannase supplemented diets had higher metabolisable energy (P<0.001) compared to un-supplemented diets (table 4). For some reasons non tannase supplemented control diet had higher NR (P=0.004) than supplemented diet, but no difference (P>0.05) in DEE was
observed. Overall, tannase supplemented diets had higher NR (P<0.001) and DEE (P=0.002), than un-supplemented diets.

Birds fed Maris Bead had a higher daily weight gain (P<0.001), and a higher determined metabolisable energy (P<0.05) compared to those fed Sultan. There was a three way interaction (bean x enzyme x micronizing; P=0.033) for FCR, as diet containing non-micronized Maris Bead with tannase had a lower FCR although the response of the rest of the diets was inconsistent.

There was bean by micronizing interaction (P=0.043) in TD, as the TD for Maris Bead was reduced with micronizing although no changes were observed for Sultan.

Maris Bead based diets had lower CTD (P<0.001), that Sultan based diets. Micronized diets had lower CTD (P<0.001), than non-micronized diets.

The results on endogenous mucin losses secretion, measured as SA, in excreta responses to the experimental diets have been summarised in table 5. The SA concentration was reduced in bean containing diets (P=0.042), Sultan based diets (P=0.009) and in non-micronized diets (P=0.034), compared to controls, Maris Bead and micronized diets, respectively (table 5).

The weight of the TGI was reduced by feeding Sultan compared to Maris Bead containing diets (P=0.018) and tannase supplemented compared to none supplemented diets (P=0.020). When expressed as a percent from the body weight the GIT was increased by feeding bean containing diets compared to controls (P<0.001), Sultan compared to Maris Bead based diet (P=0.011) and enzyme non-supplemented compared to those with tannase (P=0.003).

The weight of the PG was increased by feeding bean containing diets compared to controls (P=0.010) and when compare enzyme free to tannase supplemented diets (P=0.003). Similarly, the PG% was increased by feeding bean containing diets compared to controls (P<0.001), Sultan compared to Maris Bead based diet (P=0.031) and non-supplemented compared to tannase supplemented diets (P=0.001).
The weight of the SI was reduced by feeding bean containing compared to control diets (P<0.001) and Sultan compared to Maris Bead containing diet (P=0.003). For SI% only tendencies were observed.

The weight of the pancreas was not affected (P>0.05) by any of the treatments. However, the Pan% was increased by feeding bean containing diets compared to controls (P<0.001).

4. DISCUSSION

The purpose of the experiment reported in this paper was to determine whether heat treatment (micronizing) of field beans and exogenous tannase could be used to improve available energy and nutrient utilisation in diets for broilers. It was important to evaluate these treatments using different 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 a higher tannin content compared to Maris Bead sample. Tannins can form strong complexes with proteins, starch, cellulose, and minerals (Lekha and Lonsane, 1997). However, Sultan also had a lower AMEn, most probably due its higher NSP content, than Maris Bead. In addition Sultan has a lower CP content. The lower metabolisable energy and CP content of these diets may have directly affected growth performance. Reduced mucin endogenous losses in birds fed cultivar Maris Bead compared to Sultan could be associated with a reduced irritation of the gut due to lower dietary tannin content.

The experiment showed that there were no differences in nutritional value between the raw and heat treated field beans. Alonso et al. (2000) demonstrated that heat treatment (extrusion) gave a two-fold reduction in CT in faba beans. However, in the present study heat treatment
only gave approximately 9% reduction in CT. However, there is a difference between the process of autoclaving and micronizing, as extruding requires higher temperature, some water and relatively more time, compared to micronizing (Lashkari et al. 2015). The reduced CTD of micronized diets, and the observed interactions where micronizing reduced feed efficiency and TD of Maris Bead based diet only, were not expected. Bellido et al. (2006) reported that micronizing legumes, e.g. cowpea flour, at 130 ºC changed its functional properties, including reduced foaming capacity, increase in the surface hydrophobicity and cross-linking of the protein, formation of disulphide bonds and possibly Maillard cross-links. It is possible that the two cultivar samples reacted differently to the heat treatment applied in this experiment.

Abdulla et al. (2016a) showed that exogenous tannase was effective in improving the nutrient availability and performance of broilers fed a diet containing field beans. It was expected that the efficacy of tannase would be limited in the control diet as it was a low tannin feed. The two field bean containing diets had different tannin contents thus different responses between these two diets to tannase was also expected. However, a part from the interaction for FCR, no other enzyme by diet interactions were observed in the present study, thus showing that exogenous tannase improved the feeding value of all diets with the same magnitude. In addition tannase supplementation did not influence tannin degradability. Chamorro et al. (2015) found no effect of tannase on growth performance in chickens fed diet rich in polyphenols. The tannase used in the present experiment also had alpha-amylase, xylanase, and pectinase activities. It is possible that these enzyme activities may have been partially responsible for the observed improvements in nutrient availability and feed efficiency in the study.

The most noticeable response to dietary tannase was in increasing DEE by 7.1%, followed by 4.4% for dietary metabolisable energy and DMD, and by 2.9% for dietary N retention. The
results are similar to those reported by Abdulla et al. (2016b). Although there was an increased dietary N retention when tannase was fed, N retention is influenced not only by protein digestibility, but also by metabolic N excretion (Souffrant, 2001). It is generally accepted that part of the anti-nutritional effect of field beans is also mediated by its NSP constituents (Longstaff and McNab, 1991a,b; Nalle et al. 2010) that raise the viscosity of gut contents and may alter the microflora (Smits et al. 1998; Langhout et al. 1999). An increase in intestinal viscosity associated with enhanced bacterial fermentation can also depress fat digestion (Danicke et al. 1999).

The weight of the GIT decreased with tannase supplementation by 6.0%, which is in the range of values reported by Gracia et al. (2003) (4.0%) and Wu et al. (2004) (7.9%), when feeding α-amylase or a mixture of phytase and xylanase to broilers. The weight of the PG was particularly affected and decreased by 8.9%, a decrease that is in similar range (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 changes in GIT expressed as % of the weight of the birds were similar to the absolute values. 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).

5. Conclusion

The results from this study demonstrate that there can be large differences in the nutritional value of different field bean samples that are available to the poultry feed industry. Application of heat treatment (micronizing) did not improve the nutritional value of either
bean sample, but other heat treatment processes such as extrusion may be more effective. Addition of a commercial tannase enzyme preparation (that additionally had alpha-amylase, xylanase, and pectinase activities) proved to be a highly effective in improving dietary available energy and nutrient utilisation in chickens. Further research is warranted to elucidate the effectiveness of tannase supplementation in poultry diet formulations by dose response trials with purified tannase preparations. Similarly, more research is needed on the temperature and the processing time applied to field beans.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References


Longstaff MA, McNab JM. 1991b. The effect of concentration of tannin-rich bean hulls (Vicia faba L.) on activities of lipase (EC 3.1.1.3) and α-amylase (EC 3.2.1.1) in digesta and pancreas and on the digestion of lipid and starch by young chicks. Brit J Nutr. 66:139-147.


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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maris beads</th>
<th>Sultan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>400.0</td>
<td>404.2</td>
<td>404.2</td>
</tr>
<tr>
<td>Maris beads</td>
<td>-</td>
<td>300.0</td>
<td>-</td>
</tr>
<tr>
<td>Sultan</td>
<td>-</td>
<td>-</td>
<td>300.0</td>
</tr>
<tr>
<td>SBM (CP=48%)</td>
<td>190.4</td>
<td>27.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Full fat Soya meal</td>
<td>127.0</td>
<td>127.5</td>
<td>127.5</td>
</tr>
<tr>
<td>Maize gluten meal</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Washed sand</td>
<td>119.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soya oil</td>
<td>82.5</td>
<td>65.0</td>
<td>65.0</td>
</tr>
<tr>
<td>L-Lysine-HCL</td>
<td>6.0</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>6.8</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>14.0</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Salt</td>
<td>2.8</td>
<td>2.8</td>
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</tr>
<tr>
<td>Vitamin/mineral premix</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Calculated values

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Maris beads</th>
<th>Sultan</th>
</tr>
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<tbody>
<tr>
<td>ME (MJ/kg)</td>
<td>12.83</td>
<td>13.12</td>
<td>12.65</td>
</tr>
<tr>
<td>CP</td>
<td>221</td>
<td>217</td>
<td>201</td>
</tr>
<tr>
<td>Fat</td>
<td>113</td>
<td>97</td>
<td>97</td>
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</table>

Analysed values (as-fed)

<table>
<thead>
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<th></th>
<th>Control</th>
<th>Maris beads</th>
<th>Sultan</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>855</td>
<td>877</td>
<td>876</td>
</tr>
<tr>
<td>GE (MJ/kg)</td>
<td>16.21</td>
<td>17.57</td>
<td>17.52</td>
</tr>
<tr>
<td>CP</td>
<td>197</td>
<td>198</td>
<td>183</td>
</tr>
<tr>
<td>Fat</td>
<td>112</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Total phenols(^a)</td>
<td>1.31</td>
<td>2.76 (2.66)</td>
<td>3.78 (3.63)</td>
</tr>
<tr>
<td>Tannins(^a)</td>
<td>0.45</td>
<td>1.98 (1.77)</td>
<td>2.54 (2.42)</td>
</tr>
<tr>
<td>Condensed tannins(^b)</td>
<td>0.00</td>
<td>1.15 (0.95)</td>
<td>1.86 (1.54)</td>
</tr>
</tbody>
</table>

\(^a\) Vitamin and mineral premix provided (units · kg\(^{-1}\) feed): \(\mu g\): 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

\(^b\) As leucocyanidin equivalent

The contents of total phenols, tannins and condensed tannins in the ingredients of diets containing field beans was 1.42 g/kg, 0.60 g/kg and 0.00 g/kg, respectively.
Table 2. Chemical composition of the experimental field bean cultivar samples (DM basis)*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Field bean cultivar</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maris Bead</td>
<td>Sultan</td>
</tr>
<tr>
<td>Dry matter (g/kg)</td>
<td>854 (883)</td>
<td>851 (887)</td>
</tr>
<tr>
<td>Ether extract (g/kg)</td>
<td>10.5</td>
<td>11.7</td>
</tr>
<tr>
<td>Crude protein (g/kg)</td>
<td>304.5</td>
<td>244.6</td>
</tr>
<tr>
<td>Gross energy (MJ/kg)</td>
<td>18.41</td>
<td>18.27</td>
</tr>
<tr>
<td>Total phenols (g/kg)*</td>
<td>6.9 (6.3)</td>
<td>10.9 (9.9)</td>
</tr>
<tr>
<td>Tannins (g/kg)*</td>
<td>6.1 (5.1)</td>
<td>8.3 (7.5)</td>
</tr>
<tr>
<td>Condensed tannins (g/kg)*</td>
<td>4.5 (3.6)</td>
<td>7.3 (5.8)</td>
</tr>
</tbody>
</table>

*As tannic acid equivalent

*As leucocyanidin equivalent

*Note: The information in brackets is for the micronized bean samples; all analyses were performed in triplicate.
Table 3. Carbohydrate contents (g/kg DM) of the studied field bean cultivars*

<table>
<thead>
<tr>
<th>Bean cultivar</th>
<th>Fraction</th>
<th>Maris Bead</th>
<th>Sultan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble sugar</td>
<td>Insoluble sugar</td>
<td>Total sugar</td>
</tr>
<tr>
<td>NSP constituent sugars</td>
<td>Glucose</td>
<td>1.5</td>
<td>80.9</td>
</tr>
<tr>
<td></td>
<td>Galacturonic acid</td>
<td>10.1</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>Arabinose</td>
<td>7.6</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Xylose</td>
<td>2.8</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>4.9</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Mannose</td>
<td>1.4</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Rhamnose</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Fucose</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Total NSPs</td>
<td>30.0</td>
<td>125.5</td>
<td>155.5</td>
</tr>
<tr>
<td>Total starch</td>
<td>443</td>
<td>467</td>
<td></td>
</tr>
</tbody>
</table>

*Note: All data are the results of a chemical analysis conducted in duplicate.
Total-NSPs = total non-starch polysaccharides.
Table 4. Performance, dietary available energy, nutrient and tannin retention coefficients*
<table>
<thead>
<tr>
<th>Diet</th>
<th>FI (DM g/b)</th>
<th>WG (g/b)</th>
<th>FCR</th>
<th>AME a (MJ/kg DM)</th>
<th>DMD</th>
<th>NR</th>
<th>DEE</th>
<th>TD</th>
<th>CTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>39.7</td>
<td>28.9</td>
<td>1.377</td>
<td>12.66</td>
<td>0.611</td>
<td>0.678</td>
<td>0.758</td>
<td>0.362</td>
<td>0.483</td>
</tr>
<tr>
<td>2 Maris Beads raw</td>
<td>40.7</td>
<td>31.0</td>
<td>1.314</td>
<td>12.67</td>
<td>0.614</td>
<td>0.653</td>
<td>0.737</td>
<td>0.281</td>
<td>0.483</td>
</tr>
<tr>
<td>3 Sultan raw</td>
<td>36.8</td>
<td>26.6</td>
<td>1.386</td>
<td>12.95</td>
<td>0.642</td>
<td>0.629</td>
<td>0.695</td>
<td>0.351</td>
<td>0.504</td>
</tr>
<tr>
<td>4 Maris Beads micronized</td>
<td>34.2</td>
<td>26.4</td>
<td>1.298</td>
<td>13.45</td>
<td>0.662</td>
<td>0.652</td>
<td>0.727</td>
<td>0.330</td>
<td>0.499</td>
</tr>
<tr>
<td>5 Sultan micronized</td>
<td>37.0</td>
<td>26.9</td>
<td>1.377</td>
<td>12.95</td>
<td>0.642</td>
<td>0.624</td>
<td>0.708</td>
<td>0.169</td>
<td>0.363</td>
</tr>
<tr>
<td>6 Control + Enzyme</td>
<td>35.4</td>
<td>26.4</td>
<td>1.343</td>
<td>13.49</td>
<td>0.666</td>
<td>0.642</td>
<td>0.718</td>
<td>0.243</td>
<td>0.395</td>
</tr>
<tr>
<td>7 Maris Beads raw + Enzyme</td>
<td>34.8</td>
<td>23.7</td>
<td>1.471</td>
<td>12.68</td>
<td>0.625</td>
<td>0.635</td>
<td>0.661</td>
<td>0.301</td>
<td>0.532</td>
</tr>
<tr>
<td>8 Sultan raw + Enzyme</td>
<td>35.3</td>
<td>23.8</td>
<td>1.492</td>
<td>13.16</td>
<td>0.647</td>
<td>0.643</td>
<td>0.712</td>
<td>0.393</td>
<td>0.577</td>
</tr>
<tr>
<td>9 Maris Beads micronised + Enzyme</td>
<td>35.1</td>
<td>23.5</td>
<td>1.495</td>
<td>12.65</td>
<td>0.609</td>
<td>0.622</td>
<td>0.682</td>
<td>0.348</td>
<td>0.485</td>
</tr>
<tr>
<td>10 Sultan micronised + Enzyme</td>
<td>33.8</td>
<td>23.6</td>
<td>1.440</td>
<td>13.43</td>
<td>0.652</td>
<td>0.643</td>
<td>0.748</td>
<td>0.360</td>
<td>0.511</td>
</tr>
<tr>
<td>SEM (n=7)*</td>
<td>1.35</td>
<td>1.10</td>
<td>0.0209</td>
<td>0.131</td>
<td>0.0060</td>
<td>0.0058</td>
<td>0.0213</td>
<td>0.0484</td>
<td>0.0302</td>
</tr>
</tbody>
</table>

Specific orthogonal contrasts
Beans x Enzyme x Micronizing
Bean cultivar
Maris Beads (n=28) 35.8 26.6 1.351 13.21 0.653 0.637 0.703 0.273 0.440
Sultan (n=28) 34.8 23.7 1.471 12.98 0.633 0.636 0.701 0.350 0.527
Enzyme
No enzyme (n=28) 35.9 25.2 1.432 12.81 0.629 0.627 0.677 0.292 0.471
Enzyme (n=28) 34.7 25.1 1.393 13.38 0.657 0.645 0.726 0.331 0.496
Micronizing
No micronized (n=28) 35.3 25.1 1.412 13.06 0.644 0.640 0.690 0.344 0.528
Micronized (n=28) 35.3 25.1 1.414 13.13 0.642 0.633 0.714 0.280 0.439
SEM (n=28)* 0.675 0.548 0.0105 0.066 0.0030 0.0029 0.0106 0.0242 0.0151
Beans vs Controls
Beans (n=56) 35.3 25.1 1.413 13.10 0.643 0.636 0.702 0.312 0.483
Control (n=14) 40.2 30.0 1.345 12.66 0.612 0.665 0.748 0.322 0.483

SEM (min – max replicate)* 0.96-0.48 0.78-0.39 0.0148-0.0074 0.093-0.046 0.0043-0.0021 0.0041-0.0021 0.0150-0.0750 0.0342-0.0171 0.0214-0.0107

Probabilities of differences
Bean cultivar (B) 0.261 <.001 <.001 0.017 <.001 0.814 0.880 0.028 <.001
Enzyme (E) 0.209 0.887 0.011 <.001 <.001 <.001 <.001 <.002 <.258 <.260
Micronized (M) 0.966 0.989 0.902 0.455 0.671 0.108 0.113 0.067 <.001
B x E 0.383 0.773 0.147 0.557 0.213 0.472 0.528 0.703 0.605
B x M 0.483 0.787 0.278 0.607 0.346 0.943 0.803 0.043 0.128
E x M 0.833 0.909 0.728 0.356 0.137 0.598 0.469 0.913 0.840
B x E x M 0.463 0.952 0.033 0.460 0.333 0.283 0.232 0.205 0.523

Probabilities of other specific contrasts
Control 1 (n=7) vs Control 2 (n=7) 0.598 0.183 0.037 0.928 0.771 0.004 0.472 0.244 <.001
Beans (n=56) vs Control (n=14) <.001 <.001 <.001 <.001 <.001 <.001 <.001 0.009 0.800 0.260

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Notes: FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; AMEn, N-corrected apparent metabolisable energy; DMR, dry matter retention coefficient; NR, nitrogen retention coefficient; FD, coefficient of fat digestibility; TD, coefficient of total tannin digestibility; CTD, coefficient of condensed tannin digestibility.

Each mean represents values from 7 replicate pens of 2 chicks each; bird performance was determined from 6 to 16 d age; dietary AME, AMEn, DMR, NR, FD, TD and CTD were determined from 12 to 16 d age.

*Notes: SEM, Standard error of the mean; There is statistically significant difference between treatments when $P \leq 0.05$. 
<table>
<thead>
<tr>
<th>Diet</th>
<th>SAc mg/g</th>
<th>SAt mg</th>
<th>GIT g</th>
<th>GIT%</th>
<th>PG g</th>
<th>PG%</th>
<th>Pancreas g</th>
<th>Pancreas%</th>
<th>SI g</th>
<th>SI%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>1.19</td>
<td>0.18</td>
<td>36.54</td>
<td>8.67</td>
<td>13.50</td>
<td>3.04</td>
<td>1.95</td>
<td>0.44</td>
<td>21.09</td>
<td>5.18</td>
</tr>
<tr>
<td>2 Maris Beads raw</td>
<td>1.15</td>
<td>0.18</td>
<td>37.50</td>
<td>8.57</td>
<td>14.23</td>
<td>3.09</td>
<td>1.91</td>
<td>0.42</td>
<td>21.36</td>
<td>5.06</td>
</tr>
<tr>
<td>3 Sultan raw</td>
<td>1.13</td>
<td>0.16</td>
<td>38.36</td>
<td>9.71</td>
<td>16.96</td>
<td>4.07</td>
<td>2.21</td>
<td>0.53</td>
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</tr>
<tr>
<td>4 Maris Beads micronized</td>
<td>1.14</td>
<td>0.16</td>
<td>36.06</td>
<td>9.46</td>
<td>14.93</td>
<td>3.70</td>
<td>2.09</td>
<td>0.52</td>
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<tr>
<td>5 Sultan micronized</td>
<td>1.19</td>
<td>0.17</td>
<td>38.04</td>
<td>9.85</td>
<td>15.97</td>
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<td>2.18</td>
<td>0.53</td>
<td>19.89</td>
<td>5.42</td>
</tr>
<tr>
<td>6 Control + Enzyme</td>
<td>1.14</td>
<td>0.17</td>
<td>36.33</td>
<td>9.39</td>
<td>14.75</td>
<td>3.62</td>
<td>2.06</td>
<td>0.50</td>
<td>19.52</td>
<td>5.27</td>
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<tr>
<td>7 Maris Beads raw + Enzyme</td>
<td>1.06</td>
<td>0.16</td>
<td>36.75</td>
<td>10.17</td>
<td>15.98</td>
<td>4.18</td>
<td>2.10</td>
<td>0.55</td>
<td>18.67</td>
<td>5.44</td>
</tr>
<tr>
<td>8 Sultan raw + Enzyme</td>
<td>1.11</td>
<td>0.17</td>
<td>34.01</td>
<td>9.78</td>
<td>14.58</td>
<td>3.97</td>
<td>1.89</td>
<td>0.51</td>
<td>17.54</td>
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</tr>
<tr>
<td>9 Maris Beads micronized + Enzyme</td>
<td>1.13</td>
<td>0.17</td>
<td>35.56</td>
<td>10.38</td>
<td>14.89</td>
<td>4.12</td>
<td>2.01</td>
<td>0.56</td>
<td>18.65</td>
<td>5.70</td>
</tr>
<tr>
<td>10 Sultan micronized + Enzyme</td>
<td>1.12</td>
<td>0.20</td>
<td>33.24</td>
<td>9.65</td>
<td>13.85</td>
<td>3.80</td>
<td>2.02</td>
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<tr>
<td>SEM (n=7)</td>
<td>0.024</td>
<td>0.012</td>
<td>1.339</td>
<td>0.210</td>
<td>0.650</td>
<td>0.124</td>
<td>0.118</td>
<td>0.025</td>
<td>0.823</td>
<td>0.144</td>
</tr>
</tbody>
</table>

Specific orthogonal contrasts
Beans x Enzyme x Micronizing
Bean cultivar
Maris Beads (n=28)  
1.15 0.17 37.20 9.60 15.65 3.82 2.13 0.52 19.41 5.26
Sultan (n=28)  
1.10 0.17 34.89 10.00 14.82 4.02 2.01 0.54 18.06 5.44
Enzyme
No enzyme (n=28)  
1.13 0.17 37.17 10.03 15.95 4.07 2.12 0.54 19.10 5.42
Enzyme (n=28)  
1.13 0.18 34.91 9.57 14.53 3.77 2.02 0.52 18.37 5.28
Micronizing
No micronized (n=28)  
1.11 0.16 36.29 9.78 15.61 3.98 2.07 0.53 18.61 5.27
Micronized (n=28)  
1.15 0.18 35.79 9.82 14.87 3.86 2.07 0.54 18.86 5.42
SEM (n=28)  
0.012 0.006 0.669 0.105 0.325 0.062 0.059 0.013 0.412 0.072
Beans vs Controls
Beans (n=56)  
1.13 0.17 36.04 9.80 15.24 3.92 2.07 0.53 18.73 5.35
Control (n=14)  
1.17 0.18 37.02 8.62 13.86 3.07 1.93 0.43 21.22 5.12
SEM (min – max replicate)*  
0.017-0.009 0.009-0.004 0.947-0.473 0.149-0.074 0.460-0.230 0.088-0.044 0.083-0.042 0.018-0.009 0.582-0.291 0.102-0.051

Probabilities of differences
Bean cultivar (B)  
0.009 0.624 0.018 0.011 0.077 0.031 0.133 0.231 0.024 0.090
Enzyme (E)  
0.897 0.264 0.020 0.003 0.003 0.000 0.200 0.284 0.212 0.163
Micronized (M)  
0.034 0.099 0.597 0.785 0.112 0.184 0.956 0.629 0.676 0.147
B x E  
0.339 0.281 0.781 0.501 0.664 0.729 0.902 0.885 0.418 0.213
B x M  
0.784 0.669 0.615 0.997 0.727 0.981 0.763 0.440 0.558 0.879
E x M  
0.081 0.718 0.791 0.377 0.531 0.950 0.527 0.968 0.876 0.216
B x E x M  
0.920 0.712 0.964 0.831 0.807 0.589 0.505 0.492 0.981 0.973

Probabilities of other specific contrasts
Control 1 (n=7) vs Control 2 (n=7)  
0.222 0.791 0.613 0.749 0.433 0.771 0.825 0.574 0.814 0.537
Beans (n=56) vs Control (n=14)  
0.042 0.281 0.360 <.001 0.010 <.001 0.150 <.001 <.001 0.052
Notes: SAc, concentration of endogenous mucin losses as sialic acid in excreta; SAt, total excreted endogenous mucin losses as sialic acid over 96 hours (12-16d); GIT, gastrointestinal tract weight (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG, proventriculus and gizzard weight; SI, small intestine weight (including duodenum, jejunum and ileum); GIT%, gastrointestinal tract as a proportion to the body weight; PG%, proventriculus and gizzard as a proportion to the body weight; SI%, small intestine as a proportion to the body weight; SEM, standard error of the means; Each mean represents values from 7 replicate pens; gastrointestinal tract development were determined at 16 d old using heavier bird in each pen; endogenous mucin losses as sialic acid in excreta was measured in excreta collected from 12-16 d of age; there is statistically significant difference between treatments when $P \leq 0.05$. 