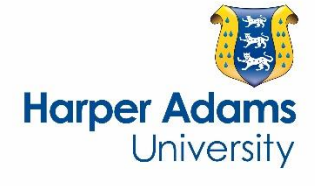
Nutritional value of raw and micronized field beans (*Vicia faba* L. var. *minor*) with and without enzyme supplementation containing tannase for growing chickens

by Abdulla, J.M., Rose, S.P., Mackenzie, A.M., Ivanova, S.G., Staykova, G.P. and Pirgozliev, V.R.

DOI: 10.1080/1745039X.2016.1214344



Abdulla, J.M., Rose, S.P., Mackenzie, A.M., Ivanova, S.G., Staykova, G.P. and Pirgozliev, V.R. 2016. Nutritional value of raw and micronized field beans (*Vicia faba* L. var. *minor*) with and without enzyme supplementation containing tannase for growing chickens. *Archives of Animal Nutrition,* 70 (5), 350-363.

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

Jalil Mahmwd Abdulla[a], Paul Rose[a], Alexander Mackay Mackenzie[a], Sonya G. Ivanova[b], Genoveva P. Staykova[b], and Vasil Pirgozliev[a]

[a]National Institute of Poultry Husbandry, Harper Adams University, Newport, Shropshire, TF10 8NB, UK

[b]Agricultural Institute, Shumen, 3 Simeon Veliki blvd., 9700, Bulgaria

Corresponding author: V. Pirgozliev. E-mail: vpirgozliev@harper-adams.ac.uk

T: +44 (0) 1952 820280 F: +44 (0) 1952 814783

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 covocine) that reduce egg size in laying hens (Mateos and Puchal, 1981). However, the antinutritional influence of vicine and covocine 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 (130ºC, 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 aplha-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 m^2 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^{o}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

240 only gave approximately 9% reduction in CT. However, there is a difference between the
241 process of autoclaving and micronizing, as extruding requires higher temperature, some water
242 and relatively more time, compared to micronizing (Lashkari et al. 2015).
243 The reduced CTD of micronized diets, and the observed interactions where micronizing
244 reduced feed efficiency and TD of Maris Bead based diet only, were not expected. Bellido et
245 al. (2006) reported that micronizing legumes, e.g. cowpea flour, at 130 ºC changed its
246 functional properties, including reduced foaming capacity, increase in the surface
247 hydrophobicity and cross-linking of the protein, formation of disulphide bonds and possibly
248 Maillard cross-links. It is possible that the two cultivar samples reacted differently to the heat
249 treatment applied in this experiment.
250 Abdulla et al. (2016a) showed that exogenous tannase was effective in improving the nutrient
251 availability and performance of broilers fed a diet containing field beans. It was expected that
252 the efficacy of tannase would be limited in the control diet as it was a low tannin feed. The
253 two field bean containing diets had different tannin contents thus different responses between
254 these two diets to tannase was also expected. However, a part from the interaction for FCR,
255 no other enzyme by diet interactions were observed in the present study, thus showing that
256 exogenous tannase improved the feeding value of all diets with the same magnitude. In
257 addition tannase supplementation did not influence tannin degradability. Chamorro et al.
258 (2015) found no effect of tannase on growth performance in chickens fed diet rich in
259 polyphenols. The tannase used in the present experiment also had alpha-amylase, xylanase,
260 and pectinase activities. It is possible that these enzyme activities may have been partially
261 responsible for the observed improvements in nutrient availability and feed efficiency in the
262 study.
263 The most noticeable response to dietary tannase was in increasing DEE by 7.1%, followed by
264 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.

Acknowledgements

We thank Richard James, Rose Crocker, Amjad Ali, Kevin Jones and Waseem Mirza for their technical support. We also thank Kerry Ingredients and Flavours (Ireland) for providing us with tannase enzyme and Askew & Barrett (Pulses) Ltd which donated the field bean samples for this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This experiment is a part of a PhD project funded by the Ministry of Higher Education and Scientific Research - Kurdistan Regional Government - Iraq.

References

[AOAC] Association of Official Analytical Chemists. 2000. Official Methods of Analysis of AOAC. Vol. II. 17th ed. Gaithersburg, MD: Association of Official Analytical Chemists.

Abdulla J, Rose SP, Mackenzie AM, Mirza W, Pirgozliev V. 2016a. Exogenous tannase improves feeding value of a diet containing field beans (*Vicia faba*) when fed to broilers. Brit Poult Sci, 57: 246–250.

315 Abdulla J, Rose SP, Mackenzie AM, Mirza W, Llamas-Moya S, Pirgozliev V. 2016b. The
316 effect of exogenous tannase on dietary energy, nutrient availability, gastrointestinal tract
317 development and performance when different field bean cultivars are fed to chicks.
318 International Poultry Scientific Forum, 25-27 January, Atlanta, GA, USA
319 (http://www.ippexpo.org/ipsf/docs/2016SPSS_Program.pdf).

320 Alonso R, Aguirre A, Marzo F. 2000. Effects of extrusion and traditional processing methods
321 on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans.
322 Food Chem. 68: 159-165

323 Amerah AM, Ravindran V. 2008. Influence of method of whole-wheat feeding on the
324 performance, digestive tract development and carcass traits of broiler chickens. Anim Feed
325 Sci Technol. 147:326-339.

326 Bedford MR. 2006. Effect of non-starch polysaccharidases on avian gastrointestinal function.
327 In: Perry GC, editor. Avian gut function in health and disease. Oxon: Wallingford; p. 159-
328 170.

329 Bellido G, Arntfield SD, Cenkowski S, Scanlon M. 2006. Effects of micronization
330 pretreatments on the physicochemical properties of navy and black beans (*Phaseolus*
331 *vulgaris* L.). LWT-Food Sci Technol. 39:779-787.

332 Chamorro S, Viveros A, Rebolé A, Rica BD, Arija I, Brenes A. 2015. Influence of dietary
333 enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape
334 pomace. Food Res Intern. 73:197–203.

335 Cowieson AJ, Acamovic T, Bedford MR. 2003. Supplementation of diets containing pea
336 meal with exogenous enzymes: effects on weight gain, feed conversion, nutrient
337 digestibility and gross morphology of the gastrointestinal tract of growing broiler chicks.
338 Brit Poult Sci. 44:427-437.

339 Crépon K, Marget P, Peyronnet C, Carrouée B, Arese P, Duc G. 2010. Nutritional value of
340 faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Res. 115:329-339.

341 Dänicke S, Simon O, Jeroch H, Keller K, Gläser K, Kluge H, Bedford MR. 1999. Effects of
342 dietary fat type, pentosan level and xylanase supplementation on digestibility of nutrients
343 and metabolizability of energy in male broilers. Arch Tierernahr. 52:245-61.

344 Duc G, Marget P, Esnault R, Le Guen J, Bastianelli D. 1999. Genetic variability for feeding
345 value of faba bean seeds (*Vicia faba L.*): Comparative chemical composition of isogenics
346 involving zero-tannin and zero-vicine genes. J Agri Sci. 133:185-196.

347

348 Englyst HN, Quigley ME, Hudson GJ. 1994. Determination of dietary fibre as non-starch
349 polysaccharides with gas–liquid chromatographic, high-performance liquid
350 chromatographic or spectrophotometric measurement of constituent sugars. Analyst.
351 119:1497-1509.

352 Englyst KN, Hudson GJ, Englyst HN. 2000. Starch analysis in food. In: Meyers RA, editor.
353 Encyclopaedia of Analytical Chemistry. Chichester: John Wiley and Sons; p. 4246–4262.

354 Gatta D, Russo C, Giuliotti L, Mannari C, Picciarelli P, Lombardi L, Giovannini L,
355 Ceccarelli N, Mariotti L. 2013. Influence of partial replacement of soya bean meal by faba
356 beans or peas in heavy pigs diet on meat quality, residual anti-nutritional factors and
357 phytoestrogen content. Arch Anim Nutr. 67:235-247.

358 Gracia MI, Aranı´bar M, La´zaro R, Medel P, Mateos GG. 2003. α-Amylase
359 Supplementation of Broiler Diets Based on Corn. Poult Sci. 82:436-442.

360 Grosjean F, Bourdillon A, Rudeaux F, Bastianelli D, Peyronnet C, Duc G, Lacassagne L.
361 2000. Valeur alimentaire pour la volaille de féveroles isogéniques (Vicia faba L.) avec ou
362 sans tannins et avec ou sans vicine–convicine. Sci Technol Avic. 32:17–23.

363 Hill FW, Anderson DL. 1958. Comparison of metabolizable energy and productive energy
364 determinations with growing chicks. J Nutr. 64:587-603.

365 Jourdian GW, Dean L, Roseman S. 1971. The sialic acids XI. A periodate-resorcinol method
366 for the quantitative estimation of free sialic acids and their glycosides. J Biol Chem.
367 246:430-435.

368 Lashkari S, Azizi O, Jahani-Azizabadi H. 2015. Effects of different processing methods of
369 flaxseed on ruminal degradability and in vitro post-ruminal nutrient disappearance. Arch
370 Anim Nutr. 69: 177-186.

371 Langhout DJ, Schutte JB, Van Leeuwen P, Wiebenga J, Tamminga S. 1999. Effect of dietary
372 high-and low-methylated citrus pectin on the activity of the ileal microflora and
373 morphology of the small intestinal wall of broiler chicks. Brit Poult Sci. 40:340-347.

374 Lekha PK, Lonsane BK. 1997. Production and application of tannin acyl hydrolase: state of
375 the art. In: Advances in Applied Microbiology. London: Academic Press Limited; Volume
376 44: p. 216-260.

377 Longstaff MA, McNab JM. 1991a. The inhibitory effects of hull polysaccharides and tannins
378 of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid and on
379 digestive enzyme activities in young chicks. Brit J Nutr. 65:199-216.

380 Longstaff MA, McNab JM. 1991b. The effect of concentration of tannin-rich bean hulls
381 (*Vicia faba* L.) on activities of lipase (EC 3.1. 1.3) and α-amylase (EC 3.2. 1.1) in digesta
382 and pancreas and on the digestion of lipid and starch by young chicks. Brit J Nutr. 66:139-
383 147.

384 Makkar HP, Blümmel M, Borowy NK, Becker K. 1993. Gravimetric determination of tannins
385 and their correlations with chemical and protein precipitation methods. J Sci Food Agri.
386 61:161-165.

387 Mateos GG, Puchal F. 1981. The nutritional value of broad bean for laying hens. Br Poult Sci
388 23:1–6.

389 Metayer JP, Barrier-Guillot B, Skiba F, Crepon K, Bouvarel I, Marget P, Duc G, Lessire M.
390 2003. Nutritional value of three faba bean cultivars for broiler chickens and adult
391 cockerels. Br Poult Sci. 44:816-817.

392 Muduuli, D., Marquardt, R., Guenter,W., 1981. Effect of dietary vicine on the productive
393 performance of laying chickens. Can. J. Anim. Sci. 61:757–764.

394

395 Nalle CL, Ravindran G, Ravindran V. 2010. Influence of dehulling on the apparent
396 metabolisable energy and ileal amino acid digestibility of grain legumes for broilers. J
397 Sci Food Agri. 90: 1227-1231.

Porter LJ, Hrstich LN, Chan BG. 1985. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry. 25:223-230.

Smits CH, Veldman A, Verkade HJ, Beynen AC. 1998. The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine. Poult Sci. 77:1534-1539.

Souffrant WB. 2001. Effect of dietary fibre on ileal digestibility and endogenous nitrogen losses in the pig. Anim Feed Sci and Technol. 90: 93–102.

Van der Poel AFB, Gravendeel S, Boer H. 1991. Effect of different processing methods on tannin content and in vitro protein digestibility of faba bean (*Vicia faba* L.). Anim Feed Sci Technol. 33:49-58.

Vilarino M, Metayer JP, Crepon K, Duc G. 2009. Effects of varying vicine, convicine and tannin contents of faba bean seeds (Vicia faba L.) on nutritional values for broiler chicken. Anim Feed Sci Technol 150:114–121.

Wiryawan KG, Dingle JG. 1999. Recent research on improving the quality of grain legumes for chicken growth. Anim Feed Sci Technol. 76:185-193.

Woyengo TA, Nyachoti CM. 2012. Ileal digestibility of amino acids for zero-tannin faba bean (*Vicia faba* L.) fed to broiler chicks. Poult Sci. 91:439-443.

Wu YB, Ravindran V, Thomas DG, Birtles MJ, Hendriks WH. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Brit Poult Sci. 45:76-84.

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

	Control	Maris beads	Sultan
Wheat	400.0	404.2	404.2
Maris beads	-	300.0	-
Sultan	-	-	300.0
SBM (CP=48%)	190.4	27.0	27.0
Full fat Soya meal	127.0	127.5	127.5
Maize gluten meal	35.0	35.0	35.0
Washed sand	119.1	-	-
Soya oil	82.5	65.0	65.0
L-Lysine-HCL	6.0	2.3	2.3
Methionine	6.8	5.8	5.8
Threonine	2.4	2.4	2.4
Monocalcium phosphate	10.0	10.0	10.0
Limestone	14.0	14.0	14.0
Salt	2.8	2.8	2.8
Vitamin/mineral premix	4.0	4.0	4.0
Total	1000	1000	1000
Calculated values			
ME (MJ/kg)	12.83	13.12	12.65
CP	221	217	201
Fat	113	97	97
Analysed values (as-fed)			
DM	855	877	876
GE (MJ/kg)	16.21	17.57	17.52
CP	197	198	183
Fat	112	95	95
Total phenols[a]	1.31	2.76 (2.66)	3.78 (3.63)
Tannins[a]	0.45	1.98 (1.77)	2.54 (2.42)
Condensed tannins[b]	0.00	1.15 (0.95)	1.86 (1.54)

* 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

[a] As tannic acid equivalent

[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)*

	Field bean cultivar	
Ingredient	Maris Bead	Sultan
Dry matter (g/kg)	854 (883)	851 (887)
Ether extract (g/kg)	10.5	11.7
Crude protein (g/kg)	304.5	244.6
Gross energy (MJ/kg)	18.41	18.27
Total phenols (g/kg)[a]	6.9 (6.3)	10.9 (9.9)
Tannins (g/kg)[a]	6.1 (5.1)	8.3 (7.5)
Condensed tannins (g/kg)[b]	4.5 (3.6)	7.3 (5.8)

[a] As tannic acid equivalent

[b] 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*

Bean cultivar		Maris Bead			Sultan		
Fraction		Soluble sugar	Insoluble sugar	Total sugar	Soluble sugar	Insoluble sugar	Total sugar
NSP constituent sugars	Glucose	1.5	80.9	82.3	15.4	96.1	111.5
	Galacturonic acid	10.1	12.7	22.8	17.1	11.6	28.7
	Arabinose	7.6	12.5	20.1	9.7	11.4	21.0
	Xylose	2.8	11.4	14.3	3.7	8.2	11.9
	Galactose	4.9	3.3	8.2	5.4	3.1	8.5
	Mannose	1.4	4.2	5.6	2.1	4.6	6.6
	Rhamnose	0.9	0.2	1.1	1.0	0.0	1.0
	Fucose	0.7	0.2	0.9	0.4	0.5	0.9
Total NSPs		30.0	125.5	155.5	54.8	135.4	190.2
Total starch			443			467	

*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*

Diet	FI (DM g/b)	WG (g/b)	FCR	AME n (MJ/kg DM)	DMD	NR	DEE	TD	CTD
1 Control	39.7	28.9	1.377	12.66	0.611	0.678	0.758	0.362	0.483
2 Maris Beads raw	40.7	31.0	1.314	12.67	0.614	0.653	0.737	0.281	0.483
3 Sultan raw	36.8	26. 6	1.386	12.95	0.642	0.629	0.659	0.351	0.504
4 Maris Beads micronized	34.2	26.4	1.298	13.45	0.662	0.652	0.727	0.330	0.499
5 Sultan micronized	37.0	26.9	1.377	12.95	0.642	0.624	0.708	0.169	0.363
6 Control + Enzyme	35.4	26.4	1.343	13.49	0.666	0.642	0.718	0.243	0.395
7 Maris Beads raw + Enzyme	34.8	23.7	1.471	12.68	0.625	0.635	0.661	0.301	0.532
8 Sultan raw + Enzyme	35.3	23.8	1.492	13.16	0.647	0.643	0.712	0.393	0.577
9 Maris Beads micronised + Enzyme	35.1	23.5	1.495	12.65	0.609	0.622	0.682	0.348	0.485
10 Sultan micronized + Enzyme	33.8	23.6	1.440	13.43	0.652	0.643	0.748	0.360	0.511
SEM (n=7)*	1.35	1.10	0.0209	0.131	0.0060	0.0058	0.0213	0.0484	0.0302
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	1.474	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	0.002	0.258	0.260
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	<0.001
Beans (n=56) *vs* Control (n=14)	<.001	<.001	<.001	<.001	<.001	<.001	0.009	0.800	0.260

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 5. Endogenous mucin losses as sialic acid secretion in excreta and gastrointestinal tract development responses to the experimental diets*

Diet	SAc mg/g	SAt mg	GIT g	GIT%	PG g	PG%	Pancreas g	Pancreas%	SI g	SI%
1 Control	1.19	0.18	36.54	8.67	13.50	3.04	1.95	0.44	21.09	5.18
2 Maris Beads raw	1.15	0.18	37.50	8.57	14.23	3.09	1.91	0.42	21.36	5.06
3 Sultan raw	1.13	0.16	38.36	9.71	16.96	4.07	2.21	0.53	19.19	5.12
4 Maris Beads micronized	1.14	0.16	36.06	9.46	14.93	3.70	2.09	0.52	19.04	5.24
5 Sultan micronized	1.19	0.17	38.04	9.85	15.97	3.91	2.18	0.53	19.89	5.42
6 Control + Enzyme	1.14	0.17	36.33	9.39	14.75	3.62	2.06	0.50	19.52	5.27
7 Maris Beads raw + Enzyme	1.06	0.16	36.75	10.17	15.98	4.18	2.10	0.55	18.67	5.44
8 Sultan raw + Enzyme	1.11	0.17	34.01	9.78	14.58	3.97	1.89	0.51	17.54	5.29
9 Maris Beads micronized + Enzyme	1.13	0.17	35.56	10.38	14.89	4.12	2.01	0.56	18.65	5.70
10 Sultan micronized + Enzyme	1.12	0.20	33.24	9.65	13.85	3.80	2.02	0.55	17.36	5.30
SEM (n=7)	0.024	0.012	1.339	0.210	0.650	0.124	0.118	0.025	0.823	0.144
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	0.018-0.09	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	0.001	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 $P \leq 0.05$.