

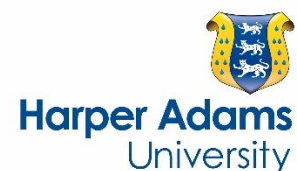
# Nutritional value of raw and micronized field beans (*Vicia faba* L. var. *minor*) with and without enzyme supplementation containing tannase for growing chickens

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

3

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12

13 **ABSTRACT**

14 An experiment examined the effects of two field bean cultivar samples with different tannin  
15 contents, the effect of heat treatment (micronizing), and the effect of dietary enzyme  
16 containing tannase, pectinase and xylanase activities on N-corrected dietary apparent  
17 metabolisable energy (AMEn), coefficients of total tract dry matter (DMD) and ether extract  
18 digestibility (EDD), nitrogen retention (NR), tannin degradability, gastrointestinal tract (GIT)  
19 development, and endogenous mucin losses excretion in broiler chickens. A control diet was  
20 prepared that contained 221 g/kg crude protein and 12.83 MJ/kg metabolizable energy. Four  
21 additional diets containing 300 g/kg of each of two untreated or micronized experimental  
22 field bean cultivar samples were also mixed. Each diet was then split into two batches and  
23 one of them was supplemented with 3400 units/kg of proprietary tannase enzyme resulting in  
24 ten diets in total. Each diet was fed to seven pens that contained two randomly selected male  
25 broilers. Birds fed the high tannin bean sample had a lower weight gain ( $P < 0.001$ ), and a

26 lower determined metabolisable energy ( $P<0.05$ ), and DMD ( $P<0.001$ ) but a higher tannin  
27 degradability ( $P<0.001$ ). Compared to the control diet, feeding field beans increased  
28 ( $P<0.001$ ) the weights of the proventriculus and gizzard of the birds, and also increased  
29 endogenous mucin losses ( $P<0.05$ ). Supplementing diets with tannase-containing enzyme  
30 improved dietary AMEn ( $P<0.001$ ), DMD ( $P<0.001$ ), NR ( $P<0.001$ ) and DEE ( $P<0.05$ ), but  
31 did not change ( $P>0.05$ ) tannin digestibility. Heat treatment of the beans reduced the  
32 degradability of condensed tannins and increased endogenous mucin losses ( $P<0.05$ ). This  
33 experiment has shown that there are differences in the feeding value of different field bean  
34 samples and these are not improved by heat treatment. Enzyme supplementation improved  
35 the feeding value of all diets regardless of the bean samples or heat treatment (no treatment  
36 factor interactions,  $P>0.05$ ). Further research is warranted to study the effectiveness of  
37 tannase supplementation in poultry diet formulations by dose response trials with purified  
38 tannase preparations.

39 Field bean; tannase; heat treatment; broiler chicken; ME; digestibility

40

41 **1. Introduction**

42 Grain legumes, including field beans (*Vicia faba* L. var. *minor*), are considered possible  
43 alternative protein sources to soybean meal because of the similarity of their amino acid  
44 profiles (Wiryawan and Dingle, 1999; Gatta et al. 2013). Large amount of field beans can be  
45 produced in many parts of Europe because of their adaptation to the climate in addition to  
46 their cultivar diversity that allows them to be cultivated in winter and spring (Crépon et al.  
47 2010; Duc et al. 1999). The poultry industry has been reluctant to use field beans in diet  
48 formulations due to the presence of antinutritional factors including oligosaccharides, soluble  
49 non-starch polysaccharides (NSP) and tannins (Longstaff and McNab, 1991a,b). **Field beans**  
50 **also contain some pyrimidine glucosides (vicine and covocine) that reduce egg size in laying**  
51 **hens (Mateos and Puchal, 1981). However, the antinutritional influence of vicine and**  
52 **covocine in broilers is not consistent (Grosjean et al., 2000; Metayer et al., 2004; Vilarino et**  
53 **al., 2009).** In order to alleviate the negative impact of antinutritional factors in field beans,  
54 different practices with various successes have been suggested, including genetic selection,  
55 mechanical processing, heat treatments, and exogenous fibre degrading enzyme  
56 supplementation (Van der Pole et al. 1991; Cowieson et al. 2003; Woyengo and Nyachoti,  
57 2012).

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

62 The main objective of this experiment, therefore, was to determine the effect of heat  
63 treatment (micronizing) and exogenous tannase on dietary metabolisable energy, nutrient  
64 utilisation, and gastrointestinal tract development when feeding diets containing two different

65 field bean cultivar samples to chickens. The overall feed intake, weight gain and feed  
66 conversion efficiency of the birds were also measured.

67

## 68 **2. Materials and methods**

69

### 70 **2.1. *Experimental samples***

71

72 This report is focused on the nutritional value for broilers of two UK grown field bean  
73 samples that were fed either as raw or as micronized to broiler chickens. The two field bean  
74 samples used in the study were Maris Bead (Spring cultivar) and Sultan (Winter cultivar).  
75 Both cultivar samples were produced in the UK during 2013 harvest year, and were stored in  
76 porous synthetic bags at ambient air temperatures in a dark, dry store. The samples were  
77 chosen because of their different tannin contents, although there were differences in their  
78 proximate composition. The stored field bean samples did not experience any freezing  
79 temperatures during this period. The bean samples were milled through a 4 mm screen. Each  
80 sample was then split on two and half of it was micronized (130°C, 90 sec, 2 microns wave  
81 length; Heraeus Noblelight GmbH, Germany).

82

### 83 **2.2. *Diet preparation***

84 Birds were fed one of ten mash diets. A control diet was prepared that had major ingredients  
85 of 404.2 g/kg wheat and 127.5 g/kg soybean meal (SBM), and contained 221 g/kg CP and  
86 12.83 MJ/kg **metabolizable energy** in agreement with breeder's recommendation (Aviagen  
87 Ltd., Edinburgh, UK) (Table 1). To reduce nutrient density the control diet also contained  
88 119.1 g/kg washed sand. Another four diets containing 300 g/kg of each of two untreated or  
89 micronized experimental field bean cultivar samples in replacement for soybean meal and

90 sand were also mixed in order to have **metabolisable energy** and CP in a range similar to the  
91 control diet (Table 1).

92

93 Each diet was then split into two batches and one of them was supplemented with the  
94 proprietary tannase (Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland)  
95 resulting in ten diets in total. The determined enzyme activities of the proprietary tannase  
96 were; tannase 3400 units / kg, pectinase 6220 units/kg; xylanase 6100 units/kg, and there  
97 were some additional amylase and alpha-galactosidase activities. The enzyme preparation  
98 was based on tannase produced by *Aspergillus niger* in a submerged fermentation  
99 methodology. The enzyme was in a liquid form and 17ml/kg was sprayed on the top of diets.

100 The dry matter content of non-supplemented diets was adjusted by spraying of 17ml water  
101 per kg of diet. Additional water was added to diets containing micronized beans to adjust for  
102 the water loss during heat treatment. The diets were thoroughly mixed in a horizontal mixer.

103

104 ***2.3. Animal husbandry, determination of dietary metabolisable energy, nutrient utilisation,***  
105 ***tannin degradability, endogenous mucin losses and comparison of broiler growth***  
106 ***performance***

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

109 One hundred and forty male Ross 308 broiler chickens in total were obtained from a  
110 commercial hatchery. During the pre-study period, from day old to 6 days of age, the birds  
111 were reared in a single floor pen and fed a proprietary wheat-based diet without coccidiostats  
112 or antimicrobial growth promoters, or other similar additives. At the beginning of the study,  
113 at 7 days of age, 140 chicks were allocated to 70 small pens with 0.160 m<sup>2</sup> solid floors area,

114 two birds in each pen. Feed and water was offered *ad libitum* to birds throughout the  
115 experimental period. Each diet was offered to birds in 7 pens in a randomised block design.  
116 Information on growth and feed intake was obtained from 7 to 16 days of age. The  
117 temperature was kept at 29°C at 7d age and was gradually reduced to 22°C at the end of the  
118 10 d feeding period (16 days of age). The light regimen was 18 h light and 6 h dark. At 12  
119 days of age, the solid floor of each pen was replaced with a wire mesh and excreta samples  
120 were collected for four consecutive days from each pen, immediately dried at 60°C and then  
121 milled for further analyses. The feed intake for the same period was also measured. The gross  
122 energy, dry matter, nitrogen, and fat of each dried excreta sample and the experimental diets  
123 were determined as described in Chapter 2.5. The AMEn of the diets was calculated as  
124 described by Hill and Anderson (1958). The coefficients of total tract ether extract (DEE) and  
125 dry matter (DMD) digestibility, and nitrogen retention (NR) were determined as the  
126 difference between intake and excretion of the nutrient divided by its respective intake. The  
127 degradation in the GIT of tannins was described as tannin degradability (TD), when tannins  
128 were presented as tannic acid equivalent, and as condensed tannin degradability (CTD), when  
129 tannins were presented as leucocyanidin equivalent. The endogenous mucin losses in excreta  
130 were measured using the concentration of the sialic acid (SA) as a marker, following the  
131 periodate-resorcinol method (Jourdian et al. 1971).

#### 132 **2.4. Gastrointestinal tract development**

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

137

#### 138 **2.5. Proximate analysis of samples**

139 Dry matter (DM) was determined by drying samples in a forced draft oven at 105°C to a  
140 constant weight. Crude protein (6.25 X N) in samples was determined by dry combustion  
141 method (AOAC, 2000) using a Leco (FP-528 N, Leco Corp., St. Joseph, MI). Oil (as ether  
142 extract) was extracted with diethyl ether by the ether extraction method (AOAC, 2000), using  
143 a Soxtec system (Foss UK Ltd.). The gross energy (GE) value of the samples was determined  
144 in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL), and benzoic acid was  
145 used as the standard. Total starch (TS) was determined following the method of Englyst et al.  
146 (2000). The non-starch polysaccharides (NSPs) content was determined by the method of  
147 Englyst et al. (1994), whereby starch is completely dispersed and then hydrolysed  
148 enzymatically. The NSP is isolated by precipitation in 80% ethanol then hydrolysed by  
149 sulphuric acid and the released sugars measured by gas chromatography as their alditol  
150 acetate derivatives.

151 The total phenol, non-tannin phenol, total tannin (all as tannic acid equivalent) in the  
152 representative samples of excreta, as well as freshly milled raw and micronized studied field  
153 bean cultivars, the control diet and other feed ingredients were determined by applying the  
154 procedure used by Makkar et al. (1993). The condensed tannins in the same samples were  
155 determined as leucocyanidin equivalent as described by Porter et al. (1985).

156  
157

## 158 **2.6. Statistical analysis**

159  
160 The experiment was arranged as a randomised block analysis of variance with 10 treatments  
161 each with 7 replicates. The treatments were arranged 2 x 2 x 2 factorial with a further two  
162 specific orthogonal contrasts for the control diets. The 2 x 2 x 2 factorial arrangement had  
163 field bean cultivar (Maris Bead or Sultan), enzyme (with and without tannase) and  
164 micronizing (with and without). The first specific orthogonal contrasts was Control 1 (no  
165 enzyme) vs Control 2 (with enzyme), and the second contrast was mean of all bean diets vs



166 mean of the two control diets. In all instances, differences were reported as significant at  $P$   
167  $\leq 0.05$ . Tendencies towards significance ( $0.05 < P \leq 0.1$ ) were also reported.

168  
169 **3. Results**

170 Overall, with the exception of total starch content, Maris Bead contained higher nutrient and  
171 lower anti-nutrient comparing to Sultan field bean cultivar, and the crude protein content  
172 (CP) was more variable than the oil and GE. Crude protein varied from 244.6 (Sultan) to  
173 304.5 (Maris Bead) g/kg DM. The total phenols and tannins, as tannic acid equivalent, and  
174 condensed tannins, as leucocyanidins, differ from 6.9 to 10.9, 6.1 to 8.3, and 4.5 to 7.3 g/kg  
175 DM for Maris Bead and Sultan (Table 2). Micronizing slightly reduced the tannin contents of  
176 the beans. The carbohydrate content of the field bean samples has been illustrated in table 3,  
177 as Sultan contained more carbohydrates than Maris Bead. The total starch concentration, as  
178 g/kg DM, was 443 and 467, the total NSPs 155.4 and 190.1 including 30.0 and 54.4 soluble  
179 and 125.5 and 135.4 insoluble sugars in Maris Bead and Sultan, respectively. Glucose,  
180 galacturonic acid, arabinose, xylose, galactose and mannose were the main NSP constituent  
181 sugars in the field bean samples.

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

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

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

190 observed. Overall, tannase supplemented diets had higher NR ( $P<0.001$ ) and DEE ( $P=0.002$ ),  
191 than un-supplemented diets.

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

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

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

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

205 The weight of the TGI was reduced by feeding Sultan compared to Maris Bead containing  
206 diets ( $P=0.018$ ) and tannase supplemented compared to none supplemented diets ( $P=0.020$ ).

207 When expressed as a percent from the body weight the GIT was increased by feeding bean  
208 containing diets compared to controls ( $P<0.001$ ), Sultan compared to Maris Bead based diet  
209 ( $P=0.011$ ) and enzyme non-supplemented compared to those with tannase ( $P=0.003$ ).

210 The weight of the PG was increased by feeding bean containing diets compared to controls  
211 ( $P=0.010$ ) and when compare enzyme free to tannase supplemented diets ( $P=0.003$ ).

212 Similarly, the PG% was increased by feeding bean containing diets compared to controls  
213 ( $P<0.001$ ), Sultan compared to Maris Bead based diet ( $P=0.031$ ) and non-supplemented  
214 compared to tannase supplemented diets ( $P=0.001$ ).

215 The weight of the SI was reduced by feeding bean containing compared to control diets  
216 ( $P<0.001$ ) and Sultan compared to Maris Bead containing diet ( $P=0.003$ ). For SI% only  
217 tendencies were observed.

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

220

#### 221 **4. DISCUSSION**

222

223 The purpose of the experiment reported in this paper was to determine whether heat treatment  
224 (micronizing) of field beans and exogenous tannase could be used to improve available  
225 energy and nutrient utilisation in diets for broilers. It was important to evaluate these  
226 treatments using different bean cultivar samples because of the large variation in the  
227 agronomic production and chemical composition of beans available to the animal feed  
228 industry.

229 The sample of bean cultivar Sultan had a higher tannin content compared to Maris Bead  
230 sample. Tannins can form strong complexes with proteins, starch, cellulose, and minerals  
231 (Lekha and Lonsane, 1997). However, Sultan also had a lower **AMEn**, most probably due its  
232 higher NSP content, than Maris Bead. In addition Sultan has a lower CP content. The lower  
233 metabolisable energy and CP content of these diets may have directly affected growth  
234 performance. Reduced mucin endogenous losses in birds fed cultivar Maris Bead compared  
235 to Sultan could be associated with a reduced irritation of the gut due to lower dietary tannin  
236 content.

237 The experiment showed that there were no differences in nutritional value between the raw  
238 and heat treated field beans. Alonso et al. (2000) demonstrated that heat treatment (extrusion)  
239 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 °C 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

265 results are similar to those reported by Abdulla et al. (2016b). Although there was an  
266 increased dietary N retention when tannase was fed, N retention is influenced not only by  
267 protein digestibility, but also by metabolic N excretion (Souffrant, 2001). It is generally  
268 accepted that part of the anti-nutritional effect of field beans is also mediated by its NSP  
269 constituents (Longstaff and McNab, 1991a,b; Nalle et al. 2010) that raise the viscosity of gut  
270 contents and may alter the microflora (Smits et al. 1998; Langhout et al. 1999). An increase  
271 in intestinal viscosity associated with enhanced bacterial fermentation can also depress fat  
272 digestion (Danicke et al. 1999).

273 The weight of the GIT decreased with tannase supplementation by 6.0%, which is in the  
274 range of values reported by Gracia et al. (2003) (4.0%) and Wu et al. (2004) (7.9%), when  
275 feeding  $\alpha$ -amylase or a mixture of phytase and xylanase to broilers. The weight of the PG was  
276 particularly affected and decreased by 8.9%, a decrease that is in similar range (6.1%)  
277 reported by Abdulla et al. (2016a) when fed the same enzyme to broilers of similar age. Wu  
278 et al. (2004) also reported a reduced weight of the PG by 7.4% when feeding a mixture of  
279 phytase and xylanase to broilers. A similar trend was observed by Gracia et al. (2003) after  
280 feeding  $\alpha$ -amylase to broilers at similar age. The changes in GIT expressed as % of the  
281 weight of the birds were similar to the absolute values. In general, if the efficiency of  
282 digestion is consistently suboptimal, whether due to ingredient quality, microbial interaction  
283 of anti-nutritive factors, the GIT responds by increasing in both size (surface area) and  
284 digestive enzyme output (Bedford, 2006).

285

## 286 **5. Conclusion**

287 The results from this study demonstrate that there can be large differences in the nutritional  
288 value of different field bean samples that are available to the poultry feed industry.  
289 Application of heat treatment (micronizing) did not improve the nutritional value of either

290 bean sample, but other heat treatment processes such as extrusion may be more effective.  
291 Addition of a commercial tannase enzyme preparation (that additionally had alpha-amylase,  
292 xylanase, and pectinase activities) proved to be a highly effective in improving dietary  
293 available energy and nutrient utilisation in chickens. Further research is warranted to  
294 elucidate **the effectiveness of tannase supplementation in poultry diet formulations by dose**  
295 **response trials with purified tannase preparations**. Similarly, more research is needed on the  
296 temperature and the processing time applied to field beans.

297

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302 samples for this study.

### 303 **Disclosure statement**

304 No potential conflict of interest was reported by the authors.

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308

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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 <sup>a</sup>	1.31	2.76 (2.66)	3.78 (3.63)
Tannins <sup>a</sup>	0.45	1.98 (1.77)	2.54 (2.42)
Condensed tannins <sup>b</sup>	0.00	1.15 (0.95)	1.86 (1.54)

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434 \* Vitamin and mineral premix provided (units · kg<sup>-1</sup> feed): µg: retinol 2160, cholecalciferol 75; mg: alpha-tocopherol 25,  
435 menadione 1.5, riboflavin 5, pantothenic acid 8, cyanocobalamin 0.01, pyridoxine 1.5, thiamine 1.5, folic acid 0.5, niacin 30,  
436 biotin 0.06, I 0.8, Cu 10, Fe 80, Se 0.3, Mn 80, Zn 80. Diets were not supplemented with coccidiostat

437 <sup>a</sup> As tannic acid equivalent

438 <sup>b</sup> As leucocyanidin equivalent

439 The contents of total phenols, tannins and condensed tannins in the ingredients of diets containing field beans was 1.42 g/kg,  
440 0.60 g/kg and 0.00 g/kg, respectively.

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

Ingredient	Field bean cultivar	
	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) <sup>a</sup>	6.9 (6.3)	10.9 (9.9)
Tannins (g/kg) <sup>a</sup>	6.1 (5.1)	8.3 (7.5)
Condensed tannins (g/kg) <sup>b</sup>	4.5 (3.6)	7.3 (5.8)

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448 <sup>a</sup> As tannic acid equivalent

449 <sup>b</sup> As leucocyanidin equivalent

450 \*Note: The information in brackets is for the micronized bean samples; all analyses were performed in triplicate.

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

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Bean cultivar		Maris Bead			Sultan		
		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		

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

474 Total-NSPs = total non-starch polysaccharides.

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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  $P \leq 0.05$ .