



**HAL**  
open science

## **Sensitivity of broiler starters to three doses of an enzyme cocktail in maize-based diets.**

Aaron Joell Cowieson, Velmurugu Ravindran

► **To cite this version:**

Aaron Joell Cowieson, Velmurugu Ravindran. Sensitivity of broiler starters to three doses of an enzyme cocktail in maize-based diets.. *British Poultry Science*, 2008, 49 (03), pp.340-346. <10.1080/00071660802126669>. <hal-00545333>

**HAL Id: hal-00545333**

**<https://hal.science/hal-00545333v1>**

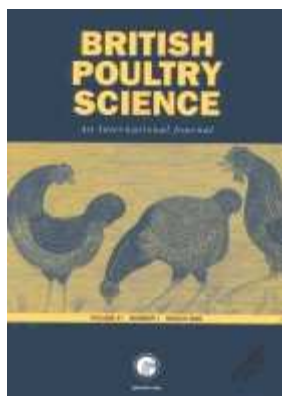
Submitted on 10 Dec 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



**Sensitivity of broiler starters to three doses of an enzyme cocktail in maize-based diets.**

Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2007-303.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	11-Jan-2008
Complete List of Authors:	Cowieson, Aaron; Danisco Animal Nutrition, Research and Development Ravindran, Velmurugu; Massey University, Institute of Food, Nutrition and Human Health
Keywords:	Broilers, Enzymes, Metabolisable energy, Maize, Dose response



CBPS-2007-303

ed. MacLeod, April 2008

1  
2  
3 1  
4  
5  
6 2  
7  
8 3  
9  
10 4 **Sensitivity of broiler starters to three doses of an enzyme cocktail in maize-based diets**  
11  
12  
13 5

14  
15 6 A.J. COWIESON<sup>1</sup> AND V. RAVINDRAN<sup>2</sup>  
16

17  
18 7 <sup>1</sup>Danisco Animal Nutrition, Marlborough, Wiltshire, SN8 1XN, UK, and <sup>2</sup>Institute of Food,  
19  
20 8 *Nutrition and Human Health, Massey University, Palmerston North, New Zealand*  
21  
22  
23 9

24  
25 10  
26  
27 11 **RUNNING TITLE: DOSE RESPONSE TO DIETARY ENZYMES**  
28  
29  
30 12

31  
32 13 <sup>1</sup>To whom correspondence should be addressed: Aaron Cowieson, PO Box 777, Marlborough,  
33  
34 14 Wiltshire, SN8 1XN, UK.

35  
36 15 Phone: +44 (0)1672 517787

37  
38  
39 16 E-mail: [aaron.cowieson@danisco.com](mailto:aaron.cowieson@danisco.com)  
40  
41  
42  
43 17  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Accepted for publication 14<sup>th</sup> February 2008

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Abstract** 1. The aim of the present study was to investigate the effect of an enzyme cocktail composed of xylanase, amylase and protease on the performance and nutrient utilisation of broiler starters fed on a maize-based diet. A maize/soy-based control diet was formulated to be marginal in apparent metabolisable energy (AME) and amino acids and this diet was fed without or with two concentrations (250 and 500 g/tonne) of the exogenous enzyme cocktail.

2. Growth performance was measured over a 21d period after which ileal contents were collected for measurement of digestible energy, nitrogen and dry matter. Excreta were collected from d 17 to 20 post-hatch and used to determine the AME and, retention of nitrogen and dry matter.

3. Supplementation of the control diet with 250 and 500g/ tonne of the enzyme cocktail resulted in enhanced performance of the chicks (1.4% and 8.3% respectively for weight gain and, 1.2% and 2.2% respectively for feed per gain), but the improvements were significant ( $P < 0.05$ ) only at 500g/ tonne.

4. Addition of both 250 and 500g/tonne improved AME, nitrogen-corrected AME and dry matter retention compared with the control though the improvements were greater with the higher dose. These effects were also reflected in the apparent ileal digestibility coefficients of dry matter, nitrogen and energy, with 500g/tonne of the enzyme cocktail improving ileal digestible energy by 3.4%.

5. These results demonstrate that a cocktail of exogenous enzymes containing xylanase, amylase and protease is effective in improving the performance of broiler chicks fed on a maize-soybean meal based diet. However, these benefits may be dose-dependent and so an economic analysis involving ingredient and enzyme pricing and the relative performance and digestibility enhancements could allow the end user to select the most economical dose to maximise return on investment.

## INTRODUCTION

The responsiveness of maize-based diets to exogenous enzymes has received considerable attention in recent years due to increasing pressure on feed formulators from rising ingredient prices. With the biofuel boom showing little evidence of slowing, the consumption of nutritionally-important starch sources such as maize and wheat by ethanol plants instead of animals is likely to continue apace. As the price of starch and fat sources increases so too does the economic potential of micro-ingredients that offer energy value to the diet *via* sparing mechanisms or digestibility enhancements. These micro-ingredients, of which enzymes are an example, are included in least cost formulation through the use of matrix values for energy, amino acids, calcium and phosphorus. Furthermore, the use of empirical models to predict the magnitude of response can assist in optimising both the enzyme type and dose (Cowieson *et al.*, 2006a) through the use of regression equations that consider the responsiveness of the diet and the relative cost of the ingredients.

Increasing the inclusion concentration of an exogenous enzyme in a poultry diet can improve performance and digestibility in a linear fashion but a more typical response can be described by a logarithmic equation which follows the law of diminishing return. Rosen (2002) summarised 1,869 independent datasets on the use of various non-phytase enzymes for poultry and, using log to base 10 enzyme units/kg of feed, described a significant positive effect of increasing dose concentrations. However, of the 1,869 datasets used, only 346 (18.5%) contained dose titrations, illustrating the paucity of information on dose response to exogenous carbohydrase enzymes in the literature. More recently, positive responses to increasing enzyme dose have been demonstrated both for phytase (Cowieson *et al.*, 2006b) and carbohydrases (Mikulski *et al.*, 2006). Dose titration experiments for exogenous enzymes will not necessarily follow a 'convenient' dose response curve. The effect of increasing the dose rate of an enzyme

1  
2  
3 67 product will be dependent on the relative sensitivity of the system, this being influenced by a  
4  
5 68 plethora of interacting factors, including the susceptibility and concentration of substrate, the  
6  
7 69 maturity of the host animal and the balance of nutrients in the diet.  
8  
9

10 It was the purpose of the experiment reported in this paper to investigate the effect of  
11  
12 70 increasing the dose of an enzyme cocktail composed of xylanase, amylase and protease on the  
13  
14 71 performance and nutrient utilization of broiler starter chicks fed a nutritionally marginal maize-  
15  
16 72 based diet.  
17  
18  
19

## 20 74 MATERIALS AND METHODS

### 21 22 75 Enzyme

23  
24  
25 76 A commercial enzyme preparation containing endoxylanase,  $\alpha$ -amylase and subtilisin protease  
26  
27 77 (Avizyme™ 1502; Danisco Animal Nutrition, Marlborough, UK), supplied in dry form, was used.  
28  
29  
30 78 The enzyme was added in diets to provide a guaranteed minimum of 300 U xylanase, 400 U  
31  
32 79 amylase and 4,000 U protease per kg of feed at 500g/tonne inclusion.  
33

### 34 35 80 Diets

36  
37 81 The study was conducted as a completely randomised design to evaluate the effects of doses (0,  
38  
39 82 250 or 500g/tonne) of the exogenous enzyme cocktail. A diet (Table 1), based on maize and  
40  
41 83 soybean meal and formulated to meet the requirements for all nutrients, except AME and  
42  
43 84 essential amino acids, for broiler starters as per breeder recommendations (Ross, 2007), served as  
44  
45 85 the control. This control diet was formulated to contain 0.67 MJ/kg less AME and 3% less in  
46  
47 86 total sulphur-containing amino acids and lysine than the breeder recommendations. Titanium  
48  
49 87 oxide (3 g/kg) was added to all diets as an inert digesta marker. The diets were fed in mash form  
50  
51 88 and, both feed and water were available to allow *ad libitum* consumption throughout the  
52  
53  
54  
55  
56 89 experiment.

Table 1 near here

1  
2  
3 90 **Bird management**  
4

5  
6 91 All experimental procedures were reviewed and approved by the Massey University Animal  
7  
8 92 Ethics Committee. Male broiler (Ross 308) chicks were obtained as d-olds from a commercial  
9  
10 93 hatchery (Golden Coast Commercial, New Plymouth, New Zealand). The chicks were  
11  
12 94 individually weighed and allocated to 24 brooder cages (8 chicks per cage) so that the average  
13  
14 95 bird weight per cage was similar. The three dietary treatments were then randomly assigned to 6  
15  
16 96 replicate cages each. On d 12, the birds were transferred to grower cages. The space allocation  
17  
18 97 per bird in the brooder and grower cages was 530 and 640 cm<sup>2</sup>, respectively. The brooder and  
19  
20 98 grower cages were housed in environmentally controlled rooms. The temperature was maintained  
21  
22 99 at 31 °C in the first week and then gradually reduced to 22 °C by the third week. The birds  
23  
24 100 received 24-h fluorescent illumination and the light intensity at the height of birds was 160 lux.  
25  
26 101 The trial lasted 21 d.

27  
28  
29  
30  
31  
32 102 Body weights and feed intake were recorded at weekly intervals. Mortality was recorded  
33  
34 103 daily. Any bird that died was weighed and the weight was used to adjust feed conversion ratio.  
35  
36 104 Feed:gain ratio was calculated by dividing total feed intake by weight gain of live plus dead  
37  
38 105 birds.

39  
40  
41 106 From d 17 to 20 post-hatching, feed intake and total excreta output were measured  
42  
43 107 quantitatively per cage over four consecutive days. Excreta were pooled within a cage, mixed  
44  
45 108 using a blender and representative samples per pen were taken. The samples were lyophilised  
46  
47 109 and dried samples were ground to pass through a 0.5 mm sieve. Samples of diets, and excreta  
48  
49 110 were analysed for dry matter (DM), nitrogen (N) and gross energy (GE).  
50

51  
52  
53 111 On d 21, all birds were killed by intracardial injection of sodium pentobarbitone and  
54  
55 112 contents of the distal half of the ileum were expressed by gentle flushing with distilled water. The  
56  
57  
58  
59  
60

1  
2  
3 113 ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum  
4  
5  
6 114 to a point 40 mm proximal to the ileo-caecal junction. The ileum was divided into two halves  
7  
8 115 and the digesta was collected from the distal half towards the ileo-caecal junction (Ravindran *et*  
9  
10 116 *al.*, 1999). Digesta from birds within a cage were pooled, resulting in six samples per dietary  
11  
12 117 treatment. The digesta samples were frozen immediately after collection, subsequently  
13  
14 118 lyophilised and dried samples were ground to pass through a 0.5-mm-sieve. Samples of diets and  
15  
16 119 ileal digesta were assayed for DM, N, GE and titanium.

### 120 **Chemical analysis**

121 Dry matter determination was carried out according to AOAC (1994) procedures. Gross energy  
122 was determined using an adiabatic bomb calorimeter (Gallenkamp, London, UK), standardised  
123 with benzoic acid. Nitrogen content was determined by the Dumas method (Sweeney, 1989)  
124 using a CNS-2000 carbon, nitrogen and sulphur analyser (LECO Corporation, St. Joseph, MI).  
125 Titanium content was measured on a UV spectrophotometer following the method of Short *et al.*  
126 (1996).

127 All diet samples were analysed for the activities of xylanase, amylase and protease.  
128 Xylanase activity in feed was measured using a modified method based on the Megazyme  
129 xylanase assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Amylase activity in  
130 feed was measured using phadebas tablets based on the method by McCleary and Sheehan (1989)  
131 and Barnes and Blakeney (1974). Protease activity in feed was determined by a Megazyme  
132 method where the extraction and assay buffers used were pH10 Tris/HCl.

### 133 **Calculations**

134 The AME values of the diets were calculated using the following formula, with appropriate  
135 corrections for differences in moisture content.

136

$$\text{AME} = \frac{(\text{Feed intake} \times \text{Gross energy}_{\text{diet}}) - (\text{Excreta output} \times \text{Gross energy}_{\text{excreta}})}{\text{Feed intake}}$$

Nitrogen-corrected AME ( $\text{AME}_N$ ) were determined by correction for zero nitrogen retention by simple multiplication by 36.54 kJ per g nitrogen retained in the body, as described by Hill and Anderson (1958).

The apparent ileal digestibility coefficient of nutrients was calculated by the following formula using the titanium marker ratio in the diet and ileal digesta.

$$\text{Digestibility coefficient of nutrients} = \frac{(\text{NT/Ti})_d - (\text{NT/Ti})_i}{(\text{NT/Ti})_d}$$

where,  $(\text{NT/Ti})_d$  = ratio of nutrient and titanium in diet, and

$(\text{NT/Ti})_i$  = ratio of nutrient and titanium in ileal digesta.

### Statistical analysis

All data were subject to analysis of variance using the General Linear Model procedure of SAS (1997) using pen as the experimental unit. Differences were considered significant at  $P < 0.05$  and when a significant F-test was detected, means were separated using the least significant difference test.

## RESULTS

The recovery of enzyme activity in the diets was in line with expectations (Table 2), being above the guaranteed minima for the products at both inclusion concentrations and close to targets (targets being 700, 1800 and 4000 U/kg finished feed for xylanase, amylase and protease respectively). Increasing the inclusion rate from 250g to 500g/tonne increased the recovered activity of xylanase, amylase and protease by 68, 89 and 109%, respectively, in line with expectations when mixing and assay errors are considered.

The effect of enzyme dose on the performance of broiler starters is presented in Table 3. Inclusion of the enzyme improved ( $P < 0.05$ ) weight gain and reduced ( $P < 0.05$ ) feed per unit gain

1  
2  
3 162 in a dose-dependent manner. The inclusion of the enzyme at 500g/tonne resulted in  
4  
5 163 improvements in weight gain and feed intake, being significantly ( $P<0.05$ ) higher than the  
6  
7 164 control or the 250g/tonne inclusion rate. Feed conversion and weight gain were improved  
8  
9 165 ( $P<0.05$ ) by 8.3 and 2.1%, respectively, with the addition of 500g/tonne enzyme compared with  
10  
11  
12  
13 166 the control. Tables 2 & 3 near here

15 167 The influence of enzyme dose on total tract nutrient retention and AME is presented in  
16  
17 168 Table 4. Supplementation of the control diet with both 250g and 500g/tonne of the enzyme  
18  
19 169 cocktail improved ( $P < 0.05$ ) DM retention and AME, compared with the control. The highest  
20  
21 170 dose of the enzyme resulted in the greatest improvements in DM retention, being significantly  
22  
23 171 higher than the lower dose. The addition of 500g/tonne of the enzyme improved ( $P < 0.05$ ) AME  
24  
25 172 and AMEn by 2.3% (0.26 MJ/kg) compared with the control. There was no effect ( $P > 0.05$ ) of  
26  
27 173 enzyme addition at either concentration on total tract N retention.

31 174 The effect of enzyme dose on apparent ileal digestibility coefficients is presented in Table  
32  
33 175 5. The greatest improvement in ileal digestibility of DM, N and energy were associated with the  
34  
35 176 highest dose of enzyme, being 3.4, 3.8 and 3.4%, respectively, compared with the control. The  
36  
37 177 improvements in ileal digestible energy with 250g and 500g/tonne inclusions were 0.22MJ  
38  
39 178 (1.9%) and 0.40MJ (3.4%) respectively, compared with the unsupplemented diet.  
40  
41 179 Supplementation with the lower enzyme inclusion rate resulted in intermediate improvements  
42  
43 180 which differed ( $P<0.05$ ) from the control only for ileal N digestibility. Tables 4 & 5 near here

## 181 DISCUSSION

50 182 The usefulness of exogenous enzymes for poultry production is ultimately described by return on  
51  
52 183 investment equations that assign an economic benefit to the improved nutritional value of the diet  
53  
54 184 or ingredient, and also considers the net change in the feed cost with regard to both ingredient  
55  
56 185 and supplement pricing. As feed ingredient prices have increased dramatically in recent years,  
57  
58  
59  
60

1  
2  
3 186 driven by energy prices (biofuel and crude oil), it has become increasingly difficult to provide  
4  
5  
6 187 growing animals with cost effective starch, fat, mineral and protein sources (Bedford, 2000;  
7  
8 188 Choct, 2006). Traditional starch and fat sources, such as maize and wheat, and vegetable oils,  
9  
10 189 have increased in price to the point where profitability of poultry production has been seriously  
11  
12 190 challenged. In this context, feed additives, such as enzymes, that can enhance the energy, protein  
13  
14 191 and mineral 'value' of a diet have an important role to play. Furthermore, flexibility in dosing of  
15  
16 192 enzyme products is preferable to fixed doses so that effective economic optimization of the  
17  
18 193 product is possible. It was to this end that the current study was conducted to assess the  
19  
20 194 sensitivity of broiler chickens to a cocktail of xylanase, amylase and protease, provided at two  
21  
22 195 inclusion rates.  
23  
24  
25

26  
27 196 Dose titration experiments, relative to fixed inclusion contrasts, are scarce. Making firm  
28  
29 197 conclusions based on so few data is difficult but a significant beneficial effect of enzyme dose  
30  
31 198 rate was established by Rosen (2002) using a log to the base 10 enzyme units/kg feed. His  
32  
33 199 analysis showed that improved efficacy with higher doses was offset by a reduction in the  
34  
35 200 efficacy of enzymes *per se* over the years, which was attributed to the improvements in  
36  
37 201 husbandry practices, nutrition and genetics. These factors effectively serve to provide a genetic  
38  
39 202 and nutritional backdrop to enzymes that chronologically mutes response, prompting the use of  
40  
41 203 higher doses to mitigate risk. Extrapolating from the empirical model (Rosen, 2002) it can be  
42  
43 204 seen that higher doses of enzyme may be required in the future to maintain consistency of  
44  
45 205 bioefficacy against temporal shifts in poultry paradigms. Fortunately, the financial burden  
46  
47 206 associated with increased enzyme doses is offset by enzyme price erosion, estimated to be around  
48  
49 207 4% per annum, allowing end users more flexibility in economic optimisation. Further, though  
50  
51 208 improvements in genetics, husbandry, disease, biosecurity and nutritional understanding  
52  
53 209 continues, the feed industry faces unprecedented pressure from feed ingredient prices that force  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 210 nutritionists to use unconventional ingredients that typically have a lower quality and more  
4  
5 211 variable nutritional profile, and are arguably more responsive to exogenous enzymes because of  
6  
7  
8 212 higher substrate concentrations. Thus an updated empirical model to reflect current feeding  
9  
10 213 practice seems to be justified.

11  
12  
13 214 In the present study, improvements in feed efficiency with enzyme addition were  
14  
15 215 observed to be dose related, being 1.2% for 250g and 2.2% for 500g/tonne. However, increasing  
16  
17 216 dose rates resulted in dramatic changes in feed intake from -0.1% reduction with 250g to 5.4%  
18  
19  
20 217 increase with 500g/tonne. This response is difficult to explain but it may be that the higher  
21  
22 218 concentration of amylase and protease provided by 500g of the enzyme supplement increased  
23  
24 219 passage rate through enteroglucagon feedback mechanisms or via an improvement in digestibility  
25  
26  
27 220 of starch and protein. An increase in feed intake with supplemental protease and amylase has  
28  
29 221 been reported previously (Ghazi *et al.*, 2002; Gracia *et al.*, 2003; Onderci *et al.*, 2006), but is in  
30  
31 222 contrast to a previous report where digestibility of a maize-soy based diet was improved but feed  
32  
33  
34 223 intake was maintained relative to the control (Cowieson and Ravindran, 2008). Nevertheless, the  
35  
36 224 increase in feed intake would appear to be justified by the improved feed efficiency and weight  
37  
38 225 gain associated with the highest dose of enzyme, which suggests that the enzyme did not cause  
39  
40 226 any imbalance the diet in terms of limiting nutrients. In fact, this contention is borne out by  
41  
42 227 proportionately similar improvements in the ileal digestibility of both energy and N, suggesting  
43  
44 228 that the enzyme preparation improved both energy and protein digestibility. These results have  
45  
46 229 also been confirmed elsewhere (Zanella *et al.*, 1999; Douglas *et al.*, 2000; Cowieson and  
47  
48 230 Ravindran, 2008), where a cocktail of xylanase, amylase and protease improved ileal N, amino  
49  
50  
51 231 acid and energy digestibility.

52  
53  
54  
55 232  
56  
57  
58  
59  
60

1  
2  
3 233 Instructively, the relative improvement in energy digestibility at the ileal level was greater  
4  
5  
6 234 than that measured in the excreta. Though there was no difference between the ileal digestible  
7  
8 235 energy and AME<sub>N</sub> in the control diet (11.64 vs. 11.68 MJ/kg dry matter), supplementation with  
9  
10 236 the highest dose of the enzyme improved the ileal digestible energy by 0.40 MJ/kg (3.4%) but  
11  
12 237 AME<sub>N</sub> was improved by only 0.26 MJ/kg (2.2%). This apparent discrepancy in responses may  
13  
14  
15 238 be explained, at least in part, by the modifying effects of hindgut microflora and suggests that  
16  
17 239 ileal measurements may be a more accurate method to detect enzyme effects than on a total tract  
18  
19 240 basis where the contribution of the microbial populations may lead to underestimation of the true  
20  
21 241 effect of the enzyme product. It is also likely that digestible and metabolisable energy  
22  
23 242 measurements underestimate the true caloric value of enzyme supplements and overlook the  
24  
25 243 genuine 'net' effects *e.g.* altered nutrient requirements through a reduced gastrointestinal tract  
26  
27 244 mass as proposed by Cowieson *et al.* (2003).

28  
29 245 The effect of the enzyme cocktail on ileal N digestibility is of interest and is supported by  
30  
31 246 previous work on N and amino acid digestibility (Zanella *et al.*, 1999; Cowieson and Ravindran,  
32  
33 247 2008). There are a number of possible explanations for these effects, including improved  
34  
35 248 solubility and digestibility of dietary protein, reduced secretion of endogenous proteins and also a  
36  
37 249 reduction in ileal microbial load. Cowieson and Ravindran (2008) reported an improvements of  
38  
39 250 around 3% in the ileal digestibility of N and amino acids with a cocktail of xylanase, amylase and  
40  
41 251 protease, which is in general agreement with the results presented herein. However, the reported  
42  
43 252 effects were dependent on the amino acid in question, for example, with improvements in cystine  
44  
45 253 and threonine being proportionally greater than for methionine. It is possible that the enzyme  
46  
47 254 product alleviates endogenous loss via a reduction in the secretion of pancreatic  $\alpha$ -amylase and  
48  
49 255 proteases. Importantly, significant relationships between enzyme-generated responses in ileal  
50  
51 256 amino acid digestibility and the amino acid profile of key endogenous proteins have been  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 257 established (Cowieson and Ravindran, 2007). These effects have been implied previously with  
4  
5 258 reductions in pancreatic mass associated with amylase supplementation (Onderci *et al.*, 2006) and  
6  
7  
8 259 a reduction in pancreatic  $\alpha$ -amylase activity in duodenal contents with glucanase supplementation  
9  
10 260 (Leslie *et al.*, 2007). Recently, some doubt has been shed on the endogenous loss mechanism by  
11  
12 261 Rutherford *et al.* (2007), who failed to demonstrate a reduction in endogenous lysine flow with a  
13  
14 262 mixture of xylanase, amylase and glucanase. Nevertheless, a reduction in endogenous loss  
15  
16 263 associated with exogenous carbohydrases cannot be discounted, and more evidence is required to  
17  
18 264 elucidate the mechanisms by which amino acid and N digestibility are improved. If endogenous  
19  
20 265 protein loss is reduced by supplemental enzymes, the implications for both digestible and net  
21  
22 266 energy are considerable.

267 It can be concluded that a combination of xylanase, amylase and protease can improve the  
268 nutritional value of maize-soybean meal based diets in a dose-dependent manner. The dose  
269 sensitivity may be related to the concentration of substrates in the diet, ingredient quality and  
270 enzyme combinations in the cocktail. In the present study, the higher doses offered the greatest  
271 enhancements in weight gain, feed efficiency, ileal N digestibility and DM retention, but this may  
272 not always be the most economically attractive choice. The decision on enzyme dose should  
273 therefore be made using economic optimisation tools which reflect ingredient pricing and the  
274 benefit of the enzyme on energy utilisation and nutrient digestibility.

## 275 REFERENCES

- 276 AOAC (1994). Official Methods of Analysis. 15<sup>th</sup> Edition. Association of Official Analytical  
277 Chemists, Arlington, VA.
- 278 BARNES, W.C. & BLAKENEY, A.B. (1974) Determination of cereal alpha-amylase. *Starch* **26**,  
279 193-197.

- 1  
2  
3 280 BEDFORD, M.R. (2000) Exogenous enzymes in monogastric nutrition – their current value and  
4 future benefits. *Animal Feed Science and Technology* **86**: 1-13.  
5 281  
6  
7 282 CHOCT, M. (2006) Enzymes for the feed industry: past, present and future. *World's Poultry*  
8 *Science Journal* **62**: 5-15.  
9 283  
10  
11 284 COWIESON, A.J. & RAVINDRAN, V. (2007). Effect of phytic acid and microbial phytase on  
12 the flow and amino acid composition of endogenous protein at the terminal ileum of growing  
13 broiler chickens. *British Journal of Nutrition*. **98**: 745-752.  
14 285  
15 286  
16  
17 287 COWIESON, A.J. & RAVINDRAN, V. (2008) Effect of exogenous enzymes in maize-based  
18 diets varying in nutrient density for young broilers: growth performance and digestibility of  
19 energy, minerals and amino acids. *British Poultry Science*, in press.  
20 288  
21 289  
22  
23 290 COWIESON, A.J., ACAMOVIC, T. & BEDFORD, M.R. (2003) Supplementation of diets  
24 containing pea meal with exogenous enzymes: effects on weight gain, feed conversion,  
25 nutrient digestibility and gross morphology of the gastrointestinal tract of growing broiler  
26 chickens. *British Poultry Science*, **44**: 427-437.  
27 291  
28 292  
29 293  
30  
31 294 COWIESON, A.J., SINGH, D.N. & ADEOLA, O. (2006a) Prediction of ingredient quality and  
32 the effect of a combination of xylanase, amylase, protease and phytase on the performance  
33 of broiler chicks. I. Growth performance and digestible nutrient intake. *British Poultry*  
34 *Science* **47**: 477-489.  
35 295  
36 296  
37 297  
38 298 COWIESON, A.J., ACAMOVIC, T. & BEDFORD, M.R. (2006b) Supplementation of corn-soy  
39 based diets with an *Escherichia coli*-derived phytase: effects on broiler chick performance  
40 and the digestibility of amino acids and the metabolizability of minerals and energy. *Poultry*  
41 *Science* **85**: 1389-1397.  
42 300  
43 301  
44  
45  
46 302 DOUGLAS, M.W., PARSONS, C.M. & BEDFORD, M.R. (2000) Effect of various soybean  
47 meal sources and Avizyme on chick growth performance and ileal digestible energy.  
48 *Journal of Applied Poultry Research* **9**, 74-80.  
49 303  
50 304  
51  
52 305 GHAZI, S., ROOKE, J.A., GALBRAITH, H. & BEDFORD, M.R. (2002) The potential for the  
53 improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks  
54 and broiler cockerels. *British Poultry Science*, **43**: 70-77.  
55 306  
56 307  
57  
58  
59  
60

- 1  
2  
3 308 GRACIA, M.I., ARANIBAR, M.J., LAZARO, R., MEDEL, P. & MATEOS, G.G. (2003) Alpha-  
4  
5 309 amylase supplementation of broiler diets based on corn. *Poultry Science*, 82: 436-442.  
6  
7 310 HILL, F. W. & ANDERSON, D.L. (1958). Comparison of metabolizable energy and productive  
8  
9 311 energy determinations with growing chicks. *Journal of Nutrition*. 64: 587-603.  
10  
11 312 LESLIE, M.A., MORAN, E.T. & BEDFORD, M.R. (2007) The effect of phytase and glucanase  
12  
13 313 on the ileal digestible energy of corn and soybean meal fed to broilers. *Poultry Science*, **86**:  
14  
15 314 2350-2357.  
16  
17 315 MCCLEARY, B.V. & SHEEHAN, H. (1989) Measurement of cereal  $\alpha$ -amylase: A new assay  
18  
19 316 procedure. *Journal of Cereal Science* **6**, 237-251.  
20  
21 317 MIKULSKI, D., JANKOWSKI, J., ZDUNCZYK, Z., JUSKIEWICZ, J., KABUKOWSKA, L. &  
22  
23 318 MIKULSKA, M. (2006) Performance and gastrointestinal responses to different levels of  
24  
25 319 enzyme (xylanase and glucanase) in a diet. *Medycyna Weterynaryjna* **62**: 887-892.  
26  
27 320 ONDERCI, M., SAHIN, N., SAHIN, K., CIKIM, G., AYDIN, A., OZERCAN, I. & AYDIN, S.  
28  
29 321 (2006) Efficacy of supplementation of  $\alpha$ -amylase-producing bacterial culture on the  
30  
31 322 performance, nutrient use, and gut morphology of broiler chickens fed a corn-based diet.  
32  
33 323 *Poultry Science*, **85**: 505-510.  
34  
35 324 ROSEN, G. (2002) Exogenous enzymes as pro-nutrients in broiler diets. In: *Recent Advances in*  
36  
37 325 *Animal Nutrition*. P.C. GARNSWORTHY & J. WISEMAN (Eds.), Nottingham University  
38  
39 326 Press, Nottingham, UK. pp. 89-104.  
40  
41 327 ROSS. (2007). Ross 308 Broiler Nutrient Specification Manual. Aviagen Ltd, Midlothian,  
42  
43 328 Scotland. June 2007.  
44  
45 329 RAVINDRAN, V., HEW, L.I., RAVINDRAN, V. & BRYDEN, W.L. (1999). A comparison of  
46  
47 330 ileal digesta and excreta analysis for the determination of amino acid digestibility in feed  
48  
49 331 ingredients for poultry. *British Poultry Science*, **40**: 266-274.  
50  
51 332 RUTHERFURD, S.M., CHUNG, T.K. & MOUGHAN, P.J. (2007) The effect of a commercial  
52  
53 333 enzyme preparation on apparent metabolizable energy, the true ileal amino acid digestibility,  
54  
55 334 and endogenous ileal lysine losses in broiler chickens. *Poultry Science*, **86**: 665-672.  
56  
57 335 SAS. (1997). SAS/STAT<sup>®</sup> User's Guide: Statistics. Version 6.12. SAS Institute Inc., Cary, NC.  
58  
59  
60

1  
2  
3 336 SHORT, F.J., GORTON, P., WISEMAN, J. & BOORMAN, K.N. (1996) Determination of  
4  
5 337 titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed*  
6  
7 338 *Science and Technology*, 59: 215-221.

8  
9 339 SWEENEY, R.A. (1989) Generic combustion method for determination of crude protein in  
10  
11 340 feeds: Collaborative study. *Journal of the Association of Official Analytical Chemists*, 72:  
12  
13 341 770-74.

14  
15 342 ZANELLA, I., SAKOMURA, N.K., SILVERSIDES, F.G., FIQUEIRDO, A. & PACK, M.  
16  
17 343 (1999) Effect of enzyme supplementation of broiler diets based on maize and soybeans.  
18  
19 344 *Poultry Science* **78**: 561-568.

20  
21  
22 345

23  
24  
25 346

26  
27  
28 347  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1.** *Ingredient composition and analysis (g/kg air dry basis) of the control diet<sup>1</sup>*

Ingredient	Control diet
Maize	604.0
Soybean meal	353.0
Soybean oil	5.0
Limestone	11.5
Dicalcium phosphate	15.0
DL Methionine	1.5
Salt	4.0
Vitamin- trace mineral premix <sup>2</sup>	3.0
Titanium oxide	3.0
Calculated and analysed provision	
Dry matter <sup>3</sup>	915
ME (MJ/kg)	12.23
Gross energy (MJ/kg) <sup>3</sup>	16.72
Crude protein	218.0 (231.2) <sup>3</sup>
Calcium	9.0
Total phosphorus	6.8
Available phosphorus	4.0
Fat	57.9
Fibre	25.6
Methionine	5.9
Total SAA	9.5
Lysine	13.0
Arginine	14.5
Threonine	8.3
Tryptophan	2.5
Na	1.8
K	9.3

<sup>1</sup> The control diet was offered without or with enzyme (Avizyme<sup>TM</sup> 1502; Danisco Animal Nutrition, Marlborough, UK) at either 250 or 500g/tonne of finished feed.

<sup>2</sup> Provided the following per kg diet: ethoxyquin, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl- $\alpha$ -tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

<sup>3</sup> Analysed values.

361 **Table 2.** *Recovery of xylanase, amylase and protease activities in the experimental diets*<sup>1,2</sup>

Diet	Xylanase (U/kg)	Amylase (U/kg)	Protease (U/kg)
Control	<100	<100	<100
Control + 250g/tonne enzyme <sup>1</sup>	377	1010	2010
Control + 500g/tonne enzyme <sup>1</sup>	634	1917	4217

362 <sup>1</sup> The enzyme cocktail was added to provide a guaranteed minimum of 300 U xylanase, 400 U  
 363 amylase and 4,000 U protease per kg of feed at 500g/tonne inclusion.

364 <sup>2</sup> In-feed assays were carried out in duplicate.

365  
 366

367 **Table 3.** *Influence of enzyme dose rates on the performance of male broilers fed nutritionally*  
 368 *marginal maize-soy diets (1-21 d post-hatching)<sup>1</sup>*

Treatment	Weight gain (g/bird)	Feed intake (g/bird)	Feed per gain <sup>2</sup> (g/g)	Mortality, deaths/total <sup>3</sup>
No enzyme	674 <sup>a</sup>	966 <sup>a</sup>	1.433 <sup>a</sup>	0/48
+250g/tonne	684 <sup>a</sup>	956 <sup>a</sup>	1.416 <sup>ab</sup>	1/48
+500g/tonne	730 <sup>b</sup>	1018 <sup>b</sup>	1.402 <sup>b</sup>	1/48
Pooled SEM	11.5	17.7	0.0099	-
Probability, <i>P</i> <	0.01	0.05	0.05	-

369 <sup>a,b</sup>Means in the same column not sharing a common superscript are significantly different  
 370 (*P*<0.05).

371 <sup>1</sup> Each mean represents values from six replicates (8 birds/replicate).

372 <sup>2</sup> Corrected for mortality.

373 <sup>3</sup> Number of deaths per total number of birds (6 replicates x 8 birds per replicate).

386 **Table 4.** Influence of enzyme dose rates on total tract dry matter retention, N retention, AME and  
 387 N-corrected AME in male broilers fed nutritionally marginal maize-soy diets<sup>1</sup>  
 388

Treatment	Total tract DM retention coefficient	Total tract N retention, g/kg DM intake	AME, MJ/kg DM	AME <sub>n</sub> , MJ/kg DM
No enzyme	0.739 <sup>a</sup>	25.9	12.63 <sup>a</sup>	11.68 <sup>a</sup>
+250g/tonne	0.749 <sup>b</sup>	26.6	12.79 <sup>b</sup>	11.82 <sup>ab</sup>
+500g/tonne	0.759 <sup>c</sup>	26.3	12.90 <sup>b</sup>	11.94 <sup>b</sup>
Pooled SEM	0.003	0.33	0.041	0.042
Probability, <i>P</i> <	0.000	NS	0.000	0.000

389 <sup>a,b</sup>Means in the same column not sharing a common superscript are significantly different  
 390 (*P*<0.05).

391 <sup>1</sup> Each mean represents values from six replicates (8 birds/replicate).  
 392  
 393  
 394  
 395

396 **Table 5.** Influence of enzyme dose rates on ileal digestibility coefficients of DM, N and GE, and  
 397 ileal digestible energy in male broilers fed nutritionally marginal maize-soy diets<sup>1</sup>

398

Treatment	Ileal digestibility coefficient			Ileal digestible energy, MJ/ kg DM <sup>2</sup>
	Dry matter	Nitrogen	Gross energy	
No enzyme	0.678 <sup>a</sup>	0.817 <sup>a</sup>	0.696 <sup>a</sup>	11.64 <sup>a</sup>
+250g/tonne	0.693 <sup>ab</sup>	0.832 <sup>b</sup>	0.709 <sup>ab</sup>	11.86 <sup>ab</sup>
+500g/tonne	0.701 <sup>b</sup>	0.848 <sup>c</sup>	0.720 <sup>b</sup>	12.04 <sup>b</sup>
Pooled SEM	0.005	0.002	0.004	0.041
Probability, <i>P</i> <	0.05	0.001	0.01	0.01

399 <sup>a,b</sup>Means in the same column not sharing a common superscript are significantly different  
 400 (*P*<0.05).

401 <sup>1</sup> Each mean represents values from six replicates (8 birds/replicate).

402 <sup>2</sup> Ileal digestibility coefficient of gross energy x gross energy of diet.

403