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## The effect of dietary xylanase on energy, amino acid and mineral metabolism and egg production and quality in laying hens

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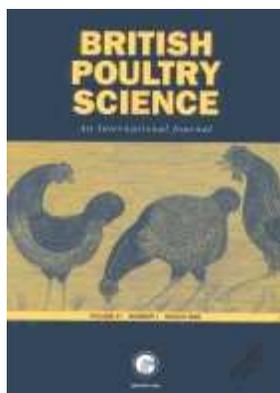
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**The effect of dietary xylanase on energy, amino acid and mineral metabolism and egg production and quality in laying hens**

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Manuscripts

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3 1 **The effect of dietary xylanase on energy, amino acid and mineral metabolism and egg**  
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6 2 **production and quality in laying hens**  
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23 **RUNNING TITLE: Xylanase in hen diets**  
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3  
4 26 **Abstract** 1. The aim of the present study was to examine the effect of dietary xylanase on the  
5  
6 27 availability of nutrients for laying hens when fed on wheat-rye-soy-based diets. The basal diet  
7  
8 28 was formulated to contain 11.03 MJ/kg apparent metabolisable energy (AME) and the  
9  
10 29 experimental diets were formulated by supplementing the basal diet with four different  
11  
12 30 activities of xylanase (400, 800, 1200 and 1600 xylanase units (XU) /kg).

13  
14  
15 31 2. The AME and nitrogen metabolisability coefficient of xylanase supplemented diets was  
16  
17 32 1.2% and 7.1% respectively; these were significantly higher than the control diet.

18  
19  
20 33 3. Supplementary xylanase significantly improved the coefficients of metabolisability of  
21  
22 34 indispensable, dispensable and total amino acids by 8.2, 6.9 and 7.8% respectively, and led to  
23  
24 35 a significantly linear response of total amino acid metabolisability coefficient to xylanase.  
25  
26 36 There was a range of effects within the indispensable amino acids with xylanase  
27  
28 37 supplementation (1600 XU/kg) significantly improving the metabolisability of threonine by  
29  
30 38 4.9%, but having no have effect on lysine. The response of total amino acid retention to  
31  
32 39 added xylanase was a significant quadratic function and suggests that 800 XU/kg is the  
33  
34 40 optimum supplementary dosage.

35  
36  
37 41 4. Supplementary xylanase significantly improved sulphur metabolisability in a linear fashion  
38  
39 42 to a maximum of 2.3% higher than that of the control diet. In terms of daily retention, most of  
40  
41 43 the minerals responded in a quadratic manner to dietary xylanase, as the suggested optimal  
42  
43 44 supplementary level was between 800 and 1200 XU/kg.

44  
45 45 5. The yolk colour of the birds receiving 1200 and 1600 XU/kg was 0.33 and 0.28 units  
46  
47 46 (Roche score), these were 4.1 and 3.5%, respectively, darker than the yolk of the birds given  
48  
49 47 the control diet.

50  
51 48 6. Birds receiving xylanase had a significantly higher weight gain than those fed on the  
52  
53 49 unsupplemented diet. Feed intake, number of eggs per hen.d, dirty and cracked eggs, and feed  
54  
55 50 conversion ratio for egg production (FCR) were not affected by xylanase. These data suggest  
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2  
3 51 that use of a xylanase may improve the metabolisability of many nutrients but that such  
4  
5 52 effects may not always benefit production parameters.  
6  
7

## 8 53 INTRODUCTION

9  
10 54 Wheat is an important source of energy and protein in UK poultry feeds and can be used as  
11  
12 55 the sole cereal source in poultry diets. Barley, rye and other cereals can also be included in  
13  
14 56 diets at lower concentrations in diet formulations. However, the cell walls of cereals are  
15  
16 57 primarily composed of carbohydrate complexes known as non-starch polysaccharides  
17  
18 58 (NSPs). NSPs have a structural function and account for approximately 100 g/kg of the whole  
19  
20 59 grain in UK wheat cultivars (Austin *et al.*, 1999; Rose *et al.*, 2001; Pirgozliev *et al.*, 2003).  
21  
22 60 The majority of NSPs in wheat and rye are arabinoxylans (pentosans), whilst mixed-linked  $\beta$ -  
23  
24 61 D-glucans are the most nutritionally significant in barley (Henry, 1985; Annison, 1991). The  
25  
26 62 physicochemical properties of the soluble, higher molecular weight NSPs result in increased  
27  
28 63 digesta viscosity which is correlated with reduced bird performance (Bedford and Classen,  
29  
30 64 1992; Choct *et al.*, 1999). In particular, soluble viscous NSPs depress the digestibility of  
31  
32 65 protein, starch and fat in broiler diets, an effect which is easily overcome through use of the  
33  
34 66 appropriate enzyme (Annison, 1991; Bedford and Classen, 1992). In addition to the effect on  
35  
36 67 viscosity, the positive effect of exogenous NSP'ases has been associated with degradation of  
37  
38 68 cell wall NSPs and release of encapsulated nutrients in the gut (Bedford and Schulze, 1998;  
39  
40 69 Rotter *et al.*, 1990; Annison, 1991). The beneficial effect of dietary NSP'ases is well  
41  
42 70 recognised and their use is an established part of the animal feed industry (Bedford, 2000;  
43  
44 71 Acamovic, 2001; Choct, 2006).  
45  
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53 72 In comparison with the broiler literature, there is relatively little research reported on the  
54  
55 73 effects of xylanases on energy, amino acid and mineral metabolism in laying hens. It is  
56  
57 74 already known that the positive effects of dietary xylanase decreases with the age of the bird  
58  
59 75 and its action is more noticeable in early stage of growth (Salih *et al.*, 1991; Campbell and  
60

1  
2  
3 76 Bedford, 1992). Compared to mature birds, the digestive capacity of chickens during the first  
4  
5 77 weeks of life is less than optimal, the physiological development of the gastro-intestinal tract  
6  
7  
8 78 is not complete, and chicks are unable to consistently produce an adequate array of digestive  
9  
10 79 enzymes (Leeson and Summers, 2005). Unlike chickens, hens have much bigger, well  
11  
12 80 developed digestive tract which is considered capable of dealing with viscosity (Annison *et*  
13  
14 81 *al.*, 1968).

15  
16  
17 82 The aim of the present study was to examine the effect of dietary xylanase on  
18  
19 83 performance, metabolisable energy, amino acid and mineral metabolisability, and egg  
20  
21 84 production and quality when fed to Lohmann Brown layer hens between 28 and 32 weeks of  
22  
23 85 age.  
24  
25

## 26 27 86 MATERIALS AND METHODS

### 28 29 87 **Diet formulation**

30  
31 88 Five wheat-rye-soybean-based diets were prepared and supplemented with an exogenous  
32  
33 89 xylanase sourced from *Bacillus* (EC 3.2.1.8; Syngenta Biotechnology Inc., Research Triangle  
34  
35 90 Park, NC). Diets were manufactured to be nutritionally adequate but designed to be relatively  
36  
37 91 high in NSPs and low in metabolisable energy and crude protein (Table 1). The basal diet  
38  
39 92 was supplemented with enzyme at 400, 800, 1200 or 1600 XU/kg diet respectively. The  
40  
41 93 enzyme was added to the diets in powder form and all diets fed as mash with 5g/kg of  
42  
43 94 titanium dioxide included as a marker. Table 1 near here

### 44 45 46 47 95 **Experimental design**

48  
49 96 In order to maximise chances of detecting responses to the enzyme, the experiment was  
50  
51 97 undertaken during a time of high energy requirement for egg production. Eight hundred and  
52  
53 98 ten Lohmann Brown hens were housed 3 per cage in 270 layer cages (3 tiers, 833 cm<sup>2</sup> per  
54  
55 99 bird) between 28 and 32 weeks of age. Each cage was equipped with a separate feeder in  
56  
57 100 front and a nipple drinker inside. The experiment was conducted as a randomised block

1  
2  
3 101 design with 54 blocks located over three tier levels (18 blocks per tier, each block consisting  
4  
5  
6 102 of 5 cages given different experimental diets). The temperature was maintained at 21°C and  
7  
8 103 relative humidity was between 50 and 70%. The birds had *ad libitum* access to feed and water  
9  
10  
11 104 and were given 14 h of light each 24 h.

12  
13 105 The Animal Experimental Committee of SAC approved the study.

#### 14 15 106 **Hen performance, egg production and egg quality determination**

16  
17  
18 107 The hens in each cage were bulk weighed at the beginning and the end of the four week  
19  
20 108 experimental period. Feed intake (FI) of each cage was recorded and calculated per hen per  
21  
22 109 day. Eggs were collected daily for the whole experimental period and their weight was  
23  
24  
25 110 recorded. The number of dirty and cracked eggs, eggs with double yolk, eggs with soft shell  
26  
27 111 and hen mortality were also recorded daily. The feed conversion ratio for egg production  
28  
29 112 (FCR) was calculated as grams of feed intake/gram of eggs laid.

30  
31  
32 113 Egg and egg shell quality analyses were performed on a total of 270 eggs which had been  
33  
34 114 collected, one egg from each cage, on the last day of the experiment (32 weeks old). The  
35  
36 115 analyses of the eggs were completed on the day of collection. The eggs were individually  
37  
38 116 weighed, and the external and internal quality was determined by using an egg multimeter  
39  
40  
41 117 instrument (QSM-System, TSS, York, YO1 5SE). Shell colour was assessed by determining  
42  
43 118 the shell reflectivity (an indication of the lightness of colour of the eggshell). All eggs were  
44  
45  
46 119 then broken and their contents removed. The internal quality of the eggs was assessed as  
47  
48 120 albumen height, Haugh Units (HU) and yolk colour. Shell thickness without the membrane in  
49  
50  
51 121 place was the mean value of measurements at three locations on the egg's equator. The shell  
52  
53 122 thickness index was calculated by the system using the data on whole egg weight and dried  
54  
55 123 shell weight. The incidence of blood and meat spots on the eggs was determined by a single  
56  
57  
58 124 controller.

1  
2  
3 125 **Apparent metabolisable energy (AME), dry matter digestibility, nitrogen, amino acid**  
4  
5 126 **and mineral metabolisability determination**  
6  
7

8 127 During the final 2 d of the experimental period, at 32 weeks of age, a representative sample  
9  
10 128 of excreta was collected from the belt under each cage, freeze dried and milled (0.75 mm  
11  
12 129 mesh). The gross energy (GE) of feed and excreta was determined using a Parr adiabatic  
13  
14 130 bomb calorimeter (Parr-1755, Parr Instruments Company, MO, USA). Sample dry matter was  
15  
16 131 determined by drying for 24 h in a force draft oven at 100°C. The nitrogen in feed and excreta  
17  
18 132 was determined by the method of Sweeney (1989) using an FP-200 nitrogen analyser  
19  
20 133 (LECO<sup>®</sup>, St. Joseph, MI, USA). Amino acids in samples were determined by high  
21  
22 134 performance liquid chromatography with fluorimetric detection of the orthophthaldehyde  
23  
24 135 derivatives (Jones *et al.*, 1981; Alltech, Associates, Carnforth, UK; Oduguwa *et al.*, 2007).  
25  
26 136 Since the method of hydrolysis destroys methionine, cystine and tryptophan, data on these  
27  
28 137 amino acids are not reported. Excreta digestibility for glycine is not presented because of the  
29  
30 138 glycine yield from acid hydrolysis of uric acid in excreta (Soares *et al.*, 1971). Minerals,  
31  
32 139 including Ti, were determined by inductively coupled plasma emission spectrometry (Optima  
33  
34 140 4300 DV Dual View ICP-OE spectrometer, Perkin Elmer, Beaconsfield, UK) (Tanner *et al.*,  
35  
36 141 2002).  
37  
38  
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42

43 142 Dietary AME, dry matter digestibility coefficient (DMD), metabolisability coefficient and  
44  
45 143 retention of N, amino acids, and minerals were calculated using the recovered marker content  
46  
47 144 in feed and excreta (McDonald *et al.*, 1994).  
48  
49

50 145 The excretion of cations from the hens is expressed as milli-equivalents (mEq) of cations  
51  
52 146 measured in the excreta as previously explained (Pirgozliev *et al.*, 2009).  
53  
54

55 147 **Statistical analyses**  
56

57 148 The observational unit was the cage. Statistical analyses were performed by *Genstat 11th*  
58  
59 149 *edition* (Lawes Agricultural Trust, 2008). Differences in blood and meat spots, yolk and shell  
60

1  
2  
3 150 colour, cracked and dirty eggs, eggs with double yolk, eggs with soft shell, and mortality  
4  
5  
6 151 were tested using Generalised Linear Mixed Model analysis. Other data were analysed by  
7  
8 152 ANOVA with random term block and fixed term treatment. Pre-planned comparisons and a  
9  
10 153 polynomial contrast tests were performed to test for overall difference between all xylanase  
11  
12 154 supplemented diets vs control diet and for linear and quadratic responses to xylanase  
13  
14  
15 155 supplementation across diets.

## 17 156 RESULTS

19  
20 157 The birds remained healthy and the overall mortality was less than 1% throughout the  
21  
22 158 experiment, with no significant differences between treatment groups (data not included).  
23  
24 159 Although hens receiving the 1200XU/kg diet tended ( $P=0.089$ ) to consume more feed  
25  
26 160 compared to hens given the other diets, overall, dietary xylanase supplementation did not  
27  
28 161 have a significant effect on the daily feed intake of the birds (Table 2). Over the experimental  
29  
30 162 period hens receiving xylanase supplemented diets gained significantly more weight (about  
31  
32 163 14 g) than birds fed on the control only. FCR for egg production was not significantly  
33  
34 164 affected by xylanase supplementation. The AME of diets supplemented with xylanase  
35  
36 165 supplemented diets was 1.2% significantly higher than the AME of the control, although the  
37  
38 166 intake of dietary AME was not affected by enzyme inclusion. Nitrogen metabolisability was  
39  
40 167 7.1% significantly higher in xylanase supplemented diets than the control diet. The difference  
41  
42 168 was even more pronounced, i.e. 10.5%, when a specific comparison was made between the  
43  
44 169 control diet and the 1200XU/kg supplemented diet. Overall, birds receiving xylanase retained  
45  
46 170 5.3% significantly more N than the control-fed hens, with those fed on the 1200 XU/kg diet  
47  
48 171 retaining 8.3% more. There was a significant linear response of N metabolisability and of N  
49  
50 172 retention (NR) ( $P < 0.001$ ) to xylanase supplementation. However, the quadratic response of  
51  
52 173 NR to xylanase supplementation ( $P < 0.001$ ) suggested that the optimum xylanase addition for  
53  
54 174 NR is near 1200 XU/kg feed. In addition, DMD of the xylanase-supplemented diets was  
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2  
3 175 1.2% significantly greater than that of the control. The yolk colour of the birds receiving  
4  
5 176 1200 and 1600 XU/kg was 0.33 and 0.28 units higher (Roche scale), i.e. 4.1 and 3.5%  
6  
7  
8 177 respectively ( $P < 0.001$ ), than the yolk of the birds fed on the control diet (Table 3). There was  
9  
10 178 a linear ( $P < 0.001$ ) response of yolk colour to supplementary xylanase. Other egg production  
11  
12 and quality parameters were affected by treatment. Table 2 near here

13  
14  
15 180 Exogenous xylanase supplementation improved the coefficients of metabolisability of  
16  
17 181 most of the measured dietary amino acids (Table 4). Compared to the control, dietary  
18  
19 182 xylanase significantly improved the overall metabolisability coefficients for indispensable,  
20  
21 183 dispensable and total amino acids by 8.2%, 6.9% and 7.8% respectively. These improvements  
22  
23 184 were the result of a significant linear improvement in total amino acid metabolisability in  
24  
25 185 accord with xylanase administration. Specifically, there was a significant linear improvement  
26  
27 186 in lysine and threonine metabolisability with the use of xylanase. Tables 3, 4 & 5 near here

28  
29  
30 187 Compared to the control, dietary xylanase significantly improved overall daily retention  
31  
32 188 of indispensable amino acids by 9.3%, though the response was quadratic in nature (Table 5).  
33  
34 189 This relationship suggested that the optimum xylanase dosage for indispensable amino acid  
35  
36 190 retention is close to 800 XU/kg feed.

37  
38  
39 191 Feeding xylanase significantly improved overall S metabolisability in a linear fashion; the  
40  
41 192 average effect being approximately 2.3% greater than the control (Table 6). Supplemental  
42  
43 193 xylanase tended ( $P = 0.074$ ) to improve Mg metabolisability but did not significantly affect  
44  
45 194 any other determined minerals.

46  
47  
48 195 Retention of the following minerals was significantly enhanced, relative to the control  
49  
50 196 diet, by feeding xylanase; K by 2.8%, Mg by 5.2% ( $P < 0.001$ ), Mn by 4.9% ( $P < 0.001$ ), Na  
51  
52 197 by 2.1% and S by 1.9% (Table 7). This resulted in a quadratic response ( $P < 0.001$ ) of the  
53  
54 198 retention of most of the minerals to added xylanase, with each relationship suggesting an

1  
2  
3 199 optimum xylanase addition close to 800 XU/kg. No significant changes in the daily retention  
4  
5 200 of dietary Ca and P or the excretion of cations were detected. Tables 6 & 7 near here

## 201 DISCUSSION

202 Poultry do not produce enzymes capable of digesting dietary NSPs and so it is not surprising  
203 to observe an improvement in nutrient availability and bird performance when NSP'ases are  
204 added to diets based on wheat and rye. Although the exact anti-nutritive role of NSPs is not  
205 completely understood, it is assumed that the mode of action combines encapsulation of  
206 dietary nutrients and/or increasing the viscosity of the intestinal digesta (Bedford, 2000;  
207 Acamovic, 2001; Choct, 2006). Addition of exogenous xylanase has been shown to hydrolyse  
208 the high molecular mass sub-fraction of arabinoxylan and decrease the formation of viscous  
209 solution in the digestive tracts of broiler chickens (Bedford and Classen, 1992). Nevertheless,  
210 older birds are able to cope better with higher viscosity and hence respond to a lesser extent  
211 on supplementation with a xylanase compared with younger birds (Huyghebaert and de  
212 Groote, 1995; Mahagna *et al.*, 1995; Smulikowska, 1995). Although digesta viscosity was not  
213 specifically determined in this study, the lack of difference in the number of dirty eggs laid, a  
214 problem that is seen consistently and can be related to viscosity in layers (Chesson, 2001),  
215 suggests that the benefit of using dietary xylanase in this study came primarily from changes  
216 in characteristics than other viscosity.

217 Whilst it is possible that the lack of a performance response to the enzyme in this study  
218 may have been due to the relatively short period of investigation coupled with the ability of  
219 the laying hen to use reserves to maintain egg production over moderate periods of time  
220 (Morris, 1969), Novak *et al.* (2008) also saw no differences in egg production during 20  
221 weeks of lay (18 to 38 weeks of age) when layers were given diets with different xylanase  
222 activity, suggesting that the length of the feeding period may not necessarily have been the  
223 reason for lack of response.

1  
2  
3 224 Performance and dietary AME and DMD were in the expected range for wheat-soy layer  
4  
5 225 diets (Pan *et al.*, 1998; Silversides *et al.*, 2006). Pan *et al.* (1998) found an improvement in  
6  
7 226 dietary AME when wheat and rye based diets were supplemented with NSP degrading  
8  
9 227 enzymes, which is in agreement with the current work. Silversides *et al.* (2006), however, did  
10  
11 228 not observe a relationship between dietary xylanase and the AME values, and suggested that  
12  
13 229 the NSP content of the cereals used in his experimental diet was too low to elicit a response  
14  
15 230 from the addition of a xylanase. The variable responses reported in the literature may be a  
16  
17 231 result of using different diet formulations, different strains of birds at different ages, different  
18  
19 232 products and different experimental lengths between studies (Pan *et al.*, 1998; Jaroni *et al.*,  
20  
21 233 1999).

22  
23  
24  
25  
26 234 Diets with a high NSP content can reduce the rate of feed passage (Acamovic, 2001) and  
27  
28 235 this may encourage the proliferation of microflora in the small intestines. Not only can such  
29  
30 236 bacteria ferment and utilise carbohydrate and protein, therefore competing with the host for  
31  
32 237 nutrients (Choct *et al.*, 1996), but some species secrete enzymes which lead to deconjugation  
33  
34 238 of bile acids resulting in an impairment of lipid digestion and absorption (Feighner and  
35  
36 239 Dashkevicz, 1987; Smits *et al.*, 1998). The significantly improved growth rate of the birds  
37  
38 240 and dietary AME and DMD in the presence of the xylanase may be a consequence of  
39  
40 241 decreasing microbial colonisation in the gut, thereby improving the availability of dietary  
41  
42 242 starch, fat and protein. Moreover, improved fat digestion, through reduced bile salt  
43  
44 243 deconjugation, may be responsible for the improvement in yolk colour noted as a result of the  
45  
46 244 pigments co-migrating with fat. This is in agreement with previous reports (Aimonen & Uusi-  
47  
48 245 Rawa, 1991; Çiftci *et al.*, 2003), where feeding high dosages of a xylanase increased the  
49  
50 246 density of the yolk colour compared to the control diet.

51  
52  
53 247 The improvements in dietary nitrogen and amino acid metabolisability were also in the  
54  
55 248 expected range and in accordance with previous reports (Brenes *et al.*, 1993a; Oloffs *et al.*,

1  
2  
3 249 1999; Mathlouthi *et al.*, 2003; Silversides *et al.*, 2006). Bedford *et al.* (1998) also noted that  
4  
5  
6 250 xylanase supplementation improved the metabolisability of threonine, which is prevalent in  
7  
8 251 endogenous secretion, but did not affect the metabolisability of lysine, which is primarily of  
9  
10 252 dietary origin. Threonine comprises about two-thirds of the amino acid composition of  
11  
12 253 mucin, and over half of the absorbed dietary threonine is used by the enterocytes (Neutra and  
13  
14  
15 254 Forstner, 1987). The decrease in the quantity of threonine excreted by the birds offered the  
16  
17 255 xylanase suggests that less mucin is being excreted by these birds. One suggestion is that  
18  
19  
20 256 enhanced mucin secretion in the control birds may be a result of the higher microbial loading  
21  
22 257 of these birds, since Meslin *et al.* (1999) demonstrated that microbes can enhance mucin  
23  
24  
25 258 secretion in rodents.

26  
27 259 A further consideration is that if the concentration of available dietary nutrients is  
28  
29 260 improved by feeding a xylanase (Bedford *et al.*, 1998; Mathlouthi *et al.*, 2003; Silversides *et*  
30  
31 261 *al.*, 2006), the GIT may respond by proportionately decreasing in both size and digestive  
32  
33  
34 262 enzyme output, i.e. reducing endogenous inputs and losses (Brenes *et al.*, 1993b; Esteve-  
35  
36 263 Garcia *et al.*, 1997; Bedford, 2006).

37  
38 264 The results for dietary mineral metabolisability for the control birds were in the expected  
39  
40  
41 265 range for layers (van der Klis *et al.*, 1997; Um and Paik, 1999), but the effect of xylanase was  
42  
43  
44 266 not consistent. Only S metabolisability was improved and this may relate more to  
45  
46 267 metabolisability of S containing amino acids, which is in agreement with the most of the  
47  
48 268 other amino acids. The lack of excreta-cation concentration response to dietary xylanase is in  
49  
50  
51 269 accord with the mineral digestibility data. However, the relatively different responses to  
52  
53 270 dietary xylanase in terms of digestibility coefficients vs retention for amino acids and  
54  
55 271 minerals indicates that data on digestibility only should be interpreted carefully. The  
56  
57  
58 272 quadratic responses of dietary amino acids and minerals to xylanase suggested that substrate  
59  
60 273 was limiting in the GIT of the birds once 800 to 1200 XU/kg had been dosed.

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2  
3 274 The results from this study demonstrated that dietary xylanase improves the nutritional  
4  
5  
6 275 value of wheat-rye-based diets when given to Lohmann Brown laying hens between 28 and  
7  
8 276 32 weeks of age. Supplemental xylanase improved bird weight and yolk colour, which was  
9  
10 277 generally associated with an improvement in dietary nutrient metabolisability and retention.  
11  
12 278 The improved dietary N utilisation suggests that the use of dietary xylanases may be an  
13  
14  
15 279 efficient way to reduce pollution from animal production. The lack of egg production  
16  
17  
18 280 response may relate to the short period of study and the relatively long period of time  
19  
20 281 required for changes in nutrition to deliver changes in performance in laying hens.

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**Table 1.** *Ingredient composition ('as fed') of the control diet*

Ingredients	kg/ 100kg		
Wheat	56.68		
Rye	10.00		
Wheat bran	3.75		
Maize gluten meal	1.00		
Soybean meal (48)	2.90		
Soybean meal (full fat)	15.16		
Limestone	8.65		
Mono DCP	0.95		
Salt	0.22		
Methionine	0.19		
Vitamin mineral premix <sup>1</sup>	0.50		
Calculated values (as fed)			
ME (MJ/kg)	11.03		
CP (N x 6.25), g/kg	161		
Ca, g/kg	34.1		
P, g/kg	6.3		
Determined values (DM) <sup>2</sup>			
GE (MJ/kg)	16.78		
CP (N x 6.25) g/kg	174		
DM (g/kg)	928		
Determined amino acid and mineral composition of the control diet (DM) <sup>2</sup>			
Amino acids (g/kg)	Minerals (g/kg)		
Alanine	11.57		
Arginine	14.68	Calcium	48.04
Aspartate	23.94	Potassium	7.53
Glutamate	62.67	Magnesium	1.39
Histidine	5.76	Sodium	1.12
Isoleucine	12.36	Phosphorus	6.9
Leucine	20.99	Sulphur	3.02
Lysine	12.37		
Phenylalanine	13.14		
Serine	8.76		
Threonine	7.83		
Tyrosine	7.39		
Valine	13.63		

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425 <sup>1</sup>Vitamin and mineral premix provided (units per kg/feed): retinol 2160 µg; cholecalciferol 75 µg; α-tocopherol

426 25 mg; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; cyanocobalamin 0.01 mg; pyridoxine, 1.5

427 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80

428 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg.

429 <sup>2</sup>Analyses were performed in duplicate and are presented on a dry matter basis.

**Table 2.** *The effect of dietary xylanase activity on growth performance and feed conversion ratio for egg production (FCR) in Lohmann Brown laying hens between 28 to 32 weeks of age, dietary apparent metabolisable energy (AME), daily intake of AME, dietary nitrogen metabolisability coefficient (NM), daily nitrogen retention (NR) and dietary dry matter digestibility coefficient (DMD), determined on the final 2 d of the study*

Results	C <sup>1</sup>	C+400XU <sup>2</sup>	C+800XU <sup>2</sup>	C+1200XU <sup>2</sup>	C+1600XU <sup>2</sup>	SED	P <sup>3</sup>	P <sup>4</sup>	L <sup>5</sup>	Q <sup>6</sup>
Feed intake (g DM/hen.d)	111.4	111.1	110.5	112.8	109.9	1.073	0.089	NS	NS	NS
Weight hen (kg start study)	1.796	1.788	1.808	1.814	1.778	0.0147	NS	NS	NS	NS
Weight hen (kg end study)	1.817	1.812	1.843	1.849	1.816	0.0172	0.100	NS	NS	0.078
Weight gain (g/hen)	21	28	34	39	39	7.3	0.062	<0.05	NS	NS
FCR eggs	1.965	1.959	1.994	1.962	1.939	0.0317	NS	NS	NS	NS
AME (MJ/kg DM)	12.96	13.16	13.12	13.01	13.18	0.142	NS	<0.05	NS	NS
AME intake (MJ/hen.d)	1.43	1.44	1.46	1.47	1.44	0.026	NS	NS	NS	NS
NM	0.400 <sup>a</sup>	0.408 <sup>ab</sup>	0.434 <sup>bc</sup>	0.442 <sup>c</sup>	0.429 <sup>bc</sup>	0.0140	<0.05	<0.05	<0.05	NS
NR (g/hen.d)	1.001 <sup>a</sup>	1.031 <sup>b</sup>	1.058 <sup>c</sup>	1.084 <sup>d</sup>	1.043 <sup>bc</sup>	0.0113	<0.001	<0.001	<0.001	<0.001
DMD	0.738	0.744	0.746	0.745	0.751	0.0063	NS	<0.05	0.063	NS

<sup>1</sup> Control diet.

<sup>2</sup> Xylanase activity (units/kg) in diet.

<sup>3</sup> Significance between treatments determined by ANOVA. Means within a row with no common superscript differ significantly.

<sup>4</sup> Significance between the C vs all xylanase supplemented diets determined by comparisons contrast tests.

<sup>5</sup> Linear effects of added dietary xylanase activities.

<sup>6</sup> Quadratic effects of added dietary xylanase activities.

**Table 3.** *The effect of dietary xylanase activity on total egg production of Lohmann Brown laying hens between 28 and 32 weeks of age, and egg quality determined on eggs collected for the last day of the 32nd week of age*

Egg parameters	C <sup>1</sup>	C+400XU <sup>2</sup>	C+800XU <sup>2</sup>	C+1200XU <sup>2</sup>	C+1600XU <sup>2</sup>	SED	P <sup>3</sup>	P <sup>4</sup>	L <sup>5</sup>	Q <sup>6</sup>
Total eggs laid (28 d/cage)	80.4	81.0	80.6	80.1	81.4	1.21	NS	NS	NS	NS
Average egg weight (g)	58.1	58.8	58.9	59.6	59.1	0.767	NS	NS	NS	NS
Egg weight (g/hen.d)	56.7	57.0	55.9	57.0	57.2	1.004	NS	NS	NS	NS
Albumen height (mm)	8.39	8.78	8.55	8.76	8.56	0.229	NS	NS	NS	NS
Haugh units	91.50	93.57	92.21	93.11	92.35	1.118	NS	NS	NS	NS
Shell thickness (μ)	376	369	372	373	375	4.71	NS	NS	NS	NS
Shell weight (g)	5.92	5.90	5.93	5.99	5.97	0.084	NS	NS	NS	NS
Shell thickness index (mg/cm <sup>2</sup> )	84.52	83.58	83.88	84.14	84.28	0.938	NS	NS	NS	NS
Blood spots (%)	0.18	0.12	0.18	0.09	0.07	0.617	NS	NS	NS	NS
Meat spots (%)	0.29	0.26	0.24	0.28	0.28	0.452	NS	NS	NS	NS
Yolk colour score	8.11 <sup>a</sup>	7.94 <sup>a</sup>	8.06 <sup>a</sup>	8.44 <sup>b</sup>	8.39 <sup>b</sup>	0.041	<0.001	NS	<0.001	NS
Shell colour lightness (% reflectivity)	28.78	28.70	27.52	27.91	27.91	0.035	NS	NS	NS	NS

<sup>1</sup> Control diet.

<sup>2</sup> Xylanase activity (units/kg) in diet.

<sup>3</sup> Significance between treatments determined by ANOVA. Means within a row with no common superscript differ significantly.

<sup>4</sup> Significance between the C vs all xylanase supplemented diets determined by comparisons contrast tests.

<sup>5</sup> Linear effects of added dietary xylanase activities.

<sup>6</sup> Quadratic effects of added dietary xylanase activities.

**Table 4.** *The effect of dietary xylanase activity on the metabolisability coefficient of dietary amino acids when fed to Lohmann Brown laying hens between 28 and 32 weeks of age as determined on the final 2 d of the 32nd week of age*

Amino acids	C <sup>1</sup>	C+400XU <sup>2</sup>	C+800XU <sup>2</sup>	C+1200XU <sup>2</sup>	C+1600XU <sup>2</sup>	SED	P <sup>3</sup>	P <sup>4</sup>	L <sup>5</sup>	Q <sup>6</sup>
<i>Indispensable</i>										
Arginine	0.247 <sup>a</sup>	0.340 <sup>bc</sup>	0.392 <sup>c</sup>	0.298 <sup>ab</sup>	0.315 <sup>abc</sup>	0.0420	<0.05	<0.05	NS	<0.05
Histidine	0.374	0.443	0.423	0.399	0.420	0.0377	NS	0.115	NS	NS
Isoleucine	0.616 <sup>a</sup>	0.651 <sup>abc</sup>	0.680 <sup>c</sup>	0.630 <sup>ab</sup>	0.657 <sup>bc</sup>	0.0196	<0.05	<0.05	NS	NS
Leucine	0.525 <sup>a</sup>	0.571 <sup>bc</sup>	0.601 <sup>bc</sup>	0.558 <sup>ab</sup>	0.573 <sup>bc</sup>	0.0229	<0.05	<0.05	NS	<0.05
Lysine	0.540	0.564	0.526	0.584	0.598	0.0298	0.105	NS	<0.05	NS
Phenylalanine	0.469	0.516	0.543	0.518	0.527	0.0257	0.064	<0.05	<0.05	NS
Threonine	0.729 <sup>a</sup>	0.741 <sup>ab</sup>	0.759 <sup>b</sup>	0.740 <sup>a</sup>	0.765 <sup>b</sup>	0.0119	<0.05	<0.05	<0.05	NS
Valine	0.657 <sup>a</sup>	0.687 <sup>abc</sup>	0.715 <sup>c</sup>	0.665 <sup>ab</sup>	0.695 <sup>bc</sup>	0.0166	<0.05	<0.05	NS	0.074
<i>Dispensable</i>										
Alanine	0.729 <sup>a</sup>	0.756 <sup>ab</sup>	0.781 <sup>b</sup>	0.756 <sup>ab</sup>	0.773 <sup>b</sup>	0.0147	<0.05	<0.05	<0.05	NS
Aspartic acid	0.635	0.658	0.658	0.647	0.663	0.0230	NS	NS	NS	NS
Glutamic acid	0.269 <sup>a</sup>	0.307 <sup>ab</sup>	0.358 <sup>b</sup>	0.249 <sup>a</sup>	0.272 <sup>a</sup>	0.0360	<0.05	NS	NS	0.051
Serine	0.464 <sup>ab</sup>	0.408 <sup>a</sup>	0.490 <sup>bc</sup>	0.552 <sup>cd</sup>	0.594 <sup>d</sup>	0.0316	<0.001	0.062	<0.001	<0.05
Tyrosine	0.510 <sup>a</sup>	0.562 <sup>b</sup>	0.613 <sup>bc</sup>	0.588 <sup>bc</sup>	0.622 <sup>c</sup>	0.0256	<0.001	<0.001	<0.001	NS
Average indispensable	0.523	0.564	0.580	0.551	0.569	0.0237	NS	<0.05	NS	NS
Average dispensable	0.529 <sup>a</sup>	0.537 <sup>a</sup>	0.580 <sup>b</sup>	0.561 <sup>ab</sup>	0.585 <sup>b</sup>	0.0199	<0.05	<0.05	<0.05	NS
Average total	0.525	0.554	0.580	0.554	0.575	0.0218	0.105	<0.05	<0.05	NS

<sup>1</sup> Control diet.

<sup>2</sup> Xylanase activity (units/kg) in diet.

<sup>3</sup> Significance between treatments determined by ANOVA. Means within a row with no common superscript differ significantly.

<sup>4</sup> Significance between the C vs all xylanase supplemented diets determined by comparisons contrast tests.

<sup>5</sup> Linear effects of added dietary xylanase activities.

<sup>6</sup> Quadratic effects of added dietary xylanase activities.

**Table 5.** The effect of dietary xylanase activity on the daily retention of dietary amino acids (g/hen.d) when fed to Lohmann Brown laying hens between 28 and 32 weeks of age

Amino acids	C <sup>1</sup>	C+400XU <sup>2</sup>	C+800XU <sup>2</sup>	C+1200XU <sup>2</sup>	C+1600XU <sup>2</sup>	SED	P <sup>3</sup>	P <sup>4</sup>	L <sup>5</sup>	Q <sup>6</sup>
<i>Indispensable</i>										
Arginine	0.395 <sup>a</sup>	0.511 <sup>ab</sup>	0.593 <sup>b</sup>	0.465 <sup>ab</sup>	0.470 <sup>ab</sup>	0.0637	<0.05	<0.05	NS	<0.05
Histidine	0.220	0.260	0.252	0.241	0.244	0.0229	NS	NS	NS	NS
Isoleucine	0.780	0.820	0.869	0.817	0.821	0.0286	0.055	<0.05	NS	<0.05
Leucine	1.131 <sup>a</sup>	1.222 <sup>ab</sup>	1.306 <sup>b</sup>	1.229 <sup>ab</sup>	1.216 <sup>ab</sup>	0.0543	<0.05	<0.05	NS	<0.05
Lysine	0.685	0.710	0.674	0.757	0.747	0.0404	NS	NS	0.060	NS
Phenylalanine	0.632	0.691	0.739	0.714	0.699	0.0370	0.067	<0.05	0.060	<0.05
Threonine	0.585	0.591	0.615	0.610	0.606	0.0139	NS	0.064	0.053	NS
Valine	0.917 <sup>a</sup>	0.954 <sup>a</sup>	1.009 <sup>b</sup>	0.952 <sup>a</sup>	0.957 <sup>ab</sup>	0.0281	<0.05	<0.05	NS	<0.05
<i>Dispensable</i>										
Alanine	0.864 <sup>a</sup>	0.891 <sup>ab</sup>	0.935 <sup>b</sup>	0.919 <sup>b</sup>	0.905 <sup>ab</sup>	0.0230	<0.05	<0.05	<0.05	<0.05
Aspartic acid	1.555	1.606	1.636	1.630	1.602	0.0659	NS	NS	NS	NS
Glutamic acid	1.806 <sup>a</sup>	1.971 <sup>ab</sup>	2.315 <sup>b</sup>	1.632 <sup>a</sup>	1.723 <sup>a</sup>	0.2336	<0.05	NS	NS	0.061
Serine	0.417 <sup>a</sup>	0.364 <sup>a</sup>	0.444 <sup>a</sup>	0.508 <sup>b</sup>	0.524 <sup>b</sup>	0.0299	<0.001	0.070	<0.001	NS
Tyrosine	0.387 <sup>a</sup>	0.423 <sup>ab</sup>	0.469 <sup>c</sup>	0.456 <sup>bc</sup>	0.465 <sup>bc</sup>	0.0212	<0.001	<0.001	<0.001	<0.05
∑ indispensable	5.345	5.758	6.057	5.785	5.760	0.2689	NS	<0.05	NS	<0.05
∑ dispensable	5.029	5.255	5.799	5.145	5.219	0.3072	NS	NS	NS	0.069
∑ total	10.374	11.013	11.856	10.930	10.979	0.5260	NS	0.069	NS	0.051

<sup>1</sup> Control diet.

<sup>2</sup> Xylanase activity (units/kg) in diet.

<sup>3</sup> Significance between treatments determined by ANOVA. Means within a row with no common superscript differ significantly.

<sup>4</sup> Significance between the C vs all xylanase supplemented diets determined by comparisons contrast tests.

<sup>5</sup> Linear effects of added dietary xylanase activities.

<sup>6</sup> Quadratic effects of added dietary xylanase activities.

**Table 6.** *The effect of dietary xylanase activity on the metabolisability coefficient of dietary minerals when fed to Lohmann Brown laying hens, determined on the last 2 d of the 32nd week of age*

Minerals	C <sup>1</sup>	C+400XU <sup>2</sup>	C+800XU <sup>2</sup>	C+1200XU <sup>2</sup>	C+1600XU <sup>2</sup>	SED	P <sup>3</sup>	P <sup>4</sup>	L <sup>5</sup>	Q <sup>6</sup>
Ca	0.645	0.637	0.651	0.642	0.646	0.0097	NS	NS	NS	NS
K	0.145	0.136	0.151	0.136	0.140	0.0099	NS	NS	NS	NS
Mg	0.202	0.222	0.226	0.214	0.218	0.0127	NS	0.074	NS	NS
Mn	0.095	0.092	0.114	0.106	0.106	0.0097	NS	NS	0.092	NS
Na	0.736	0.719	0.757	0.736	0.727	0.0155	NS	NS	NS	NS
P	0.376	0.367	0.386	0.369	0.374	0.0126	NS	NS	NS	NS
S	0.649	0.658	0.663	0.668	0.666	0.0073	0.082	<0.05	<0.05	NS

<sup>1</sup> Control diet.

<sup>2</sup> Xylanase activity (units/kg) in diet.

<sup>3</sup> Significance between treatments determined by ANOVA.

<sup>4</sup> Significance between the C vs all xylanase supplemented diets determined by comparisons contrast tests.

<sup>5</sup> Linear effects of added dietary xylanase activities.

<sup>6</sup> Quadratic effects of added dietary xylanase activities.

**Table 7.** The effect of dietary xylanase activity on the daily retention of dietary minerals (g/hen.d) and the cation concentration in excreta when fed to Lohmann Brown laying hens between 28 and 32 weeks of age

Minerals	C <sup>1</sup>	C+400XU <sup>2</sup>	C+800XU <sup>2</sup>	C+1200XU <sup>2</sup>	C+1600XU <sup>2</sup>	SED	P <sup>3</sup>	P <sup>4</sup>	L <sup>5</sup>	Q <sup>6</sup>
Ca	3.177	3.298	3.206	3.244	3.191	0.0314	NS	NS	NS	NS
K	0.106 <sup>a</sup>	0.107 <sup>a</sup>	0.116 <sup>b</sup>	0.108 <sup>a</sup>	0.105 <sup>a</sup>	0.0015	<0.001	<0.05	NS	<0.001
Mg	0.029 <sup>a</sup>	0.031 <sup>c</sup>	0.031 <sup>c</sup>	0.030 <sup>b</sup>	0.030 <sup>b</sup>	0.0003	<0.001	<0.001	NS	<0.001
Mn	1.031 <sup>a</sup>	1.019 <sup>a</sup>	1.160 <sup>a</sup>	1.101 <sup>d</sup>	1.046 <sup>c</sup>	0.0001	<0.001	<0.001	<0.05	<0.001
Na	0.083 <sup>a</sup>	0.084 <sup>a</sup>	0.086 <sup>b</sup>	0.086 <sup>b</sup>	0.083 <sup>a</sup>	0.0009	<0.001	<0.05	NS	<0.001
P	0.287	0.283	0.296	0.287	0.283	0.0101	NS	NS	NS	NS
S	0.202 <sup>a</sup>	0.205 <sup>ab</sup>	0.205 <sup>ab</sup>	0.209 <sup>b</sup>	0.204 <sup>a</sup>	0.0020	<0.05	<0.05	NS	<0.05
mEq/kg excreta DM <sup>7</sup>	3998	4055	4088	4210	4115	93.1	NS	0.109	0.063	NS

<sup>1</sup> Control diet.

<sup>2</sup> Xylanase activity (units/kg) in diet.

<sup>3</sup> Significance between treatments determined by ANOVA. Means within a row with no common superscript differ significantly.

<sup>4</sup> Significance between the C vs all xylanase supplemented diets determined by comparisons contrast tests.

<sup>5</sup> Linear effects of added dietary xylanase activities.

<sup>6</sup> Quadratic effects of added dietary xylanase activities.

<sup>7</sup> The cation concentration measured as milli-equivalent/kg of excreta dry matter.