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Short communication

Dietary xylanase increases hepatic vitamin E concentration of chickens fed wheat based diet

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ABSTRACT. The study examined the effect of xylanase supplementation on apparent metabolizable energy (AME) and hepatic vitamin E and carotenoids in broiler chickens fed wheat based diets. A total of one hundred forty four male Ross 308 chickens were used in this study. Birds were randomly assigned to 3 dietary treatments (8 cages per treatment of 6 male broilers each) for 14 days from 7 to 21 day old. The control treatment was based on wheat-soyabean meal and was either unsupplemented or supplemented with either 1000 or 2000 xylanase units per kg diet. Orthogonal polynomial contrasts were used to test linear response to dietary xylanase activity. There was a positive linear relationship ($P < 0.05$) between dietary AME and doses of supplementary xylanase. A linear relationship ($P < 0.05$) was also observed between dosage of xylanase supplementation and hepatic vitamin E concentration and retention. In conclusion, xylanase supplementation improved dietary AME and increased hepatic vitamin E concentration which may have positive effects on the antioxidative status of the birds.

Introduction

Wheat is an important source of energy in poultry diets and is often the only cereal used in poultry feed formulations in many countries of north-west Europe. However, the nutritive quality of wheat may vary depending on the carbohydrate complexes, known as non-starch polysaccharides (NSPs), which compose the cell wall of the wheat (Choct et al., 1996; Amerah et al., 2009a). NSPs have a structural function and account for approximately 10% of the whole grain in some UK wheat cultivars (Pirgozliev et al., 2003). The physicochemical properties of the soluble, higher molecular weight NSPs result in increased digesta viscosity which is correlated with reduced nutrient availability and bird performance (Bedford and Classen, 1992). The beneficial effect of exogenous xylanases in poultry nutrition has been perceived to be due to the direct hydrolytic effects on dietary NSPs, reduced intestinal viscosity and the subsequent release of encapsulated nutrients in the gut (Choct, 2006). It has also been suggested that xylanase in poultry diets improves gut health as indicated by reduced endogenous losses from the gastrointestinal tract (Bedford and Schulze, 1998). Wheat also contains about 75 mg of vitamin E (Panfili et al., 2003) and 2 mg of carotenoids (Hidalgo et al., 2006) per kg dry matter, which may contribute

in a significant amount of total dietary vitamin E and carotenoids. Biological activities of vitamin E and carotenoids are generally believed to be due to their antioxidant action that improves the animal health status (Surai, 2002; Karadas et al., 2014). Although much research has been conducted on the effect of xylanase on performance and nutrient availability, there is a lack of information on the effect of exogenous xylanase on the utilization of dietary vitamin E and carotenoids. Therefore, the main objective of this experiment was to determine the effect of varying doses of dietary xylanase on vitamin E and carotenoid content of the liver of broilers fed wheat-based diets from 7 to 21 d of age. Birds performance and dietary apparent metabolizable energy (AME) were also determined.

Material and methods

Diet formulation

One basal wheat-soyabean-based diet used for all diets formulations was made as one batch. The diet was manufactured to be adequate in protein but relatively low in energy (223 g · kg^{-1} crude protein; 12.13 MJ · kg^{-1} ME; Table 1) compared to breeders' recommendation (Aviagen, Edinburgh, UK). Five per cent wheat dried distillers grains with solubles were included in diets to increase the content of NSP. The basal diet was then split on three and supplemented with either 0, 1000 or 2000 units (XU · kg^{-1}) of exogenous xylanase (Danisco Xylanase, Danisco Animal Nutrition, Wiltshire, UK) sourced from fungal production system (*Trichoderma reesei*). The enzyme was added to the diets in powder form and no coccidiostat was added. All diets were fed as mash.

Table 1. Ingredient composition of the experimental control diet

Indices	
Ingredients, g · kg^{-1}	
wheat	588.2
wheat DDGS	50.0
soyabean meal (48)	250.6
rapeseed meal	50.0
soyabean oil	26.2
L-Lysine HCL	1.8
DL-Methionine	2.3
NaCl	3.0
limestone	10.7
dicalcium phosphate	14.1
vitamin mineral premix[1]	3.0
Calculated analysis, as fed basis	
ME, MJ · kg^{-1}	12.13
crude protein, g · kg^{-1}	223
lysine (available)	10.5
Meth + Cys (available)	8.5
Ca	8.8
P available	4.2

DDGS – distillers dried grains with solubles; [1] vitamin and mineral premix provided (units · kg^{-1} feed): µg: retinol 2160, cholecalciferol 75; mg: alpha-tocopherol 25, menadione 1.5, riboflavin 5, pantotenic acid 8, cyanocobalamin 0.01, pyridoxine 1.5, thiamine 1.5, folic acid 0.5, niacin 30, biotin 0.06, I 0.8, Cu 10, Fe 80, Se 0.3, Mn 80, Zn 80. Diets were not supplemented with coccidiostat

Birds, husbandry and sample collection

The Harper Adams University Animal Experimental Committee approved the study protocol.

All birds were fed a commercial starter ration until 7 d of age at which time one hundred and forty four male broiler chickens (Ross 308) were randomly placed in wire-mesh metabolism cages (six birds in a cage). The house temperature at day old was 33°C and was gradually reduced to 20°C after birds were 20 d of age. A standard lighting programme for broilers was used, decreasing from 23:1 (hours light:dark) from day old to 18:6 at 7 days of age, which was maintained until the end of the study. Each diet was fed *ad libitum* to birds in eight cages in a randomised block design. Total excreta collection was made over a 96 h period (between 17 and 21 d age). At the end of the experiment, at 21d age, one bird with a body weight that was similar to the group mean was selected from each cage, killed by cervical dislocation and its liver was collected and stored at –20°C for further analysis.

Metabolizable energy, dry matter digestibility and hepatic antioxidants determination

The analytical methods used in this work were described elsewhere and are summarized briefly here (Dei et al., 2008). Diets and oven-dried excreta were ground to pass through a 0.75 mm sieve and then dry matter (DM) was determined by drying of samples in forced draft oven at 105°C to constant weight. Gross energy (GE) in feed and excreta samples was determined using an adiabatic isoperibol bomb calorimeter (Parr-6200, Parr Instrument Company, Moline, IL,USA).

The AME value of the diets and total tract dry matter retention (DMR) coefficients was calculated using standard procedures (Wu et al., 2004).

The concentration of hepatic vitamin E and carotenoids was determined as described by Karadas et al. (2006, 2010) using high-performance liquid chromatography system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co. Ltd.).

Table 2. The effect of dietary xylanase activity on performance, liver weight, dietary apparent metabolizable energy (AME), dietary dry matter retention (DMR) and hepatic vitamin E and carotenoids content

Indices	Diets			Pooled SEM	Probability	
	C[1]	C+1000XU[2]	C+2000XU[2]		treatment effect	L[3]
Feed intake, g/bird/day	59.2	56.4	57.2	0.91	NS	NS
Weight gain, g/bird/day	35.0	35.5	35.4	0.61	NS	NS
Feed conversion efficiency	0.592	0.632	0.619	0.0120	0.092	NS
Body weight, g	640	645	649	8.4	NS	NS
Liver weight, g	14.4	15.1	14.6	0.42	NS	NS
Liver as % body weight	2.3	2.4	2.2	0.08	NS	NS
AME, MJ · kg^{-1}	13.09	13.02	13.39	0.097	0.040	0.048
DMR	0.782	0.772	0.779	0.0064	NS	NS
Vitamin E, µg · g^{-1} liver	81.1	99.8	130.7	12.12	0.038	0.013
Vitamin E, µg retained in liver	1176	1539	1903	212.7	0.090	0.031
Carotenoids, µg · g^{-1} liver	0.25	0.32	0.36	0.039	NS	0.077
Carotenoids, µg retained in liver	3.56	4.81	5.19	0.551	NS	0.057

[1] control; [2] xylanase activity, units · kg^{-1} diet; [3] linear effects of dietary xylanase activities. There were 8 observations per treatment. Bird performance was determined from 8 to 21 d age; dietary AME and DMR were determined between 17 and 21 d age; body and liver weights and hepatic vitamin E and carotenoids were determined at 21 d old birds. Results are statistically significant when $P < 0.05$ (the P values between 0.05 and 0.1 are described to show a tendency); NS – not significant

Statistical analysis

Statistical analyses were performed with Genstat 15 statistical software package (IACR Rothamstead, Hertfordshire, England). The comparison between the experimental results was performed by ANOVA, testing orthogonal polynomials for the linear response to dietary xylanase activity. The overall differences were reported as significant at $P \leq 0.05$ and trends were noted when the P values were < 0.1.

Results

All birds were healthy throughout the study period and there was no mortality. There was no effect of treatment on feed intake, weight gain and feed efficiency (Table 2). There was no effect of dietary treatment on liver weight, but a significant treatment effect ($P = 0.04$) on dietary AME was observed. That indicated that there was a positive linear response ($P = 0.048$) of dietary xylanase on AME (Table 2). An increase of 1000 xylanase units increased dietary AME by 150 joules (estimated within the range of doses used in the present experiment) (Table 3).

Increasing xylanase supplementation did not influence ($P > 0.05$) DMR.

There was a significant treatment difference ($P = 0.038$) in hepatic vitamin E concentrations. Increasing levels of exogenous xylanase linearly increased the hepatic vitamin E concentration ($P = 0.013$) and total retention in liver ($P = 0.031$). Each 1000 increase in xylanase units gave 23.7 and 339 µg increase in hepatic vitamin E concentration and total retention, respectively, estimated within the range of doses used in the present experiment (Table 3).

Table 3. Relationship between supplementary xylanase activity and dietary apparent metabolizable energy (AME), hepatic vitamin E concentration and total retention of vitamin E in liver

Dependant variates	Constant	Xylanase activity	r^2	RSD[1]
AME, MJ · kg^{-1}	13.02 ± 0.100	0.00015 ± 0.000078	0.11	0.311*
vit E, µg · g^{-1} liver	80.8 ± 10.10	0.0237 ± 0.00762	0.29	28.3*
vit E, µg retained in liver	1217 ± 175.0	0.339 ± 0.1330	0.21	512*

statistical significance of regression equation: *$P < 0.05$; [1] residual standard deviation

Discussion

Dietary enzymes have received a lot of attention recently as a result of their health and growth-promoting properties (Choct, 2006). However, while enzymes are involved in the hydrolysis of various antinutrients, their mode of action in poultry physiology and nutrition remains unclear. The benefits of using fibre-degrading enzymes in broiler feed has been associated with reduced intestinal viscosity, degradation of cell wall NSP and the release of encapsulated nutrients in the gut (Bedford and Classen, 1992). Wheat and soyabean meal, the principal components of the experimental diets, contain up to 13% and 29% of NSP, respectively (Malathi and Devegowda, 2001; Pirgozliev et al., 2003). This supports the improved dietary AME in the present study. Similar responses to supplementary xylanase have been observed in other studies (Wu et al., 2004; Amerah et al., 2009b). A number of studies have demonstrated a beneficial effect of dietary xylanase on weight gain and feed efficiency in broilers (Steenfeldt et al., 1998; Choct, 2006). There was no statistically significant effect

on these growth performance variables in the present study probably because a relatively short growing period was used.

The concentration of total vitamin E and carotenoids in the liver of chickens was comparable with previously reported values for broiler chickens (Koutsos et al., 2003; Karadas et al., 2006). The present study also demonstrated that the inclusion of xylanase in broiler diet improved hepatic vitamin E content of birds and the carotenoid content tended $P < 0.1$ to follow a similar trend. These results are in line with previous observations that feeding a low viscosity, in comparison to a high viscosity, diet improves the hepatic antioxidant content of broilers (Pirgozliev et al., 2014). There are a number of factors that could explain this effect: first, there could have been improved bioavailability of these fat soluble compounds. Although dietary fat digestibility was not examined in the present study, high viscous diets thicken the unstirred water layer of the mucosa which has been suggested (Palliyeguru and Rose, 2014) to thicken the intestinal mucous layer and reduce nutrient absorption. Second, a decreased digesta viscosity may have reduced dysbacteriosis in the small intestine. Dysbacteriosis is a disease condition caused by imbalance of the normal microbial flora in the distal part of the small intestine and the pathogenesis can be initiated by a mixture of opportunistic pathogens (Palliyeguru and Rose, 2014). An increased dysbacteriosis may result in more toxins produced in the gut of the birds, which will be absorbed across the intestinal mucosa and stimulate the immune system and increase the demand for vitamin E (Teirlynck et al., 2009).

In conclusion, the results of this study showed that supplementing diets with xylanase increased hepatic vitamin E in broilers. Thus, feeding xylanase to broiler chickens reared under commercial conditions has the potential to improve their antioxidant status which could increase their resistance to disease and stress challenges.

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