

Effects of xylanase inhibitors on nutritive value of diets and performance of broilers fed wheat diets plus different xylanases

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The aim of trial was to evaluate the effects of different microbial xylanases added to wheat diets on their nutritive value for poultry and broiler performance. Six experimental treatments were tested, according xylanase enzymes used (inhibited or un-inhibited by proteinaceous xylanase-inhibitors present in wheat).

288 Ross 308 male broilers were used. A basal wheat diet in mash form was used. Six dietary treatments replicated 8 times were evaluated: T-1 (control diet), T-2 (wild xylanase 1), T-3 (un-inhibited xylanase 1), T-4 (wild xylanase 2), T-5 (un-inhibited xylanase 2) and T-6 (commercial xylanase). Performance parameters were recorded between 7 and 25 days. The AME of diets and nutrient digestibilities were evaluated at 21 days using titanium oxide as digestibility marker. Freeze-dried excreta were analysed for energy, starch and lipids while protein and viscosity were determined in ileal samples. Data were analysed as a randomized block design ANOVA (SAS). Treatment means were compared using Duncan multiple range test.

Xylanase enzymes statistically increased feed efficiency ($P<0.001$), energy of diets ($P<0.0001$) and nutrient digestibilities ($P<0.01$) of diets. Intestinal digesta of broilers fed diets with commercial xylanase presented statistically lower viscosities than those fed control diet. No differences were found between treatments including inhibited or un-inhibited xylanases.

Keywords: xylanase; xylanase inhibitors; broiler performance; wheat diets.

Introduction

The non starch polysaccharides (arabinoxylans and β -glucans) present in diets based on wheat, barley or rye are known to contribute to the anti-nutritive effects of these diets in chickens due to their property of increasing intestinal viscosity where absorption of nutrients takes place, resulting in an impaired growth of animals. Supplementing wheat or rye diets with microbial xylanases increase the performance of broiler chickens and nutritive value of diets (Steenfeldt et al., 1998).

In some *in vivo* experiments, the expected improvements due to the addition of xylanase enzymes to wheat diets have not been obtained. Several researchers have demonstrated the presence in wheat of different proteinaceous xylanase-inhibitors (Rouau and Surget, 1998; Debyser et al., 1999; McLauchlan et al., 1999; Furniss et al., 2002) and studies about the characterization of these inhibitors in wheat cultivars (Gebruers et al., 2001) and the interactions enzyme-inhibitor have been undertaken (Flatman et al., 2002).

The content and type of xylanase inhibitors is variable in different wheat varieties, and different wheat cultivars could react in different way against xylanases of different origin (bacterial or fungi) (Perez-Vendrell and Brufau, 2003). Mutants of target enzymes, such as *B. subtilis* and *A. niger* xylanases have been produced, which showed resistance to the xylanase inhibitors. These engineered enzymes remained fully active even in presence of endoxylanase-inhibitors.

The aim of this digestibility trial was to evaluate the effects of different microbial xylanases (inhibited or not by proteinaceous xylanase-inhibitors) present in wheat diets on their nutritive value for poultry and broiler performance.

Materials and methods

288 day-old male broiler chickens of the Ross 308 breed were bulk weighed at arrival to the farm. During the first week, all birds were fed the same diet, based on maize and soybean. At day 7, chicks were weighed and distributed into eight weight groups or blocks. Afterwards, the experimental diets were distributed at random into each block. A single wheat based diet in mash form was used from 7 to 25 days. The composition of basal diets used in this assay is presented in Table 1. Both feed and water were provided *ad libitum*. Six dietary treatments replicated 8 times were evaluated: T-1 (control diet), T-2 (wild xylanase 1), T-3 (un-inhibited xylanase 1), T-4 (wild xylanase 2), T-5 (un-inhibited xylanase 2) and T-6 (commercial xylanase).

Performance parameters (body weight gain, feed intake and feed to gain ratio) were recorded between 7 and 25 days. The AME of diets and nutrient digestibilities were evaluated from 21 to 23 days using 0.5% titanium oxide as digestibility marker. Freeze-dried excreta were analysed for energy, starch and lipids while protein and viscosity were determined in ileal samples. At the end of balance period, two birds per cage were euthanatized by intravenous injection of sodium pentobarbital (according to the procedure num. 688 approved by the Ethical Commission of IRTA) to collect digesta samples in the last 15 cm before ileo-cecal conjunction. Data were analysed as a randomized block design ANOVA (SAS). Treatment means were compared using Duncan multiple range test.

Results and discussion

Performance parameters of broilers obtained between 7 and 25 days are presented in Table 2. No statistical significant differences were found in final weight or feed consumption, but all xylanase enzymes tested statistically increased feed efficiency ($P < 0.001$).

The apparent metabolizable energy and nutrient digestibilities obtained during the balance period are exposed in Table 3. The inclusion of xylanases in diets statistically increased the energy of diets ($P < 0.0001$) and digestibility of starch, lipid and protein fractions ($P < 0.01$) of diets. The effect of xylanases on viscosity of intestinal digesta of broilers was variable. Intestinal viscosity of broilers fed xylanase 1 (wild or mutant) were higher than control, while intestinal viscosity measured in broilers fed commercial xylanase was significantly lower ($P < 0.0001$). No differences were found between treatments including inhibited or un-inhibited xylanases, for any of the productive or digestibility parameters evaluated.

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Table 1. Composition and calculated nutritive value of basal diets

Ingredient	Pre-experimental (%)	Experimental (%)
Maize	58.347	-
Wheat	-	62.220
Soybean meal 48 %	33.377	27.410
Lard	3.000	5.290
Minerals, vitamins and aminoacids	5.27	5.08
<u>Calculated nutrient composition:</u>		
Metabolizable energy (kcal/kg)	3000	3000
Crude protein (%)	20.74	21.50
Crude fibre (%)	3.09	2.97
Crude fat (%)	5.60	6.77
Ash (%)	7.18	6.78
Lysine (%)	1.20	1.20
Met + Cys (%)	0.92	0.92
Calcium (%)	1.31	1.17
Total phosphorus (%)	0.77	0.77
Inorganic phosphorus (%)	0.45	0.45

¹ One kg of feed contains: Vitamin A, 12000 IU; Vitamin D₃, 2400 IU; Vitamin E, 30 mg; Vitamin K₃, 3 mg; Vitamin B₁, 2.2 mg, Vitamin B₂, 8.0 mg; Vitamin B₆, 5.0 mg; Vitamin B₁₂, 11.0 µg; Folic acid, 1.5 mg; Biotin, 150 µg; Calcium pantothenate, 25 mg; Nicotinic acid, 65 mg; Ethoxyquin, 150 mg; Fe, 80 mg; Cu, 8 mg; Zn, 40 mg, Mn, 60 mg, Se, 0.15 mg, I, 0.33 mg.

Table 2. Performance of broiler chicks between 7 and 25 days .

Treatment	Xylanase type	Final Weight (g)	ADG (g/d)	ADC (g/d)	FE (g/g)	
T-1	-	792.70	37.64	61.94	1.651 a	
T-2	Wild inhibited xylanase 1	837.23	39.81	61.16	1.573 b	
T-3	Mutant un-inhibited xylanase 1	827.67	39.31	61.60	1.569 b	
T-4	Wild inhibited xylanase 2	800.41	38.00	60.00	1.575 b	
T-5	Mutant un-inhibited xylanase 2	785.09	37.08	59.01	1.592 b	
T-6	Commercial xylanase 3	811.80	38.55	59.98	1.563 b	
		Pr>F	0.31	0.26	0.52	0.0015
		St.Err.	23.40	1.16	1.72	0.019

Table 3. EMA and Nutrient digestibility of diets

Treat ment	Xylanase type	AME (kcal/kg)	AME n (kcal/kg)	Starch dig. (%)	PB dig. (%)	Lipid dig. (%)	Viscosity (mPa.s)	
T-1	-	3014 e	2867 c	91.46 b	75.73 b	65.28 c	2.94 b	
T-2	Wild inhibited xylanase 1	3266 ab	3119 ab	97.07 a	79.95 a	75.77 ab	4.31 a	
T-3	Mutant un-inhibited xylanase 1	3246 ab	3097 ab	96.93 a	80.21 a	72.15 b	4.18 a	
T-4	Wild inhibited xylanase 2	3256 ab	3103 ab	96.27 a	80.21 a	75.74 ab	2.93 b	
T-5	Mutant un-inhibited xylanase 2	3212 b	3065 b	96.03 a	80.62 a	74.81 ab	2.92 b	
T-6	Commercial xylanase 3	3287 a	3136 a	97.29 a	81.21 a	78.06 a	2.16 c	
		Pr>F	0.0001	0.0001	0.0001	0.009	0.0001	0.0001
		St.Err.	25.7	24.9	0.74	1.16	1.68	0.22