

# A simple in vitro test for evaluation of the usefulness of industrial enzymes as additives to broiler diet based on rye

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## ABSTRACT

The effect of 7 commercially produced enzyme preparations on reducing the viscosity of rye grain substances in water extract (in vitro) and in the digestive tract of chicken (in vivo) was studied. The enzyme preparations tested were: FC-1 and FC-2 (amylase, protease,  $\beta$ -glucanase), DP-2885 and DP-2985 (amylase, protease,  $\beta$ -glucanase, cellulase), WF-078 ( $\beta$ -glucanase), Zymine xbp (xyylanase,  $\beta$ -glucanase, pentosanase, cellulase, pectinase) and Avizyme ( $\beta$ -glucanase, cellulase, protease). Rye var. Dańkowskie Złote, containing 12.9% dietary fiber and 3.1% soluble fiber was used.

The most efficient in reducing the viscosity of a water extract of rye flour (in vitro) and digesta (in vivo) was Zymine xbp (by 44 and 77% respectively), followed by DP-2885 (by 31 and 55% respectively), the effects of the remaining enzyme preparations were statistically insignificant.

AME<sub>N</sub> of unsupplemented rye was  $10.97 \pm 0.73$  and supplemented with 0.1% Zymine xbp,  $11.87 < 0.76$  MJ per kg of dry matter.

It seems that the usefulness of commercial enzyme preparations as additives to broiler diets based on rye may be preliminary checked by means of a simple in vitro test.

**KEY WORDS:** industrial enzymes, rye, broiler chicken

## INTRODUCTION

Feeding trials with young chicks indicate that substances which depress growth and cause sticky faeces are present in rye. Antoniou and Marquardt (1981) and Antoniou et al. (1981) found that the principal antinutritional factors in rye grains are highly viscous water-soluble and water-insoluble pentosans and pectins. According to Raczynska-Bojanowska et al. (1983) rye grain contains 17.5% non-starchy polysaccharides in which: 6.8% are pentosans; 8.4% pectins and 4.3% betaglucan. Due to their high water binding properties, all of these substances form highly viscous solution in the digestive tract of chicken and increase the weight and bulk of the digesta.

The above effects support proliferation of intestinal microflora which is

detrimental to the chick and interferes with digestion and absorption of such nutrients as fat, protein, Na ions, vitamin D<sub>3</sub> and others. Several authors reported that the growth-depressing properties of rye were overcome by addition of enzymes capable of hydrolysing pentosanes. The current studies were conducted to compare the effect of different industrial enzymes on reducing the viscosity of rye grain substances in water solutions (in vitro) and in the digestive tract of chickens (in vivo).

## MATERIALS AND METHODS

Rye var. Dańkowskie Żłote was used in all of the experiments. The composition of the rye is given in Table 1. The commercially produced enzyme preparations tested were:

- FC-1 and FC-2 (Dalgeti, U.K.) containing amylase, protease and beta-glucanase,

TABLE 1  
Chemical composition of rye (in % of DM)

Item	Dańkowskie Żłote
Dry matter	87.43
Crude protein (N × 6.25)	9.44
Crude fat	1.74
Crude fiber	1.79
Crude ash	1.73
NFE	85.30
Water-insoluble fiber:	
Rhamnose	0.07
Arabinose	1.71
Xylose	2.47
Mannose	0.41
Galactose	0.27
Glucose	2.99
Uronic acids	0.11
Klason lignin	1.75
Total	9.78
Water soluble fiber:	
Arabinose	0.81
Xylose	1.37
Mannose	0.12
Galactose	0.16
Glucose	0.50
Uronic acids	0.14
Total	3.10
Total fiber	12.88

- DP-2885 and DP-2985 (Dalgeti, U.K.) containing amylase, protease, beta-glucanase and cellulase,
- WF-078 (Woodward Dickerson, USA) containing beta-glucanase,
- Zymine (Revedura GmbH, Germany, Interchemia, Austria) xbp containing xylanase, beta-glucanase, pentosanase, cellulase and pectinase,
- Avizyme (Finland) containing beta-glucanase, cellulase and protease.

### Experiment 1

Rye grain was ground until it passed a 0.49 mm sieve. Rye flour was mixed with distilled water in a proportion of 1:9 by weight and slowly agitated for one hour. The mixture was centrifuged at  $1000 \times g$  for 10 min. This extraction time allows for optimal extraction of the water-soluble fraction of rye pentosans (Fernandez et al., 1973, Raczyńska-Bojanowska et al., 1983). Twenty five ml of supernatant were taken into an Erlenmayer flask and 0.5 ml of distilled water (control sample) or 0.5 ml freshly prepared enzyme solution (10 mg/ml) was added. The flasks were closed and kept for 1 h in a slowly shaking water bath at  $40^{\circ}\text{C}$ . Next they were cooled to  $30^{\circ}\text{C}$  and the relative viscosity was immediately measured using Baker's „Jelometer” viscosity pipette.

### Experiment 2

Twenty four Cornish-White Rock broiler male chicks aged 5 weeks were divided into 6 groups of 4 birds each. After 6 h of fasting the birds were offered the experimental diet containing 70% rye and 100 mg per kg of one of the 5 enzyme preparations used in Experiment 1 (experimental groups) or starch (control group). Feed and water were supplied ad libitum. After 16–18 h the chickens were killed by decapitation, the content of the small intestine extracted into weighed beakers, mixed with the same amount of distilled water, agitated for 3 min. and centrifuged at  $1000 \times g$  for 10 min. The relative viscosity of the supernatant was measured immediately as in Experiment 1.

### Experiment 3

Two groups of 9 male Cornish  $\times$  White Rock broiler chickens aged 4 weeks were fed ground rye with or without the addition of 100 mg/kg of Zymine xbp. After 2 days the birds were kept fasting for 24 h, fed the same diets for the next 48 h. Excreta were collected during this 48 h period and the following 24 h fast. Dry matter, total N and gross energy content were determined in the samples of rye and excreta and  $\text{AME}_N$  values for rye with or without enzyme added were calculated.

## RESULTS AND DISCUSSION

The rye used in the experiments contained 12.88% dietary fiber in which 3.1% was soluble fiber that gives a solution 3–4 times more viscous than soluble wheat fiber (Karlsson, 1985). Incubation of the water extract of rye grain for 1 h at 40°C reduced its relative viscosity by 34%, probably due to the action of endogenous grain pentosanases, which according to Karlsson (1985) are 3 types: endoxylanase, beta-xylosidase and arabinosidase. They did not, however, decompose all of the viscous substances because incubation with enzyme preparations added in amounts corresponding to 200 mg/kg of flour caused further reduction of relative viscosity (see Table 2). The most efficient was Zymine xbp containing the broadest spectrum of enzymes, followed by DP 2885, DP 2985, FC-2 and Avizyme.

The results of the test *in vivo* were highly correlated with those obtained *in vitro*. Zymine xbp and DP-2885 were the most effective in reducing digesta viscosity. This reduction had a positive effect on digestion and utilization of rye grain. AME<sub>N</sub> of unsupplemented rye (Experiment 3) was  $10.97 \pm 0.73$  MJ and rye with 0.1% of Zymine xbp added  $11.87 \pm 0.76$  MJ per kg of dry matter.

The enzyme preparations tested in these experiments were also used by Nierodzik (1987) in broiler fattening trials. They were added in amounts of 0.05 or 0.012% to diets composed of locally produced components and containing 10 or 20% rye. Only 2 preparations, Zymine xbp and DP. 2885, significantly improved the weigh gain of chicken and feed efficiency.

Jackisch and Jeroch (1990) obtained marked improvement in weight gain and

TABLE 2  
Relative viscosity of supernatant samples  
obtained *in vitro* (exp. 1) and *in vivo* (exp. 2)

Enzyme addition	<i>in vitro</i>		<i>in vivo</i>	
	relative viscosity	% of control	relative viscosity	% of control
None (control) <sup>1)</sup>	3.14 <sup>a</sup>	100	14.27 <sup>a</sup>	100
FC-1	2.77 <sup>ab</sup>	88		
FC-2	2.47 <sup>bc</sup>	79	12.45 <sup>a</sup>	87
DP-2885	2.18 <sup>c</sup>	69	6.36 <sup>b</sup>	45
DP-2985	2.39 <sup>bc</sup>	76		
WF-078	3.12 <sup>a</sup>	99	14.02 <sup>a</sup>	98
Zymine xbp	1.76 <sup>d</sup>	56	3.27 <sup>b</sup>	23
Avizyme	2.73 <sup>ab</sup>	87	16.26 <sup>a</sup>	114

<sup>1)</sup> Relative viscosity of initial supernatant liquid prior to incubation was 4.74.

a, b, c means in the same column not having a common superscript are significantly different ( $P \leq 0.05$ )

feed efficiency after supplementation of a 50% rye diet with Zymine xbp preparate. Scholtyssek and Knorr (1987) supplementing a 30% rye diet with an enzyme mixture containing cellulase, beta-glucanase, xylanase and pectinase obtained better performance of chicken. A balance trial showed that enzyme supplementation had a positive effect on the digestibility of organic matter, particularly its non-starchy carbohydrate fraction.

All the results show clearly that addition of enzymes, which accelerate decomposition of viscous rye pentosans and pectines, improves utilization of feed mixtures containing a high proportion of rye grain.

The ability of different industrial enzyme preparations to modify the viscosity of rye pentosans may be measured precisely by means of the proposed simple, fast and inexpensive *in vitro* test. However, the results of the test should be applied with great care. In practical broiler rations, containing relatively small amounts of rye, only enzyme preparations showing the greatest activity may significantly improve chicken performance. The results of the fattening trials by Nierodzik (1987) with the same set of preparations demonstrate that at least a 30% reduction in viscosity for of rye pentosans in solution, prepared as described above, is necessary practical classifying an enzyme preparation as useful for application in rye-containing rations for broilers.

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#### STRESZCZENIE

**Prosty test *in vitro* do oceny przydatności enzymów paszowych w mieszankach dla kurcząt brojlerów zawierających żyto**

Badano wpływ 7 produkowanych przemysłowo enzymów paszowych na obniżenie lepkości substancji zawartych w ziarnie żyta w roztworach wodnych (*in vitro*) i w przewodzie pokarmowym kurcząt (*in vivo*). Testowano następujące preparaty enzymatyczne: FC-1 i FC-2 (amylaza, proteaza,  $\beta$ -glukanaza), DP-2885 i DP-2985 (amylaza, proteaza,  $\beta$ -glukanaza, celulaza), WF-078 ( $\beta$ -glukanaza).

za), Zymine xbp (ksylanaza,  $\beta$ -glukanaza, pentozanaza, celulaza, pektynaza) i Avizyme ( $\beta$ -glukanaza, celulaza, proteaza). Żyto odm. Dańkowskie Złote, zawierało 12,9% włókna pokarmowego, w tym 3,1% stanowiło włókno rozpuszczalne. Najbardziej aktywny w obniżaniu lepkości wodnych wyciągów z mąki żytniej (in vitro) oraz lepkości treści przewodu pokarmowego (in vivo) był preparat Zymine xbp (odpowiednio o 44 i 77%) oraz DP-2885 (odp. o 31 i 55%), wpływ pozostałych preparatów był statystycznie nieistotny.  $EM_N$  ziarna żyta wynosiła 10,97, a po dodaniu 0,1% preparatu Zymine xbp wzrosła do 11,87 MJ na kg suchej masy.

Wydaje się, że użyteczność produkowanych przemysłowo preparatów enzymatycznych proponowanych jako dodatki do mieszanek pełnoporcjowych dla kurcząt brojlerów zawierających żyto, może być wstępnie sprawdzana za pomocą prostego testu in vitro.