



Effects of whole wheat with or without xylanase supplementation on performance of layers and digestive organ development

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ABSTRACT

An experiment was conducted using Bovans White layers to investigate the effects of 30% whole-wheat inclusion in a standard layer diet supplemented with xylanase, on laying performance, digestive organs and ileal mucosa development. Three dietary treatments were used: 1) control diet (30% ground wheat); 2) 30% whole wheat; 3) whole wheat+wheat xylanase. Xylanase was added to whole wheat at 150g/ton. Including the pre-experimental period the trial lasted for 13 weeks.

Xylanase supplementation to whole wheat significantly ($P<0.05$) improved egg production and feed conversion rate compared to the ground wheat and whole wheat fed groups. Gizzard pH was not affected by dietary treatments, while whole wheat feeding significantly ($P<0.05$) reduced jejuno-ileal pH and increased gizzard and jejuno-ileal viscosity compared to the ground wheat fed and xylanase supplemented groups. Proventriculus, gizzard, duodenum, jejunum, ileum, caecum, colon and liver weights were not significantly affected by dietary treatments. Feeding whole wheat w/wo xylanase supplementation significantly ($P<0.05$) increased crypt depth but did not affect lamina *muscularis mucosae* thickness.

Key words: Whole wheat, Xylanase, Gut histomorphology, Viscosity, Layers.

RIASSUNTO

EFFETTI DEL FRUMENTO INTEGRALE CON O SENZA SUPPLEMENTO DI XYLANASE SULLE PERFORMANCE PRODUTTIVE E SULLO SVILUPPO DELL'APPARATO DIGERENTE DI GALLINE OVAIOLE

Il presente studio è stato condotto al fine di valutare gli effetti sulle performance produttive e sullo sviluppo dell'apparato digerente e della mucosa ileale in galline ovaiole di razza Bovans White in seguito all'introduzione del 30% di frumento integrale in una dieta standard addizionata con xylanase,. Sono stati utilizzati tre diversi trattamenti alimentari: 1) dieta di controllo (30% farina di frumento); 2) 30% fru-

mento integrale; 3) frumento integrale+frumento con xylanase. Lo xylanase è stato aggiunto al frumento integrale in quantità pari a 150g/t. Il periodo di prova sperimentale, includendo la parte pre-sperimentale, è durato tredici settimane.

L'aggiunta di xylanase al grano integrale ha migliorato significativamente ($P<0,05$) la produzione di uova e l'indice di conversione dell'alimento rispetto ai gruppi alimentati con farina di frumento o con frumento integrale. Il pH del ventriglio non è stato influenzato dai diversi trattamenti, mentre l'alimentazione con il frumento integrale ha ridotto in maniera significativa ($P<0,05$), rispetto ai gruppi alimentati con farina di frumento o con l'integrazione di xylanase, il pH nel tratto digiuno-ileale incrementando la viscosità sia in quest'ultimo tratto sia nel ventriglio. Il peso di proventricolo, ventriglio, digiuno, ileo, cieco, colon e fegato non è stato significativamente influenzato dal trattamento alimentare. I gruppi alimentati con frumento integrale con o senza il supplemento di xylanase hanno evidenziato un incremento significativo della profondità delle cripte intestinali ($P<0,05$) senza modifiche a carico dello spessore della muscularis mucosae.

Parole chiave: *Frumento integrale, Xylanase, Viscosità, Isto-morfologia intestinale, Galline ovaiole.*

Introduction

Wheat is a major feed ingredient in poultry diets and is available in almost all countries of the world. The birds are able to utilize whole-grain wheat because they can generate high grinding pressure in their gizzards (Sturkie, 1965). It has been suggested that feeding whole grain not only improves bird health (Cumming, 1994) and gizzard function (Ferket, 2000; Bennett *et al.*, 2002; Hetland *et al.*, 2005), but is also a beneficial application in that it reduces feed milling and transportation costs (Rose, 1996; Hetland *et al.*, 2002; Bennett and Classen, 2003). Moreover, as observed by Forbes and Covasa (1995), poultry naturally select whole grains and pellets from the feed in the proportions that best meet their individual nutritional needs. Replacement of ground wheat with whole wheat has been found to improve feed utilization, increase starch digestibility and markedly increase gizzard size (Forbes and Covasa, 1995; Hetland *et al.*, 2003).

On the other hand, the supplementation of microbial enzymes in poultry diets is now common practice in many countries where the predominant cereals are wheat or barley. Enzymes are used mainly to achieve consistency in performance and to reduce formulation costs by easing constraints on the inclusion level of some ingredients, such

as wheat and barley. Xylanase has been reported to alleviate the negative effects of arabinoxylans, present in wheat as the dominant cell-wall constituent, by lowering gut viscosity and increasing protein, fat and starch digestibility (Bedford and Morgan, 1996). Improved starch digestibility, for instance, has been reported to account for up to 35% of the improvement in available energy (AME) as a result of xylanase supplementation (Carre *et al.*, 1992).

Although whole-wheat feeding is becoming a useful practice, particularly in broiler production, this application has not yet been established in layer feeding because of insufficient studies using layers as a model. Moreover, using xylanase in combination with whole wheat might also yield beneficial nutritional consequences.

Therefore, the present study attempts to investigate the effect of whole-wheat feeding w/wo xylanase supplementation on laying performance and digestive organs in layers at their first cycle of production.

Material and methods

Bird housing and management

A total of 144 fifty-week-old Bovans White layers, still yielding high levels of egg production (90% hen day), were supplied from a commercial layer farm and ran-

domly allocated to battery type wire cages (50x44x46cm). Four layers were placed in each of 2 consecutive cages with 6 replicates and assigned to receive one of the 3 dietary treatments. Battery cages were equipped with nipple drinkers. Each set of 2 consecutive cages formed one experimental unit in which 8 birds were housed. Laying hens were maintained in an environmentally controlled experimental house with air ventilation and window and received additional artificial light to provide 16.5h light and 7.5h dark daily.

A three-week pre-experimental period was implemented to get the layers accustomed to the diets. The birds were weighed at the start (53 week of age) and end (63 week of age) of the trial. All experimental diets were fed *ad libitum* to the laying hens and water was available all the time throughout the experimental period. Feed intake was recorded

weekly and egg production daily. Egg weight was measured weekly by weighing all of the eggs collected from the experimental groups. Feed conversion rate was calculated as gram of feed consumption per day per hen divided by gram egg mass per day per hen.

Dietary treatments

The three dietary treatments were the following: 1) control diet (30% ground wheat), 2) 30% whole wheat diet, 3) 30% whole wheat diet + wheat xylanase.

Feed ingredients were obtained from the local feed market, and they were ground in a hammer mill to pass a 3-mm sieve and mixed through a horizontal mixer (200kg capacity) in the feed-mixing unit of the department. Experimental diets were formulated isocaloric and isonitrogenous to contain 17.5% CP and 11.51MJ/kg AME (Table 1). The whole-wheat diet, was prepared by

Table 1. Ingredients and chemical composition of ground or whole wheat diets (as fed).

Ingredients	%	Nutrients		
Corn	37.45	AME	MJ/kg	11.51
Wheat	30.00	Crude protein	%	17.50
Soybean meal (48% CP)	14.96	Ether extract	"	4.01
Limestone	9.72	Crude fibre	"	2.22
Poultry by-product meal	2.00	Calcium	"	4.00
Feather meal	2.00	Available phosphorus	"	0.38
Fish meal	1.00	Sodium	"	0.16
Soya oil	1.463	Lysine	"	0.88
Monocalcium phosphate	0.599	Methionine	"	0.41
Vitamin and mineral premix ¹	0.250	Met + Cys	"	0.76
Common salt	0.152			
NaHCO ₃	0.150			
DL-Methionine	0.149			
L-Lysine HCl	0.107			

¹Provided per kg of diet: retinyl acetate, 8000 U; cholecalciferol, 2500 U; DL- α -tocopheryl acetate, 30 mg; menadione, 2.5 mg; thiamine, 2 mg; riboflavin, 5 mg; pyridoxine, 2 mg; vit. B₁₂ cobalamin, 0.01 mg; niacin, 30 mg; calcium-D-pantothenate, 8 mg; folic acid, 0.5 mg; D-biotin, 0.045 mg; choline chloride, 800 mg; vit. C, 50 mg; Mn, 70 mg; Fe, 35 mg; Zn, 70 mg; Cu, 8 mg; I, 1 mg; Co, 0.2 mg; Se, 0.25 mg.

AME: Apparent Metabolizable Energy.

replacing the 30% ground wheat with whole wheat on a w/w basis. The third dietary treatment consisted of the second dietary treatment supplemented with a microbial wheat xylanase, Ronozyme® WX (CT) obtained from *Thermomyces lanuginosus* with main endo-1,4- α -xylanase (IUB No.3.2.1.8) activity and was layered on top of the whole-wheat diet at 150g/ton diet.

Viscosity and pH measurements

At the end of the 63rd week of age (10-week experimental period) 18 layers in total, 1 layer per replicate and 6 layers per dietary treatment groups, were randomly taken from each experimental unit and killed by cranial blow followed by cervical dislocation for dissection. Viscosity determination was made according to Dusel *et al.* (1998). The gizzard, jejunal and ileal segments were quickly ligated in order to prevent post-mortem digesta movement. Total intestinal contents from the entrance of the bile ducts to the ileocaeco-colic junction (jejunum, ileum) were squeezed out and collected in a beaker. The viscosity of the intestinal digesta samples was measured in an aqueous extract; approximately 2g of the fresh and homogenized digesta were immediately placed in a microfuge tube and centrifuged at 10,000xg for 15min. The supernatants were stored on ice until the viscosity measurements were performed with a cone and plate viscometer (model DV-II+LV, Brookfield Engineering Laboratories, Inc., Middleboro, MA). Viscosity measures were expressed as centipoise (cPs).

To measure the pH, the gizzard was weighed separately with its contents. A cut was made between the two openings of the gizzard, and the electrode of the pH meter (Inolab level 1, WTW GmbH, Weilheim, Germany) was inserted inside for digital pH measurement. To measure the pH of the jejunum-ileum content, the same pH meter,

after the electrode had been rinsed with distilled water and dried with tissue paper, was inserted inside the collected digesta which was stored in the beaker.

Gut organs and histomorphology

Following dissection, emptied gastrointestinal organs (proventriculus, gizzard, duodenum, jejunum, ileum, caecum, colon) and liver were individually weighed as g/kg BW. The lengths of the gut segments were measured as cm/100g BW.

For the histological examination, villus height and depth and crypt depth were measured according to the procedure described by Ritz *et al.* (1995). For each sampling, ileal tissues, from the middle of the ileum were cut into 5-mm sections and put into tissue cassettes. The tissues were processed, embedded in paraffin, and subsequently cut and placed onto a slide at 5- μ m thickness. They were then exposed to hematoxylin and eosin fixation stain. Six tissue samples were prepared for each of the treatments. Pictures of villus height, villus depth, crypt depth and Lamina muscularis mucosae thickness were taken with a digital camera and measured by using linear micrometer.

Statistical analysis

All the data were recorded on a weekly basis and statistically analyzed by ANOVA using the GLM (General Linear Model) procedure in a Windows-based statistical package program (SAS, 1996). The differences among group means were separated by Duncan's Multiple Range Test. The significance level for the group comparisons was set at $P < 0.05$.

Results

The results presented in Table 2 indicate that layer production performance was significantly affected by the dietary treatments.

Table 2. Effects of whole wheat and xylanase on laying performance (53-63 wk).

Treatments	Egg production	Feed intake	Egg weight	Egg mass	FCR
	(%)	(g)	(g)	(g)	(g feed/g egg)
Ground wheat	85.0 ^b	106.3 ^b	63.2 ^b	53.7 ^b	1.980 ^a
Whole wheat (WW)	87.4 ^b	112.7 ^a	64.7 ^a	56.6 ^a	1.991 ^a
WW+xylanase	90.5 ^a	107.0 ^{ab}	64.2 ^a	58.1 ^a	1.842 ^b
Pooled SEM	0.730	1.231	0.203	0.524	0.026
P level	0.004	0.064	0.003	0.001	0.024

^{a-b}: Means within a column with different superscripts differ significantly ($P < 0.05$).

FCR: Feed conversion rate.

Means represent six replicates, eight birds per replicate.

There was a significant increase (5.5%) in egg production with the whole-wheat diet supplemented with xylanase relative to the ground-wheat diet (control group). There was also a tendency toward increased egg production in response to the whole-wheat diet. Feeding layers with 30% whole wheat significantly ($P < 0.05$) increased feed intake compared to the group fed ground wheat, implying an effect of choice feeding. The addition of xylanase to the whole-wheat diet, however, did not significantly affect feed intake. Feeding whole wheat w/wo xylanase addition significantly increased egg

weight or egg mass compared to the control group. A clear and significant increase in egg mass was observed in dietary treatments over the control group. For example, in the group fed whole wheat+xylanase, mean egg mass reached 58.1g/hen d, compared to 53.7g/hen d observed in the control group. Therefore, feed conversion rate was significantly ($P < 0.05$) higher in the whole wheat+xylanase group than in the other groups (Table 2).

None of the dietary treatments significantly affected gizzard pH (Table 3). On the other hand, the jejunum-ileal and gizzard vis-

Table 3. Effects of whole wheat and xylanase on pH and viscosity of gizzard and jejunum-ileum content.

Treatments	pH		Viscosity cPs	
	Gizzard	Jejunum-ileum	Gizzard	Jejunum-ileum
Ground wheat	4.68	6.21 ^{ab}	1.60 ^b	2.72 ^b
Whole wheat (WW)	4.35	5.98 ^b	2.90 ^a	4.59 ^a
WW+xylanase	4.31	6.39 ^a	1.72 ^b	1.93 ^b
Pooled SEM	0.097	0.077	0.230	0.380
P level	0.342	0.050	0.563	0.014

^{a-b}: Means within a column with different superscripts differ significantly ($P < 0.05$).

Means represent six replicates, one bird per replicate.

cosity were significantly affected by the dietary treatments. Jejunum-ileal digesta pH significantly ($P < 0.05$) increased from 5.98 to 6.39 by addition of xylanase to whole-wheat diet. Conversely, jejunum-ileal digesta viscosity significantly ($P < 0.05$) increased in response to whole wheat feeding compared to ground-wheat feeding or xylanase supplementation. Viscosity of gizzard content revealed a similar effect as jejunum-ileal digesta viscosity.

Measurements of empty intestinal-segment weights showed that dietary treatments did not significantly affect proventriculus, gizzard, duodenum, jejunum, ileum, caecum, colon or liver weights (Table 4). Whole-wheat feeding w/wo xylanase supplementation did not significantly alter duodenum, jejunum, ileum, caecum, or colon lengths (cm/kg BW) as shown in Table 5.

However, only crypt depth was significant-

ly affected by whole-wheat feeding regardless of w/wo xylanase supplementation (Table 6). Crypt depth was increased from 71.0 to 92.9 and 87.6 μ m, respectively, by whole-wheat feeding and xylanase supplementation. Dietary treatments had no significant effect on villus height, villus depth or thickness of lamina muscularis mucosae.

Discussion

Replacement of ground wheat with whole wheat in layer diets resulted in a substantial increase in feed intake. This is in agreement with results obtained using broilers, where complete diets were offered in mash form (Olver and Jonker, 1997; Svihus *et al.*, 1997). However, whole-wheat feeding has been shown to reduce feed intake in some other studies (Heland *et al.*, 2002; Wu *et al.*, 2004a).

Table 4. Effects of whole wheat, and xylanase on digestive-organ relative weights (g/kg BW).

Treatments	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Caecum	Colon	Liver
Ground wheat	3.7	13.0	7.9	9.2	7.7	3.4	0.26	22.0
Whole wheat (WW)	3.7	13.6	7.9	10.0	8.1	3.4	0.30	22.9
WW+xylanase	3.6	13.4	7.6	10.1	7.9	3.5	0.23	23.1
Pooled SEM	0.092	0.419	0.349	0.336	0.246	0.190	0.150	0.629

Means represent six replicates, one bird per replicate.

Table 5. Effects of whole wheat, and xylanase on relative intestinal lengths (cm/kg BW).

Treatments	Duodenum	Jejunum	Ileum	Caecum	Colon
Ground wheat	16.6	35.0	31.1	7.3	4.3
Whole wheat (WW)	15.7	31.5	29.2	7.1	5.3
WW+xylanase	16.7	33.8	30.1	6.7	4.7
Pooled SEM	0.664	1.019	1.048	0.355	0.258

Means represent six replicates, one bird per replicate.

Table 6. Effects of whole wheat, and xylanase on ileal histomorphology.

Treatments	Villus height (μm)	Villus depth (μm)	Crypt depth (μm)	Thickness of lamina <i>muscularis mucosae</i> (μm)
Ground wheat	653.9	80.9	71.0 ^b	274.2
Whole wheat (WW)	701.0	70.5	92.9 ^a	309.1
WW+xylanase	586.8	75.2	87.6 ^a	269.2
Pooled SEM	31.32	4.74	3.55	35.86

^{a-b}: Means within a column with different superscripts differ significantly ($P < 0.05$).

Means represent six replicates, one bird per replicate.

In the present study, feeding whole wheat to laying hens supplemented with wheat xylanase resulted in improved layer performance with respect to egg production and feed conversion rate. The beneficial effect of whole-wheat feeding supplemented with xylanase may be associated with the lowered viscosity of the gizzard and jejuno-ileal content. However, gizzard content and jejuno-ileal digesta viscosity significantly increased in response to whole wheat feeding compared to ground-wheat feeding or xylanase supplementation. Bedford and Classen (1992) have demonstrated a significant correlation between digesta viscosities measured *in vivo* and BW gain and feed conversion rate. Intestinal viscosity has been shown to account for as much as 70 to 80% of the variation observed in BW gain and feed conversion rate when poultry are fed wheat- and rye-based diets. The reduction in intestinal viscosity is generally attributed to the partial depolymerization of wheat pentosans following xylanase supplementation (Bedford and Morgan, 1996; Dusek *et al.*, 1998). Starch digestibility has also been reported to account for up to 35% of the improvement in available energy (AME) as a result of xylanase supplementation, although fat accounts for 35% and protein for 30% (Carre *et al.*, 1992). Moreover, an improvement in overall protein digestibility,

including lysine, methionine and cysteine, of dietary components other than wheat, such as soybean, has also been reported (Bedford and Morgan, 1996). All of these explanations could possibly be valid for the improved performance obtained in the present study.

In the present study, dietary treatments did not alter gizzard size or gut-segment weights or lengths. The lack of the effect on gizzard size and gut-segments might probably be related to the age (50-63 weeks) of hens used in this study. Nevertheless, increased gizzard size has been reported (Cumming, 1994; Ferket, 2000; Hetland *et al.*, 2005) by whole-wheat feeding or insoluble fibre addition, whereas other treatments (Wu *et al.*, 2004a) did not reveal any such effects. Likewise, modifications in gastrointestinal-tract weight or length by these factors have been confirmed by some authors (Hetland *et al.*, 2003; Wu *et al.*, 2004b), but not by others (Wu *et al.*, 2004a). The fact that no gastrointestinal changes were observed here could be attributed to the layers' age (50-63 weeks), as they were already mature in terms of their body development and were nearing the end of their first laying cycle, although their egg production level was relatively high (85-90% hen day). It is clear that gastrointestinal development occurs during the early stages of life (Sturkie, 1965; Uni *et al.*, 1999).

Similar results have also been reported in recent studies (Wu *et al.*, 2004a, 2004b) conducted with broilers, in which whole wheat (at 20% inclusion) supplemented with microbial phytase or xylanase was tested. In the former study, phytase was found to be as effective as xylanase in improving the performance of broilers fed wheat-based diets. The combination of phytase and xylanase increased villus height in the ileum and crypt depth in the jejunum and ileum. Improved performance was found to be generally associated with reduced digesta viscosity, increased AME and reduced relative small-intestinal weight and length. In the latter study, birds fed whole wheat exhibited improved weight gain, feed efficiency and AME relative to those fed diets containing ground wheat. Xylanase supplementation also resulted in improved bird performance in terms of growth and feed efficiency, and reduced viscosity in the gizzard and three segments of the small intestine. However, neither weight nor length of the small intestine was modified by the dietary treatments, with the exception of increased ileal villus height following xylanase supplementation.

Conclusions

The clear improvement in performance, namely increased egg production

and feed conversion rate, observed in the present study can be attributed to whole-wheat-based diet following xylanase addition. Supplementation of xylanase to the whole-wheat diet resulted in a significant decrease in gizzard and jejunal-ileal digesta viscosity. Xylanase has been reported to alleviate the negative effects of arabinoxylans, which are present in wheat as the dominant cell-wall constituent. One of the mechanisms by which xylanase has been reported to alleviate the antinutritive activity of arabinoxylan in wheat is by lowering viscosity. In other words, xylanase lowers the gut viscosity caused, in particular, by the soluble arabinoxylan fraction in the ingested feed, thereby increasing nutrient diffusion and consequently, improving bird performance. The results obtained in the present experiment demonstrate that whole-wheat feeding of laying hens with xylanase supplementation can be advantageous and even beneficial in terms of improving egg production, egg mass and feed conversion rates, probably via the positive effects of xylanase on digestion and absorption.

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