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Effects of Supplemental Phytase and Xylanase on Phytate Phosphorus Degradation, Ileal Protein and Energy Digestibility of a Corn-soybean-wheat Bran Diets in Broiler Chicks.

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ABSTRACT

Effects of supplemental phytase and xylanase in a corn-soybean meal based diet containing 250 g/Kg wheat bran, were investigated. on broiler performance and phosphorus degradability and nitrogen digestibility. Phytase (500 and 1000 FTU) and xylanase (2700 and 5400 EXU) individually and in combination were added to the basal diet and given to 6 replicate groups (4 birds per replicate). At 21 days of age, birds were weighed and killed , ileal contents were collected and analyzed for P, N, chromic oxide and gross energy. Added phytase significantly (P<0.01) improved feed conversion ratio (FCR) (1.59 vs 1.62 g /g) and tibia ash (464.4 vs 444.3 g/Kg). Protein digestibility was increased (81.7 vs 79.4%) significantly (P<0.01) by 500 FTU/Kg added phytase. Supplemental xylanase significantly (P<0.01) improved FCR (1.58 vs 1.63 g/g) and protein digestibility (82.0 vs 80.4%). Phytate P degradability was significantly (P<0.02) improved by added phytase (41.4 vs 27.8%). Combination of supplemental phytase and xylanase had some beneficial effects on improving the nutritive value of diets containing wheat bran for broilers.

Keywords: Broiler, Phytase, Wheat bran, Xylanase.

INTRODUCTION

Wheat bran may be included in layer diets, but only in smaller amount in broiler diets owing to its high content of fiber and other antinutritional factors. Wheat and rice brans are two phosphorus-rich by-products that have feeding value for non-ruminant animals (Kratzer *et al.,* 1974; Farrell and Martin, 1998b). Phosphorus content of wheat bran is high (NRC, 1994) but most of it is present as phytate which is not available to poultry (Corley *et al.,* 1980). Phytic acid in wheat bran not only reduces P availability (<23%, Corley *et al.,* 1980), but also complexes with some other minerals and reduces their availability. In recent years, much attention has been focused on improving nutrient utilization of low quality or inferior feed ingredients with the aim of reducing feed costs (Farrell and Martin,1998a). Such improvement has been achieved by exogenous enzyme supplementation to the diets of poultry (Scott *et al.,* 1988, Farrell *et al*., 1993;Yi *et al*., 1996a;).

Non-starch polysaccharides, phytate, tannins and other antinutritional factors in cereal grains and their by-products reduce their digestibility and nutrient availability, and hence their feeding value (Annison *et al.* 1995). Results of many experiments indicated that enzyme supplementation of poultry diets improved the nutritional value of

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cereal grains and their by-products. Improvements in AME (Bedford *et al.,* 1998), starch digestibility (Choct and Annison, 1992), and phytate utilization (Simons *et al.,* 1990; Kornegay *et al*., 1996) due to added enzymes have been reported.

Data on the effect of enzyme supplementation of wheat bran for poultry are limited. Addition of phytase and other enzymes could improve phytate degradability of wheat bran and such improvement may allow wheat bran to be used at higher levels in poultry diets and to supply considerable proportions of phosphorus requirements and also reduce feed cost and environmental pollution.

The objective of this study was to investigate the effects of different combinations of xylanase and phytase on performance, phosphorus, protein and energy utilization in broiler chickens fed on a high wheat bran basal diet.

MATERIALS AND METHODS

Birds and Diets

Two hundred and sixteen-day old male broiler chickens (Hubbard x Peterson) were weighed individually and divided into 54 groups of 4 (6 replicates per treatment) and each group was assigned to one of nine dietary treatments. Initial body weight of all replicates was nearly identical. The chicks were housed in battery cages and whole

Table 1. composition of the basal diet.

Ingredient	g/kg
Wheat bran	250.0
Yellow com	375.0
Soybean meal (48% protein)	285.0
Canola oil	50.0
Dicalcium phosphate [a]	6.0
Limestone	17.0
Vitamins & minerals premix [b]	5.0
Sodium chloride	2.2
Choline chloride 70%	1.0
DL- Methionin	2.0
L- Lysine HCl	0.3
Coccidiostat	1.0
Growth promotant	0.5
Chromic oxide	5.0
Enzymes [c]	
Calculated analysis	
ME (Kcal/kg)	2910
Crude protein (g/kg)	209.0
Calcium (g/kg)	9.0
Non-phytate P (g/kg)	2.9
Methionine+cystine (g/kg)	8.15
Lysine (g/kg)	10.6
Linoleic acid (g/kg)	14.0
Crude fibre (g/kg)	47.0

[a]Dicalcium phosphate 220 g/Kg Ca and 187 g/Kg P.
[b]Supplied per kilogram of diet: vitamin A (retinyl acetate+retinyl palmitate), 11000 IU;vitamin D, 2200 IU; vitamin E , 30 IU; menadione, 2.0 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; niacin, 60.0 mg; pyridoxine, 4.0 mg; vitamin B12, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; biotin, 0.15 mg; iron, 80.0 mg; zinc, 80.0 mg; manganese, 80.0 mg; copper, 10.0 mg; iodine, 0.8 mg; and selenium, 0.3 mg.
[c]Natuphos was added at the levels of 0.0, 0.1 and 0.2 g/Kg to provide 0.0, 500 and 1000 FTU/kg of phytase respectively. Avizyme 1300 was added at the levels of 0.0, 1 and 2 g/kg to provide 0.0, 2700 and 5400 EXU/kg of xylanase, respectively.

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room brooding was used. Initial brooding temperature was 35°C and gradually reduced to 28°C by 21 d of age. Chicks had free access to feed and nipple drinkers during a 21 day experimental period.

A corn-soybean meal based diet contained 250g wheat bran/Kg (Table 1) supplemented with two kinds of phytase (Natuphos. 5000 FTU/g: BASF Corp., 3000# Continental Drive North, Mount Olive, NJ, U.S.A 07828) and xylanase (Avizyme 1300, 2700 EXU/g: Finnfeeds International, Box 777, Marlborough, Wiltshire, UK, SN8 1XN), each given individualy at three defferent levels (0, 500 and 1000 FTU/Kg phytase and 0, 2700 and 5400 EXU/Kg xylanase) and their combinations were tested. Dietary available P level was formulated at 2.9g/Kg, which was 30% below the current National Research Council (NRC, 1994) recommendations, to ensure a maximum response with phytase additions. Diets were otherwise formulated to meet the requirements of broiler chickens as established by NRC (1994).

Measurements and Analytical Methods

At 21 d of age, birds were weighed out and feed consumption and conversion during this period were measured out. All birds were killed by cervical dislocation and the lower section of ileal contents were gently squeezed, pooled and frozen for analysis.

The viscosity of fresh ileal contents was determined using a Brookfield (Brookfield Engineering Labs, Stoughton, MA 0207) viscometer (model LVDVII+Cp). The viscosity of five ileal samples were not determined because of the lack of supernatant after centrifugation, and those samples were therefore considered as missing values. Left tibia of two birds in each replicate were removed, pooled and stored at –20°C for tibia ash determination.

Ileal digesta were freeze-dried and then ground for gross energy, nitrogen, phytate and chromic oxide determination. Diets and digesta were ground to pass a 0.5 mm sieve. Chromic oxide was determined according to the method of Fenton and Fenton (1979). Gross energy was measured with a Parr 1720 Automatic Calorimeter (Parr Instrument Company, 21 Fifty Third Street, Moline, Illinois, U.S.A. 61265,) and nitrogen was measured using a nitrogen analyzer, lesed on the procedures of AOAC (1995). Apparent energy digestibility coefficients were calculated by the following formula.

Apparent digestible enegy = $(GE/Cr203)d-(GE/Cr203)i/(GE/Cr_{203})i$.

Where (GE/Cr203) d= the ratio of GE and Cr203 in diet and (GE/Cr203)i =the ratio of GE and Cr203 in ileal digesta.

Phytate was extracted by the method of Lehrfeld (1989) and measured using a Beckman (Beckman Instruments, 1045 Tristar Drive, Mississuaga, Ontario, Canada, L5T 1W5) HPLC anion-exchange chromatography with post-column reagent for detection (Rounds and Nielsen, 1993; Newkirk and Classen, 1997).

The bones were thawed and fat extracted in a Soxhlet extractor for 18 h with petroleum ether, then dried at 110°C for two h and ashed at 600°C for 24 h.

Statistical Analysis

All data were analyzed as a 3*3 factorial arrangement in a completely randomized design using the General Linear Model Procedure of SAS (1985) and if significant Duncan's multiple range test was used to compare means.

RESULTS

Body Weight, Feed Conversion and Tibia Ash

The effects of phytase and xylanase on body weight gain, feed intake, and feed conversion ratio (FCR), tibia ash and ileal viscosity are shown in table 2. Only FCR and tibia ash were improved significantly ($P<0.01$) by 500 FTU/Kg phytase. There

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Table 2. Effects of wheat bran and dietary phytase and xylanase supplementation on body weight gain, feed consumption, feed conversion ratio, ileal digesta viscosity , tibia ash, phytate P degradability, energy and protein digestibility of male broiler chicks at 21 d of age.

Deit No	Enzyme U/kg		Body Wt.gain	Feed intake	FCR	Viscosity	Tibia ash	DE	Phytate P degradability b coefficient	Protein digestibility coefficient
	Phytase (FTU)	Xylanase (EXU)	(g)	(g)	(Feed/gian)	(ceps)	(g/kg)	(Kcal/kg)	%	%
1	0	0	499	828	1.66a[1]	4.02	437b	2723.2	22.2bc	80.2ab
2	500	0	523	831	1.60ab	2.95	466a	2830.0	46.5a	81.8a
3	1000	0	507	830	1.63ab	3.16	453ab	2727.2	43.4a	80.0ab
4	0	2700	484	780	1.61ab	2.92	455ab	2815.0	39.5ab	80.5a
5	0	5400	505	806	1.60ab	2.62	453ab	2727.0	10.7c	77.6b
6	500	2700	508	806	1.58ab	2.57	453ab	2878.0	43.2a	83.0a
7	500	5400	530	838	1.58ab	2.70	466a	2743.3	34.6ab	80.6a
8	1000	2700	528	861	1.63ab	2.52	452ab	2915.3	37.8ab	82.6a
9	1000	5400	511	800	1.56b	4.16	457ab	2871.7	24.9bc	81.7a
SE			± 21.4	30.0	0.022	1.22	8.0	63.8	5.7	0.97
Main effect										
Phytase FTU/Kg										
	0	0	496.2	829.5	1.62a	3.24	444.3b	2755.2	27.8b	79.4b
	500	0	520.3	825.1	1.59b	2.75	464.4a	2817.0	41.4a	81.7a
	1000	0	515.5	830.2	1.61ab	4.60	435.2ab	2838.0	454.0ab	81.4a
SE			12.60	17.40	0.013	4.60	0.93	36.80	3.30	0.56
Xylanase EXU/Kg										
	0	0	509.7	829.5	1.63a	3.40	452.0	2760.0	37.4a	80.4
	0	2700	506.6	816.0	1.61ab	3.67	453.3	2869.5	39.7a	82.0a
	0	5400	515.7	814.6	1.58b	4.44	458.4	2780.7	23.4b	80.0b
SE			12.60	17.40	0.0130	4.60	0.93	36.80	3.30	0.56
Probability (P<)										
Phytase			NS	NS	**	NS	**	NS	*	**
Xylanase			NS	NS	*	NS	NS	NS	**	*
Phytase x xylanase			NS	NS	*	NS	*	NS	**	*

1 Values in each colums followed by the same letter are not significantly different.
* (P< 0.01)
** (P< 0.001)
NS. Not significant

was no significant difference between 500 and 1000 FTU/Kg phytase levels. Supplemental xylanase (5400 EXU/Kg) improved FCR significantly (P<0.01). The interaction of phytase and xylanase regarding FCR and tibia ash was significant (P<0.05) (table 2).The best FCR was obtained with dietary combination of 1000 FTU/Kg and 5400 EXU/Kg of phytase and xylanase, respectively. Tibia ash from the birds that consumed diets containing phytase and xylanase had a higher ash content. There was no mortality during the experimental period.

DE, Protein Digestibility and Phosphorus Utilization

The effect of phytase supplementation on DE of diets was not significant, but added phytase at 500 FTU improved DE (table 2). The highest but not significant DE was obtained with 1000 FTU/Kg phytase and 2700 EXU/Kg xylanase respectively. Xylanase supplementation tended to improve dietary DE. The interaction of phytase and xylanase for dietary DE was not significant. The effects of phytase and xylanase on ileal protein digestibility were significant (P<0.01). Phytase added at 500 FTU/Kg improved protein digestibility significantly (P<0.01), but increasing the supplement to 1000 FTU/Kg had no further effect. Protein digestibility was improved significantly (P<0.01) by

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2700 EXU/Kg xylanase. The difference between 5400 EXU/Kg and the control diet was not significant in this regard.

Supplemental phytase improved phytate P degradability significantly (P<0.02). Phytate P degradability was not improved significantly by xylanase. The interaction between phytase and xylanase regarding phytate P degradability was significant (P<0.01). Xylanase alone or in combination with 1000 FTU/Kg phytase significantly (P<0.01) reduced phytate P degradability. 5400 EXU/Kg Xylanase alone or in combination with 1000 FTU/Kg phytase decreased FCR, DE, protein digestibility and phytate P degradability when compared with 2700 EXU/Kg.

DISCUSSION

Phytase supplementation improved FCR and tibia ash, thus indicating a better utilization of phytate and other dietary constituents particularly energy and nitrogen. These results are in agreement with the previous findings in broilers (Kornegay *et al.* 1996; Yi *et al.* 1996a; Qian *et al.* 1996a; Sebastian *et al.* 1996; Cabahug *et al.* 1999; Ravindran *et al.* 2000), and in turkeys (Qian *et al.* 1996a; Yi *et al.* 1996b) suggesting an improvement in AME and N retention due to supplemental phytase. Farrell and Martin (1998a) found significant increases in dry matter, N and P retention by adding phytase to diets containing rice bran for ducks. Improvement in energy value of wheat due to supplemental phytase and xylanase individually and in combination was reported by Ravindran *et al*. (1999). This is in agreement with the results of our experiment.

Xylanase improved FCR, energy and protein digestibility. It seems that xylanase breaks the cell wall and liberates more nutrients for digestion and absorption. The combination of these two enzymes gave a better response regarding FCR, DE and protein digestibility. The low ileal viscosity found in this experiment (table 2) supported the idea that NSP's present in wheat bran contained no anti-nutritional factors and are not detrimental to broiler performance. Such a conclusion was reached by Farrell and Martin (1998b) with rice bran. This may be the reason for the lack of any significant interaction between phytase and xylanase regarding DE and body weight gain.

Improvement in phytate P degradability by the addition of phytase is in agreement with the findings of Farrell and Martine (1998b), Simons *et al*. (1990), Yi *et al.* (1996b) and Ravindran *et al.* (2000). The reason for the negative effects of 5400 EXU/Kg xylanase on phytate P degradation is unclear.

In conclusion, there was some beneficial response to phytase addition to all experimental diets with wheat bran. In addition, xylanase supplementation had some beneficial effects on FCR, DE and N digestibility. Added phytase improved DE and N digestibility in addition to P utilization. Feed intake was not increased by added phytase; therefore, improvements in FCR, DE and P utilization could be due to positive effects of supplemental phytase on these parameters. Combination of supplemental phytase and xylanase had some discauble benefits in improving the nutritional value of diets containing wheat bran for broilers.

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اثرات فیتاز و زایلاناز بر قابلیت استفاده از فسفر فیتاتی، قابلیت هضم پروتئین و انرژی قابل سوخت و ساز جیره‌های حاوی سبوس گندم در جوجه‌های گوشتی

چکیده

اثرات مکمل‌های فیتاز و زایلاناز در جیره پایه ذرت- کنجاله سویا حاوی ۲۵درصد سبوس گندم بر عملکرد و قابلیت هضم فسفر و ازت و جوجه‌های گوشتی مورد بررسی قرار گرفت. در یک طرح کاملاً تصادفی به ترتیب سه سطح آنزیم فیتاز (۵۰۰ و ۱۰۰۰ واحد در کیلوگرم) و سه سطح انزیم زایلاناز (۲۷۰۰ و ۵۴۰۰ واحد در کیلوگرم) و ترکیب آنها به جیره پایه اضافه شد. هر یک از ۹ جیره آزمایشی به مدت ۲۱ روز به شش تکرار، در هر تکرار حاوی داده شد. در پایان آزمایش، جوجه‌های هر تکرار به صورت گروهی توزین و با جابجایی مهره گردن کشته شدند. محتویات ایلئوم جمع‌آوری و میزان ازت، فسفر، اکسید کرم و انرژی خام مورد بررسی قرار گرفت. مکمل فیتاز باعث بهبود معنی‌دار ($P<$ ۰/۰۱) ضریب تبدیل غذا و خاکستر استخوان شد. قابلیت هضم پروتئین دراثر افزودن ۵۰۰ واحد فیتاز به هر کیلوگرم غذا به طور معنی داری ($P<$ ۰/۰۱) افزایش یافت. مکمل زایلاناز، باعث بهبود معنی دار ($P<$ ۰/۰۱) ضریب تبدیل غذا، انرژی قابل هضم و قابلیت هضم پروتئین شد. مکمل فیتاز و زایلاناز دارای اثرات مفیدی در بهبود ارزش غذایی جیره‌های حاوی سبوس گندم برای جوجه‌های گوشتی بودند.

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