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Effect of Wheat Bran Phytase Subjected to Different Conditioning Temperatures on Phosphorus Utilization by Broiler Chicks Based on Body Weight and Toe Ash Measurements

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Abstract: Cereal grains and oilseed byproducts are major components of diets commercially fed to poultry and swine. Although phosphorus levels are relatively high in these feedstuffs, most of it is presented as phytate and thus, unavailable to monogastric species. Wheat bran is known for having high endogenous phytase enzyme activity. This study was conducted to investigate the ability of wheat bran endogenous phytase to withstand the high temperatures associated with the pelleting process. Additionally, to evaluate the efficacy of this enzyme source in improving phytate phosphorus utilization by broiler chicks. Wheat bran was subjected to steam conditioning/pelleting at temperatures between 50 °C and 90 °C. Test diets were formulated with levels of available phosphorus (AvP) below those recommended by the NRC. Wheat bran was incorporated into test diets at 5% inclusion level in either the unprocessed form or after being subjected to steam conditioning at 60 °C and 80 °C followed by pelleting. Live weight gain, feed efficiency and toe ash measurements were observed as response variables to available phosphorus in the diets. Considerable losses in endogenous phytase activity in wheat bran were observed after steam conditioning at the aforementioned temperatures. However, based on the observations, the inclusion of wheat bran in the unprocessed form positively influenced the bird's ability to utilize phosphorus present in the diet.

Key words: Wheat bran endogenous phytase, pelleting, broilers

Introduction

Cereal grains and oilseed byproducts are extensively used as ingredients in commercial feed formulations for swine and poultry. These feedstuffs usually exhibit relatively high total phosphorus content. However, about two thirds of the phosphorus is presented in the phytate form, which impedes its digestion and absorption by monogastric species. In order to meet required levels of available phosphorus (AvP) in the diets, nutritionists make use of inorganic sources, which represents significant increases in the cost of formulations. From an environmental standpoint, high levels of phosphorus in manure applied onto crops as fertilizer constitute a potential hazard to surrounding waterways (Ullah *et al*., 2000).

Phytase is an enzyme naturally found in plant seeds and also produced by some microorganisms (Ullah, 1988). As compared to other cereals, significantly higher concentrations of endogenous phytase is found in the bran of both rye and wheat (Greiner and Egli, 2003; Viveros *et al*., 2000). The enzyme releases phytate bound phosphorus, therefore increasing levels of AvP in traditional corn-soy diets fed to poultry an swine (Mitchell and Edwards, Jr., 1996; Huff *et al*., 1998; Zhang *et al*., 2000). Commercially, most of the feed used in poultry and swine operations is consumed in the pelleted form. That implies that during the manufacturing process the meal has been subjected to temperatures usually in excess of 80 °C, through direct exposure to steam. However, the phytase molecule has a limited thermal stability and studies have demonstrated that losses in activity begin to occur at around 60 °C (Ullah and Mullaney, 1996). Earlier studies have suggested that high endogenous-phytase cereals and their by-products can effectively enhance phosphorus utilization by monogastric species (Saveur, 1984; Pointillart *et al*., 1987; Pointillart, 1991). However, diets utilized in those experiments were not subjected to steam conditioning or pelleting, and the question remains regarding the survivability of those enzymes under such circumstances. Therefore, a study was conducted to investigate if any losses in endogenous phytase activity in wheat bran occurred when it was subjected to steam conditioning at different temperature levels and subsequent pelleting. And furthermore, to evaluate the ability of endogenous phytase in wheat bran to improve phosphorus availability in corn-soy based diets fed to broiler chicks.

Materials and Methods

A portion of the wheat bran available for the study was not subjected to hydrothermal processing (WB, unprocessed), while the remaining (processed) was subjected to steam conditioning in a single pass conditioner (Bliss Industries, Ponca City, OK 74602) to final target temperatures of 50°, 60° (WB60), 70°, 80°

(WB80) and 90 °C respectively. Retention time inside the conditioner was 15 seconds. Subsequent to steam conditioning, the wheat bran was pelleted (Master Model HD 1000, California Pellet Mill, Crawfordsville, IN 47933) using a 3.97- x 38.1-mm die. As the wheat bran was expelled through the die, samples were collected in a thermally insulated container and the temperatures of the hot pellets were recorded . The temperature gradient between hot pellet and conditioning temperatures (ΔT °C) represents the frictional heat generated as the wheat bran was forced through the die orifice. Samples of unprocessed and processed wheat bran were analyzed for endogenous phytase activity at the cereal chemistry laboratory, Dept. of Grain Science and Industry, Kansas State University (Manhattan, KS 66506). Two samples of processed wheat bran (WB60, WB80) were re-ground into a meal in an experimental hammermill (Bliss Industries, Ponca City, OK) prior to incorporation into the experimental diets.

A total of eight diet treatments were utilized in the experiment and diets were formulated to be isocaloric and isonitrogenous. Treatments 1 (base) through 4 were formulated to contain increasing levels of Pav (0.35, 0.40, 0.45 and 0.50%, respectively) and used as references in establishing the birds' response to additional AvP provided as monocalcium phosphate (Table 1). Treatments 5 through 8 were formulated to contain 0.35% calculated AvP and supplemented with a source of phytase . Treatments 5, 6 and 7 contained 5 percent wheat bran, as WB, WB60 and WB80 respectively. For treatment 8, a liquid source of microbial origin was added to the base diet at the dilution recommended to deliver 500 FTU/kg of feed. The ingredient composition of all diets is provided in Table 2. A total of 70 kg of each diet was manufactured and fed as mash.

Male Cobb-Vantress 1-day old chicks were reared in battery pens (Petersime, Belgium) during the 0-3 week period. Individual pens were used as the experimental unit and eight birds were randomly assigned to each pen. A total of 384 birds were used in a complete randomized block design with six replicate pens/treatment. Blocking criterion was pen level within the battery. An average initial body weight was obtained at the beginning of the experiment. Feed consumption and mortality were recorded for each pen. At the end of week 3, birds from each pen were weighed and euthanized. The middle toes were excised, pooled and analyzed for toe ash content (expressed as percent of dry matter) according to a previously adopted procedure (Perney *et al.*, 1993; Qian *et al.*, 1997).

Statistical analysis for differences (α = 0.05) in live weight gain (LWG), feed efficiency (FE) and toe ash (TA) among treatments was performed using the GLM procedure of SAS (SAS Institute, 2000). Regression analysis of TA in response to known increments in AvP (treatment 1 through 4) was performed with the REG procedure of SAS.

Results

A comparison of the analyzed enzyme activity levels in the unprocessed and processed wheat bran samples suggests a great degree of sensitivity of the endogenous phytase to the hydrothermal conditions employed (Table 3). Conditioning temperature of 90 °C resulted in complete destruction of the enzyme whereas following exposure to 80 °C, only 12 percent of the original 5.7 FTU/g remained in the active form. However, even the lowest conditioning temperature used in the experiment (50 °C) was sufficient to reduce the activity of the endogenous phytase to slightly over half of what was present in the unprocessed sample . Lower temperature gradient values (Δ T °C) were observed as the conditioning temperature increased. Inside the conditioning cylinder, the feed temperature rises as steam condenses onto the surface of the particles. Consequently, the greater the conditioning temperature, the lower the degree of friction and generation of heat that takes place as the meal is expelled through the die. Significant differences between treatments were observed for LWG ($p<0.0076$) and TA ($p<0.0001$) (Table 4). Among the birds receiving the reference treatments (1 through 4), a clear separation in LWG response appears between those fed AvP levels below (1 and 2) and above (3 and 4) the NRC (1994) recommendation of 0.45 percent for the 1-21 day period. LWG of birds on treatment 8 were similar to those on treatments 3 and 4. Although statistically undifferentiated from treatments 3, 4 and 8, LWG results of treatment 5 was substantially lower and numerically closer to the results from treatments 6 and 7. Additionally, no significant differences were observed in FE ($p<0.1475$). Hence, the lower LWG pattern observed among birds receiving diets with wheat bran could be perhaps attributed to the effect that this ingredient has on bulk density of the feed, thus limiting feed intake.

Linear increases in bone mineralization (as measured by TA) were observed as a response to additional levels of AvP in treatments 1 through 4 ($p<0.0001$, $r^2=0.6974$). Among treatments containing 0.35% AvP and a supplemental source of phytase (5 through 8), similar results were obtained for treatments 5 and 8, while lower measurements were observed for treatments 6 and 7. A comparison with the reference treatments may suggest that an additional 0.05% AvP was yielded by the endogenous and microbial phytases in diets 5 and 8, respectively. In contrast, TA measurements from treatments 6 and 7 provide no indication that additional phosphorus was released from unavailable forms (*e.g.* phytate). Similarly, two earlier reports (Corley *et al.*, 1980; Edwards, Jr. *et al.*, 1999) had come to the conclusion that neither pelleting or extrusion of individual

Table 1: Treatment outline

Treatment	Available phosphorus (AvP)	Description
Ref 0.35	0.35%	base, 0.35% AvP
Ref 0.40	0.40%	base, 0.35% AvP + 0.05 % AvP
Ref 0.45[a]	0.45%	base, 0.35% AvP + 0.10 % AvP
Ref 0.50	0.50%	base 0.35% AvP + 0.15 % AvP
WB	0.35%	wheat bran phytase, no steam conditioning
WB-60	0.35%	wheat bran phytase, steam conditioning at 60°C
WB-80	0.35%	wheat bran phytase, steam conditioning at 80°C
MP	0.35%	base, 0.35% AvP + microbial phytase[b]

[a]NRC recommended level for broilers 0-21 d. period
[b]Natuphos® phytase, BASF Corporation, Mount Olive, NJ 07828

Table 2: Composition of Experimental Diets (as % of total)[1]

Ingredient	Treatments				
	Base (0.35% P_{AV})	Base + 0.05% P_{AV}	Base + 0.10% P_{AV}	Base + 0.15% P_{AV}	Base + wheat bran
Corn	50.536	50.254	49.972	49.690	44.322
SBM 46.5%	39.378	39.428	39.479	39.529	38.837
Soy oil	6.207	6.301	6.394	6.487	7.984
Wheat bran	------	------	------	------	5.000
Limestone	1.875	1.774	1.673	1.572	1.871
Monocalcium phosphate	1.062	1.300	1.539	1.778	1.043
Salt	0.463	0.463	0.462	0.462	0.460
Vitamin/mineral premix[2]	0.250	0.250	0.250	0.250	0.250
DL-methionine 98%	0.172	0.173	0.173	0.173	0.175
Tylan 40[3]	0.050	0.050	0.050	0.050	0.050
Thiamin premix[4]	0.005	0.005	0.005	0.005	0.005

[1]All diets were formulated to contain 3200 Kcal/kg and 23% crude protein. Essential amino-acids levels were adjusted to meet or exceed those recommended by the NRC.
[2]Supplied per kilogram of diet: manganese, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B_6, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; menadione, 0.83 mg; vitamin B_{12}, 0.01 mg; vitamin E, 16.53 IU; vitamin D_3, 2,133 ICU; vitamin A, 7,716 IU.
[3]Tylosin Phosphate - 0.1 lb/tonne inclusion (40g/lb) Elanco Animal Health, Indianapolis, IN 46240.
[4]Supplied per kilogram of diet: 1.1025 mg thiamin.

ingredients and whole diets could enhance the utilization of phytate P by birds. Instead, this study indicates that steam conditioning/pelleting resulted in destruction of the endogenous phytase in wheat bran to levels below those necessary before any beneficial effects can be realized by the birds.

Discussion

The endogenous phytase activity levels reported in the unprocessed wheat bran samples in this experiment (5.7 FTU/g) seem compatible with data from earlier reports (Pointillart, 1991; Viveros *et al.*, 2000). The addition of phytases of microbial origin to feeds, followed by pelleting under conditions routinely applied in the industry have resulted in considerably poor recovery of the enzyme (Wyss *et al.*, 1998; Simon and Igbasan, 2002). Rates as low as 11.8 and no higher 57 percent recovery were reported following steam conditioning temperatures between 80 °C and 85 °C. Likewise, our results seem to indicate that the wheat bran endogenous phytase is highly sensitive to the hydrothermal conditions involved in the pelleting process (12.3 percent recovery at 80 °C), rendering it an impractical source for pre-pelleting inclusion.

It is generally accepted that bone mineralization, usually measured as ash content present in the toes or tibia, is a good indicative of dietary Ca and P utilization (Perney *et al.*, 1993). Toe ash measurements in this study provide a clear suggestion that the endogenous phytase resulting from the inclusion of wheat bran in the unprocessed form to low AvP diet (0.35%) was able to improve P utilization by the birds when compared to the reference base diet. This is in agreement with several investigations on the effects of endogenous phytases from different cereal sources on P utilization by monogastric species (Sauveur, 1984; Pointillart *et al.*,

Table 3: Effect of steam conditioning and pelleting on wheat bran endogenous phytase activity

Conditioning Temperature °C[a]	Hot Pellet Temperature °C[b]	Temperature Gradient- ΔT °C [c]	Phytase Activity FTU/g
Untreated	n/a	n/a	5.7
50	79	29	3.3
60	83	23	3.0
70	84	14	1.9
80	91	11	0.7
90	93	3	0

[a]Fifteen seconds of retention time inside conditioning cylinder.
[b]Samples collected immediately after pelleting and placed inside insulated container prior to measurement.
[c]Temperature rise due to friction generated as bran is expelled through pellet die.

Table 4: Influence of available phosphorus (Pav) on broiler performance and toe ash measurements, 0-3 week data*

Treatment	Live Wt. Gain (g)	Feed Efficiency (g/g)	Toe Ash(%)
1- SC 0.35	602.16[d]	0.7350	11.88[c]
2- SC 0.40	607.90[d]	0.7260	12.37[bc]
3- SC 0.45	710.02[ab]	0.7765	12.89[ab]
4- SC 0.50	714.77[a]	0.7717	13.32[a]
5- WB/C	644.71[bcd]	0.7440	12.28[bc]
6- WB/60	635.66[d]	0.7265	11.91[c]
7- WB/80	640.73[cd]	0.7480	11.86[c]
8- MP	704.23[abc]	0.7648	12.35[bc]
SEM	25.74	0.0147	0.2162
P-value	0.0076	0.1270	<0.0001

*Results followed by same superscript letters mean no significant differences were found ($\alpha = 0.05$).

1987; Pointillart, 1991; Han *et al.*, 1998). The supplementation of an equally low AvP diet with microbial phytase also resulted in higher TA measurements. A comparison of TA observations from the both endogenous and microbial phytase groups with the reference treatments reveals a close similarity to TA values from birds receiving 0.40% Pav in the diet as monocalcium phosphate.

When provided adequate amounts of AvP (0.45%) in the diet, birds exhibited an average 15 percent higher LWG than when fed reference AvP deficient diets (0.4% and 0.35% AvP, respectively). Equally superior LWG was observed with the group receiving 500 FTU/kg of microbial phytase in addition to a diet low in AvP (0.35%). However, all the groups presented with diets containing wheat bran, regardless of the amount of endogenous phytase in the bran, exhibited lower LWG. As previously speculated, lower bulk density of these treatments might have imposed a physical limitation to the amount of feed the birds were able to consume. Moreover, as suggested by Han *et al.*, 1998, the young age of the animals could also have contributed to their inability in taking full advantage of such a fibrous ingredient.

In summary, hydrothermal treatment of wheat bran irreversibly damages the endogenous phytase present in that ingredient, compromising its ability to enhance P availability in the experimental diets. However, the inclusion of unprocessed wheat bran or microbial phytase to the diets resulted in an estimated 0.05% additional dietary Pav. This study indicates that in situations where pelleting is not required and inclusion of wheat bran at five percent or higher levels do not represent a difficulty due to formulation constraints or physiological state (*e.g.* layers, breeders), wheat bran can be utilized as a viable source of phytase, thus lowering the need for sources of inorganic P.

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