Supplemental wheat bran and microbial phytase could replace inorganic phosphorus in laying hen diets

J.H. Yao[1], J.C. Han[1], S.Y. Wu[1], M. Xu[1], L.L. Zhong[1], Y.R. Liu[2], Y.J. Wang[2]

[1]College of Animal Science and Technology, Northwest A and F University, Yangling, Shaanxi, China

[2]Shenzhen Kondarl (Gaoling) Feed Co., Ltd., Gaoling, Shaanxi, China

ABSTRACT: An experiment was conducted to determine effects of wheat bran (WB) phytase on production performance and nutrient utilization in laying hens. Three hundred and seventy-five Lohmann hens at 32 weeks of age were randomly allotted to treatments of fifteen hens per pen with five pens per treatment. Five experimental diets were formulated. Diet one (control) contained 0.19% inorganic phosphate (Pi) from dicalcium phosphate. On the basis of diet 1, diet 2 and 3 were formulated to contain WB 5% and 10%, respectively. In diet 4 and 5, the WB was fixed at the level of 10% with Pi level adjusted to 0.14% in diet 4 and completely replaced with 500 U/kg microbial phytase in diet 5. The results showed that treatment three improved egg yield ($P = 0.142$), feed conversion ratio (FCR) ($P = 0.011$), utilization of crude protein (CP) ($P = 0.060$) and total phosphorus (tP) ($P < 0.001$), and serum Pi concentration ($P = 0.016$) compared with the control. Ten percent of WB replacing 0.05% Pi did not influence either egg yield or nutrient utilization. Compared with the control, treatment five improved FCR ($P = 0.011$) and utilization of CP ($P = 0.060$) and tP ($P < 0.001$), but did not influence either performance or serum parameters. The current study suggests that wheat bran could be used successfully in laying hen diets and wheat bran and microbial phytase supplemented together could replace inorganic phosphate completely.

Keywords: wheat bran phytase; laying hens; production performance; nutrient utilization

Approximately two-thirds of phosphorus in plants are in the form of phytate (Punna and Roland, 1999; Viveros et al., 2000) and are unavailable to or poorly utilized by poultry. This unavailability is due to the very low phytase activity found in the digestive tract (Pallauf et al., 1994). Therefore, phytase has been added to poultry diets to improve the utilization of phytate phosphorus. Phytase initiates dephosphorylation of phytate complexes at either the 3- or the 6-position, of which the commercial phytases Natuphos and Ronozyme, respectively, are examples. The microbial 6-phytase improved phytate P digestibility (Augspurger et al., 2003) and true ileal amino acid digestibility for a maize-soybean meal diet in broiler chicks (Rutherfurd et al., 2004).

Previous research showed that cereals such as wheat, rye and triticale contain phytase activities (McCance and Widdowson, 1944; Simgh and Sedeh, 1979) and that triticale phytase increased growth performance and phytate phosphorus utilization in pigs (Pointillart et al., 1987). Further studies revealed that by-products of cereals such as wheat bran (Eeckhout and Depaepe, 1994) and rye bran (Viveros et al., 2000) are rich in phytase, and researches of wheat bran (WB) (Han et al., 1997), wheat middlings (Han et al., 1998) and rye bran (Pointillart, 1991) demonstrated the positive effects of phytase from cereal by-products on phosphorus retention, bone density and growth performance in pigs.

Wheat bran contains 6-phytase activity ranging between 2 349 and 9 945 U/kg (Eeckhout and Depaepe, 1994; Han et al., 1997; Viveros et al., 2000; Steiner et al., 2007), which is high enough to be con-

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sidered in feed formulation (Paik, 2003). Research in poultry revealed that WB phytase increased growth rate and phosphorus utilization in turkeys (Roberson et al., 2005) and broilers (Paik, 2003; Cavalcanti and Behnke, 2004). However, no reports about WB phytase supplementation in laying hens were found. Therefore, the objective of this experiment was to measure effects of WB phytase on production performance and nutrient utilization and to determine whether inorganic phosphate could be replaced by supplemental WB phytase and microbial phytase in laying hens.

MATERIAL AND METHODS

Birds and management

The animal protocol for this research was approved by the Animal Care and Use Committee of Northwest A and F University. Three hundred and seventy-five Lohmann laying hens, 32 weeks old, were randomly allotted to five treatments with five replications of fifteen birds each. The area of cage was 0.043 square meters per bird. All birds were allowed free access to mesh feed and water. A 16-h photoperiod from 06.00 to 22.00 was scheduled over the period of the experiment. In a 12-week of experiment, egg production was calculated daily and feed intake was determined weekly.

Wheat bran and microbial phytase

Wheat bran, a by-product of wheat processing, is the outer layer including bran (hull), germ and endosperm. When processed to wheat flour in China, about sixteen percent of wheat is discarded, which is wheat bran. As a feed ingredient bought from wheat flour companies, wheat bran is stored in cylindric storage houses of feed companies for feed formulation as quickly as possible. Wheat bran used in this experiment was supplied by Shenzhen Kondarl (Gaoling) Feed Co., Ltd. The analyzed nutritive value of WB was as follows: dry matter (DM) 88.57%, crude protein (CP) 15.52%, total phosphorus (tP) 0.89%, calcium (Ca) 0.13%, crude ash 4.70%, crude fibre 8.34% and phytase activity 2 400 U/kg.

Microbial phytase was Ronozyme P (Roche) with activity 2 500 U/g when assayed using the method described by Roche Vitamins (1999). One unit was defined as the amount of enzyme required to release 1 μmol of inorganic phosphorus per minute from 0.00015 mol/l sodium phytate at pH 5.5 and 37°C.

Experimental diets

Five diets were formulated to contain similar metabolizable energy (ME) 10.90 MJ/kg, crude protein (CP) 14.90%, Ca 3.60%, lysine 0.70%, methionine 0.36%, Met+Cys 0.65% and threonine 0.54%, which are the typical nutrient levels used in Northwest China and meet the nutrient requirements of laying hen. On the basis of diet 1 including Pi 0.19% from dicalcium phosphate, diet 2 and 3 were formulated to contain WB 5% and 10%, respectively. In diet 4 and 5, the WB was fixed at the level of 10% with Pi level adjusted to 0.14% in diet 4 and completely replaced with 500 U/kg microbial phytase in diet 5. The total phytase levels in diets 1 to 5 were 0, 120, 240, 240 and 740 U/kg, respectively.

Sample collection and analysis

At 44 weeks of age, five hens per treatment were selected and housed individually in stainless steel metabolism cages to measure effects of dietary treatments on Ca, P and N balances. Total collection of excreta was run for four days at 12-h intervals. Excreta was weighed, and subsequently frozen at –20°C for chemical analysis. Blood samples were taken via the wing vein from five hens per treatment at 44-weeks-old hens. The serum was separated by centrifugation, and stored at –20°C for subsequent determination of Ca, Pi and alkaline phosphatase (AP). Feed and excreta samples were dried and ground to pass a 1 mm screen.

Dry matter of diets and excreta was determined by weighing a 5.0 g sample and placing it into a drying oven at 100°C for 24 h. Total phosphorus was determined spectrophotometrically after a reaction with ammonium molybdate and aminonaphtholsulphonic acid. Calcium content was determined using a potassium permanganate titration method. Crude protein was determined by the Kjeldhal method using a Kjeltec 2300 analyzer (Foss Tecator AB, Hoeganaes, Sweden). Gross energy (GE) was determined with an automatic bomb calorimeter (Shimadzu Corporation, Tokyo, Japan). Serum Ca, Pi and AP were analyzed using

Table 1. Ingredients and nutrient composition (%) of experimental diets

Item		Treatments				
	inorganic phosphorus (%)	0.19	0.19	0.19	0.14	–
	wheat bran (%)	–	5	10	10	10
	microbial phytase (U/kg)	–	–	–	–	500
Ingredients (%)						
Maize		66.44	63.42	57.90	58.25	59.23
Wheat bran		–	5.00	10.00	10.00	10.00
Lard		–	0.50	1.70	1.60	1.20
Soybean meal		9.80	8.50	7.80	7.70	7.50
Cottonseed meal		7.00	7.00	7.00	7.00	7.00
Rapeseed meal		5.00	5.00	5.00	5.00	5.00
Limestone		8.50	8.50	8.50	8.70	9.15
Dicalcium phosphate		1.20	1.20	1.20	0.86	–
Lysine		0.16	0.17	0.18	0.18	0.18
Methionine		0.12	0.13	0.14	0.13	0.14
Sand		1.20	–	–	–	–
Salt		0.30	0.30	0.30	0.30	0.30
Mineral premix[a]		0.13	0.13	0.13	0.13	0.13
Choline chloride		0.12	0.12	0.12	0.12	0.12
Vitamin premix[b]		0.03	0.03	0.03	0.03	0.03
Microbial phytase		–	–	–	–	0.02
Nutrient composition (%)						
ME (MJ/kg)**		10.96	10.91	10.99	10.94	10.96
CP*		14.96	14.94	15.03	14.97	14.89
Ca*		3.60	3.59	3.55	3.52	3.47
tP*		0.53	0.59	0.63	0.53	0.44
AP**		0.33	0.33	0.33	0.28	0.14
Lys**		0.70	0.70	0.70	0.70	0.70
Met**		0.36	0.36	0.36	0.36	0.36
Met+Cys**		0.65	0.65	0.65	0.65	0.65
Thr**		0.56	0.55	0.54	0.54	0.54

[a]Provided as milligrams per kg diet: manganese, 30 from manganese oxide; iron, 60 from iron sulphate; zinc, 35 from zinc oxide; copper, 4 from copper sulphate; iodine, 0.4 from calcium iodate; selenium, 0.3 from sodium selenite
[b]provided per kg diet: vitamin A 7 625 IU; vitamin D 1 437 IU; nicotinic acid 2.5 mg; vitamin K 250 µg; vitamin B_1 187.5 µg; vitamin B_2 375 µg; vitamin B_6 62.5 µg; vitamin B_{12} 0.875 µg; pantothenic acid 250 µg; folic acid 12.5 µg and vitamin C 125 µg
*analyzed value
**calculated value

an automatic biochemistry analyzer (CL8000, Shimadzu, Japan).

Utilizations of Ca, P and N were calculated by the following formula:

Nutrient utilization (%) = (feed intake × $nutrient_{diet}$ – excreta output × $nutrient_{excreta}$) × 100/(feed intake × $nutrient_{diet}$).

The dietary AME was calculated using the following formula:

AME = ((feed intake × GE_{diet}) – (excreta output × $GE_{excreta}$))/feed intake.

Statistical analysis

Analysis of variance was performed on all data using the General Linear Models procedure of SAS (SAS, 2001) appropriate for a randomized block

Table 2. Effects of dietary treatments on the production performance of laying hens

Treatment	Inorganic P (%)	Wheat bran (%)	Phytase (U/kg)	Egg production (%)	Egg weight (g/egg)	Egg yield (g/day per hen)	Feed intake (g/day per hen)	Feed intake/egg yield
1	0.19	–	–	89.27^{a}	59.30^{b}	52.93^{b}	114.9	2.17^{a}
2	0.19	5	–	90.32^{a}	60.90^{ab}	55.00^{ab}	112.8	2.05^{b}
3	0.19	10	–	90.44^{a}	61.44^{ab}	55.56^{a}	112.6	2.02^{b}
4	0.14	10	–	86.68^{b}	61.66^{a}	53.04^{ab}	112.9	2.11^{ab}
5	–	10	500	89.31^{a}	61.24^{ab}	54.69^{ab}	113.0	2.06^{b}
SEM				0.38	0.34	0.38	0.46	0.02
Probabilities[2]				0.003	0.175	0.142	NS	0.011

Values represent the mean of 5 replicated pens per treatment with 15 hens in each pen;
means with different superscripts within a column are significantly ($P < 0.05$) different and NS represents $P > 0.05$

design. Treatment means were compared by Duncan's multiple range test (Duncan, 1955). Statistical tests and comparisons were considered significant at $P < 0.05$.

RESULTS

Production performance

Effects of dietary treatments on the production performance of hens are summarized in Table 2. Results showed that treatment three improved egg yield (EY) ($P = 0.142$) and feed conversion ratio (FCR) ($P = 0.011$) compared with the control. Compared with the control, treatment four resulted in lower egg production (EP) ($P = 0.003$) but higher egg weight (EW) ($P = 0.175$), and no significant differences were found in EY between the two treatments. When Pi was replaced completely by WB and microbial phytase, FCR in treatment five was improved compared with the control ($P = 0.011$), but EP, EW and EY were not affected.

Nutrient utilization

Effects of dietary treatments on nutrient utilization are summarized in Table 3. Results showed that treatment three and five enhanced the utilization of total phosphorus (tP) ($P < 0.001$) and CP ($P = 0.060$) compared with the control, but not treatment four. There were no significant differences between treatments in AME and utilization of dry matter and Ca.

Serum parameters

Effects of dietary treatments on serum parameters are summarized in Table 4. Results showed that treatment three increased serum Pi concen-

Table 3. Effects of dietary treatments on apparent nutrient utilization in laying hens

Treatment	Inorganic P (%)	Wheat bran (%)	Phytase (U/kg)	AME (MJ/kg)	Dry matter (%)	Crude protein (%)	Calcium (%)	Total P (%)
1	0.19	–	–	11.02	72.11	51.89^{b}	49.20	29.73^{c}
2	0.19	5	–	10.96	72.17	54.26^{ab}	48.15	32.70^{bc}
3	0.19	10	–	11.04	72.93	56.58^{a}	48.12	35.19^{b}
4	0.14	10	–	10.91	72.44	54.67^{ab}	49.80	31.28^{c}
5	–	10	500	10.99	72.64	55.42^{a}	50.40	40.93^{a}
SEM				0.03	0.15	0.54	0.41	0.90
Probabilities				NS	NS	0.060	NS	< 0.001

Values represent the mean of 5 replicated pens per treatment with one hen in each pen
AME = Apparent metabolizable energy;
means with different superscripts within a column are significantly ($P < 0.05$) different and NS represents $P > 0.05$

Table 4. Effects of dietary treatments on serum parameters

Treatment	Inorganic P (%)	Wheat bran (%)	Phytase (U/kg)	Calcium (mmol/l)	Inorganic P (mmol/l)	Alkaline phosphatase (IU/l)
1	0.19	–	–	7.32	1.63[b]	479.14
2	0.19	5	–	7.60	1.71[ab]	466.84
3	0.19	10	–	7.37	1.78[a]	467.92
4	0.14	10	–	7.55	1.64[b]	482.28
5	–	10	500	7.71	1.72[ab]	473.52
SEM				0.07	0.02	2.83
Probabilities				NS	0.016	NS

Values represent the mean of 5 hens per treatment with one hen in each pen means with different superscripts within a column are significantly ($P < 0.05$) different and NS represents $P > 0.05$

tration compared with the control ($P = 0.016$). At a WB level of 10%, lowering Pi content from 0.19% to 0.14% decreased serum Pi concentration ($P = 0.016$). No significant differences between treatments were present in serum Ca concentration and AP activity.

DISCUSSION

Previous research has revealed that cereals contain phytase activities (McCance and Widdowson, 1944; Simgh and Sedeh, 1979). Further studies showed that phytase activities were lowest in legume seeds and oats (262–496 U/kg) (Steiner et al., 2007), intermediate in wheat, barley, rye and triticale (582–6 016 U/kg) and highest in cereal by-products, such as wheat bran, wheat middlings and rye bran (2 957–9 945 U/kg DM) (Eeckhout and Depaepe, 1994; Viveros et al., 2000; Steiner et al., 2007). The wheat bran phytase activity in this experiment was similar to that reported by Eeckhout and Depaepe (1994) and Han et al. (1997) but lower than reported by Viveros et al. (2000) and Steiner et al. (2007). Differences in the phytase activity of cereals and their by-products may come from cultivars, processing and measurement methods (Steiner et al., 2007).

Previous experiments with phytase from cereals and their by-products were conducted in pigs (Pointillart et al., 1984, 1987; Pointillart, 1991; Han et al., 1997, 1998), and in the last years more research was conducted in poultry (Paik, 2003; Cavalcanti and Behnke, 2004; Roberson et al., 2005). Wheat bran phytase increased growth rate and bone strength in pigs (Han et al., 1997) and turkeys (Roberson et al., 2005), and research of wheat middlings (Han et al., 1998) and rye bran (Pointillart, 1991) in pigs obtained similar results. In this experiment, treatment three improved EY, FCR and serum Pi concentration compared with the control, which was attributed to the WB phytase. Han et al. (1997) found that pigs fed the WB-containing diet had similar responses to those fed Pi. Similar results were found in this experiment. Egg yield and serum Pi concentration in treatment four were equal to the control, which indicated that 10% WB (or WB phytase at 240 U/kg) could replace about 0.05% Pi. No differences were found in performance and serum parameters except for EP between treatment five and four, which indicated that about 0.14% Pi was released by microbial phytase at 500 U/kg and WB and microbial phytase supplemented together could replace completely Pi in laying hen diets.

In this experiment, the dietary AME value in laying hens was not affected by WB phytase. However, Ravindran et al. (2001) reported that microbial phytase increased dietary AME in broiler chicks. No reports whether cereal phytase affected dietary energy utilization were found and it should be further studied.

Wheat bran phytase enhanced the digestion and utilization of dietary protein in pigs (Han et al., 1997). Similar results were obtained in this experiment and treatment three and five improved crude protein utilization compared with the control. It is possible that the inclusion of WB in diets might have enhanced the secretion of digestive enzyme and juices that are beneficial to protein digestion (Langlois et al., 1987; Valette et al., 1989). Research indicated that microbial phytase

increased the dietary nitrogen retention (Ketaren et al., 1993; Mroz et al., 1994). The positive effects of microbial phytase on protein digestibility are a result of the hydrolysis of phytate complexes by the enzyme and the release of phytate-bound proteins or amino acids.

Wheat bran phytase improved the phosphorus retention in growing pigs (Han et al., 1997) and decreased its content in turkey litter (Roberson et al., 2005). The present study revealed similar results and treatment three increased tP utilization and serum Pi concentration compared with the control. Pointillart et al. (1984, 1987), Pointillart (1991) and Han et al. (1998) indicated positive effects of phytase from cereals or their by-products on the utilization of dietary phytate phosphorus by pigs. Further studies demonstrated that WB phytase was almost as effective as supplemental microbial phytase in improving the phytate phosphorus utilization by pigs (Han et al., 1997). In this experiment, WB phytase facilitated the phytate phosphorus hydrolysis and increased Pi absorption and tP retention.

Previous studies indicated that cereal phytase from rye bran (Pointillart, 1991) or WB (Han et al., 1997) did not affect the serum Ca concentration. In this experiment, WB phytase had no significant effect on Ca absorption or retention in laying hen diets, which was in agreement with Pointillart (1991) and Han et al. (1997).

In this experiment wheat bran phytase did not significantly influence the serum AP activity, the increase of which induced by the osteoblast activity could be related to intestinal lesions, skeletal disorders or liver dysfunctions.

The present study demonstrated that wheat bran phytase improved the production performance of laying hens and utilization of total phosphorus and crude protein. It is feasible to completely replace the addition of inorganic phosphorus by wheat bran and microbial phytase in laying hen diets. Further studies should be conducted to determine the interaction of phytase from cereal and microbial sources in the performance and nutrient utilization in poultry.

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Corresponding Author

J.H. Yao, College of Animal Science and Technology, Northwest A and F University, Yangling, 712100 Shaanxi, China
Tel. +86 029 870 921 02, fax +86 029 870 921 64, e-mail: yaojunhu2004@sohu.com