Bioavailability of Phosphorus in Low Phytic Acid Barley[1]

Y. C. LI, D. R. LEDOUX,[2] and T. L. VEUM
Department of Animal Sciences, University of Missouri, Columbia, Missouri 65211
Phone: (573) 882-1140
Fax: (573) 882-6827
e-mail: LedouxD@missouri.edu

V. RABOY
USDA, Agricultural Research Service,
National Small Grain Germplasm Research Facility, Aberdeen, Idaho 83210

K. ZYLA and A. WIKIERA
University of Agriculture, Krakow, Poland

Primary Audience: Nutritionists, Feed Manufacturers, Researchers

Summary

A study was conducted to determine the bioavailability of P in a low phytic acid mutant barley (*Hordeum vulgare* L.; MB) containing the *lpa* 1-1 allele and a near-isogenic wild-type hybrid barley (NB). By analysis, the MB contained 0.21% non-phytate P (nP) and 0.35% total P (tP), whereas NB contained 0.11% nP and 0.35% tP. Non-phytate P was considered to be available to poultry. Reagent grade KH_2PO_4 was used as a standard source to determine relative availability. The experimental design consisted of 10 dietary treatments fed to six replicate pens of six chicks each. Dietary treatments included a low available P (aP) basal diet (0.20% aP; 1.0% Ca; Diet 1) supplemented with 0.04, 0.08, and 0.12% P from reagent-grade KH_2PO_4 (Diets 2, 3, and 4, respectively), MB (Diets 5, 6, and 7, respectively), and NB (Diets 8, 9, and 10, respectively). Diets were fed ad libitum for 14 d. Feed intake was not affected ($P > 0.10$) by source or level of P. Body weight gain was not influenced ($P > 0.05$) by P level, but was affected ($P < 0.05$) by P source. Feed conversion was influenced ($P < 0.05$) by both P source and level. Based on non-linear regression analysis (assuming KH_2PO_4 = 100%) and using percentage toe ash as the response variable, bioavailability of P was determined to be 49.3 ± 17% for MB and 28.0 ± 15% for NB. Based on the results of toe ash, P in MB was 1.8 times more available to chicks than P in NB. These results compare favorably with analyzed nP values, which indicated that MB contains 1.9 times as much nP as NB (0.21 vs. 0.11%).

Key words: Bioavailability, phosphorus, low phytic acid, barley, broiler

2001 J. Appl. Poultry Res. 10:86–91

Description of Problem

With few exceptions, poultry rations are based primarily on cereal grains (CG) and oilseed meals (OSM). Unfortunately, about two-thirds of the P in CG and OSM is bound in the form of phytate and is poorly available to poultry [1]. As a result, rations based on CG and OSM need to be supplemented with inorganic P. One result of the poor availability of phytate P is that a significant amount of P is excreted in poultry manure. It is estimated that 250,000 tons of P are

[1] Contribution Number 13032 of the Missouri Agricultural Experiment Station
[2] To whom correspondence should be addressed

excreted annually in poultry excreta [2]. Poultry manure is usually disposed of by applying it to pastures and cropland. However, in areas of intensive poultry production, the amount applied exceeds the requirement for plant growth. When this occurs, P can pose a significant environmental problem. Phosphorus is the least mobile of the three major nutrients in soil, and leaching to ground water is not generally considered a problem; however, the possibility of P-laden soil eroding into surface waters is a primary environmental concern. The movement of P-laden soils into lakes and streams results in a stimulation of algae and other aquatic plant growth. This process is called eutrophication, which results in reduced water quality.

A number of approaches are currently being evaluated to deal with the poor availability of phytate P. One of these approaches is to increase the availability of P in CG and OSM by genetically manipulating the form of P in these feed ingredients. Recently, USDA scientists developed low phytic acid (*lpa* 1-1) corn mutants that show reductions in phytate P ranging from 50 to 60% with no reduction in tP in the grains [3]. In a recent chick study, using bone ash as the response variable, P availability from a low phytic acid corn mutant was found to be 75.4% compared with 35% from an isogenic wild-type corn [4]. A low phytic acid (*lpa*1-1) MB (*Hordeum vulgare* L.) that is phenotypically identical to the NB has also been developed by the USDA scientists [5, 6]. This low phytic acid barley shows reductions of 50 to 75% in phytic acid P with no changes in tP. Compared with NB, P in the MB has been shown to be more available to fish and swine [7, 8, 9]. Phosphorus availability in the MB has not been determined in poultry; therefore, the objective of the present study was to determine if P from MB is more available to poultry than P from NB.

Materials and Methods

Three hundred and sixty 1-d-old male chicks [10] were purchased from a commercial hatchery and were fed a conventional corn and soybean meal diet for 6 d. On Day 7, chicks were weighed, wing-banded, and randomly assigned to 1 of 10 dietary treatments (six replicate pens of six chicks each) for 2 wk. Feed and water were provided for ad libitum consumption. Dietary treatments included a low aP basal diet (0.20% aP; 1.0% Ca) supplemented with 0.04, 0.08, and 0.12% added P from a reagent-grade KH_2PO_4 standard source, MB, and NB. The basal diet consisted of dextrose, gelatin, and soybean meal with added corn oil, amino acids, vitamins, and minerals (Table 1). With the exception of Ca and P, the basal diet met or exceeded the nutrient requirements of chicks [1]. Barley and KH_2PO_4 were substituted for glucose to achieve dietary treatments.

TABLE 1. Composition of the basal diet[A]

INGREDIENT	(%)
Dextrose[B]	45.73
Soybean meal (48% CP)	40.64
Corn oil	5.16
Gelatin	4.20
Limestone	1.58
Dicalcium phosphate	0.59
Sodium chloride	0.50
DL-Methionine	0.31
Trace mineral mix[C]	0.11
Vitamin mix[D]	0.05
Se premix	0.05
Choline chloride	0.03
Ronozyme® B[E]	0.05
Celufil	1.00

[A]Basal diet contained 0.2% available P and 1.0% Ca.
[B]Barley and KH_2PO_4 substituted for glucose to achieve dietary treatments.
[C]Mineral mix provided (mg/kg diet): MnO_2, 222; ZnO, 209; $FeSO_4 \cdot 7H_2O$, 654; $CuSO_4$, 32 mg; ethylenediamine dihydroiodide, 1.9 mg; and $CaCO_3$, 160.
[D]Supplied (/kg feed): vitamin A (retinyl acetate), 8,810 IU; cholecalciferol, 3,855 IU; vitamin E (dl-α-tocopheryl acetate), 14 IU; niacin, 55 mg; calcium pantothenate, 17 mg; riboflavin, 6.6 mg; pyridoxine, 2.2 mg; menadione sodium bisulfite, 1.7 mg; folic acid, 1.4 mg; thiamin mononitrate, 1.1 mg; biotin, 0.2 mg; cyanocobalamin, 11 μg; and ethoxyquin, 83 mg.
[E]Hoffmann-La Roche Inc., Nutley, NJ; contains β-glucanase, xylanase, and α-amylase.

Both MB and NB used in the current study were provided by the USDA, Agricultural Research Service [11]. The MB used in the present study was homozygous for the low phytic acid 1-1 (*lpa*1-1) allele of the low phytic acid 1 gene and was analyzed to contain 0.21% nP and 0.35% tP, whereas NB was analyzed to contain 0.11% nP and 0.35% tP using methods described by Raboy *et al.* [12, 13]. In this study, nP was considered as aP. The analytical composition of MB and NB is presented in Table 2. To avoid

confounding effects that may be caused by the presence of non-starch polysaccharides in barley, all diets were supplemented with 500 g non-starch polysaccharide enzyme, Ronozyme® B (β-glucanase, xylanase, and α-amylase) [14] per ton of diet. In addition, because barley has been shown to contain high endogenous phytase activity, the endogenous phytase activity in both MB and NB was also analyzed [15].

On Day 21, chicks were weighed by pen, and feed consumption was determined per pen. Chicks were killed by asphyxiation with CO_2 followed by cervical dislocation. Middle toes from both feet were collected from each chick to determine toe ash. Toes were dried at 100°C for 24 hr and ashed in a muffle furnace at 600°C overnight. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

Data were analyzed by analysis of variance using the General Linear Models procedure of SAS [16]. Pen was used as the experimental unit. The model included main effects of P source, P level, and the interaction of P source by P level. Phosphorus availability was determined by regressing percentage toe ash on supplemental P using non-linear regression procedures of SAS [16] and methods described by Noll *et al.* [17]. Statistical significance was accepted at $P < 0.05$.

TABLE 2. Analyzed nutrient content of low phytic acid mutant barley (MB) and near-isogenic wild-type barley (NB)

	MB	NB
CP, %	13.09	13.31
Crude fat, %	2.22	1.86
Ash, %	2.26	2.36
Moisture, %	11.51	11.46
Amino acid content, %		
Arginine	0.69	0.65
Cysteine	0.30	0.31
Glycine	0.52	0.51
Histidine	0.30	0.29
Isoleucine	0.47	0.49
Leucine	0.90	0.95
Lysine	0.50	0.47
Methionine	0.22	0.23
Phenylalanine	0.68	0.74
Serine	0.46	0.48
Threonine	0.42	0.43
Tryptophan	0.14	0.13
Valine	0.65	0.66
Macro and micro mineral content		
Ca, %	0.051	0.052
K, %	0.42	0.36
Mg, %	0.1	0.1
Mn, mg/kg	15.1	16.7
Analyzed P content, %		
Phytate P	0.14	0.24
Nonphytate P	0.21	0.11
Total P	0.35	0.35

Results and Discussion

The analyzed nutrient compositions of both MB and NB are listed in Table 2. Results indicated that, with the exception of nP and phytate P values, MB and NB had similar CP, crude fat, amino acid profiles, and selected mineral contents, which suggests that diets containing MB and NB should have similar nutrient values, and any differences observed in any response variables should result from increased nP value in MB. In addition, on analysis, the endogenous phytase activity in MB was similar to that of NB (MB: 251 ± 46 U/kg; NB: 290 ± 18 U/kg), indicating that the results of the current study should not be confounded by the differences in endogenous phytase activity in MB and NB.

Performance data are presented in Table 3. Feed intake was not affected ($P > 0.05$) by source or level of P. Body weight gain was affected by P source ($P < 0.05$). Chicks fed diets supplemented with KH_2PO_4 had superior body weight gain ($P < 0.05$) compared with chicks fed diets containing MB and NB. Body weight gain was not affected by P level ($P > 0.05$). Feed conversion was affected ($P < 0.05$) by both source and level of P. Chicks fed diets containing KH_2PO_4 were more efficient ($P < 0.05$) in converting feed to gain compared with chicks fed diets containing MB or NB. Chicks fed a lower level of supplemental P (0.04%) were also more efficient ($P < 0.05$) in converting feed to gain compared with chicks fed diets supplemented with 0.08 and 0.12% P. No P level by source interactions ($P > 0.05$) were observed for any of the performance variables. The higher body weight gain and better feed efficiency observed in chicks fed KH_2PO_4 is expected because it has been shown that P in KH_2PO_4 is readily available for poultry. The failure of source and dietary P level to affect feed intake and the lack of a P level effect on body weight gain in the current study confirm

TABLE 3. Performance and toe ash of chicks fed dietary treatments for 14 d[A]

DIET	SUPPLEMENTAL P	FI[B]	WG[B]	F:G[B]	TOE ASH
	(%)	(g)		(g:g)	(%)
B + 0.18% KH_2PO_4[C]	0.04	717	557	1.29	9.93
B + 0.36% KH_2PO_4	0.08	727	547	1.33	10.67
B + 0.54% KH_2PO_4	0.12	758	573	1.32	11.07
B + 11.43% MB[C]	0.04	727	548	1.33	9.52
B + 22.80% MB	0.08	716	535	1.34	10.23
B + 34.29% MB	0.12	727	540	1.35	10.18
B + 11.43% NB[C]	0.04	701	527	1.33	9.63
B + 22.80% NB	0.08	704	508	1.39	9.37
B + 34.29% NB	0.12	728	537	1.36	10.13
Pooled SEM		15	10	0.02	0.13
		P			
Source		0.1927	0.0010	0.0051	0.0001
Level		0.1271	0.0707	0.0290	0.0001
Source × level		0.7530	0.7017	0.6364	0.0004

[A]Each value represents the mean of six pens of six chicks each.
[B]FI = Feed intake, WG = weight gain, and F:G = feed:gain.
[C]B = Basal, MB = low phytic acid mutant barley, and NB = near-isogenic, wild-type barley.

previous reports indicating that, unlike bone ash, growth performance may not be a sensitive response variable for determining P availability [18, 19].

Toe ash data are presented in Table 3. Percentage toe ash was affected ($P < 0.05$) by both P source and level. Percentage toe ash increased ($P < 0.05$) with increasing dietary aP. Chicks fed the KH_2PO_4 diets had the highest percentage toe ash followed by chicks fed diets containing MB, and chicks fed diets containing NB. Again, the superior bone mineralization of chicks fed KH_2PO_4 was not unexpected; KH_2PO_4 was chosen as a standard source because it is a readily available source of P. A significant P source by level interaction ($P < 0.05$) was observed for toe ash. This interaction occurred because of differences in response among the sources of P to increasing levels of dietary P.

Relative availability values of P in MB and NB are shown in Table 4. Initially, a slope-ratio assay was used to determine P availability. However, the data were curvilinear in nature and did not meet the criteria for a slope-ratio assay. Therefore, the non-linear regression procedure described by Noll *et al.* [17] was used to determine P availability. Assuming the availability of P from KH_2PO_4 is 100% and using percentage toe ash as a response variable, bioavailability of P was determined to be 49.3 ± 17% for MB and 28.0 ± 15% for NB.

Based on the toe ash data, P in MB was approximately 1.8 times more available than P in NB. These results are comparable with the analyzed nonphytate values, which indicate that MB contains 1.9 times as much nonphytate P as NB (0.21 vs. 0.11%). Results of toe ash data were consistent with the findings of Veum *et al.* [8] who reported that the estimated aP values in MB (0.21% aP) and NB (0.11% aP) correlated well with the pig performance and bone strength data.

Results of the current study clearly demonstrated that P in MB is more available than P in NB, and these results are consistent with the findings of previous studies [7, 8, 9]. Sugiura *et al.* [7] evaluated P availability in MB for rainbow trout and found that P in MB was more available to fish than was P in NB. Low phytate

TABLE 4. Bioavailability of P in low phytic acid mutant barley (MB) and near-isogenic wild-type barley[A]

BARLEY SOURCE	TOE ASH	ANALYZED NON-PHYTATE P
	(%)	
MB	49.3	0.21
NB	28.0	0.11
Relative bioavailability[B]	1.8	1.9

[A]Based on non-linear regression analysis and assuming KH_2PO_4 equals 100%.
[B]Values of MB in each column/values of NB in each column.

barley also significantly reduced fecal P excretion and increased P retention compared with NB. Similar findings were also observed in pigs [9]. In addition to MB, increased P availability has also been shown in low phytate corn (*Zea mays* L) containing the *lpa* 1-1 allele [4]. Chick studies have demonstrated that the relative bioavailability of P in low phytate corn ranged from 45 to 52% compared with 10% for wild-type corn [20]. Studies conducted in our laboratory confirmed the increased P availability in low phytate corn and the reduction in manure P excretion by chicks [21]. Results of the studies with low phytate corn suggest that substituting MB for NB should result in a reduction in manure P excretion by poultry fed barley-based diets.

In summary, results of the current study clearly showed that P in MB was more available than P in NB. The development of low phytate barley and other low phytate grains will provide the livestock industry with another tool to improve P utilization and to combat P pollution caused by poultry manure.

Conclusions and Applications

1. Results of the current study indicated that P in the MB is more available than P in the NB. Based on toe ash data, it is estimated that P in the MB is 49.3 ± 17% available, and P in the NB is 28 ± 15% available.
2. As a consequence of the increased P availability in MB, diets containing MB will not need to be supplemented with as much inorganic P. The combination of lower levels of supplementary inorganic P and increased availability of P in MB will result in a significant reduction in manure P by poultry fed MB. Reductions in manure P will significantly reduce the potential for environmental pollution caused by P.
3. The discovery of low phytic acid grains has given poultry producers another tool that can be used to continue producing high quality poultry products in an environmentally friendly manner.

References and Notes

1. **National Research Council,** 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

2. **Cromwell, G.L.,** 1994. Diet formulation to reduce the nitrogen and phosphorus in pig manure. Pages 1–22 In: Nutrient Manage. Symp. Proc., Harrisburg, PA.

3. **Raboy, V., P. Gerbasi, K.A. Young, S. Stoneberg, S.G. Pickett, A.T. Bauman, P.P.N. Murthy, W.F. Sheridan, and D.S. Ertl,** 2000. Origin and seed phenotype maize low phytic acid 1-1 and low phytic acid 2-1. Plant Physiol. 124:355–368.

4. **Ertl, D.S., K.A. Young, and V. Raboy,** 1998. Plant genetic approaches to phosphorus management in agricultural production. J. Environ. Qual. 27:299–304.

5. **Raboy, V.,** 1998. The genetics of seed storage phosphorus pathways. Pages 192–203 In: Current Topics in Plant Physiology. Am. Soc. Plant Physiol., vol. 19, Rockville, MD.

6. **Larson, S.R., K.A. Young, A. Cook, T.K. Blake, and V. Raboy,** 1998. Linkage mapping of two mutations that reduce phytic acid content of barley grain. Theor. Appl. Genet. 97:141–146.

7. **Sugiura, S.H., V. Raboy, K.A. Young, F.M. Dong, and R.W. Hardy,** 1999. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 170:285–296.

8. **Veum, T.L., D.R. Ledoux, and V. Raboy,** 1999. Low phytic acid barley for growing pigs: Pig performance and bone strength. Page 287 In: Proc. 50th Annu. Mtg. Eur. Assoc. Anim. Prod., Zurich, Switzerland.

9. **Veum, T.L., D.R. Ledoux, and V. Raboy,** 1999. Low phytic acid barley for growing pigs: Calcium and phosphorus balance. Page 287 In: Proc. 50th Annu. Mtg. Eur. Assoc. Anim. Prod., Zurich, Switzerland.

10. Stover Hatchery, Stover, MO.

11. USDA-Agricultural Research Service, 1691 S. 2700 W, P.O. Box 307, Aberdeen, ID 83210.

12. **Raboy, V., D.B. Dickinson, and F.E. Below,** 1984. Variation in seed total phosphorus, phytic acid, zinc, magnesium, and protein among lines of Glycine max and G. soja. Crop Sci. 24:431–434.

13. **Raboy, V., D.B. Dickinson, and M.G. Neuffer,** 1990. A survey of maize kernel mutants for variation in phytic acid. Maydica 35:383–390.

14. Hoffmann-La Roche Inc., Nutley, NJ.

15. A 1-g sample of grain was mixed with 20 mL of 0.2*M* citrate buffer (pH 5.5) in 1*M* NaCl solution and extracted on a magnetic stirrer at 30°C for 30 min. The slurry was centrifuged, and the clear supernatant was cooled on an ice bath and served as a source of enzyme. One milliliter of enzyme solution was pipetted into 1 mL of 5 m*M* sodium phytate solution and incubated for 120 min. The reaction was stopped by the addition of 2 mL of 15% TCA. The concentration of P was determined by the standard Fiske Subbarow method. One unit of phytase activity was defined as the amount of enzyme that frees 1 μmol inorganic P from 1.5 m*M* sodium phytate/min at pH 5.5 and 37°C.

16. **SAS Institute,** 1984. SAS User's Guide: Statistics. SAS Version 4 Edition Institute, Cary, NC.

17. **Noll, S.L., P.E. Waibel, R.D. Cook, and J.A. Witmer,** 1984. Biopotency of methionine sources for young turkeys. Poult. Sci. 63:2458–2470.

18. **Nelson, T.S., and A.C. Walker,** 1964. The biological evaluation of phosphorus compounds. Poult. Sci. 43:94–98.

19. **Yoshida, M., and H. Hoshii,** 1979. Monobasic calcium phosphate as a standard for bioassay of phosphorus availability. Jpn. Poult. Sci. 16:271–276.

20. **Cromwell, G.L., J.L. Pierce, H.L. Stillborn, D.W. Rice, D.S. Ertl, and V. Raboy,** 1998. Bioavailability of phosphorus in low phytic acid corn for chicks. Poult. Sci. 77(Suppl. 1):117.

21. **Li, Y.C., D.R. Ledoux, T.L. Veum, V. Raboy, and D.S. Ertl,** 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444–1450.

ACKNOWLEDGMENTS

Appreciation is expressed to Roche Vitamin Inc. for supplying Ronozyme® B. This study was partially supported by USDA-CSREES Fund for Rural America (Project Number 98-1A-0353-283) and the Idaho Barley Commission.