

《Research Note》

Phytase Activity in the Digesta from Different Parts of the Digestive Tract and Ileal Digestibility of Nutrients in Broilers Fed with Buckwheat Diets

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In the present study, the effects of dietary buckwheat on phytase activity in the digesta from different parts of the digestive tract, and ileal digestibility of nutrients were determined in broilers fed with buckwheat diets. Eighty male broilers (29-d-old) were divided into four groups (20 birds each), and were fed one of the following diets until they were 36-d-old: positive control (PC) diet formulated based on the NRC recommendations, negative control (NC) diet containing 0.15% lower non-phytate phosphorus (P) than that in the PC diet, and two other diets formulated by replacing corn in NC diet with either 20% non-germinated (BU) or germinated (GBU) buckwheat. At the age of 36 d, broilers were sacrificed to collect digesta from the crop, gizzard, duodenum, jejunum, ileum, and cecum. The activity of phytase was low in the PC and NC diets, which increased in the BU diet and increased further in the GBU diet. A similar trend was observed in the crop digesta; however, the phytase activity in the crop digesta of BU and GBU diets was marginally lower when compared with that in each diet. These values decreased sharply when the digesta moved to the gizzard, and then decreased gradually. The ileal digesta exhibited significantly low activity with negligible effect of dietary treatment. The result of two-way analysis of variance with germination and digestive tract parts as main factors showed that the effect of digestive tract parts and interaction between factors was significant on the phytase activity in digesta. The dietary BU and GBU did not affect the ileal crude protein digestibility; however, it increased the ileal phytate P digestibility. These results suggest that in broilers, the crop might be the primary site of phytate degradation by buckwheat phytase, and the buckwheat might have negligible adverse effect on ileal digestibility of nutrients.

Key words: broilers, buckwheat, digesta, ileal digestibility, phytase activity

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Introduction

Microbial phytases are commercially used as feed additives to improve the availability of phytate phosphorus (P) in the feed of monogastric animals. However, this approach is not entirely satisfactory because of the additional cost of enzyme additive. It is well known that wheat and barley have intrinsic phytase activity, and can be used as phytase sources. So far, the followings have been reported: 1) the retention of P improved by about 10%, when wheat or barley was included in the diet of chickens (Takemasa and Murakami, 1995), 2) the phytase of wheat and barley functioned exclusively in the crop of chickens (Takemasa and Murakami, 1995), and 3) germination increased their phytase activity (Ma and Shan, 2002; Bartnik and Szafrńska, 1987).

However, these cereals also contain high levels of β -glucans, which are indigestible polysaccharides that decrease nutrient digestibility (Havrlentová and Kraic, 2006; Moharrery, 2006; Wang *et al.*, 2005). Therefore, when wheat or barley is included in poultry diets, β -glucanase should be added to eliminate the adverse effect of β -glucans, which might increase the cost of diet. Furthermore, buckwheat is a pseudocereal with high phytase activity, but with low levels of β -glucans (Havrlentová *et al.*, 2011; Egli *et al.*, 2003).

In our previous study, we confirmed that dietary addition of buckwheat as phytase source can improve P availability in chickens (Chowdhury *et al.*, 2017). However, because of the limited number of studies, basic information on the use of buckwheat as feed source is scarce. Buckwheat is a plant belonging to the family Polygonaceae and is taxonomically different from wheat and barley, and therefore it might be impossible to consider its properties similar with wheat and barley.

The aim of the present study was to (1) measure the phytase activity in the digesta of different parts of the di-

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gestive tract and ileal digestibility of nutrients in broilers given non-germinated (BU) and germinated (GBU) buckwheat diets, (2) identify the part of digestive tract that is involved in phytate degradation by buckwheat phytase, and (3) verify if dietary buckwheat affects nutrient digestibility.

Materials and Methods

The present study was conducted in accordance with the guidelines for regulation of animal experimentation of Shinshu University, Japan.

Preparation of GBU

The seeds of buckwheat (Shinano no. 1 variety) with high phytase activity were purchased commercially, and a portion of these seeds were germinated following the method of Egli *et al.* (2002) with slight modification. The seeds were soaked in water for 12 h, and then transferred to a tray lined with wet paper and allowed to germinate for 36 h at $23 \pm 2^\circ\text{C}$ in dark. During germination, water was sprinkled on seeds every 10 h. After germination, the seeds were dried at 50°C in a forced-air oven for 7 h. Both non-germinated and germinated seeds were ground to pass through a 1.0 mm aperture, and approximately 93% of the hull was removed by sieving whole ground seeds. Ground BU and GBU were placed at room temperature (25°C) to analyze their chemical composition and phytase activity at different pH (Tables 1 and 2).

Birds, Diets, and Sampling

Eighty, 29-d-old male broilers (Ross 308) were divided into four groups (20 birds each) with an average body weight of 1,484 g, and fed one of the following four diets until 36-d-old: a positive control (PC) diet formulated according to the National Research Council (NRC) (1994) recommendations; a negative control (NC) diet containing 0.15% lower non-phytate P than that in the PC diet; and two other diets formulated by replacing corn with either 20% of BU or GBU in the NC diet (Table 3). All diets contained titanium oxide (0.5%) as an indigestible marker. At the age of 36 d, after 12 h of fasting, the birds were allowed to consume the feed for 1 h. Thirty minutes after feeding, 10 birds from each group were sacrificed to collect the crop and gizzard digesta, and the remaining 10 birds from each group were sacrificed after 60 min to collect the duodenum, jejunum, ileum, and cecum digesta, and stored at -20°C until further processing. Frozen digesta (crop, gizzard, jejunum, and ileum) were thawed, dried (at 40°C in air-draft oven), and ground to pass through a 1.0 mm screen to measure the phytase activity and ileal digestibility.

Chemical Analysis

Samples of BU, GBU, diets, and ileal digesta were analyzed for proximate composition following the standard methods (AOAC, 1990). Total P and phytate P of the samples were measured according to International Organization for Standardization (ISO) (1998) and Haug and Lantzsch (1983), respectively. Non-phytate P was calculated by subtracting the phytate P from total P. The pH of the digesta was determined immediately after collection according to the method described by Esmaeilipour *et al.* (2011). The

Table 1. Chemical composition of buckwheat (Shinano no. 1)¹

Components	Shinano no. 1 buckwheat	
	BU	GBU
Crude protein (%)	13.33	14.98
Ether extract (%)	3.19	3.25
Crude fiber (%)	5.03	6.55
Crude ash (%)	2.34	1.96
Total P (%)	0.40	0.46
Phytate P (%)	0.33	0.28
Phytase activity (PU ² /g)	2.46	2.69

¹ Values of each parameter represent the mean of triplicate analysis (in dry matter).

¹ Values are expressed in dry matter. BU=non-germinated buckwheat; GBU=germinated buckwheat.

² Phytase unit (PU) equivalent to the enzymatic activity liberating 1 μmol inorganic phosphate per min at pH 5.5 and 37°C .

Table 2. Phytase activity (PU²/g) of buckwheat (Shinano no. 1) at different pH¹

pH	Shinano no. 1 buckwheat	
	BU	GBU
3.0	1.56	1.51
4.0	1.21	1.30
4.5	1.69	1.95
5.0	2.18	2.36
5.5	2.36	2.71
6.0	1.03	1.05
6.5	0.97	0.86
7.0	0.54	0.51
8.0	0.42	0.50

¹ Values of each parameter represent the mean of triplicate analysis (in dry matter).

¹ Values are expressed in dry matter. BU=non-germinated buckwheat; GBU=germinated buckwheat.

² Phytase unit (PU) equivalent to the enzymatic activity liberating 1 μmol inorganic phosphate per min at pH 5.5 and 37°C .

phytase activity in the diets and digesta from the crop, gizzard, jejunum, and ileum were measured following the method described by Eeckhout and De Paepe (1994). Finely ground dried sample (100 mg) was mixed with sodium (Na)-phytate solution buffered with acetate at pH 5.5. The phosphate ion liberated from phytate was then measured colorimetrically. The phytase unit was defined as phytase activity liberating inorganic P from 0.0015 M Na-phytate solution at a rate of 1 μmol per min at pH 5.5 and 37°C . However, the phytase activity in the duodenum and cecum digesta was not determined because of insufficient volume of the samples. The concentration of titanium was determined according to the method described by Short *et al.* (1996).

Calculation and Statistical Analysis

The following equation was used to calculate ileal digestibility of nutrients (Meng *et al.*, 2005):

Table 3. **Ingredients and chemical composition of the experimental diets¹**

Ingredients (%)	PC	NC	NC + 20% BU	NC + 20% GBU
Corn	55.3	55.3	35.3	35.3
Soybean meal	34.5	34.7	33.3	33.3
BU	—	—	20.0	—
GBU	—	—	—	20.0
Corn oil	6.2	6.2	7.6	7.6
Ca ₃ (PO ₄) ₂	1.2	0.35	0.35	0.35
Calcium carbonate	1.2	1.85	1.85	1.85
Vitamin-mineral premix ²	1.1	1.1	1.1	1.1
Titanium oxide	0.5	0.5	0.5	0.5
Analyzed composition (as fed)				
Crude protein (%)	17.5	17.2	17.7	17.8
Total P (%)	0.66	0.49	0.48	0.50
Phytate P (%)	0.30	0.28	0.27	0.28
Non-phytate P (%)	0.36	0.21	0.21	0.22
Calcium ³ (%)	0.95	0.96	0.97	0.97
ME (kcal/kg ³)	3,190	3,195	3,185	3,185
Phytase activity (PU/kg of diet in DM)	22.5	23.4	446.6	525.7

¹ PC=positive control, NC=negative control, BU=non-germinated buckwheat, GBU=germinated buckwheat, DM=dry matter.

² Vitamin-mineral premix was provided at the following concentrations per kg of diet: vitamin A, 5,000 IU; vitamin D₃, 1,000 IU; vitamin E, 11 IU; vitamin K₃, 1.1 mg; vitamin B₁, 6 mg; vitamin B₂, 23 mg; vitamin B₆, 8 mg; vitamin B₁₂, 0.02 mg; pantothenic acid, 18 mg; niacin, 35 mg; choline chloride, 1,700 mg; folacin, 2.2 mg; iron, 95 mg; copper, 11 mg; zinc, 180 mg; manganese, 280 mg; iodine, 3.4 mg.

³ Calculated nutrient content was based on the ingredient composition data from the NRC (1994).

Table 4. **pH of the digesta from different parts of the digestive tract in broilers¹**

Dietary groups	Crop	Gizzard	Duodenum	Jejunum	Ileum	Cecum
Time after feeding (min)	30	30	60	60	60	60
PC	5.56±0.04	3.55±0.10	6.05±0.11	6.44±0.04	7.67±0.17	7.30±0.13
NC	5.49±0.04	3.71±0.13	5.98±0.08	6.33±0.04	7.75±0.18	7.00±0.14
NC + 20% BU	5.59±0.13	3.54±0.10	6.03±0.14	6.39±0.04	7.81±0.11	7.17±0.12
NC + 20% GBU	5.59±0.04	3.85±0.08	6.06±0.08	6.38±0.05	7.73±0.11	7.18±0.14
<i>P</i> -value	0.2205	0.1152	0.9752	0.3599	0.9269	0.4669

¹ Values represent mean±standard error of ten observations.

¹ PC=positive control, NC=negative control, BU=non-germinated buckwheat, GBU=germinated buckwheat.

$$\text{Ileal digestibility (\%)} = \left\{ 1 - \left[\frac{\text{TiO}_2 \text{ \% diet}}{\text{TiO}_2 \text{ \% digesta}} \right] \times \left(\frac{\text{nutrient \% digesta}}{\text{nutrient \% diet}} \right) \right\} \times 100.$$

Statistical significances among the dietary groups were determined by Tukey's multiple comparison test at a significance level of 5% after one-way analysis of variance (ANOVA) (SAS Institute, 2015). Considering germination and digestive tract parts as factors, two-way ANOVA was conducted omitting the PC and NC groups, to evaluate the effects of germination and digestive tract parts, and their interaction on the phytase activity in digesta.

Results

Digesta pH (Table 4)

In the PC group, the pH value of the digesta in the crop was 5.56, which decreased sharply to 3.55 in the gizzard. Furthermore, it increased to 6.05, 6.44, and 7.67 in the duodenum, jejunum, and ileum, respectively. The pH value decreased marginally in the cecum. The other groups also exhibited a trend similar to that of the PC group.

Digesta Phytase Activity (Table 5)

The activity of phytase in PC and NC diets, and also in all the digesta of broilers from PC and NC groups was less than

Table 5. Phytase activity (PU/kg) in the diets and digesta from different parts of the digestive tract in broilers¹

Dietary groups	Diets	Digestive tract parts			
		Crop	Gizzard	Jejunum	Ileum
PC	22.5	28.5±8.1 ^C	10.0±2.6 ^B	16.3±3.8 ^B	18.5±3.6
NC	23.4	22.7±5.6 ^C	13.5±5.0 ^B	19.2±4.1 ^B	17.3±1.8
NC + 20% BU	446.6 (100%)	392.5±20.6 ^{Ba} (87.9%)	163.6±20.0 ^{Ab} (36.6%)	77.3±9.0 ^{Ac} (17.3%)	14.6±2.8 ^d (3.3%)
NC + 20% GBU	525.7 (100%)	465.3±11.5 ^{Aa} (88.3%)	175.2±14.9 ^{Ab} (33.3%)	60.6±9.8 ^{Ac} (11.5%)	10.5±1.6 ^d (2.0%)
Main effects of					
Germination					
BU					
GBU					
Digestive tract parts					
Crop					
Gizzard					
Jejunum					
Ileum					
Source of variation		P-value			
Germination		0.0994			
Digestive tract parts		<0.0001			
Germination×Digestive tract parts		0.0094			

^{A-D} Means within a column not followed by common superscripts are different at $P < 0.05$.

^{a-d} Means within a row not followed by common superscripts are different at $P < 0.05$.

¹ Values (except for diet) represent mean±standard error of five observations.

¹ PC=positive control, NC=negative control, BU=non-germinated buckwheat, GBU=germinated buckwheat.

Table 6. Ileal digestibility (%) of dry matter (DM), crude protein (CP), total phosphorus (TP), and phytate phosphorus (PP) in broilers¹

Dietary groups	DM	CP	TP	PP
PC	90.56±0.40	69.90±2.06	28.82±1.91	20.24±1.20 ^B
NC	89.38±0.66	67.33±2.47	26.68±1.57	19.19±1.06 ^B
NC + 20% BU	90.49±0.41	72.02±1.79	35.16±3.85	28.11±1.24 ^A
NC + 20% GBU	90.56±0.31	73.37±1.79	36.84±2.32	30.31±1.76 ^A
P-value	0.2327	0.2143	0.0556	<0.0001

^{A-B} Means within a column not followed by common superscripts are different at $P < 0.05$.

¹ Values represent mean±standard error of five observations.

¹ PC=positive control, NC=negative control, BU=non-germinated buckwheat, GBU=germinated buckwheat.

30 PU/kg. The effect of these diets on the phytase activity was negligible. On the other hand, the activity of phytase in NC diet increased with the addition of BU, and more significantly with the addition of GBU. Such increased activity was observed in the crop digesta. However, the activity of phytase in the crop digesta of broilers that were fed BU and GBU diets was marginally lower when compared with that of each diet. These values decreased ($P < 0.0001$) sharply as the digesta moved into the lower parts of the digestive tract. The highest decrease in the phytase activity was observed when the digesta moved to the gizzard; the phytase activity in the gizzard digesta was 33% to 37% of the phytase activity of

diet. Similarly, all the dietary groups exhibited a low phytase activity in the ileal digesta ($P = 0.1692$). Furthermore, two-way ANOVA showed that the effect of germination was non-significant ($P = 0.0994$), whereas the effect of digestive tract parts ($P < 0.0001$) and their interaction ($P = 0.0094$) was significant on the activity of phytase in the digesta.

Ileal Digestibility (Table 6)

The digestibility of ileal dry matter (DM) and crude protein (CP) was affected marginally by the dietary BU and GBU. However, the digestibility of total P tended ($P = 0.0556$) to increase in the BU and GBU diets when compared with that in the PC and NC diets. Furthermore, the di-

gestibility of phytate P increased significantly ($P < 0.05$).

Discussion

It has been reported that the activity of dietary phytase is affected by proteolytic stability (Bedford and Partridge, 2010). For instance, Onyango *et al.* (2005) investigated microbial phytases in broilers and reported that the phytase activity was high in the digesta from the crop to ileum when *Escherichia coli*-derived phytase was provided. However, the phytase activity was high only in the crop digesta when the broilers were fed *Peniophora lycii*-derived phytase, which suggested that the former might have higher proteolytic stability than the latter. Takemasa and Murakami (1995) observed the activity of phytases of plants, such as wheat and barley, only in the crop digesta, but not in the gizzard digesta of chicks. In the present study, about 90% of the phytase activity of diet was observed in the crop digesta and 33% to 37% in the gizzard digesta. This suggests that unlike the phytases of wheat and barley, the buckwheat phytase might be partially stable to pepsin hydrolysis in the proventriculus.

In the present study, the main effect of germination on the phytase activity was not significant; however, the crop digesta exhibited increased activity with germination. On the other hand, the dietary GBU has been reported to induce higher P utilization in broilers when compared with that of dietary BU (Chowdhury and Koh, 2018). Considering these results, it is suggested that the overall phytate degradation is due to the activity of phytase in the crop digesta. The lowers parts, such as the jejunum and ileum, might be little involved in phytate degradation because of significantly decreased phytase activity in these parts. Furthermore, the involvement of gizzard might be limited, because of low phytase activity and digesta storage capacity, when compared with the crop.

On the other hand, the main effect of digestive tract parts and their interaction was significant. This suggests that the phytase activity of the digesta decreased as it moved down the digestive tract, and the rate of decrease varied with germination. The decrease might be attributed to the proteolytic enzymes, such as pepsin and trypsin. It is difficult to confirm if the rate of decrease, i.e., proteolytic resistance, in the phytase activity was varied with germination because sufficient information on the properties of buckwheat phytase was not obtained.

The digestibility of ileal DM and CP was affected marginally by the dietary BU and GBU. Broilers that were fed wheat- or barley- based diet without supplementation of β -glucanase exhibited low digestibility of DM and CP (Moharrery, 2006; Wang *et al.*, 2005). Therefore, the use of buckwheat instead of such cereals might reduce the extra cost of supplementing enzyme for commercial broiler production.

The dietary BU and GBU tended ($P = 0.0556$) to increase total P digestibility and significantly ($P < 0.05$) increased phytate P digestibility. However, the ileal digestibility of total P was as low as 35% in both BU and GBU groups, although about 60% of the ingested P was retained in the

body of the chicken in our previous study (Chowdhury and Koh, 2018). Such low ileal digestibility of P has also been reported by Woyengo *et al.* (2010) and Mutucumarana *et al.* (2014). This inconsistency might probably be due to the retention time of digesta in the crop, the possible primary site of phytate degradation. The phytate P in the feed passing through the crop quickly might be partially hydrolyzed by phytase. The time-course change in P digestibility should be determined.

In the present study, the crop digesta exhibited the highest phytase activity in broilers that were fed BU and GBU diets. This suggested that the primary site of phytate degradation by buckwheat phytase might be the crop. Furthermore, the buckwheat had negligible adverse effect on the ileal digestibility of nutrients.

Conflicts of Interest

The authors declare no conflict of interest.

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