Evaluation of Buckwheat (*Fagopyrum esculentum*) Intrinsic Phytase Activity to Improve Phosphorus Availability in Broilers

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***Abstract*—In the present study, the effect of buckwheat (*Fagopyrum esculentum*) on phosphorus (P) availability in broilers was investigated: non-germinated (BU) and germinated buckwheat (GBU) were used. Seeds samples were analyzed for proximate components, total P, phytate P and phytase activity. The efficacy of BU phytase was assessed by *in vitro* digestibility (dry matter, crude protein and phytate P) measurement and *in vivo* experiment using 20 male broilers. For both studies, a positive control (PC), negative control (NC), NC + 10% BU, and NC + 10% GBU diets were prepared where, BU and GBU were included at the rate of 10% NC diet at the expense of corn. Increased phytase activity in BU after germination resulted in decreased phytate P content which reflected through numerically increased *in vitro* phytate P digestibility. Increased (P<0.05) CP digestibility may be also a consequence of phytate P hydrolysis. Retention of nitrogen decreased (P<0.05) in broilers given NC diet, which was recovered with the addition of BU and GBU. Moreover, total P retention increased (P<0.05) in birds given BU and GBU added diet compared with NC diet. In conclusion, the results revealed that P utilization in birds could be improved when 10% of corn was replaced with BU and GBU, because of their high phytase activity.**

***Index Terms*—broilers, buckwheat, germination, phytase activity, phytate phosphorus**

I. Introduction

Recently, nutritional approaches have been made to reduce phosphorus (P) excretion by increasing the availability of P in diets, which is due to the increasing concerns about environmental pollution from chicken excreta. Many studies on this field have been conducted and suggested that one of the solution is administration of phytase to the diet, because this enzyme can release P from phytate which is the main storage form of P in grains and indigestible for poultry. However, this enzyme is expensive for feed ingredient especially in developing countries. To find alternatives of low cost, researchers have studied to find ingredients having phytase activity and reported that wheat, triticale and barley contains relatively increased phytase activity [1].

Buckwheat (*Fagopyrum esculentum*) (BU) is a non-glutinous pseudocereal and produced in many countries, which possess high nutritive values [2], and health beneficial properties [3]: its annual production in the world is 1.5 million tons (in 2010). Of course, this is produced for human consumption but part of them, which is low grade and unsuitable for human consumption, may be used for animal feed. Interestingly, this is reported to contain increased level of phytase [1] compared to some cereals and legumes commonly used as chicken feed. Taking these into account, it may be interesting to use BU as an energy source containing phytase activity.

Besides, it is noteworthy that germination can induce a substantial increase in the activity of phytase and reduce concentration of phytic acid in grains [4], suggesting that germinated BU (GBU) can lead to better utilization of phytate P in grains, comparing with non-germinated one. This improvement may increase the economical interest in its use in broilers which may also contribute to formulate environment friendly diets. Although BU had been reported to display some effects on growth performance and lipid profile of chicken [5], [6] but information on the efficacy of BU phytase to improve P availability in broilers are limited. The aim of the present study was to measure chemical composition in BU and GBU, *in vitro* digestibility of diets containing BU and GBU, retention of nitrogen and P, and tibia characteristics in broilers given the both BU and GBU added diets.

II. Materials and Methods

A. Germination

Shinano-1-go BU seeds were soaked in water in a ratio of 1:5 (w/v) for 12h and then allowed to germinate in a tray lined with wet paper for 36h at room temperature (22±2 ℃) maintaining a dark condition. After germination, seeds were dried at 50 ℃ in a forced air draft oven for 7h

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and then, both BU and GBU seeds were ground to pass through 1.0mm aperture and subjected to chemical analysis (Table I). No physical differences were found in BU and GBU samples (Fig. 1).

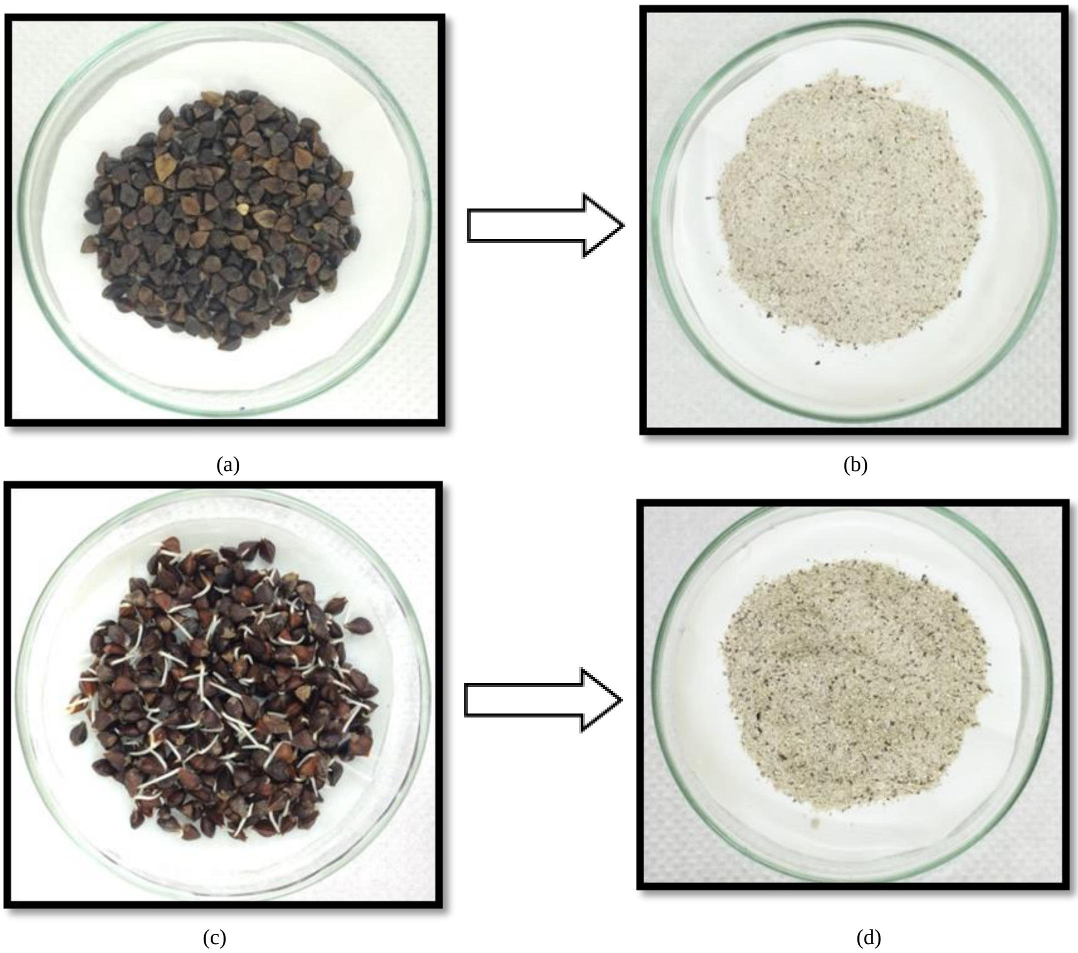

Figure 1. (a) non-germinated buckwheat seed; (b) non-germinated buckwheat meal; (c) germinated buckwheat seed; (d) germinated buckwheat meal

TABLE I. CHEMICAL COMPOSITION OF SHINANO-1-GO BUCKWHEAT

Components, %	Shinano-1-go buckwheat	
	BU[1]	GBU[2]
Crude protein	15.47	17.29
Ether extract	3.13	3.22
Crude fiber	4.73	6.65
Crude ash	2.06	2.23
Total P	0.39	0.44
Phytate P	0.33	0.29
Phytase activity, U/g	2.23	2.71

[1]BU= non-germinated; [2]GBU= germinated

B. Diet Preparation and in Vitro Study

A positive control (PC), negative control (NC), NC + 10% BU and, NC + 10% GBU diets were prepared. The PC diet (around 3200kcal/kg of energy, 20% of CP, 0.8% of Ca and 0.35% of non-phytate P) was formulated to meet or exceed the NRC (1994) nutrient requirement for growing broilers (Table II). The NC diet was same as the PC diet except non-phytate P, which was 0.25%. Both BU and GBU were included at the rate of 10% of NC diet in expense of corn. Prepared diets were ground to pass through 1.0 mm aperture and exposed to an *in vitro* dry matter, crude protein and Phytate P digestibility measurement by pepsin-pancreatin method [7].

C. Broiler Experiment

A total of 20 male broilers (29 d of age, Ross 308) were randomly distributed into four dietary groups (5 birds each). The diets were same as used for *in vitro* study. Birds had free access to feed and water throughout the experimental period (29 to 42 d of age). Feed intake and body weight were recorded daily and weekly, respectively. Excreta samples were collected from 39 to 41 d of age and stored at -20 ℃ in a freezer until analysis. The differences in the nitrogen and total P content of the feed consumed and the feces was used to calculate their relative retention. At the end of experiment, all birds were slaughtered by cervical dislocation. The left and right tibia of each bird were collected, de-fleshed and cartilage caps were removed. The length of tibia was determined with a ruler, and the width was measured with a digital caliper at the calculated midpoint (50% of length). Tibias were subsequently dried at 105 ℃ for 24h, then placed in desiccator and weight was recorded.

D. Chemical Analysis

Proximate components were analyzed according to AOAC standard methods. Total P and phytate P were measured according to Ref. [8] and [9], respectively. Non-phytate P was calculated as total P minus phytate P. The Phytase Reference Standard Method described by Ministry of Agriculture, Forestry and Fisheries, Japan [10] was followed to measure the phytase activity where, one unit of phytase activity was defined as that the quantity of enzyme required to liberates 1 μmol of inorganic P per min from 0.0041 mol/L sodium phytate at a pH of 5.5 and a water bath temperature of 37±0.5 °C.

TABLE II. INGREDIENTS AND CHEMICAL COMPOSITION OF EXPERIMENTAL DIETS

Ingredients , %	Diets[1]			
	PC	NC	NC + 10% BU	NC + 10% GBU
Commercial diet	50.0	48.5	48.3	48.3
Corn	31.4	32.6	22.3	22.3
BU	-	-	10.0	-
GBU	-	-	-	10.0
Soybean meal	14.5	15.05	13.9	13.9
Corn oil	2.05	1.85	3.5	3.5
$Ca_3(PO_4)_2$	0.35	-	-	-
$CaCO_3$	0.80	1.1	1.1	1.1
Vit-min premix[2]	0.90	0.90	0.90	0.90
Analyzed composition				
Crude protein, %	20.00	20.02	20.07	20.17
Total P, %	0.64	0.55	0.55	0.55
Phytate P, %	0.30	0.31	0.30	0.30
Non-phytate P, %	0.34	0.24	0.25	0.25
ME, kcal/kg[3]	3289	3285	3283	3283

[1]PC= positive control; NC= negative control; BU= non-germinated buckwheat; GBU= germinated buckwheat.
[2]According to NRC (1994).
[3]Calculated Value.

E. Statistical Analysis

Data were analyzed by one-way ANOVA. Significant differences among the treatment means were separated by Tukey's multiple comparison tests with a 5% level of probability.

III. RESULTS AND DISCUSSION

A. Chemical Composition of Buckwheat

Crude protein, ether extract, crude fiber, crude ash and total P contents were increased whereas, phytate P content was decreased in GBU than BU (Table I). As expected, phytase activity was increased in GBU. Ref. [4] also found an increased phytase activity and decreased phytate P content in rice, maize, millet, sorghum and wheat after germination. The reduction of phytate P in GBU can be explained comprehensively by considering the influence of germination in increasing phytase activity. Moreover, an increased in phytase activity and the accompanying decrease in phytate content, are expected to increase P availability and utilization in broilers.

B. In Vitro Digestibility

Both CP and phytate P digestibilities were increased in BU and GBU diets (Table III). Although CP and phytate P levels were same for all the diets, but the increasing trend of CP digestibility in both BU and GBU diets were consequential to phytate hydrolysis which was confirmed by the numerically increased phytate P digestibility. It is assumed that, in vitro study results did not always reflects the full potentiality in an in vivo condition because intestinal factors (like pH, temperature) and birds individual responses may have some affect on phytate degradation [11]. However, the negative effect of phytate can be ameliorated by phytase which could lead to increased digestion and availability of dietary protein [12].

C. Performance and Nutrient Retention

At the end of the experimental period, BU and GBU added NC diet, did not show any effect on the growth performance in broilers (Table IV). According to the results of Ref. [5], addition of BU up to 60% in broiler diets, showed no significant effect on body weight gain while feed efficiency was declined. Interestingly, nitrogen and total P retention were increased ($P<0.05$) in birds given BU and GBU diets compared with NC diet (Table V). Higher phytase activity in both BU and GBU, stimulated the hydrolysis of phytate P which leads to increased retention of nitrogen as well as total P in broilers. Although significant but the lower differences in terms of retention among the groups than expected, may be due to the effects of birds age and non-phytate P level. Although Ref. [13] reported that protein and P retention were not affected due to feeding of the BU in chickens, but the positive effect of wheat and triticale phytase activity on P retention and availability in broilers were reported by Ref. [14] [15]. However, the comparable performances along with increased nitrogen and total P retention in broilers given BU and GBU added NC diet with PC diet, extended the path to use BU and GBU to formulate a diet which will provide the birds with their P needs and reduced P excretion.

TABLE III. *IN VITRO* DIGESTIBILITY OF BUCKWHEAT ADDED DIETS

Digestibility, %	Diets[2]			
	PC	NC	NC + 10% BU	NC + 10% GBU
Dry matter	75.09±0.64	74.64±0.33	75.60±0.73	75.56±0.72
Crude protein	85.21±0.52[a]	85.23±0.37[a]	86.68±0.21[ab]	88.33±0.62[b]
Phytate P	33.07±1.08	31.50±0.90	34.57±1.40	35.39±1.38

[1]Values for each parameter represent mean ± standard error (n=3); [a-b]Mean within the same row with different superscripts are significantly different (P<0.05).
[2]PC= positive control; NC= negative control; BU= non-germinated buckwheat; GBU= germinated buckwheat.

TABLE IV. GROWTH PERFORMANCE AND RETENTION IN BROILERS FED BUCKWHEAT ADDED DIETS

Performance parameters	Diets[2]			
	PC	NC	NC + 10 % BU	NC + 10 % GBU
Initial body wt., g	1509 ± 41.8	1497 ± 45.9	1505 ± 41.1	1481 ± 52.9
Final body wt., g	3130 ± 49.1	3060 ± 44.9	3074 ± 45.9	3082 ± 47.5
Body wt. gain, g	1621 ± 22.9	1563 ± 39.9	1569 ± 17.8	1610 ± 37.2
Feed intake, g/bird/d	186 ± 2.37	181 ± 5.7	180 ± 5.3	183 ± 4.2
Feed conversion ratio	01.59 ± 0.02	1.60 ± 0.04	1.57 ± 0.02	1.59 ± 0.04

[1]Values for each parameter represent mean ± standard error (n=5).
[2]PC= positive control; NC= negative control; BU= non-germinated buckwheat; GBU= germinated buckwheat.

TABLE V. RETENTION OF NITROGEN AND TOTAL PHOSPHORUS IN BROILERS GIVEN BUCKWHEAT ADDED DIETS

Retention, %	Diets[2]			
	PC	NC	NC + 10% BU	NC + 10% GBU
Nitrogen	67.72 ± 0.48^{a}	66.05 ± 0.26^{b}	67.45 ± 0.25^{a}	67.95 ± 0.3^{a}
Total P	35.46 ± 0.58^{ab}	33.59 ± 1.22^{a}	36.80 ± 0.56^{b}	37.76 ± 0.43^{b}

[1]Values for each parameter represent mean ± standard error (n=5); [a-b]Mean within the same row with different superscripts are significantly different (P<0.05).
[2]PC= positive control; NC= negative control; BU= non-germinated buckwheat, GBU= germinated buckwheat.

D. Tibia Characteristics

There were no effects of diets on weight, length and width of tibia in broilers (Table VI). There are lack of evidence regarding the direct effect of phytase as well as available P on length and width of tibia in finishing broilers. Ref. [16] reported that, dietary addition of microbial phytase did not show any effect on tibia length. In addition, Ref. [17] observed that, reduced dietary calcium and P did not affect tibia cortex thickness and length in broiler chickens.

TABLE VI. TIBIA CHARACTERISTICS OF BROILERS GIVEN BUCKWHEAT ADDED DIETS

Tibia characteristics	Diets[2]			
	PC	NC	NC + 10% BU	NC + 10% GBU
Weight, g	8.27 ± 0.30	8.04 ± 0.11	8.18 ± 0.20	8.28 ± 0.22
Length, cm	10.38 ± 0.05	10.32 ± 0.07	10.34 ± 0.10	10.42 ± 0.16
Width, mm	10.71 ± 0.19	10.15 ± 0.29	10.84 ± 0.22	10.95 ± 0.18

[1]Values for each parameter represent mean ± standard error (n=5).
[2]PC= positive control; NC= negative control; BU= non-germinated buckwheat, GBU= germinated buckwheat.

IV. CONCLUSION

The results obtain here confirmed the usefulness of BU and GBU at 10% level to increase utilization of P and maintain performance as well as tibia characteristics of broilers in low P diet. The possible reduction of mineral P supplementation by using phytase rich BU and GBU would allow a diet beneficial also for environment.

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