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The effect of enzymatic pre-treatment of corn or soybean meal on their phytate content under different *in vitro* conditions

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Abstract:

BACKGROUND: Phosphorous is one of the expensive nutrients in poultry feed. Therefore, improving the bioavailability of this nutrient in feed ingredients could be effective for lowering the cost of feed. **OBJECTIVES:** This study was performed to evaluate the effect of pre-treatment of feed ingredients by commercial enzymes and different levels of pH on the release of phosphorus from phytate under in vitro condition. **METHODS:** Three solutions including Distilled water, HCl 0.5% and HCl 1% (with pH=5.5, 2.12 and 1.88 respectively) and three enzymes (None, Bio-phytase, Rovabio Excel AP, and Rovabio Max AP) were used to determine phytate content of corn and soybean meal. First, each sample was supplemented with the enzymes and pre-treated under the above mentioned solutions for 3 h at 25 and 40 ºC. **RESULTS:** The results indicated that pre-treatment of corn samples with Bio-phytase or Rovabio Max AP and different solutions (at 25 ºC and 40 ºC for 3 h) reduced phytate content significantly. The best results were obtained with corn samples supplemented with Rovabio Max AP and mixing by HCl 1% at 25 ºC, so that phytate content decreased up to 99.5% in comparison with control. The same results were also obtained for soybean samples. The highest reduction in phytate content (up to 47.4%) was observed by adding Rovabio Max AP and HCl 1% solution at 40 ºC. **CONCLUSIONS:** It could be concluded that pre-treating corn and soybean meal using various methods such as commercial enzymes including phytase and solutions with different pH were effective to reduce phytate content, which means increasing the bioavailability of phosphorous.

Introduction

Since the major part of costs in poultry production is related to feed, minimizing this part of the costs which depends on feed ingredients is very important. On the other hand, phosphorous is usually one of the expensive nutrients in poultry feed. Therefore, the amount of nutrients in feed ingredients, especially available phosphorus, has a very important role in lowering the cost of feed.

The main form of storage of phosphorus (P) in plant feedstuffs is phytate (myo-inositol-hexakis-phosphate or IP6-P) which contains approximately 60-80% of total phosphorus in plant feed ingredients (Ders-

jant-Li, 2015; Kishor Gupta, 2015; Besline Joshi, 2014; Esmaeilipour, 2012; Ravindran, 1995).

Monogastric animals including poultry birds produce endogenous phytase to certain extent. However, this enzyme has minimum effect on hydrolysis of dietary phytate in the intestine of poultry due to a number of micro-environmental factors such as pH of the site of action (intestine), presence of other dietary nutrients (divalent cations, amino acids) and low transit time of digesta. Phytate forms chelated complex with some mineral elements like calcium and magnesium and reduces their availability. Consequently, with reducing the digestibility of phytate, considerable quantities of P are excreted in the manure and lead to polluting the environment, poor absorption of mineral and protein and then high feed cost; unless plant or microbial phytases are added in the feed. Despite the low level of phytate content in feed ingredients, determining its quantity should be considered in order to supply the adequate and necessary phosphorus and also other bivalence like calcium and magnesium. Following the use phytase and its effect on phytate molecule in feed ingredients, some nutrients e.g. protein, starch, and minerals are released better, so they could be digested and absorbed more easily and quickly by the birds (Lamid, 2014; Esmaeilipour, 2012; Tahir, 2012; Woyengo, 2010).

The amount of phytic acid in feed ingredients is varied based on their type and the environment which they grow. Some researches show that the proportion of non-phytate P of total P in feed ingredients is approximately 33% but for corn is around 40% (Tahir, 2012; Ravindran, 1999). Data presented in Table 1 show the level of phytate in different ingredients (Tahir, 2012; Selle, 2007; Godoy, 2005).

Therefore, it seems that determining the amount of phytate in feed ingredients is necessary in order to balance the level of phosphorus and other elements in ration, precisely. It allows the nutritionist to formulate the feed efficiently, lower the feed cost and reduce the amount of P pollution in poultry excreta (Tahir, 2012; Woyengo, 2010; Cowieson, 2005; Common, 1940).

Exogenous enzymes are usually used to improve digestibility and nutritive value of rations including cereals, especially for poultry. There are several studies to describe the effects of exogenous enzyme on nutritional value of nutrients like energy and protein in diets while just a few researches pay attention to the effect of enzyme on nutritional value of ingredients, so it is necessary to determine the nutritional matrix of each ingredient. Therefore, some information about the effects of enzymes on nutrients and anti-nutrients availability of feed ingredients has been shown, permitting us to improve the diet formulation and making it more cost-effective (Beslin Joshi, 2014; Dourado, 2009; Olukosi, 2007; Cowieson, 2005).

Phytase is an enzyme which hydrolyzes phytate to inositol and inorganic phosphate. Some cereals like rye, triticale, and wheat and to a lower extent barley, have some phytase activity (Viveros, 2000) and also phytase exists at low level in the chicken gastro-intestinal tract but it is not enough to digest all P bounded phytate in diet even if diets are prepared directly on-farm, without any heat-treatment. Most diets are used as pellet form with heat treatment at temperature above 80 ºC that deactivate the enzyme activity of cereals. Moreover, corn and soybean meal (SBM) which are the main ingre-

dients used in poultry diet in our territory, have no enzyme activity. Therefore, it has been proven that supplementation of diets with exogenous phytase is effective for enhancing the P availability, reducing the requirement of inorganic P to the diet and hence, the amount of P excreted into the environment (Woyengo, 2013; Selle, 2000).

Recently, there are several studies on the effects of using the exogenous phytase along with combination of other enzymes, especially the cell wall degrading enzymes. These enzymes facilitate the access of phytase to phytate and also the release of phytate phosphorus but the results of these studies were vary (Woyengo, 2010; Kim, 2005).

Non-starch polysaccharide (NSPs) enzymes degrade the cell wall of cereals then the nutrients become more available for animals. In addition, NSPs enzymes reduce the intestinal viscosity so that digestion and absorption of the nutrients are accelerated, which leads to improved poultry performance. Some researchers explained that glycosidases are able to break the NSPs of the cell wall and thus accelerate the availability of phytase to the phytate which is imprisoned in the cell wall (Olukosi, 2007). Whilst if the phytase is used alone, its accessibility to phytate will be limited due to the substrate due to the substrate, which is imprisoned by NSP matrix (Olukosi, 2007). In addition, it has been shown that the effect of xylanase could be better when it is accompanied with other exogenous enzymes including protease, amylase and phytase (Dourado, 2009; Cowieson, 2005).

Since there is not enough time for digesta to reside in poultry gastrointestinal tract, enzymes are not enable to hydrolyze the ingredients completely. Therefore, benefiting from a method which increases contact time between enzymes and their substrate results in increased possibility of the positive effect of enzymes on nutrients, and also according to some researches, pre-treatment of feedstuffs or diets may have a beneficial effect on hydrolysis of phytate and also other anti-nutritional factors existed in feedstuffs. Consequently, pre-treatment would be a great way for using the vast quantity and variety of raw materials. Besides, it can be an added value to ration for improving the quality of diets.

Therefore, the objective of this research is to evaluate the effects of pre-treatment of feed ingredients by some factors like pH and exogenous enzymes (phytase and NSP enzyme) on content of phytic acid, in order to enhance the availability and utilization of phytate phosphorus in vitro conditions.

Materials and Methods

The influence of pre-treatment factors including pH and enzyme was evaluated on the bio-availability of phytate phosphorus in feed ingredients in two separate experiments. The samples of corn and soybean meal were supplemented with phytase and/or NSP enzymes. The design of trial for both ingredients was similar. Each trial was done at two temperatures (25 º and 40 ºC).

Experiment 1: the experiment had a completely randomized design with 3×4 factorial arrangements with three replicates for each treatment. For this purpose, the effect of three solutions with different pH (distilled water, HCl 0.5%, HCl 1%), and three commercial enzymes (None, RMAP = Rovabio Max AP, REAP = Rovabio Excel AP, Bio-phy = Bio-phytase) were studied after 3 h. Bio-phytase included (5,000 FYT U/g of CbS Canadian Bio-system compa-

ny), and Rovabio AP (22000 U visco /g xylanase & 2000 U AGL/G B- gluconase of Adisseo company), and Rovabio Max AP (Phytase 10,000 FTU/g + 22000 of U visco /g xylanase + 2000 U AGL/G B- gluconase of Adisseo Company of France).

First, samples of corn were ground and randomly divided to three groups with 4 treatments in each group with 3 replications for each treatment. Then, samples of corn were supplemented with or without enzymes precisely. In the next step, 3 solutions with different pH (Distillated water, HCl 0.5% and HCl 1%) with pH=5.5, 2.12 and 1.88 respectively, were added to the mixture of corn samples and enzymes in room temperature, then were heated in oven 60ºC or kept at room temperature (25 ºC) for 3 h. Then all of the samples were kept at room temperature overnight in order to dry (with moisture not more than12%). The dried samples were prepared to measure their phytate contents. First, the samples were extracted with 0.2 normal HCl, then 0.5 ml of extract was transferred into a test tube. 1 ml of ferric solution was added to the tube and fitted with a ground-glass stopper. The tube was heated in a boiling water bath for 30 min. After cooling in ice water for 15 min. the solution was allowed to adjust to room temperature. Then 2 ml of 2, 2–Bipyridine solution was added and the contents of tube were mixed. Absorbance was measured after 0.5 min. The Bipyridine reacted with the iron phytate and the color of tube contents changed (Haug, 1983).

Experiment 2: In the second experiment, all of the above mentioned steps were conducted with SBM samples.

Statistical analysis: The experiment had a complete random design with a 3 × 4 factorial arrangement (ANOVA), R software (Version 3.0.0).The general linear model (GLM) was used to determine the main effects of factors of any possible interaction between factors using Bonferroni Postdoc test. Significance was accepted at the $P < 0.05$ level.

Table 1. Phytate phosphorus content of feed ingredients. Data derived from the study by Godoy et.al. (2005) & Sell, Ravindran et al (2007).

Feedstuff	Total P (g/kg)	Phytate P (g/kg)
Barley	2.73- 3.7	1.86- 2.2
Corn	2.3- 2.9	1.7- 2.2
Sorghum	2.6- 3.09	1.7- 2.46
Wheat	2.9- 4.09	1.8- 2.89
Canola meal	8.79- 11.5	4- 7.78
Cottonseed meal	6.4- 11.36	4.9- 9.11
Soybean meal	5.7- 6.94	3.54- 4.53

Results

Corn: The results presented in Table 2 indicated that the effects of enzymes and solutions (with different pH) on the lowering of phytate were significantly different at both temperatures (25 and 40 ºC). At 25 ºC, the effects of enzymes or solutions (different pH) alone, and also interaction effects of enzyme × solution on phytate content of corn samples were significant ($p<0.001$). According to the results presented in Table 3, adding NSP enzymes alone (REAP) to the corn samples and pre-treatment with different pH solutions for 3 h did not affect the content of phytate except with HCl 0.5%, in comparison with control. Pre-treated corn samples supplemented with phytase (Bio-phy) decreased phytate content ($p<0.001$). There were significant differences in phytate content of corn samples supplemented with phytase alone (Bio-phy) or in combination with phytase + NSP enzymes (RMAP) when compared with control and NSP enzymes alone (REAP) ($p<0.001$).

Table 2. Analysis of variance for treated corn samples with commercial enzymes and different solutions after 3 hours at 25 and 40 ºC. [a] R Squared = .950 (Adjusted R Squared = .915). [b] R Squared = .980 (Adjusted R Squared = .966).

Source	25 ºC			40 ºC		
	df	F	P Value	df	F	P Value
Solution	2 [a]	8.44	.001	2 [b]	11.306	.000
Enzyme	3	156.34	.000	3	384.370	.000
Solution × Enzyme	6	8.715	.000	6	10.329	.000
Error	11			11		

Table 3. The mean of phytate content (± SD[1]) in corn samples treated with commercial enzymes and different solutions after 3 hours at 25 ºC. [a-d] means within the same columns with common superscripts have no significant differences (p>0.05). [1]SD = Standard deviation, * = Increasing phytate content, ** = Decreasing phytate content. Bio-phy =Bio-phytase, REAP = Rovabio Excel AP, RMAP = Rovabio Max AP.

Enzyme	Distilled Water	Variation to control (%)	HCl 0.5%	Variation to control (%)	HCl 1%	Variation to control (%)
Without Enzyme	1.32±0.25[ab]	0	1.31±0.08[b]	0	1.60±0.22[a]	0
REAP	1.43±0.14[a]	*+ 8.3	1.78±0.40[a]	+ 35.9	1.90±0.09[a]	+ 18.8
Bio-phy	0.94±0.04[bc]	**- 28.8	0.08±0.02[c]	- 94.2	0.35±0.03[b]	- 78.1
RMAP	0.62±0.57[c]	- 53.0	0.21±0.08[c]	- 84.0	0.01±0.00[b]	- 99.4

Table 4. The mean of phytate content (± SD[1]) in corn samples treated with commercial enzymes and different solutions after 3 hours at 40 ºC. [a-d] means within the same columns with common superscripts have no significant differences (p>0.05). [1] SD = Standard deviation, * = Increasing phytate content, ** = Decreasing phytate content. Bio-phy =Bio-phytase, REAP = Rovabio Excel AP, RMAP = Rovabio Max AP.

Enzyme	Distilled Water	Variation to control (%)	HCl 0.5%	Variation to control (%)	HCl 1%	Variation to control (%)
Without Enzyme	0.49±0.00b	0	1.27±0.08a	0	1.26±0.02a	0
REAP	1.15±0.04[a]	**- 12.6	1.11±0.01[a]	- 12.6	1.30±0.05[a]	*+ 3.2
Bio-phy	0.29±0.19[b]	- 40.8	0.16±0.07[b]	- 87.4	0.14±0.10[b]	- 88.9
RMAP	0.12±0.04[b]	- 75.5	0.02±0.01[b]	- 98.4	0.07±0.03[b]	- 94.4

Table 5. Analysis of variance for treated soybean meal samples with different solutions, enzymes after 3 hours at 25 and 40 ºC. [a] R Squared = .950 (Adjusted R Squared =.927). [b] R Squared = .969 (Adjusted R Squared = .947).

Source	25 ºC			40 ºC		
	df	F	P value	df	F	P value
Solution	2 [a]	74.379	.000	2	11.915	.000
Enzyme	3	28.202	.000	3	74.371	.000
Solution × Enzyme	6	6.993	.000	6	2.523	.041
Error	11			11		

Adding Bio-phy or RMAP and different pH solutions to corn samples caused a significant reduction of phytate content ($p<0.001$).

The results presented in Table 4 indicated that pre-treating corn samples supplemented with REAP and mixed with distilled water (pH=5.5) at 40 ºC, increased phytate content in comparison with control while it had no significant effects with respect to 0.5 and 1% HCl. Adding Bio-phy or RMAP to corn samples and treatment with HCl 0.5 and 1% decreased phytate contents significantly ($p<0.001$) in comparison with control. The best results were obtained with corn samples by adding RMAP (Rovabio Max AP) and pre-treated with HCl 1% at 25 ºC, that phytate content decreased up to 99.5% in comparison with control.

Table 6. The mean of phytate content (± SD[1]) in soybean meal samples treated corn samples with commercial enzymes and different solutions after 3 hours at 25 ºC. [a-d] Means within the same columns with common superscripts have no significant differences (p>0.05). [1] SD = Standard deviation, * = Increasing phytate content, ** = Decreasing phytate content. Bio-phy =Bio-phytase, REAP = Rovabio Excel AP, RMAP = Rovabio Max AP.

Enzyme	Distilled Water	Variation to control (%)	HCL 0.5%	Variation to control (%)	HCL 1%	Variation to control (%)
Without Enzyme	3.55±0.54[a]	0	2.05±0.16[ab]	0	2.23±0.03[a]	0
REAP	2.39±0.02[b]	**- 32.7	2.18±0.03[a]	*+ 6.3	1.95±0.60[a]	- 12.6
Bio-phy	2.32±0.23[b]	- 34.6	1.87±0.11[ab]	- 8.78	1.94±0.12[a]	- 13.0
RMAP	2.18±0.12[b]	- 38.6	1.71±0.08[b]	- 16.6	1.95±0.12[a]	- 12.6

Table 7. The mean of phytate content (± SD[1]) in soybean meal samples treated corn samples with commercial enzymes and different solutions after 3 hours at 40 ºC. [a-d] Means within the same columns with common superscripts have no significant differences (p>0.05). [1] SD=Standard deviation, * = Increasing phytate content, ** = Decreasing phytate content, Bio-phy =Bio-phytase, REAP = Rovabio Excel AP, RMAP = Rovabio Max AP.

Enzyme	Distilled Water	Variation to control (%)	HCL 0.5%	Variation to control (%)	HCL 1%	Variation to control (%)
Without Enzyme	3.46±0.00[a]	0	3.65±0.00[a]	0	3.4±0.04[a]	0
REAP	3.55±0.00[a]	**+ 2.6	3.76±0.00[a]	+ 3.0	3.51±0.18[a]	+ 3.2
Bio-phy	2.92±0.19[b]	*- 15.6	2.39±0.15[b]	- 34.5	2.41±0.01[b]	- 29.1
RMAP	2.83±0.16[b]	- 18.2	2.42±0.13[b]	- 33.7	1.79±0.61[b]	- 47.4

Soybean Meal: At two temperatures (25 and 40 ºC), the effect of solutions and enzymes alone, and also interaction effect of solution with enzyme on phytate contents of soybean meal samples were significant ($p<0.001$, Table 5).

According to the results indicated in Table 6, adding all of the commercial enzymes to SBM samples and mixing with distilled water for 3 h at 25 ºC reduced the content of phytate significantly ($p<0.001$) when compared with control, while no significant differences were observed for two other solutions (0.5 and 1% HCl) ($p>0.05$). A significant difference was observed between REAP and RAMP enzymes to reduce phytate content of soybean meal samples.

At 40 ºC, supplementing soybean meals with REAP and different pH solutions did not affect phytate contents of samples after 3 h ($p>0.05$, Table 7). SBM samples with Bio-Phy or RMAP enzymes and mixing them with different pH solutions decreased phytate content. The highest reduction in phytate content (up to 47.4%) was observed by adding RMAP and treating with HCl 1% solution and at 40 ºC.

Discussion

Chemical compositions of grains worldwide were measured during several studies. The results of those researches indicated some variations in composition of feed ingredients. For example, the amounts of phytate-P in different plants are very different. The reason of these variations could be due to different factors e.g. genetics, kind of plants, environmental conditions of growing plants, process, which are effective on availability of phosphorus in poultry. Similar results had been reported for chemical composition of feed ingredients which were used for preparation of poultry rations in Iran. For example, crude protein, calcium, and non-phytate phosphorus of corn samples could be in the range of 7.5 to 9.0, 0.1 to 0.2, and 0.8 to 1.2 gr/kg, respectively (Tahir, 2012; Liu, 2011; Steiner, 2007; Ravin-

dran, 2006; Godoy, 2005; Kwanyuen, 2005; Lumpkins, 2005).

Corn and soybean meal are the two main ingredients in poultry rations. Phytic acid is generally aggregated in aleurone layer of monocotyledons like wheat, barley, rice, etc. Among the cereals, corn is different and the greatest proportion of phytic acid is found in germ (Tahir, 2012; Godoy, 2005).

Moreover, Zhou et al. (1992) declared that using the levels of Phosphorus as fertilizer is an important factor in determination of phytate-P content in feed ingredients. Significant regression is observed between phytate-P and total P in corn and soybean meal. It has been reported that phytate-P contents in corn samples were approximately 0.034% lower than the value in NRC, while for soybean meal samples they were approximately 0.151% higher (Tahir, 2012; Steiner, 2007; Cowieson, 2005).

Hidvegi et al. (2002) reported that cereals had a lower phytic acid content while its amount was higher in legume; so that maximum contents were 1.42g/100g and 1.75 g/100g, respectively. In addition, interaction between protein and phytic acid depends on pH and aggregation of protein (Rajendran, 1989; Schwenke, 1989), not the amount of phytic acid or this effect is very negligible (Hidvegi, 2002; Rajendran, 1989; Schwenke, 1989).

The results of present study indicated that the effect of solutions (with different pH) and commercial enzymes on the amount of non-phytate phosphorus released from phytate was significantly different at both temperatures (25 and 40 ºC). According to some researches, in vitro pre-treatment of feedstuffs or diets may have beneficial effect on hydrolysis of phytate (Newkirk, 1998) and also other anti-nutritional factors existed in feedstuffs. Esmaeilipour et al. (2013) showed that soaking of broiler diet (in vitro conditions) improves phytate degradation, especially with increasing the time of soaking. Also, the result of present study indicates that pre-treating with HCl solutions had a better positive effect on decreasing the phytate content of corn and SBM sample when compared with distilled water. Other studies also determined the soaking of feed ingredients in citric acid was more effective in comparison with deionized water. Dephytinization or pre-treatment of feedstuffs with phytase could also be a good way, as soaking of feedstuffs prior to feeding might improve digestibility of P compared with dry feeding, because of providing a longer time for contact between phytate and phytase. Indeed, the dephytinization of individual feed ingredients has been shown to increase nutrients availability in chickens. However, the conditions of pre-treatment, like higher temperature and duration of incubation might affect the stability of enzyme (Esmaeilipour, 2012). On the other hand, Denstadli et al. (2006) reported that combination of low temperature and high moisture degraded the highest amount of phytate during incubation while moisture at high temperature had negative effect on enzyme stability.

The results of this study were consistent with the previous researches which showed that the effect of phytase along with soaking of feed ingredients on breaking of phytate phosphorus was dependent on the type of feedstuffs. Soaking of feed ingredients in water increased digestibility of P due to increasing contact time of phytate-P and exogenous phytase (Esmaeilipour, 2012; Blaabjerg, 2010). These findings are completely in agreement with the results of pres-

ent study. According to the results of current experiment, combination of exogenous enzyme with HCl solutions had a better effect on decreasing phytate content of corn in comparison with SBM in the similar condition. Moreover, it has been demonstrated that interaction between phytase and soaking time (in water) had significant effects on breaking the phytae-P in SBM, while phytase with or without xylanase had no significant effect on degrading of phtate-P in wheat (Esmaeilipour, 2012; Blaabjerg, 2010).

In addition, it has been reported that cereals intrinsic phytases were stable at pH=3 - 5.5, while they were more sensitive to pH=8, so were degraded more easily. Increasing temperature reduced stability of intrinsic phytase of cereals. Incubation time of cereals in high temperatures was also a very important factor for stability of intrinsic phytase leading to decrease in the enzyme activity. Therefore, the availability of phosphorus in diets increased (Esmaeilipour, 2012). The results of present study are approved with Esmaeilipour et al. (2012), because the reaction of feed ingredients is generally different at various conditions. It could be due to several reasons including the location of phytate-P and phytase in grain and also their quantities.

Using the exogenous phytase along with the combination of other enzymes may facilitate the release of phytate phosphorus (Lu, 2013; Slominski, 2011). For example, phytate is concentrated in the aleurone layer of wheat (Ravindran, 1995) and xylanase may simplify availability of phytase to substrate in the aleurone. The combinations of phytase and various enzymes may be similarly advantageous for other feed ingredients (Cowieson, 2005). Researchers documented that combination of carbohydrase and phytase could facilitate the access of phytase to its substrate (Juanpere, 2005). In corn, phytic acid is located in the germ but in SBM is found in protein bodies of the seed. Solubility and accessibility of phytate salts and proteins from different feed ingredients and their effects on the extent of protein-phytate complex formation, coupled with variation in effectiveness of phytase in different diets (Selle, 2007). Thus using protease enzymes along with phytase may reduce the variability in phytase efficacy and maximize release of P from phytate.

However, the current research shows that the combination of phytase with NSPase enzyme had a better effect on decreasing the phytate content of corn and SBM but this difference was not significant. Supplementation of poultry diets with NSP-degrading enzymes and phytase might decrease the anti-nutritional effects of NSPs and phytic acid. NSPs can limit the accessibility of phytase to phytate (Kim, 2005). Therefore, NSP enzymes and phytase may act synergistically for improving nutrients utilization in poultry diets. NSP enzymes can hydrolyze NSPs to increase the availability of phytate for phytase. Cowieson et al. (2005) reported that using NSP enzymes like xylanase may hydrolyze the arabinoxylans in cell walls, so the release of encapsulated nutrients and their absorption will be increased (Olukosi, 2007; Cowieson, 2005). In addition, it seems that the lack of significant response to combination of phytase and NSPs enzymes (Xylanase, B-glucanase), might be due to low availability of substrate or inactivate of enzymes. It has been reported that glycosidases break down non-starch polysaccharides present in cell walls, then phytase would be able to access intrinsic phytate in

cell wall. If phytase is used alone, accessibility to phytate will decrease, because it has been surrounded by NSP matrix. Besides, xylanase can hydrolyze the bond of soluble fiber. Then phosphorous and other minerals might be released and absorbed easily by animals (Olukosi, 2007; Coweison, 2005, 2010).

There are several reports that indicate adding the carbohydrases and phytase to ration increases the nutrients bioavailability and also performance of poultry (Adeola, 2010; Woyengo, 2010; Jia, 2008; Mori, 2007). Further, Juanpere et al. (2005) and Cowieson et al. (2010) declared that phytase along with β-glucanases decreased the excretion of calcium in the rations based on corn and barley. In addition, it may better to combine xylanase and glucanase in corn-soy based diets in broiler for maximizing the result of using NSP enzymes.

Dourado et al. (2009) determined that combination of phytase, xylanase, amylase and protease increased apparent metabolizable energy of corn but they were not effective on true metabolizable energy of SBM. Our result also shows that the effect of phytase and also phytase combined with NSPase decrease the phytate content in corn more than SBM.

Pre-treatment of corn in the solutions with different pH (including distilled water, and 0.5 or 1% HCl) at 25 and 40 ºC could enhance the release of phosphorous from phytic acid and increase its bioavailability when supplemented with Bio-phytase (Bio-Phy) or Rovabio Max AP (RMAP).

With respect to SBM samples the highest bio-availability of phosphorous was observed by pre-treatment with HCl 1% solution at 40 ºC and supplementing Rovabio Max AP.

According to the results of this study it could be concluded that pre-treating of feed ingredients (corn and SBM) of poultry diets using various methods such as using some commercial enzymatic products (including phytase) and solutions with different pH were effective to decrease the remaining phytate content in feed ingredients. It means that bioavailability of phosphorous increased.

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References

Adeola, O., Jendza, J. A., Southern, L. L., Powell, S., Owusu-Asiedu, A. (2010) Contribution of exogenous dietary carbohydrases to the metabolizable energy value of corn distillers grains for broiler chickens. J Poult Sci. 89: 1947-1954.

Beslin Joshi, J. (2014) Phytase- A key to unlock phytate complex. Int J Pure Appl Biosci. 2: 304-313.

Blaabjerg, K., Carlsson, N.G., Hansen- Moller, J., Poulsen, H.D. (2010) Effect of heat-treatment, phytase, xylanase and soaking time on inositol phosphate degradation in vitro in Wheat, soybean meal and rapeseed cake. J Anim Feed Sci Technol. 162: 123-134.

Common, R.H. (1940) The phytic acid content of some poultry feeding stuffs. Analyst. 65: 79-83.

Cowieson, A.J., Bedford, M.R., Ravindran, V. (2010) Interactions between xylanase and glucanase in maize-soy-based diets for broilers. Br J Poult Sci. 51: 246-257.

Cowieson, A.J. (2005) Factors that affect the nutritional value of maize for broilers. J Anim

Feed Sci Technol. 119: 293- 305.

Cowieson, A.J., Adeola, O. (2005) Carbohydrase, Protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. J Poult Sci. 84: 1860-1867.

Denstadli, V., Vestre, R., Svihus, B., Skrede, A., Storebakken, T. (2006) Phytate degradation in a mixture of ground wheat and ground defatted soybeans during feed processing: effects of temperature, moisture level, and retention time in small- and medium-scale incubation systems. J Agric Food Chem. 54: 5887–5893.

Dersjant-Li, Y., Awati, A., Schulze, H., Patridge, G. (2015) Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. J Sci Food Agric. 95: 878-896.

Dourado, L.R.B., Sakomura, N.K., Barbosa, N. A.A., Bonato, M.A., Kawuauchi, I.M., Fernandes, J.B.K., Costa, F.G.P. (2009) Corn and soybean meal metabolizable energy with the addition of Exogenous Enzymes for poultry. Braz J Poult Sci. 11: 51- 55.

Esmaeilipour, O., Van Krimpen, M. M., Jongbloed, A. W., De Jonge, L. H., Bikker, P. (2013) The effects of temperature, moisture, duration of incubation time, calcium level, and soaking with water or citric acid on in vitro phytate degradation in a wheat–barley–rye–soybean meal-based diet. J Anim Feed Sci Technol. 183: 168-174.

Esmaeilipour, O., Van Krimpen, M. M., Jongbloed, A. W., De Jonge, L. H., Bikker, P. (2012) Effects of temperature, pH, incubation time and pepsin concentration on the in vitro stability of intrinsic phytase of wheat, barley and rye. Anim Feed Sci Technol. 175: 168-174.

Godoy, S., Chicco, C., Meschy, F., Requena, F. (2005) Phytic phosphorus and phytase activity of animal feed ingredients. Interciencia 30: 24-28.

Haug, W., Lantzsch, H. J. (1983) Sensitive Method for the Rapid Determination of Phytate in Cereals and Cereal Products. J Sci Food Agric. 34: 1423-1426.

Hidvegi, M., Radomir, L. (2002) Phytic Acid content of Cereal and Legumes and Interaction with Proteins. Period J Polytech Chem Eng. 46: 59-64.

Jia, W., Slominski, B.A., Guenter, W., Humphreys, A., Jones, O. (2008) The effect of enzyme supplementation on egg production parameters and omega-3 fatty acid deposition in laying hens fed flaxseed and canola seed. J Poult Sci. 87: 2005-2014.

Juanpere, J., Perez-Vendrell, A.M., Angulo, E., Brufau, J. (2005) Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. J Poult Sci. 84: 571-580.

Kim, J.C., Simmins, P. H., Mullen, B. P., Pluske, J. R. (2005) Effect of wheat phosphorus content and supplemental enzymes on digestibility growth performance of weaner pigs. J Anim Feed Sci Technol. 118: 139-152.

Kishor Gupta, R., Singh Gangoliya, S., Kumar Singh, N. (2015) Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J Food Sci Technol. 52: 676-684.

Kwanyuen, P., Burton, J. W., (2005) A simple and rapid procedure for phytate determination in soybeans and soy products. J Am Oil Chem Soc. 82: 81-85.

Lamid, M., Tri Puspaningsih, N.N., Asmarani, O. (2014) Potential of phytase enzymes as Biocatalysts for improved nutritional value of rice bran for broiler feed. J Appl Environ Biol Sci. 4: 377-380.

Liu, K., Han, J. (2011) Changes in mineral concentrations and phosphorus profile during

dry-grind processing of corn into ethanol. J Bioresour Technol. 102: 3110–3118.

Lu, H., Adedokun, S.A., Preynat, A., Legrand-Defretin, V., Geraert, P.A., Adeola, O., Ajuwon, K.M. (2013) Impact of exogenous carbohydrases and phytase on growth performance and nutrient digestibility in broilers. Can J Anim Sci. 93: 243-249.

Lumpkins, B.S, Batal, A.B. (2005) The bioavailability of lysine and phosphorus in distillers dried grains with soluble. Poult Sci. 84: 581-586.

Newkirk, R. W., Classen, H. L. (1998) In vitro hydrolysis of phytate in canola meal with purified and crude sources of phytase. J Anim Feed Sci Technol. 72: 315-327.

Olukosi, O. A., Cowieson, A. J., Adeola, O. (2007) Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broiler. J Poult Sci. 86: 77-86.

Rajendran, S., Prakesh, V. (1989) Interaction of phytic acid with Alfa-Globulin from sesame seed (*Sesamus indicus* L.). In: Abhandlungen der Akademie der Wissenschaften der DDR. Schwenke, K.D., Raab, B. (eds.). Abteilung Mathematik, Naturwissenschaften, Technik Akademie Verlag Berlin. p. 247-251.

Ravindran, V, Bryden, W. L., Kornegay, E. T. (1995) Phytates: occurrence, bioavailability and implications in poultry nutrition. J Avian Poult Biol Rev. 6: 125-143.

Ravindran, V., Cabahug, S., Ravindran, G., Bryden, W. L. (1999) Influence of microbial phytase on apparent ileal amino acid digestibility in feedstuffs for broilers. J Poult Sci. 78: 699–706.

Ravindran, V., Morel, P. C., Partridge, G. G., Hruby, M. Sands, J. S. (2006) Influence of an *Escherichia coli*-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. J Poult Sci. 85: 82–89.

Schwenke, K. D., Mothes, R., Gast, K., Zirwer, D. (1989) Rapeseed protein- protein Interactions. In: Schwenke, K.D., Raab, B. (eds.). Abhandlungen der Akademie der Wissenschaften der DDR, Abteilung Mathematik, Naturwissenschaften, Technik Akademie Verlag Berlin. p. 105-114.

Selle, P. H., Ravindran,V. (2007) Microbial phytase in poultry nutrition. J Anim Feed Sci Technol. 135: 1–41.

Selle, P. H., Ravindran, V., Caldwell, A., Bryden, W. L. (2000) Phytate and phytase: consequences for protein utilization. Nutr Res Rev. 13: 255-278.

Slominski, B.A. (2011) Recent advances in enzymes for poultry diets. J Poult Sci. 90: 2013-2023.

Steiner, T., Mosenthin, R., Zimmermannb, B., Greiner, R., Roth, S. (2007) Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. J Anim Feed Sci Technol. 133: 320-324.

Tahir, M., Shim, M. Y., Ward, N. E., Pesti, G. M. (2012) NIRS calibrations for total and phytate P in poultry feed ingredients. In proceedings: International Poultry Scientific Forum, Georgia World Congress Center, Atlanta, USA.

Tahir, M., Shim, M.Y., Ward, N.E., Smith, C., Foster, E., Guney A.C., Pesti, G.M. (2012) Phytate and other nutrient components of feed ingredients for poultry. J Poult Sci. 91: 928-935.

Viveros, A., Centeno, C., Brenes, A., Canales, A., Lozano, A. (2000) Phytase and acid phosphatase activities in plant feedstuffs. J Agric Food Chem. 48: 4009-4013.

Woyengo, T.A., Nyachoti C.M. (2013) Review: Anti-nutritional effects of phytic acid in di-

ets for pigs and poultry-current knowledge and directions for future research. Can J Anim Sci. 93: 9-12.

Woyengo, T.A., Slominski, B.A., Jones, R.O. (2010) Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. J Poult Sci. 89: 2221- 2229.

Zhou, J.R., Fordyce, E.J., Raboy, V., Dickinson, D.B., Wong, M.S., Burns, R.A., Erdman, J.W. Jr. (1992) Reduction of phytic acid in soybean products improves zinc bioavailability in rats. Nutrition. 122: 2466-2473.

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تأثیر پیش فرآوری آنزیمی بر محتوای فیتات در ذرت یا کنجاله سویا تحت شرایط آزمایشگاهی

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چکیده

زمینه مطالعه: فسفر یکی از گرانترین مواد مغذی موجود در تغذیه طیور می‌باشد. بنابراین، افزایش زیستفراهمی این ماده مغذی می‌تواند در کاهش هزینه خوراک موثر باشد. **هدف:** این مطالعه جهت ارزیابی تأثیر پیشفرآوری اجزاء خوراک تحت تأثیر مقادیر مختلف pH و آنزیم‌های تجاری بر میزان فیتات در شـرایط آزمایشـگاهی است. **روش کار:** سه محلول (آب مقطر، اسید هیدرو کلریدریک ۰/۵ ٪ و ۱٪ (بـه ترتیـب دارای pH مسـاوی ۵/۵، ۲/۱۲ و ۱/۸۸)، سـه آنزیم تجاری (فاقد آنزیـم، Bio-، Rovabio Excel AP، Rovabio Max AP، phytase) برای تعیین میزان فیتات در ذرت و کنجاله سویا استفاده شد. ابتدا هر یک از نمونه‌ها با فراورده‌های آنزیمی مخلوط و بعد از افزودن محلول‌های فوق، برای مدت ۳ ساعت و در دو دمای ۲۵ °C و ۴۰ پیشفرآوری شدند و در نهایت میزان فیتات در نمونه‌های مواد غذایی اندازهگیری شد. **نتایج:** نتایج نشان دادند که پیشفرآوری ذرت از طریق افزودن آنزیم Bio-phytase یا Rovabio Max AP و محلول‌های دارای سطوح مختلف pH (در دمای ۲۵ °C و ۴۰ به مدت ۳ ساعت) مقدار فیتات را به طور معنی‌داری کاهش داد. این امر به معنی افزایش آزادسازی فسفر از فسفر فیتاته و بهبود قابلیت زیست فراهمی آن می‌باشد. بهترین نتیجه با افزودن Rovabio Max AP در دمای ۲۵ °C و محلول اسید هیدروکلریدریک ۱٪ به نمونه‌های ذرت بدست آمد، به‌طوریکه در مقایسه با گروه شاهد میزان فیتات را تا حدود ۹۹/۵ ٪ کاهش داد. در ارتباط با نمونه‌های کنجاله سویا نیز نتایج تقریباً مشابهی بدست آمده، به‌طوریکه بیشترین میزان کاهش فیتات (تا ۴۷/۴ ٪) در نتیجه افزودن Rovabio Max AP و محلول اسید هیدروکلریدریک ۱٪ بـه نمونه‌هـا در دمای ۴۰ °C حاصل گردید. **نتیجه‌گیری نهایـی:** از نتایج حاصل می‌توان نتیجه گرفت که پیش فرآوری ذرت و کنجاله سویا با روش‌های مختلف مانند استفاده از آنزیم‌های تجاری حاوی فیتاز و محلول‌هایی با سطوح مختلف pH برای افزایش زیستفراهمی فسفر سودمند می‌باشد.

واژه های کلیدی: Bio-phytase، فیتات، پیش فرآوری، Rovabio Excel AP، Rovabio Max AP

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