ASIAN JOURNAL OF AGRICULTURE
Volume 6, Number 2, December 2022
Pages: 55-60
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g060201

Effect of phytase and citric acid supplementation in the feed quality of laying hen

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Manuscript received: 9 December 2021. Revision accepted: 1 July 2022.

Abstract. *Nuningtyas YF, Huda AN, Marjuki, Ulfah SN. 2022. Effect of phytase and citric acid supplementation in the feed quality of laying hen. Asian J Agric 6: 55-60.* This study aims to determine and evaluate the combination of phytase and high dosage of citric acid on the feed quality based on seeds. This study used data experimental at Brawijaya University, Malang, Indonesia, with five treatments and four replications. This research analyzes the nutritional feed content in vitro digestibility and phosphorus content in concentrate. The result of the study was a basal feed of laying hens followed by a dry matter of 90.63%, organic matter of 81.92%, crude protein of 16.44%, crude fiber of 4.34%, and fat of 7.49%. The basal laying hen feed without adding phytase enzyme and citric acid had a relatively low phosphorus content of 0.43%. It would be limited due to the absence of phytic acid breakdown assistance from the phytase enzyme. The nutrient phosphorus content in the residual feed digestibility is sequent T0 2.2431%, T1 2.7809%, T2 1.6225%, T3 2.0717%, and T4 2.7199%, with T4 for the higher value and the lowest in T2. The addition of the phytase enzyme and citric acid to animal feed for laying hens with various levels has a significant difference ($P<0.01$) when viewed at the end of the digestibility of phosphor. However, the T2 laying hens feed with the addition of 2% phytase enzyme + 2% citric acid had the lowest phosphorus content in residue feed digestibility in vitro. Therefore, further research should be carried out in-vivo testing, especially on laying hens, to determine the effectiveness of the added enzymes by using the in-vivo test and the amount of phosphorus.

Keywords: Citric acid, feed quality, laying hen, phytase enzyme

INTRODUCTION

Poultry feed is usually sufficient from feed ingredients in the form of grains because it contains many essential amino acids that are needed by poultry for growth. Essential amino acids in the form of lysine and sulfur-containing amino acids such as methionine and cysteine must be given in sufficient proportions to maximize poultry performance (Andri et al. 2020). One of the essential amino acids that layer hen needs are a substance in the form of phosphor. Phosphor is important in poultry's metabolic process and growth, with a fairly high economic value as a source of energy and amino acids. Phosphor (P), as much as 70% contained in raw materials, is stored in the form of Phytate. According to Vieira et al. (2016) and El-Hack et al. (2018), as much as two-thirds of phosphorus is found in grains in Phytate. Furthermore, Phytate, the main form of phosphorus in plants, has chelating properties (Kühn et al. 2016). Therefore, Phytate can be categorized as an antinutrient component (Pramita et al. 2008; Samtiya et al. 2020) in terms of binding proteins and ions of several minerals such as calcium, iron, zinc, magnesium, manganese, and copper.

Phytic acid (known as inositol hexakisphosphate (IP6), or Phytate in a salt form, is the main phosphorus in various plant tissues, especially grains and legumes (Kumar et al. 2010). Monogastric livestock such as poultry have limitations in digesting Phytate due to the lack of endogenous phytase enzymes, so they cannot hydrolyze phytase in digestion (Lamid et al. 2018). Phytases are chemically known as Myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolase, which could catalyze the release of phosphate from Phytate and liberate proteins, and other bound minerals (Kumar et al. 2010; Bhavsar et al. 2012). The principle of phytase for the utilization of nutrients is to increase the absorption of nutrients by breaking the bonds of phytate compounds so that hydrolyzed minerals and proteins can be utilized optimally in subsequent metabolic and biosynthetic processes. In addition to phytase utilization, citric acid has also been shown to reduce phytic acid in plants. Research conducted by (Rodriguez et al. 2015) with the addition of phytase enzymes and citric acid in feed can reduce phytic acid (or Phytate, the salt form of phytic acid), which is characterized by increased digestibility and reduced excretion of the P and N.

The microbial phytase in Myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolase in poultry positively affects growth performance, feed efficiency, protein digestibility, and good absorption of minerals, such as calcium and phosphorus (Lamid et al. 2018). Commercial use of phytase microbes has been used since the 1990s for poultry and pigs. Monogastric livestock's lack of ability to digest Phytate negatively impacts livestock growth and the economic side of the livestock business (Dersjant-Li and Dusel 2019). In addition, Phytate easily binds to proteins and minerals (especially calcium, Ca) in the stomach and intestinal pH (Rokhmah et al. 2009; Selle et al. 2012), forming binary (phytate protein) or ternary complexes (phytate-mineral-protein), which will further affect the lack of availability of phytate-protein. As a result, p, minerals, protein, and other nutrients that livestock should absorb for growth will be wasted through feces.

Phytase activity is expressed as a phytase unit (FTU); one phytase unit is defined as the amount of enzyme that liberates one micromole of inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at 37°C and pH 5.50 under test conditions (AOAC 2012). Studies have shown that a phytase dose of 500 FTU/kg of feed typically achieves 40 to 60% of phytate degradation in the late small intestine, compared to 10 to 40% in no dietary supplementation (Dersjant-Li et al. 2015). Growth performance increased with the addition of 500 or 1000 FTU, which could replace 1.9 g P of monocalcium phosphate. Phytate has been degraded in the digestive tract by adding microbial phytase, which can be seen from the digestibility of phosphorus, namely 32% and 49.4% in a low P diet without and with 500 FTU kg^{-1} (Dersjant-Li et al. 2015). Generally, phytase microbes can increase growth performance, feed efficacy, and digestibility of monogastric animal feed.

The purpose of this study was to determine the effect of increasing the dose of phytase (%) and citric acid (%) to reduce the phytic acid content, reduce economic costs, improve growth performance, and improve the quality of animal feed in laying hens fed with a basal diet of wheat, maize, rice bran, fish meal, and MBM without added inorganic P, compared to a basal diet.

MATERIALS AND METHODS

Study area

This research was conducted in the Faculty of Animal Science, Universitas Brawijaya, Malang, East Java, Indonesia, and BPTP Karangploso Laboratory, Malang, East Java, Indonesia. The analysis of the nutritional content of feed and in vitro digestibility experiment was carried out at the Laboratory of Nutrition and Animal Feed, Faculty of Animal Science Universitas Brawijaya, while the phosphorus test on the concentrated sample was carried out in BPTP Karangploso Laboratory, Malang, East Java, Indonesia.

Procedures

Treatments-1

In treatment I in this study, five treatments was used, containing four replications. The use of basal feed in this study consisted of several ingredients: wheat, corn, bran, fish meal, and MBM. The treatment of laying hen's feed was based on the addition of doses of the phytase enzyme and citric acid levels, which were described as follows: (i) T0: basal diet without the addition of phytase enzyme and citric acid. (ii) T1: basal diet with the addition of 1% phytase enzyme + 1% citric acid. (iii) T2: basal diet with the addition of 2% phytase enzyme + 2% citric acid. (iv) T3: basal diet with the addition of 3% phytase enzyme + 3 % citric acid. (v) T4: basal diet with the addition of 4% phytase enzyme + 4% citric acid.

How to use a spectrophotometer for a phosphorus test-2

In a study by Xu et al. (2020), phosphorus contents were obtained using a spectrophotometer (UV-752 N, China). Next, phosphorus content was measured by a spectrophotometric method using a molybdate vanadate reagent (Wiyantoko et al. 2018). Finally, the phosphorus contents were obtained using a spectrophotometer (Genesys 10 UV, USA) on yellow molybdate reagent-vanadate. For the first procedure, prepare the sample until ready in a 10 mL volumetric flask. Then added, molybdate reagent-vanadate and aquadest to 10 mL (line of the flask) for color formation. Next, measured the absorbance was using a spectrophotometer (Genesys 10 UV, USA) with a length of 400 nm wave. The absorbance value is then related using linear regression with a standard solution.

Data analysis

The obtained data were tabulated using Microsoft Excel Program, then subjected to analysis of variance (ANOVA) from a Completely Randomized Design (CRD). Finally, it was continued with Duncan's Multiple Distance Test to see if there was a treatment effect.

RESULTS AND DISCUSSION

Proximate analysis of the basal diet of laying hens-1

Table 1 shows the result of the proximate analysis of the basal diet used in this study, namely the laying hens used. Based on research on the combination of citric acid and phytase enzyme on diet quality, the good quality of animal feed can be judged by its high digestibility value. The basal diet of laying hens presented in Table 1 shows the value of dry matter at 90.63%, organic matter at 81.92%, crude protein at 16.44%, crude fiber at 4.34%, and fat at 7.49%. The nutrient content of basal feed has value according to the nutritional standards of laying hens. However, without the addition of citric acid and phytase enzymes, the nutrient content cannot be digested properly by laying hen, especially protein and minerals, resulting in reduced nutrient digestibility.

Table 1. Proximate analysis of diets of laying hens

Treatments	DM (%)	OM* (%)	CP* (%)	CF* (%)	EE* (%)
T0	90.63	81.92	16.44	4.34	7.46
T1	90.42	81.87	16.47	4.25	7.35
T2	90.38	81.76	16.59	4.21	7.24
T3	90.34	80.72	16.63	4.19	7.19
T4	90.27	80.65	16.72	4.17	7.14

Note: Results of proximate analysis at the Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Universitas Brawijaya, Malang, East Java, Indonesia 2022; *: Based on 100% dry matter

Phosphorus content of basal feed of laying hens-2

The results showed that the basal laying hen feed without the addition of phytase enzyme and citric acid had a relatively low phosphorus content of 0.43% and would be limited due to the absence of phytic acid breakdown assistance from the phytase enzyme (Table 2).

Phosphorus content in residual feed digestibility-3

Figure 1 is a standard absorbance curve obtained in testing the phosphorus content using a spectrophotometer. The sample measurement principle is to convert all metaphosphate and pyrophosphate with nitric acid into orthophosphate using a wavelength of 400 nm. Those treatments will react with these reagents and form a yellow vanadic acid-molybdic acid complex. The color intensity of the complex compound can be measured with a spectrophotometer at a wavelength of 400 nm and compared with a standard phosphor with a known concentration (Table 3).

The research results evaluating the phosphorus content in the remaining digestibility of laying hens showed that the highest phosphorus content was T1 with a level of 2.780 + 0.13 % and the lowest value with a phosphorus content of 1.622 + 0.18%. The lower levels of phosphorus in the digestibility residue indicate that livestock can digest phosphorus well due to the help of phytase enzymes, so in this study, T2 with phytase enzyme levels of 2% + 2% citric acid was the feed treatment with the best results in laying hens (Table 4).

Table 2. A test result of phosphorus content of basal feed of laying hens

Feed	Water content (%)	Phosphorus content (%)
Laying hen	7.02	0.43

Note: Test results at the Soil Laboratory, BPTP, Indonesia

Table 1. The absorbance of the feed samples of laying hens

Treatments	Absorbance (nm)
T0	0.322 + 0.22
T1	0.328 + 0.24
T2	0.313 + 0.05
T3	0.370 + 0.13
T4	0.452 + 0.14

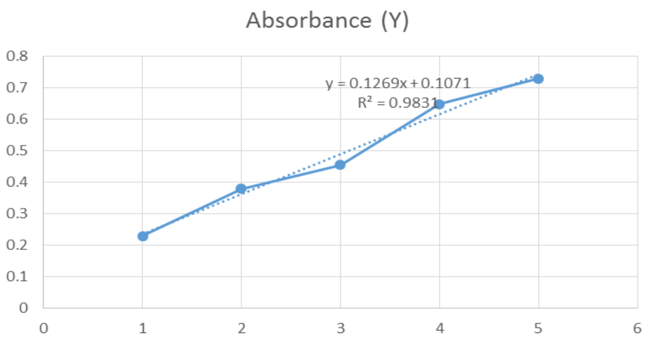

Figure 1. Standard absorbance curve

Table 4. Nutrient phosphorus content in the residual feed digestibility

Laying hen feed digestibility	
Treatments	**Phosphorus content (%)**
T0	2.243 + 0.12[bc]
T1	2.780 + 0.13[c]
T2	1.622 + 0.18[a]
T3	2.071 + 0.21[b]
T4	2.719 + 0.17[c]

Note: a-c shows a very significant difference in each treatment (P<0,01)

Discussion

Proximate analysis of laying hen feed ingredients

The proximate analysis of laying hen feed ingredients that have been formulated by adding various levels of phytase is tested for in vitro digestibility. Furthermore, the results of the residual feed digestibility of phosphorus content were tested using a quantitative spectrophotometer. Using phytases and citric acid in non-ruminant feeds is becoming increasingly relevant in conserving resources and minimizing the environmental impact of phosphorus (P) excretion. The feed's nutrient content must be of good quality to maximize productivity. Phytases are widely distributed in plants, microorganisms, and animal tissues used in animal feed to improve the nutritional value and reduce phosphorus pollution from animal waste (Cano et al. 2020). Microbial phytases are most promising for biotechnological applications to support feed digestibility (Jain et al. 2016). Intrinsic plant phytase activity varies widely among plant species and ranges from negligible in corn and soybean to very high in wheat and rye (Sommerfeld et al. 2020). Mucosal phytase activity is described for pigs and poultry (Selle and Ravindran 2007; Huber et al. 2015), but the data for ruminants are lacking (Haese 2017). The few studies describing InsP6 hydrolysis in the small intestine of ruminants, however, indicate that mucosal phytate activity seems to be physiologically irrelevant in ruminants (Humer et al. 2015).

Adding phytase enzymes and citric acid to basal feed can increase feed digestibility by meeting the nutrient requirements of laying hens. In addition to good feed digestibility, phosphorus digestibility is also very good, supporting the maximum digestibility of other nutrients is evidenced by the fulfillment of nutrient standards for laying hens in the treatment feed. Based on (SNI-01-3929-2006), the nutrient standards for layer hens are (min) CP 16%, (max) CF 7%, and (max) EE 7%. This study showed that the nutrient value of the treatment feed was by the standard nutrient requirements of laying hens. Table 1 shows the CP ranged from 16.47-16.72%, with the highest value at T4. The CF value is from 4.17-4.34%, with the lowest value at T4. And the EE value ranged from 7.14-7.46%, with the lowest score on T4. That shows that the increasing level of addition of phytase enzyme and citric acid will further affect the content of nutrients feed.

In addition to mineral substances in the form of phosphorus, could be digested properly due to phytase enzymes, there is also an increase in the digestibility of calcium (Ca) minerals. Harper et al. (1997) reported a 30%

improvement in P digestibility with grain sorghum-soybean meal based-diets supplemented with 500 FTU/kg dietary phytases. This report also indicated a 20% improvement in Ca digestibility but no response in Ca or DM digestibility due to phytase. Among the studies conducted, responses for the Ca and other nutrient digestibilities have been less consistent and small in magnitude than improvements in P digestibility.

In the present study, the nutrient content in basal feed showed the value of P according to the nutritional standards of laying hens. However, the nutrient content cannot be digested properly without adding phytase, especially crude protein and phosphor. Phytic acid is described chemically as myo-inositol-1,2,3,4,5,6-hexakisphosphate. Phytic acid is an anti-nutritional substance contained in protein bonds, so that this protein will affect the digestibility of phytic acid (Dersjant-Li et al. 2015). The formation of insoluble phytate-mineral or phytate-protein compounds can cause a decrease in the availability of minerals and the nutritional value of proteins (Lamid et al. 2018). Therefore, the presence of phytase enzymes is very good for helping maximize the digestion of laying hens in digesting nutrients in the form of minerals. That is consistent with research showing that the use of phytase and citric acid can have a positive effect on phosphorus digestibility in laying hens.

Evaluation of phosphorus content of laying hen basal feed

Phytase use with the addition of citric acid is two complementary substances that play a significant role in digestion. These two substances are needed to maximize mineral and nutrient digestion, consequently supporting productivity. The role of phosphorus to be digested optimally benefits livestock in production and from an economic point of view because this can benefit farmers by saving high-priced feed ingredients. Usually, feed ingredients with high nutritional content can be obtained at a higher price than feed ingredients with standard nutrition, which is very beneficial in cutting the cost of animal feed. Phytate can bind to protein due to its rickets, which form insoluble salts. Although salts that are difficult to dissolve are formed because of low, neutral, or high pH, phytic acid is negatively charged, so it will form bonds with proteins that have positive groups. That is reinforced by Nisa and Yuanita (2014), that citric acid can form complexes with metals bound to Phytate, so soaking corn with citric acid will reduce the amount of Phytate. In addition to using citric acid, phytate levels can also be reduced by phytase.

The degradation of Phytate by phytase occurs through a hydrolysis reaction. Hydrolysis of Phytate by phytase occurs at C3 or C6 on the Myo-inositol hexakisphosphate form to the simpler; namely, D-inositol (1,2,4,5,6) P5 becomes inositol (2,4,5,6), T4 becomes inositol (2,4,6), T3 or inositol (2,4,5) T3 or inositol (1,2,6) T3 and finally become inositol-2-P (Greiner et al. 2002). Therefore nutrients bound to phytic acid are hydrolyzed and released from their bonds. Through the enzymatic technique, it is possible to completely degrade the Phytate of cereals and legumes. That is supported by Rodriguez et al. (2015) research results; namely, the best treatment for reducing phytate levels was obtained by adding citric acid and phytase enzymes to hen's diets that, improve the digestibility and significantly reduce the excretion of P. That is to the results of the research in the form of residual phosphorus of feed digestibility value, with the best treatment being T2 1.622 + 0.18% compared to T0 2.243 + 0.12%, which has higher residual phosphorus. The lower the residual phosphorus is better because it shows that more phosphorus is being digested. A basal feed with 2% phytase enzyme and 2% citric acid (T2) compared to basal feed without the addition of phytase enzyme and citric acid (T0) was better because, with the addition of phytase enzyme and citric acid, nutrient phosphorus was digested better.

Phosphorus is one of the essential nutrients for livestock survival and optimum production. However, in livestock, there are limitations to digesting phosphorus, so it requires exogenous enzyme supplementation to help digest phosphorus. The phytase enzyme used for feed additives breaks down phytic acid in animal feed and liberates inorganic phosphorus and inositol. In addition, the phytase enzyme will work to free amino acids, proteins, minerals, and other materials attached to phytic acid. As a result, phosphorus and other nutrients such as trace minerals and protein become widely available and can be used to maintain health and promote the growth of the hen body. The addition of the phytase can also reduce and even eliminate the addition of inorganic phosphorus, which is expensive, resulting in savings in production costs (Pires et al. 2019).

Żyla (1993) proposed that acid phosphatase with optimum pH of 2.5 acts independently of phytase. The preponderance of the evidence shows the benefit of using phytase in non-ruminant animal feeding. The benefits of phytase are most manifest when feeding diets that are lower in P than required, thus making it possible to use less P in diets while meeting the P needs of the animals (Olukosi 2012). Increased understanding of the genetic makeup of the different microorganisms producing phytases has made it possible to produce newer generation phytases that can release more P per unit of enzyme supplementation and cope with high temperatures associated with some types of animal feed processing.

Evaluation of phosphorus content in residual digestibility of laying hens

Phosphorus is one of the mineral substances needed by poultry, especially laying hens, to support productivity in the form of eggs and bones (Alagawany et al. 2021). However, livestock has limitations in digesting minerals due to the presence of phytic acid in animal feed derived from plants, especially grains, because birds lack endogenous phytase (El-Hack et al. 2018). Phytic acid can interact with endogenous enzymes by rendering phytate-bound protein refractory to pepsin digestion, resulting in reduced nutrient digestibility (Dersjant-Li et al. 2015). Generally, phytic acid will be digested on the digestibility of the animal, and the rest will be disposed of in the form of residue. However, poultry productivity can be increased through the consumption of well-digested feed; therefore, it is very important to pay attention to the content of poultry feed ingredients in the form of phosphorus, especially in laying hens. Using exogenous phytase enzymes can help the digestion in poultry by degrading phytic acid into positive substances that can be digested by livestock to maintain livestock productivity.

Long et al. (2017) suggested that providing atypically high doses of phytase (up to 7,600 FTU/kg) in diets for broilers may result in the release of 94.4% of Phytate bound P. This concept is known as "super dosing" in swine and poultry diets. The added benefits observed were a 131% increase in weight gain and a 59% increase in bone ash in laying hens super dosed with phytase compared with laying hens fed no phytase. In poultry diets, phytase is sometimes included at levels up to 2,500 FTU/kg diet DM (Simons and Versteegh 1990). One FTU is defined as the amount of phytase that liberates 1 μmol of inorganic phosphate per minute from 0.0051 M Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al. 1994).

The rumen microorganisms, such as *Actinobacillus* sp. and *Bacillus pumilus* in ruminants, are considered capable of naturally producing phytase as a feed additive to produce good quality with high protein and mineral (P, Mg, Mn, Fe, Zn, and Ca) availability (Lamid et al. 2014). In research, using phytase enzymes in non-ruminant animal feed resulted in more phosphorus absorption than many ruminants. Several factors, such as optimum pH for phytase activity, influence the difference in the effectiveness of the use of phytase enzymes. Dersjant-Li et al. (2015) stated that the optimum pH range for several phytase enzymes is 2.0 to 5.00. Therefore, the lack of phosphorus absorption in ruminants is indicated as a result of the rumen pH, which tends to be neutral, namely 6.7 to 6.9, and causes the ineffective performance of the phytase.

Table 4 shows the results of the percentage value of residual phosphorus feed digestibility. As a result, there was a significant difference in residual phosphorus content (P <0.01), with the lowest post-digestion phosphorus levels, were found in the addition of laying hens concentrate + 2% phytase enzyme + 2% citric acid (T2) that has 1.622 + 0.18 % of residual feed digestibility of phosphor. Therefore, the low phosphorus content in the digestibility residue indicates that phosphorus can be properly disassembled and absorbed in the digestion of non-ruminant livestock.

ACKNOWLEDGEMENTS

We acknowledge the support from the research team of a novice research grant from the Faculty of Animal Science, Universitas Brawijaya, Malang, East Java, Indonesia that given research grant.

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