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**Submission date:** 11-Mar-2020 03:01PM (UTC+0700)

**Submission ID:** 1273531417

**File name:** The\_Physical\_and\_Chemical\_Characteristics\_of\_Eggshell.pdf (76.53K)

**Word count:** 3746

**Character count:** 18677

**LAYING HENS**

*by* Sri Kismiyati

## 9. THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF EGG SHELL WASTE AS PHOSPHORUS FORTIFICATION: ITS EFFECT ON EGG PRODUCTION AND EGG SHELL QUALITY OF LAYING HENS

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### ABSTRACT

Two experiments were conducted to investigate the physical and chemical characteristic of phosphorus fortified eggshell waste by phosphoric acid and its effect on egg production and eggshell quality of laying hens. Experiment 1, eggshell waste was washed in water temperature of 80° for 15 minutes and then divided into 4 group. Group 1, eggshell waste without phosphoric acid (control) ; group 2, eggshell waste is soaked in phosphoric acid 3% for 15 minutes; group 3 : eggshell waste is soaked in phosphoric acid 4% for 15 minutes and group 4 eggshell waste is soaked in phosphoric acid 5% for 15 minutes. The total of bacteria, the phosphorus content and breaking strength were measured to evaluate the physical and chemical character of egg shell waste. Experiment 2 is the application of the result of experiment 1 in hens feed. Forty-eight laying hens (Isa Brown strain) at 25 weeks of age were used in this study. Completely Randomized Design used in this experiment. The results of this experiment showed that the increase concentration of phosphoric acid decreases the total of bacteria, increases the phosphorus content and decreases eggshell waste breaking strength. The different concentration of phosphoric acid had not significantly effect on egg production (feed intake, calcium intake, phosphorus intake, egg weight) and egg shell quality (eggshell weight, eggshell percentage, the calcium content).

Key words: egg shell waste, bacteria, phosphorus fortification, egg production and eggshell quality.

### INTRODUCTION

The low rate of egg production and the eggshell quality accounts for highly economic losses of the egg producer. The factors influencing egg production and eggshell quality are the genetic, feed nutrition, and disease factor. N R C (1994), Squires (2003) and Lesson and Summers (2005) stated that feed is the very determining factor on egg production and eggshell quality. Egg formation need high calcium (Ca) and phosphorus (P). The requirement of calcium of hens at the laying period is 3.5%; while the requirement of phosphorus is 0.45%. Deficiency of Ca and P may decrease egg production and eggshell quality. The calcium level of feed 3% were optimum for maintaining the optimum eggshell quality in quails (Plomina and Pillia Ramakrishna, 2000). The experiment of Safaa *et al.* (2008) showed that an increase in Ca intake from 4.08 to 6.4 g/hen per day improved egg production (71.2 vs. 74.9%), egg mass (49.0 vs. 51.4 g), and feed conversion ratio (2.43 vs. 2.30 kg of feed/kg of egg). In addition, an increase in Ca intake improved shell weight (9.98 vs. 10.20%), shell thickness (0.342 vs. 0.351 mm). The study of Pelicia *et al.* (2009) showed the Ca level in feed significantly effected on egg production. The increase of Ca in feed as much as 3 – 4.5% improve the eggshell quality. Then, the research of Pelicia *et al.* (2011) showed that the calcium level of the feed as much as 4.5% produce lower egg production than 3.0 and 3.75%. Egg production at the level Ca of the feed 3.0; 3.75 and 4.5%

respectively 91.3; 90.5 and 87.6%. The requirement of phosphorus is lower than calcium; however, phosphorus has a very high price

Eggshell waste contains high calcium and little phosphorus and protein but contributes to environmental pollution. The phosphorus of eggshell waste is an inorganic phosphorus and having a high availability. Said (1996) stated that eggshell contains 37.0 – 37.4% Ca; 0.12 – 0.13% P; and 5.2 – 5.9% protein. According to Ogawa *et al.* (2004) eggshell contains 94.4% CaCO<sub>3</sub>; 0.73% Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>; 0.84% MgCO<sub>3</sub>; and 3.3% protein. Nakano *et al.* (2003) explained that chicken eggshell contained many essential amino acid in the eggshell membrane. The surface of eggshell has many bacteria. Musgrove (2005) found salmonella bacteria on the eggshell. Davis *et al.* (2008) found salmonella enteritidis and salmonella heidelberg on surface of hens eggshell. Washing eggshell using hot water is one of many methods to kill the bacteria. Middleton and Ferket (2011) reported that phosphoric acid may be used as antibacterial of chicken carcass meal that will be used as the feedstuff. Phosphoric acid increased phosphorus content of chicken carcass meal. Feed industry also uses phosphoric acid to create dicalcium phosphate. Dicalcium phosphate (DCP) is commons source of inorganic phosphorus for animal feed.

This experiment has the objectives of finding out the physical and chemical characteristics of eggshell fortified by different concentration of phosphoric acid and its effect on egg production and eggshell quality. The use of phosphoric acid would be expected to result in decrease amount bacteria, decrease eggshell strength, improve phosphorus content of eggshell waste and egg production and eggshell quality.

## MATERIAL AND METHOD

### Experiment 1.

The objective of research 1 is to find out the physical and chemical characteristics of eggshell waste phosphorus fortified with using phosphoric acid in different concentrations. The concentration of phosphoric acid is 3; 4 and 5%. Eggshell waste is collected from food industry using egg as the ingredient. The eggshell waste is soaked in the hot water with the temperature of 80 °C for 15 minutes, then it is divided into 4 groups. Group 1 : the eggshell is not soaked in phosphoric acid as the control, group 2 : the eggshell waste is soaked in phosphoric acid 3%, group 3: the eggshell waste is soaked in phosphoric acid 4%, and group 4: the eggshell waste is soaked in phosphoric acid 5%. The soaking is as long as 15 minutes.

Twelve eggshells divided into 4 treatment are used to observe the amount of bacteria. The counting of bacteria amount uses the Davis (2008) model. For the proximate and calcium and phosphor content of the eggshell analyses uses the AOAC method, used by Hall (2003). Breaking strength of eggshell waste was evaluated by Texture Analyzer TA Plus (Pelicia *et al.*, 2009).

### Experiment 2.

The experiment 2 was used the eggshell waste of experiment 1 in hens feed. The objective of experiment 2 is to find out the influence of phosphoric acid concentration used for phosphorus fortification on the eggshell waste used as hen feed on egg production and eggshell quality. Feed 1: uses eggshell waste that is not soaked in phosphoric acid (control); feed 2: uses eggshell waste soaked in phosphoric acid 3%; feed 3: uses eggshell waste soaked in phosphoric acid 4%; feed 4: uses eggshell waste soaked in phosphoric acid 5%. The experiment uses 48 isa brown strain hens with the age of 25 weeks and it is conducted for 12 weeks. As many as 12 hens are provided with feed 1, 12 hens are provided with feed 2,

12 hens are provided with feed 3, and 12 hens are provided with feed 4. The composition of feedstuff and nutrient ingredients of feed are presented in Table 1.

Table 1. The Ingredients and Calculated Composition of Diet Treatment

16 Ingredients (%)	Treatment			
	Feed 1	Feed 2	Feed 3	Feed 4
Eggshell waste	5.00 <sup>1</sup>	5.00 <sup>2</sup>	5.00 <sup>3</sup>	5.00 <sup>4</sup>
Corn	70.00	70.00	70.00	70.00
Soybean extract	10.00	10.00	10.00	10.00
Poultry Meat Meal	11.00	11.00	11.00	11.00
Topmix*	0.25	0.25	0.25	0.25
DCP **	1.10	1.00	0.80	0.75
Ca CO <sub>3</sub>	2.40	2.50	2.70	2.75
Salt	0.25	0.25	0.25	0.25
Calculated composition	100.00	100,00	100,00	100,00
ME (kcal/kg)	2892.50	2892.50	2892.50	2892.50
Crude Protein (%)	16.50	16.50	16.50	16.50
Ca (%)	3.50	3.50	3.50	3.50
P available (%)	0.50	0.50	0.50	0.50
Lycine (%)	1.10	1.10	1.10	1.10
Methionine (%)	0,44	0,44	0,44	0,44

<sup>1</sup>eggshell waste is not soaked in phosphoric acid (control), <sup>2</sup> eggshell waste is soaked in phosphoric acid 3%  
<sup>3</sup>eggshell waste 15 soaked in phosphoric acid 4%, <sup>4</sup>eggshell waste is soaked in phosphoric acid 5%;

\* Metionin, lisiin, vitamin A, D3,E , K, B1, B2, B6, B12, C, Ca-pantothenat, Niacin,Cholin Chloride Mn, Fe, I, Zn ,Co, Cu, Santoquin dan Zinc Bacitracin.

\*\* Di Calcium Phosphate

The Completely Randomized Experimental Design was used in this experiment. The observed parameters are: feed intake, calcium intake, phosphorus intake, egg production, egg weight, eggshell weight, percentage of eggshell, and eggshell thickness. The measure data of feed intake, calcium intake, phosphorus intake, and egg production are collected during the research. Egg weight, eggshell weight, percentage of eggshell, and eggshell thickness are collected for 3 days in every 4 weeks and they are conducted in the 3 last days of 4 weeks.

## RESULTS

### Experiment 1

#### **The effect of phosphoric acid concentration on physical and chemical characteristic of eggshell waste.**

The amount of bacteria, calcium content, phosphorus content, and eggshell breaking strength of eggshell waste soaked in control and soaked by phosphoric acid 3-5% are shown in Table 2. Phosphoric acid decreases the amount of bacteria and increases phosphorus content.. Phosphoric acid 5% results in lower bacteria and highest phosphorus content of eggshell waste.

Table 2. Total Bacteria, Calcium and Phosphorus Content of Eggshell Waste Fortified Phosphorus by Different Phosphoric Acid Concentration

Phosphoric acid concentration	Total bacteria (cfu/g)	Calcium (%)	Phosphorus (%)
control	$1.9 \times 10^6$	34.83	0.30
3%	$1.3 \times 10^4$	35.19	0.85
4%	$1.0 \times 10^4$	35.23	1.46
5%	$1.0 \times 10^3$	37.98	1.76

Furthermore experiment shows that phosphoric acid 5% produce the lower eggshell breaking strength. Table 3 present the effect of concentration of phosphoric acid on eggshell breaking strength.

Table 3. Eggshell Waste Breaking Strength on the Used of Different Phosphoric Acid concentration

Phosphoric acid concentration	acid	Eggshell breaking strength (kgf/second)	Breaking strength changed (kgf/second)
control		0,492	-
3%		0,478	- 0,014
4%		0,462	- 0,016
5%		0,442	- 0,020

### Experiment 2

#### **The effect of phosphoric acid concentration on egg production.**

The egg production parameters presented in Table 4. The use phosphoric acid concentration of 3 – 5% for phosphorus fortified eggshell waste is used as a source calcium and phosphorus mineral feed of laying hens did not significantly effect ( $P > 0.05$ ) on feed intake, calcium intake, phosphorus intake and egg production. Egg production tends to increase together with the increase of phosphoric acid concentration although the increase is not significant.

Table 4. The Effect of Phosphoric Acid Concentration on Laying Hens Production Parameter.

Parameters	Phosphoric acid concentration (%)				Average <sup>ns</sup>
	0	3	4	5	
Feed intake (g/hen/day)	117.42	118.68	113.10	111.68	115.22
Calcium intake (g/hen/day)	4.11	4.15	3.96	3.91	4.03
Phosphorus intake (g/hen/day)	0.59	0.59	0.57	0.56	0.58
Hen day production (%)	90.7	93.23	96.1	96.0	94.03
Egg weight (g)	59.9	59.19	58.1	59.5	59.21
	7	59.19	0	6	

<sup>ns</sup> ; not significant (P>0.05)

#### The effect of phosphoric acid concentration on egg eggshell quality.

Table 5 presented the effect of phosphoric acid on eggshell quality. The eggshell quality is not so different in control and the use of phosphate acid 3 – 5%.

Table 5. The Effect of Phosphoric Acid Concentration as Used to Soaking Eggshell Waste on Eggshell Quality of Laying Hens

Variable	Phosphoric acid concentration (%)				Average <sup>ns</sup>
	0	3	4	5	
Eggshell weight (g)	5.66	5.61	5.66	5.67	5.65
Eggshell percentage (%)	9.44	9.48	9.75	9.51	9.55
Eggshell thickness (mm)	0.38	0.37	0.37	0.37	0.37

<sup>ns</sup> ; not significant (P>0.05)

## DISCUSSION

### Experiment 1

The use of phosphoric acid decreases the amount of bacteria on the eggshell waste. The increase concentration of phosphoric acid (control; 3; 4 and 5%) decreased bacteria of eggshell waste. The amount of bacteria are  $1.9 \times 10^6$ ;  $1.3 \times 10^4$ ;  $1.0 \times 10^4$  and  $1.0 \times 10^3$  cfu/eggshell waste (Table 2). The lowest of bacteria is in the use of phosphoric acid concentration 5%. Davis *et al.* (2008) found that the eggshell has *Salmonella enteritidis* and *Salmonella Heidelberg*. Bacteria salmonella is potential pathogens of human and poultry. The research results indicate that phosphoric acid may be used as the anti-bacteria substance for eggshell waste as stated by Guinotte and Nys (1991). Middleton and Ferket (2001) used phosphoric acid for kill bacteria of poultry mortality carcasses meal. acid addition decrease the pH to prevent microbial spoilage and to destroy pathogenic organisms. Phosphoric acid

reduced significantly total aerobic counts of sausages. The maximum reduction by 1 log cfu<sub>g</sub>-1 in relation to sausages un<sup>20</sup>ated with the acid (Barros *et al.*, 2010). The research results showed that the higher the concentration of phosphoric acid is, the better it is used as anti-bacteria substance.

The calcium and phosphorus of the eggshell waste contents show<sup>7</sup>s that calcium content is relatively same; however, phosphorus content increases together with the increase of phosphoric acid concentration. The use of phosphoric acid 5% results in the highest phosphorus content. The phosphorus contents in control, the use of phosphoric acid 3, 4, and 5% are as follows (0.30; 0.85; 1.46; and 1.76%). Middleton and Ferket (2001) stated that phosphoric acid is used in the feed industry to create dicalcium phosphate.

The further experiment shows that phosphoric acid<sup>1</sup> increase the eggshell breaking strength. The eggshell breaking strength decreases together with the increase of phosphoric acid concentration (Table 3). The decrease of eggshell breaking strength is caused by the chemical reaction between Ca CO<sub>3</sub> existing in the eggshell with phosphoric acid /H<sub>3</sub>PO<sub>4</sub>. Guinotte and Nys (1991) stated that phosphoric acid may be used to soak sea shell so that it will break easily. Bain (2006) reported that the eggshell breaking strength has a correlation with fragmentation (break easily). The high eggshell breaking strength on eggshell shows that the eggshell breaks easily.

## Experiment 2<sup>24</sup>

### Egg production

Feed intake, calcium feed intake, phosphorus feed intake, egg production, and egg weight are not significantly different in the use of different phosphoric acid concentrations. The experiment uses the feed that has the same nutritional content so that feed intake, calcium intake, phosphorus intake, egg production, and egg weight are not significantly different. Leeson and Summers (2005) stated that the factor limiting feed intake is energy. The higher the feed energy is, the lower the feed intake. The research result conducted by Nahashon (2007) showed that the increase of energy as much as 2800 – 2900 kcal decreases feed intake.

Calcium and phosphorus intake are influenced by feed calcium and phosphorus level. The use of feed with different calcium levels and same energy results in different calcium intake and the feed intake is not significantly different. Calcium intake is linear to feed calcium level. The level calcium of the feed 3; 3.5; 4 and 4.5% did not significantly effect on feed intake but increased calcium intake. The intake of phosphorus increased linearly with dietary phosphorus levels of the feed (Pelicia, 2009). Squire (2003) stated that calcium and phosphorus are very important minerals for egg formation.

Soaking eggshell in phosphoric acid increases phosphorus content of the eggshell (Table 2) but all treatment feed are iso protein, energy, calcium and phosphorus. The differences in phosphorus acid concentrations cause the decrease of DCP and Ca CO<sub>3</sub> usage in feed. Squire (2003) stated that calcium and phosphorus are very important minerals for egg<sup>23</sup> mation. Saunder-Blade (2009) reported that the calcium source of the feed did not affected on egg production and egg weight. The research of Casarteli *et al.* (2005) show<sup>19</sup>ed that the different source of calcium and phosphorus did not significantly effect on egg production and egg weight of laying hens at 32 – 48 weeks. The research result shows that the use of phosphorus acid for eggshell fortification may replace DCP although it is just a little.

### Eggshell quality

Phosphoric acid concentration (3; 4; 5% and control) does not significantly influence ( $P > 0.05$ ) on the eggshell quality (egg weight, egg percentage and eggshell thickness). Robert (2004) stated that eggshell quality is influenced by genetic, feed nutrition, age, and disease factors. Calcium and phosphorus have very important roles in the process of eggshell

forming. The forming of eggshell takes place in the uterus and it requires the longest time. At that time, the gland in the uterus secretes  $\text{CaCO}_3$  and  $\text{Ca}_3(\text{PO}_4)_2$  as much as about 6 g (Squire, 2003). Calcium and phosphorus contents are the same in all treatments and so that the quality of eggshell is also not significantly different. The result of research is in accordance with the research conducted by Safaa (2008) that the same calcium and phosphorus levels do not influence on the quality of eggshell. The eggshell percentage similar with the study of Pelicia *et al.* (2009) that eggshell percentage linearly with calcium intake. Casartelli *et al.* (2005) research about the effect of calcium and sodium phosphate, micro granulated dicalcium phosphate and triple super phosphate in feed on eggshell quality. The result of the research showed that the egg quality did not different.

### CONCLUSION

The concentration of phosphoric acid 5% was the best antibacterial and the best phosphorus fortified of the eggshell waste. The concentration acid of phosphoric from 3 to 5% is sufficient to maintain the egg production and eggshell quality.

### ACKNOWLEDGEMENT

The experiment project was funded by Directorate General of Higher Education, Ministry of National Education of Republic of Indonesia (Doctor Grant). The authors would like to thank to the Director General of Higher Education, Ministry of National Education of Republic of Indonesia who have provided funding. The authors would also like to the Dean of the Faculty of Animal Science and the Rector of the Gadjah Mada University.

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