The Microstructure of Egg Shell Waste Treated with H3PO4, In vitro Solubility in Different Particle Size and the Using Effect on the Egg Shell Quality of Laying Hens

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International Journal of Poultry Science 12 (7): 421-425, 2013
ISSN 1682-8356

The Microstructure of Egg Shell Waste Treated with H_3PO_4, *In vitro* Solubility in Different Particle Size and the Using Effect on the Egg Shell Quality of Laying Hens

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Abstract: The aim of the research was to study the microstructure changes of egg shell waste treated with H_3PO_4 and its effect on *in vitro* solubility and egg shell quality of laying hens. Egg shell waste was soaked in water with the temperature of 80°C, drained and divided into 4 parts. Part 1 was sun dried (control), part 2 was soaked in H_3PO_4 3%, part 3 was soaked in H_3PO_4 4% and part 4 was soaked in H_3PO_4 5%. Furthermore, egg shells were drained, sun dried and then grinded to a particle size of 1 and 3 mm and used in the feed of laying hens. A total of 96 twenty-five weeks old of laying hens (ISA Brown strain) were divided into 8 experimental dietary treatment in 4 x 2 factorial arrangements with 3 replication. Results of the research showed that the concentration of H_3PO_4 effected on egg shell microstructure changes and interaction of H_3PO_4 3 and 4% and particle size 1 mm was increased *in vitro* solubility. Concentration of H_3PO_4 and particle size had not significantly effect (P>0.05) on calcium intake, egg shell weight and egg shell percentage but significantly effect (P<0.05) on phosphorus intake and egg shell thickness. Egg shell thickness was increased at H_3PO_4 5% and particle size 3 mm. The research concluded that H_3PO_4 resulted in microstructure changes and *in vitro* solubility but did not change the quality of the egg shell, except egg shell thickness.

Key words: Egg shell waste, H_3PO_4 concentrations, particle size, microstructure, *in vitro* solubility and egg shell quality

INTRODUCTION

Egg shells are rich in minerals and contain protein essential and non-essential amino acids. The minerals contained in the egg shell are Ca, P, Fe, Mg, I, Zn, K and Na. The content of calcium (Ca): 35-37%, while phosphorus (P): 0.03 to 0.14% and Mg: 1.07 to 1.10%. Egg shell phosphorus is an inorganic phosphorus so that it has high availability Excess calcium resulting in poor soil, while phosphorus cause damage to aquatic ecosystems/eutrophication. Egg shell is contaminated bacteria originating from *cloaca*. Davis *et al*. (2008) found the bacterium of Salmonella enteritidis and Salmonella heidelberg, then used water (80°C) to kill the bacteria. According to Guinotte *et al*. (1991), H_3PO_4 can be used as an anti-bacterial sea shells and loosen calcium bonds so that it has high solubility.

Ca and P are highly needed by the poultry for egg shell formation and egg shell quality is important for eggs producers. Improving of the egg shell quality has a positive impact on economic efficiency. According to the NRC (1994), the need for calcium and phosphorus for laying hens in laying period is 3.5% (Ca): 0.45% (P). The need for phosphorus is less than calcium but it is the most expensive mineral. The lack of calcium and phosphorus decreases egg shell quality. The decreasing quality of egg shell would adverse manufacturers because the egg will break easily.

The effectiveness of mineral resources is associated with particle size. The research results of Pizzolante *et al*. (2009), showed that limestone with a large particle size has lower *in vitro* solubility than the smaller particle size but in reverse, *in vivo* solubility showed that the bigger particles have better absorption.

The objective of this study was to investigate the microstructure changes of egg shell waste treated with H_3PO_4, the *in vitro* solubility on the particle size of 1 and 3 mm and the effect in the feed to egg shell quality of laying hens. The results are expected to be used in reference to the use of egg shell waste in poultry feed.

MATERIALS AND METHODS

The research used egg shell of laying hens obtained from the bakery industries and the *lunpia* industries (the typical food of Semarang). The egg shell waste was soaked in water with the temperature of 80°C for 15 minutes, drained and divided into 4 parts. Part 1, without the treatment of H_3PO_4 (control), part 2 soaked in H_3PO_4 3%, part 3 soaked in H_3PO_4 4%, part 4 soaked in H_3PO_4 5%. The soaking was conducted for 15 minutes and dried under the sun for 3 days. The egg shell waste was

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Table 1: The ingredients and calculated composition of diet treatment

	Dietary treatment (Feed)			
Ingredients (%)	1 and 5	2 and 6	3 and 7	4 and 8
Egg shell waste	5.00[a]	5.00[b]	5.00[c]	5.00[d]
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
Dicalcium Phosphate	1.10	1.00	0.80	0.75
Ca CO_3	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

[a]Egg shell waste is not soaked in water 80°C (control).
[B]Egg shell waste is soaked in H_3PO_4 3%.
[C]Egg shell waste is soaked in H_3PO_4 4%.
[D]Egg shell waste is soaked in H_3PO_4 5%.
1,2,3,4 Egg shell particle size 1 mm
5,6,7,8 Egg shell particle size 3 mm
*Methionine, lycine, vitamin A, D3, E, K, B1, B2, B6, B12, C, Ca-pantothenate, Niacin, Choline Chloride Mn, Fe, I, Zn, Co, Cu, Santoquin and Zinc Bacitracin.

then evaluated on the microstructure changes by Scanning Electron Microscope (SEM) according to Zamani *et al*. (2005). After that egg shell waste was grinded into 2 particle sizes 1 and 3 mm (the sieve size 1 and 3 mm) and then *in vitro* solubility test was performed using the method of Keshavarz and Scott (1993). Furthermore the egg shells were used in the feed of laying hens. A total of 96 twenty-five weeks old of laying hens (Isa Brown strain) were divided to 8 experimental dietary treatment with 3 replication. The composition of feed ingredients and nutrient content of feed used is shown in Table 1.

The variables measured were microstructure of egg shell waste, *in vitro* solubility, calcium intake, phosphorus intake, egg shell weight, egg shell percentage and egg shell thickness. Calcium and phosphorus are the average consumption per day during the research. The egg shell weight and egg shell thickness were obtained for 3 days every 4 weeks and 3 times during the research. The experimental design was Completely Randomized Design factorial pattern. The data were analyzed by analysis of variance. Duncan's Multiple Range Test was used in means comparison when found effect on treatment.

RESULTS AND DISCUSSION

The effect of H_3PO_4 concentration on microstructure of egg shells waste: The microstructure changes of egg shell waste which was soaked in water 80°C (control), soaked in H_3PO_4 3%, soaked in H_3PO_4 4% and soaked in H_3PO_4 5% can be seen in Fig. 1. The microstructure of egg shell which was not soaked in H_3PO_4, looks cohesive and massive. The higher the concentration of H_3PO_4, it causes the microstructure to be more hollow/loose. Guinotte *et al*. (1991) stated that H_3PO_4 induce microcracking of calcium mineral binding. According to Rodriguez da-Navarro *et al*. (2007) who was cited by Arpasova *et al*. (2010), egg shell microstructure changes can be caused by chemical processes.

Table 2: The *in vitro solubility of* egg shell waste soaked in H_3PO_4 3%, 4%, 5% and control on particle size 1 and 3mm

Particle size (mm)	Concentration of H_3PO_4				
	Control	3%	4%	5%	Average
1	19.84[b]	20.49[a]	20.50[a]	19.87[b]	20.18
3	13.42[f]	17.76[c]	17.19[d]	16.60[e]	16.24
Average	16.56	19.13	18.84	18.24	

Different superscript at the same raw and column indicate significantly different ($P<0.05$).

The effect of H_3PO_4 concentration and particle size on *in vitro* solubility of egg shell waste: *In vitro* solubility of egg shell waste soaked in H_3PO_4 3%, 4%, 5% and control in the particle size of 1 and 3 mm can be seen in Table 2. The results of statistical analysis show that there is an interaction effect ($P<0.05$) between H_3PO_4 concentration and particle size on *in vitro* solubility of egg shell.

The *in vitro* solubility of egg shell waste increased significantly in H_3PO_4 3-4% and the particle size of 1 mm but then decreased at a concentration of 5%. The solubility in 3 mm particle size increased along with the increase of H_3PO_4 concentrations (3-5%) but lower than the particle size 1 mm. The result agree with the observation of Guinotte *et al*. (1991) that the H_3PO_4 induce microcracking of sea shells so that it increases the solubility. Pizzolante *et al*. (2009) reported that *in vitro* solubility of limestone with the particle size 2.83 mm (10.85%) was lower than 0.185 mm (18.7%). The solubility of limestone calcium with the particle size 3.90 mm was lower than the particle size 0.18 mm. *In vitro* solubility of the particle size 3.90 mm was 22.37% and 0.18 mm was 25.56% (Pelicia *et al*., 2011). Saunders-Blades *et al*. (2009) stated that *in vitro* solubility of large limestone particles was significantly lower than the smaller particle size.

Calcium and phosphorus intake: The effect of H_3PO_4 concentration and particle size on calcium and phosphorus intake presented in Table 3. The results showed that calcium intake is not significantly different to the use differences of H_3PO_4 concentrations and particle size but phosphorus intake is effected by interaction of H_3PO_4 concentrations and particle size. Calcium intake is not significant caused the H_3PO_4 concentrations and particle size are not change the nutrition of the feed (Table 1). However, the phosphorus intake was higher in use H_3PO_4 3% particle size of 1

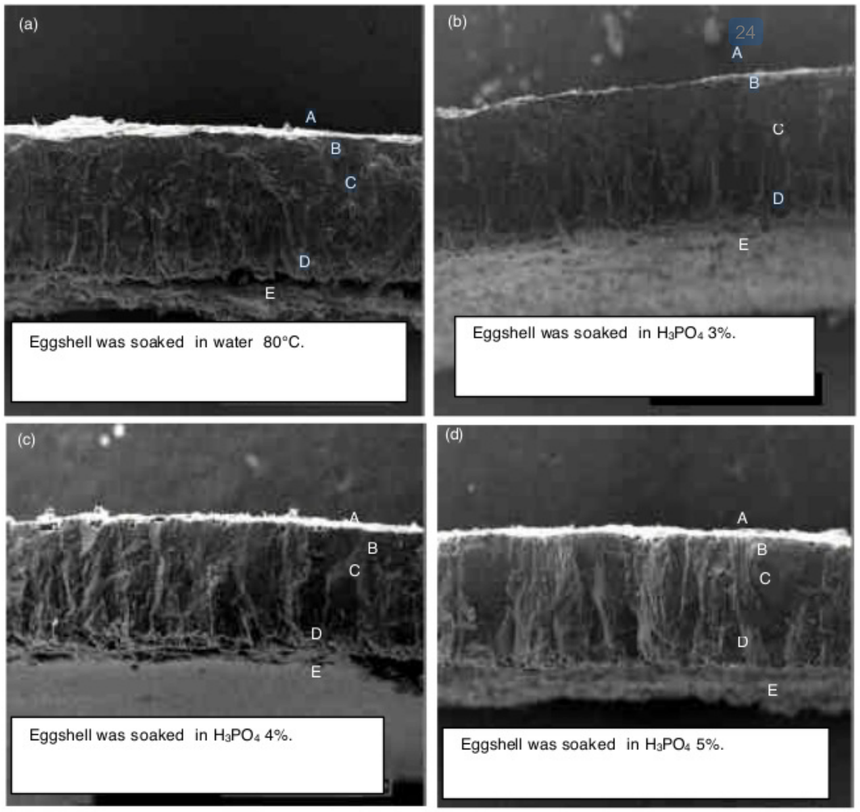

Fig. 1(a-d): Macrostructure of egg shells waste were soaked in water 80°C, H_3PO_4 3%, 4% and 5%. A: cuticula, B: vertical, C: palisade layer, D: mamilary layer, E: inner shell membrane

Table 3: The effect of H_3PO_4 concentration and particle size of egg shell waste on calcium and phosphorus intake

Variables	Particle size (mm)	Concentration of H_3PO_4 Control	3%	4%	5%	Average
Calcium intake (g/hen/day)	1	4.06	4.12	3.89	3.90	3.99
	3	3.90	4.01	4.11	4.05	4.02
Average		3.98	4.06	4.00	3.97	
Phosphorus intake (g/hen/day)	1	0.58^{a}	0.59^{a}	0.57^{ab}	0.55^{c}	0.57
	3	0.56^{bc}	0.57^{ab}	0.59^{a}	0.59^{a}	0.58

Different superscript at the same raw and column indicate significantly different ($P<0.05$).

mm, H_3PO_4 4% particle size of 3 mm, H_3PO_4 5% and particle size of 3 mm. Calcium and phosphorus intake was in the normal range. Calcium intake of laying hens aged 25-35 weeks is 3.98 to 5.21 g/hen/day (Pelicia *et al*., 2011) and phosphorus intake is 4.0 to 4.1 g/hen/day (Pelicia *et al*., 2009).

The quality of egg shell: Table 4 shows the egg shell quality. Concentrations of H_3PO_4 had no significant effect on egg shell weight and egg shell percentage but significant effect on egg shell thickness. It was due to the calcium intake and phosphorus intake (Table 3). Squires (2003), Ahmadi and Rahimi (2011) stated that calcium have the role in the eggs formation especially egg shell. According to Ogawa *et al*. (2004) and Schaafsma *et al*. (2000) egg shell contains high calcium and little phosphorus. Phosphorus intake is important in maintaining egg shell quality (Leeson and Summers, 2005). Egg shell thickness is influenced by phosphorus intake (McDonald *et al*., 2002).

The particle size of the egg shell waste had no significant effect on egg shell weight and egg shell percentage but significant effect on egg shell thickness.

Table 4: The effect of H_3PO_4 concentration and particle size of egg shell waste on egg shell quality

Variabel	Particle size (mm)	Concentration of H_3PO_4 Control	3%	4%	5%	Average
Egg shell weight (g)	1	5.72	5.67	5.72	5.70	5.70
	3	5.85	5.71	5.78	5.92	5.81
Average		5.78	5.68	5.75	5.81	
Egg shell percentage	1	9.52	9.50	9.78	9.59	9.60
	3	9.76	9.62	9.54	9.74	9.66
Average		9.64	9.56	9.66	9.66	
Egg shell thickness (mm)	1	0.377	0.367	0.375	0.371	0.372^b
	3	0.382	0.374	0.378	0.387	0.380^a
Average		0.379^a	0.370^b	0.376^b	0.379^a	

Different superscript at the same raw and column indicate significantly different ($P<0.05$)

The results correspond with the other studies. Pizzolante *et al*. (2009) reported that limestone particle size have no effect on egg shell weight. The research results of de Witt *et al*. (2009) showed that the particle size have no effect on weight and percentage. Pelicia *et al*. (2011) also stated that limestone the particle size of fine and coarse limestone have no effect on the weight. Phirinyane *et al*. (2011) stated that the limestone particle size have no effect on the character of egg shell. The research results were not in accordance with the research of Ekmay and Coon (2010) who stated that limestone particle size increases the weight of egg shells. Lichovnikova (2007) reported that particle size have effect on egg shell thickness.

The average weight of egg shell, egg shell percentage are in the normal range. According to Nys *et al*. (2011), the egg shell weight about 5.5 g or 8.5-10.5%. Zita *et al*. (2009) stated that egg shell thickness of Isa Brown strain is 0.37 to 0.38 mm.

ACKNOWLEDGEMENT

The experiment project was funded by Directorate Gneneral 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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