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Calcium deposition in egg due to substitution of limestone by eggshell flour in feed of laying hens

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ABSTRAK

Penelitian bertujuan untuk mengevaluasi deposisi kalsium pada telur ayam yang menggunakan tepung kerabang telur sebagai pengganti batu kapur dalam pakan. Dua ratus ekor ayam petelur strain Isa Brown umur 25 minggu digunakan pada penelitian ini. Perlakuan yang dicobakan adalah : penggunaan batu kapur 7,5% dalam pakan sebagai kontrol (T0), penggantian 2,5% batu kapur dengan tepung kerabang telur (T1), penggantian 5% batu kapur dengan tepung kerabang telur (T2) dan penggantian batu kapur dengan tepung kerabang telur (T3). Rancangan percobaan yang digunakan adalah Rancangan Acak Lengkap dengan 4 perlakuan dan 5 ulangan. Tiap unit percobaan terdiri 10 ekor ayam petelur. Parameter yang diukur meliputi berat telur, berat kuning telur, berat albumen, berat kerabang telur dan kalsium telur (kuning telur, albumen dan kerabang telur), panjang, berat dan Ca tulang tibia. Hasil penelitian menunjukkan bahwa penggantian batu kapur dengan tepung kerabang telur berpengaruh nyata (P<0,05) berat kerabang telur, deposisi Ca pada kuning telur, putih telur dan tulang tibia, tetapi tidak berpengaruh nyata terhadap berat telur, berat dan persentase kuning telur, berat dan persentase putih telur, persentase dan Ca kerabang telur, panjang dan berat tulang tibia. Simpulan bahwa deposisi Ca pada kuning telur tertinggi pada penggunaan 7,5% tepung kerabang telur sebagai pengganti batu kapur tetapi diperoleh Ca tulang terendah, sedangkan deposisi Ca pada putih telur tertinggi pada penggunaan 2,5% tepung kerabang telur.

Kata kunci : batu kapur, kerabang telur, deposisi kalsium

ABSTRACT

The aim of this research was to evaluate calcium deposition in egg using eggshell flour as a limestone substitute in feed. Two hundreds laying hen of Isa Brown strain of 25 weeks were used in this study. Treatments were diet with 7.5% limestone as control (T0), 2.5% limestone is substituted with eggshell flour (T1), 5% limestone is substituted with eggshell flour (T2) and limestone is substituted with eggshell flour (T3). A completly randomized design were used to allocated the treatments with 5 replications of each. Each experimental unit consists of 10 laying hens. Parameters measured were egg weight, yolk weight, albumen weight, eggshell weight, calcium of egg (yolk, albumen and eggshell), length, weight and Ca of tibia bone. The results showed that substitution of limestone with eggshell flour had significantly effect (P<0.05) on eggshell weight, Ca deposition on yolk, albumen, and Ca of tibia bone but non significantly effect on egg weight, weight and percentage of yolk, weight and percentage

of albumen, percentage and Ca of eggshell, length and weight of tibia bone. In conclusion, calcium deposition in yolk was the highest in the use of 7.5% eggshell flour to substitute limestone but obtained the lowest Ca of bone , while calcium deposition in albumen was the highest in the use of 2.5% eggshell flour.

Keywords: limestone, eggshell, calcium deposition

INTRODUCTION

Poultry industry development may give positive and negative impacts. The positive impact was nutrition sufficiency and job opportunities, the negative impact was waste product of excreta and eggshell. Excreta can be used as organic fertilizer and source of energy, while eggshell if not handled properly will effect on environmental pollution.

Egg production of Indonesia was 1401079.2 tons/year (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2016), it is estimated that there are 140107.92 tons/year eggshell waste and not utilized. The eggshell is covered with cuticles. The cuticle has a compositional with proteins, sulfated polysaccharides and phosphates (Rodríguez-Navarro *et al*., 2013). The eggshell membranes consists of highly collagens, glycoproteins and cysteine-rich**,** eggshell membrane proteins as used in the development of the embryo and protect it against pathogen invasion (Cordeiro and Hincke, 2016).

According to Panheleux *et al*. (2000), Mine *at al*. (2003), Rodríguez-Navarro *et al*. (2015)**,** Marie *et al*. (2015) and Zang *et al***. (**2016) eggshell contains minerals and a little protein. Proteins involved in mineral supply during eggshell calcification (Brionne *et al*., 2014). Yasothai and Kavithaa (2014) stated that eggshell is highly rich in calcium (90%) with little of phosphorus. Siuplapwa *et al*. (2014) reported that the eggshell contains various minerals ie Ca, P, Fe, Mg, Cu, Co, K, Na, Zn and Cr. Mineral composition of eggshell are Ca (35080 m**g**/100 g), P (150.2 mg/100 g), Mg (262mg/100g), Fe (13.06 mg/100 g**),** Zn (145.1 mg/g), K (50 mg/100 g), Cu (4.1 mg/100 g) and Mn (149.9 mg/100 g) (Hassan, 2015).

Calcium is one of minerals essential for poultry. Requirement of Ca of poultry for laying period was around 4 - 5 times compared with brooding and growing period. Ca content of the feed will affected the eggshell and bone quality of laying hens (An *et al*., 2016). Neijat *et al*. (2011) stated that Ca consumption was deposite on the bone, meat, eggshell, albumen, yolk and some other will excrete through excreta. Medullary bone acts as Ca storage for eggs formation especially eggshell (Kim *et al*., 2012) and reserve of eggshell formation when Ca deficiency in feed (Olgun and Aygun, 2016). Ca consumption of laying hen was 4000 mg and deposit to the egg 3100 mg, faeces 500 mg and urine 400 mg (Leeson dan Summers, 2005). Ca of egg is needed for embryo development (Uni *et al.*, 2012, Tahara and Obara, 2014; Scanes, 2015).

Ca source is widely used as poultry feed material is limestone. Limestone contains many minerals but no protein, while eggshell flour contains mineral and a little protein. Noviyanti *et al*. (2015) stated that limestone composed by Ca, K, Na, Al, dan Si. Limestone was composed by macro mineral Ca, P, Mg, Na, K, Cl, S and micromineral Mn, Zn, Fe, Cu, Co, and Se (Khalil and Anwar, 2009). Limestone was composed 35.7 – 36.4% of Ca (Pelicia *et al*., 2009).

Alu *et al* (2013) stated that eggshell flour may used for substitution of bone flour on broiler diet without blood hematology and nutrient digestibility changing. Olgun *et al*. (2015) stated that differences of Ca source showed significance effected on egg production and eggshell quality. Research of Cufadar *et al*. (2014) showed that used of eggshell flour, limestone, oyster shell, mixture of limestone with eggshell, limestone with oyster shell and eggshell with oyster shell showed non significance effect on the egg weight and eggshell weight. Research of Scheideler (1998) showed that Ca of eggshell has higher digestibility then of limestone. According to Dolinska *et al.* (2016), calcium carbonat ($CaCO_3$) of eggshell has a higher solubility than of synthetic $CaCO_3$. Szeleszczuk *et al*. (2015) stated that the *in vitro* solubility Ca of eggshell is higher than $CaCO_3$. The aim of this research was to evaluate a substitution of limestone with eggshell flour on the Ca deposition in egg of laying hens.

MATERIALS AND METHODS

Two hundred laying hens (Isa Brown) were used in this study. The experimental period was 12 weeks. The laying hens were distributed in a

Completely Randomized Design with 4 treatments and 5 replications. The treatment were limestone of 7.5% in feed (T0), substitution 2.5% lime limestone with eggshell flour (T1), substitution of 5% limestone with eggshell flour (T2) and the replacement of limestone with eggshell flour (T3). The composition of feedstuff and nutrient content of dietary treatment is presented in Table 1. Before use, the eggshell was immersed in water temperature 80 °C for 15 minutes, drained and then immersed into 5% phosphoric acid, dried by the sun for 3 days and grounded with 3 mm particle size.

Parameter measured were weight of egg, weight and percentage of yolk, weight and percentage of albumen, weight and percentage of eggshell, Ca of yolk, Ca of albumen, Ca of eggshell and length, weight and Ca content of tibia bone. Analysis of calcium yolk, calcium albumen and calcium eggshell using the AOAC method (Horwitz and Latimer, 2005). Data was analyzed by Analysis of Variance (ANOVA) and continued with Duncan's Multiple Range Test (DMRT) when there was significantly difference.

RESULTS AND DISCUSSIONS

Calcium Deposition on Egg

Calcium deposition on egg with feed suplementation eggshell flour as a substitution of limestones is presented in Table 2. Used of eggshell flour for substitution of limestone showed an effect ($P<0.05$) on eggshell weight, Ca of yolk, Ca of albumen but there was no effect on egg weight, weight and percentage of yolk, weight and percentage of albumen, percentage of eggshell weight and Ca of eggshell .

Egg Weight

Results of this research showed that used of eggshell flour up to 7.5% as substitution of limestone did not affect on egg weight. Eggshell flour contains Ca almost the same as limestone, so that the use of eggshell flour as a substitute for limestone had no effect on egg weight. Gongruttananun (2011) stated that Ca content of eggshell is 34.89%, while Ca content of limestone is 36.4 – 35.7% (Pelicia *et al*., 2009). The use of eggshell flour as a substitute of limestone in diet

Table 1. Composition of Feed Stuff and Nutrient Content of Dietary Treatment

Feedstuffs	T0	T1	T2	T3
		…….%……..		
Corn	70.00	70.00	70.00	70.00
Soybean meal	10.00	10.00	10.00	10.00
Poultry meat meal	11.00	11.00	11.00	11.00
Limestone	7.50	5.00	2.50	0.00
Eggshell flour	0.00	2.5	5.0	7.5
DCP	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25
Calculated Nutritional Level				
Crude protein (%)	16.17	16.31	16.45	16.59
EM (kcal/kg)	2892.50	2892.50	2892.50	2892.50
Ca (%)	3.44	3.44	3.44	3.44
P (%)	0.46	0.50	0.55	0.59
Crude fiber (%)	2.70	2.70	2.70	2.70
Lysine (%)	1.10	1.10	1.10	1.10
Methionine (%)	0.44	0.44	0.44	0.44

T0: feed using 7.5% of limestone, T1: 2.5% of limestone replaced by eggshell flour, T2: 5% limestone replaced by eggshell flour, T3: 7.5% limestone replaced by eggshell flour

Table 2. Calcium Deposition in Egg on using Eggshell Flour as Substitution of Limestone in the Feed of Laying Hens

Parameters	T0	T1	T2	T3
Egg weight(g)	51.418	50.567	53.027	52.101
Yolk weight (g)	12.536	12.317	13.292	13.681
Yolk percentage (%)	24.406	24.330	25.106	26.246
Albumen Weight (g)	33.943	32.898	34.452	33.116
Albumen percentage (%)	65.972	65.061	64.919	63.549
Eggshell weight (g)	4.938^{b}	5.352^{ab}	5.283^{a}	5.304^{a}
Eggshell percentage (%)	9.53	10.58	9.8	10.17
Ca Yolk (mg)	6.086^{d}	6.865^{c}	8.433^{b}	11.359^{a}
Ca Albumen (mg)	1.618^{c}	2.293^{a}	1.922^{b}	1.249^{d}
Ca Eggshell (g)	1.48	1.49	1.55	1.41

T0: feed using 7.5% of limestone, T1: 2.5% of limestone replaced by eggshell flour, T2: 5% limestone replaced by eggshell flour, T3: 7.5% limestone replaced by eggshell flour

of laying hen was not effect on the egg weight with the egg weight is 47.47 – 47-81 g (Gongruttananun, 2011). Result of the research of Cufadar (2014) showed that different source of Ca was not affected on the egg weight. According to Sultana *et al*. (2007) and Safaa *et al*. (2008) differences of Ca source and Ca content in diet did not effect on egg weight. An *et al*. (2016) confirmed that Ca was not the factor affecting to the egg weight. Hanusová *et al*. (2015) stated that egg weight was affected by genetic factor. Leeson and Summers (2005) and King'ori (2012) stated that factor affecting the egg weight were age, body weight and nutrition consumption. According to Adeyemo *et al*. (2012), protein content in diet was effect on the egg weight.

Weight and Percentage of Yolk

The used of eggshell flour as substitution of limestone did not affect the yolk weight and percentage of yolk. The results of the study was in agree to the results of previous studies. Saldanha *et al*. (2009) stated that percentage of yolk was not affected by source of Ca. The research of Safaa *et al*. (2008) showed that the percentage of yolk was not different between used limestone or oyster shell powder as a source of Ca in the feed. Gongruttananun (2011) stated that substitution of limestone with eggshell flour did not effect the yolk percentage. Pelicia *et al*. (2009) reported that Ca in the diet was not affected on percentage of yolk. Factor affected the percentage of yolk is genetic (Hanusová *et al*. , 2015), protein level of the feed (Gunawardana *et al*., 2008), eggs storage (Scott dan Silversides, 2000) and age of laying hens (Silversides and Budgell, 2004). Longer time of storage will increase weight of yolk (Khan *et al*., 2014). Yolk percentage was positive correlated to weight of egg and age of laying hens (Ulmer-Franco *et al*., 2012).

Weight and Prcentage of Albumen

Result of the research showed that used of eggshell flour as substitution of limestone did not affect the weight and percentage of albumen. Saldanha *et al*. (2009) and Safaa *et al*. (2009) stated that Ca source of feed did not affect percentage of albumen. The results of the study were in agree with the research of Gongruttananun (2011) that the limestone substitution with eggshell flour in feed of Rhode Island Red strain did not significantly affect the percentage of albumen. Pelicia *et al*. (2007) state that difference source of Ca (limestone or marine calcium) did not affect the weight of albumen. Ca level in the feed did not affect the albumen percentage (Pelicia *et al*. , 2009). The factor influencing albumen weight was genetic (Suk and Park, 2001; Hanusová *et al*., 2015). Silversides and Budge (2004) stated that factors affecting the

weight of albumen were age of laying hens and a long of storage.

Weight and Percentage of Eggshell

Table 2 showed that substitution of 5% and 7.5% limestone with eggshell flour will increase weight of eggshell (P<0.05). Increasing of eggshell weight was affected by protein content and minerals (Fe, Mg, Zn, Cu, and Se) of eggshell flour. Panheleux *et al*. (2000), Mine *at al*. (2003), Rodríguez-Navarro *et al*. (2015)**,** Marie *et al*. (2015) and Zang *et al***. (**2016) stated that eggshell contains many minerals and very low of protein. Schaafsma *et al*. (2000) stated that eggshell contained high Ca and other minerals such as P, Mg, Fe, Zn, Se, Cr, Na, F, Cr, V and slightly protein. Khalil and Anwar (2009) and Noviyanti *et al*. (2015) stated that limestone contains minerals but without protein. Supplementation of Mn, Zn, and Cu increased eggshell quality (Stefanello *et al*. 2014).

Eggshell percentage showed not different. Khalil and Anwar (2009) reported that eggshell persentage was not affected by different of Ca source of feed. Research of Gongruttananun (2011) showed that used of eggshell flour as substitution of limestone showed non significance effect the eggshell percentage. Olgun *et al*. (2015) stated that used of eggshell flour as substitution of limestone (66.7%) did not affect the percentage of eggshell.

Ca of Yolk

Substitution of limestone by eggshell flour affected (P<0.05) the Ca deposition in yolk. Ca deposition on yolk increased along with used of eggshell flour as substitution for limestone in feed. The result showed that Ca utilization from eggshell flour was higher compared to limestone. This result was supported by statement of Scheideler (1998) in which digestibility of eggshell flour was higher than limestone and Szeleszczuk *et al*. (2015) stated that bioavailability Ca of eggshell was high. Leeson dan Summers (2005) stated that calcium of diet was storage in bone, deposited in eggs and excreted on the excreta. Differences of Ca source was not affected the Ca of yolk (Vargas-Rodríguez *et al*., 2016).

Ca of Albumen

There was significantly effect (P<0.05) of substitution of limestone with eggshell flour on Ca deposition in albumen. Substitution of limestone with eggshell flour would increase Ca albumen. The highest of Ca deposition was found on T1 (2.5% or 33.33% eggshell flour). Kiczorowska *et al*. (2015) stated that Ca of yolk positively correlates with Ca albumen, but the results of this study Ca of albumen would increase on T1 and followed by `decreasing in T2 and T3, but T2 and T3 were still higher than T0. Calcium of albumen dereased along with increased calcium of yolk. This result was different from the result of Pastore *et al*. (2012) and Vargas-Rodríguez *et al*. (2016) that Ca of the feed did not affect the deposition Ca in albumen.

Ca of Eggshell

Ca deposition in eggshell was not significantly different, whereas Ca deposition in yolk and albumen was higher (P<0.05) on the use of eggshell flour than limestone (Table 2). Tunç and Cufadar (2015) stated that different Ca sources of feed had no significantly effect on eggshell weight, but significant on Ca deposition in eggshell. The research of Olgun *et al*. (2015) showed that the different calcium sources had significantly effect on eggshell weight and eggshell calcium content.

Length, Weight and Calcium (Ca) Content of Tibia Bone

Result of this study showed that substitution of limestone by eggshell flour significantly effect on Ca content of tibia bone but no significantly effect on the length and weight of tibia bone (Table 3).

Length and Weight of Tibia Bone

The length and weight of tibia bone were not significantly different. The results of this study were in accordance with the study of Gongruttananun (2011) that the use of eggshell flour had no significant effect on the length and weight of tibia bone.

Calcium Content of Tibia Bone

Table 3 showed that the replacement of 5% limestone with eggshell flour increased the calcium percentage and calcium content of tibia bone, but substitution of 7.5% limestone with eggshell flour decreased the percentage and weight calcium of bone. This is due to increase Ca deposition in yolks (Table 2). The results showed that the use of 7.5% eggshell flour has occurred Ca mobilization of tibia bone for egg formation, especially egg yolks. Tunç *et al*. (2015) stated

Table 3. Length, Weight and Ca Content of the Tibia Bone on Using Eggshell Flour as Substitution of Limestone in the Feed of Laying Hens

Parameters of Tibia Bone	T0	T1	T2	T3
Length (cm)	11.90	11.62	11.80	11.67
Weight (cm)	10.27	11.05	10.89	10.19
Ca (%)	12.80^{ab}	12.60^{ab}	13.61^{a}	12.03^{b}
Ca (g)	1.31^{ab}	1.39^{ab}	1.480^{a}	1.22^{b}

that the Ca content tibia bone is affected by the calcium source, but the results of this study show not significant effect.

The results of this study was different from research of Saunders-Blades *et al*. (2009) in which the weight and calcium of tibia were not affected by calcium sources. Tůmová *et al*. (2016) and Swiatkiewicz *et a*l. (2015) suggested that tibia bone Ca is affected by Ca levels of feed.

CONCLUSION

Calcium deposition in yolk was the highest in the use of 7.5% eggshell flour to substitute limestone, while calcium deposition in albumen was the highest in the use of 2.5% eggshell flour. The use of 7.5% eggshell flour as a substitute for limestone resulted in the mobilization of Ca from the tibia bone so that the Ca content of the tibia bone decreased.

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