THE PERFORMANCE OF LAYING HENS FED DIFFERENT CALCIUM SOURCE

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ABSTRACT

The research was aimed to evaluate the performance of laying hens fed different calcium sources. Two hundreds of Isa Brown laying hens were used in this study. The hens were raised in individual battery cages units for 12 weeks. Four calcium source (limestone as a control, 5% limestone + 2.5% eggshells waste, 2.5% limestone + 5% eggshells waste and 7.5% eggshell waste) were used in feed experiment. A completely randomized design was applied, with 4 treatments and 5 replications. Each experimental unit consisted of 10 laying hens. The parameters measured were feed intake, protein intake, calcium intake, phosphorus intake, egg production, egg weight and feed conversion ratio. Results of the research showed that the calcium source had significantly effect on performance productions. The use of eggshell waste 7.5% significantly increased the feed intake, calcium intake, phosphorus intake, egg production and egg weight except for feed conversion ratio. The conclusion of this research was that the use of eggshell waste as calcium source of feed resulted in better performance than using limestone or mixed limestone with eggshell waste.

Keywords: Calcium, eggshell waste, performance

INTRODUCTION

Laying hens need high calcium (Ca) especially during egg production. Calcium is essential for egg formation. Leeson and Summers (2005) stated that deficiency of calcium resulted in decrease egg production and mobilization of calcium from bone. The release of calcium from bones is along with phosphorus (P). Egg production will return to normal in 6 to 8 days after hens receiving a diet adequate in calcium. Medullary bone is a woven bone that acts as a labile source of calcium for eggshell formation (Whitehead, 2004). The nutritional role of Ca is closely linked to that of phosphorus and to the effect of vitamin D (Arajuo et al., 2005 in Pelicia...
Calcium requirement of the laying hens is 3-4.5 g/ hen/day and phosphorus/non phytate phosphorus requirement is 250 mg/ hen/day (NRC, 1994).

Couto et al. (2008) stated that calcium source effect on egg production. The use of calcium carbonate produced higher egg production than dicalcium phosphate (Silva and Santos, 2000). Supplementation of 50% limestone with oyster shell increased egg production (Ahmad and Balander, 2003). Marine calcium can replace up to 45% of calcitic limestone with no effects on performance or egg quality (Pelicia et al., 2007). Lesson and Summers (2005), Safaa et al. (2008) and Saunders-Blades et al. (2009), stated that limestone and oyster shell could be used as a source of calcium for hens feed with no effect on egg production. Oyster shell and limestone both provide Ca in the form of Ca carbonate, and each contain about 38% of Ca (NRC, 1994). Leeson and Summers (2005) stated that oyster shell is more expensive than limestone.

Ogawa et al. (2004) stated that eggshell contains 94.4% Ca CO3, 0.73% Ca3 (PO4)2, 0.84% Mg CO3 and 3.3% protein. According to Gongruttananun (2011) eggshell contains 5.35% protein, 34.89% calcium and 0.001% phosphorus. The research results of Schaafsma et al. (2000) showed that eggshell powder contains minerals (Ca, Mg, P, Sr, Zn, Fe, B, Cr, F, Se, N, F and V), protein, amino acids and hormones. The hormones of the eggshell are calcitonin (10-25 ng/g) and progesterone (0.30 to 0.33 ng/g). Nakano et al. (2001) stated that eggshell contains glycosaminoglycans and calcium. The research of Nakano et al. (2002) found galactosaminogycan in eggshell. Nakano et al. (2003) stated that amino acids contained within the eggshell are threonine, serine, glycine, methionine, alanine, isoleucine, valine, tryosine, phenylalanine, histidine, lysine, arginine, proline and hydroxyproline. Davis et al. (2008) stated that the eggshell contained Salmonella. The cooking temperature of 82.5°C can kill Salmonella of eggshell. Kismiati et al. (2012) reported that fortification phosphorus with H3PO4 5% increased phosphorus content and kill bacteria of the eggshell. Phosphorus is a critical aspect of laying hen nutrition in hot climates (Usayran et al. 2001). Phosphorus from animal products is generally considered to be well utilized (NRC, 1994). Snow et al. (2004) stated that phosphorus level of the feed affected on egg production. Kingori (2011) stated that eggshell can affect environmental pollution. In Indonesia there were about 75,112 tons of laying hens eggshell waste, 18,620 tons of indigenous hens eggshell waste and 18,986 tons of duck eggshell waste every year (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2011). Laying hen eggshell waste product was higher than eggshell waste of indigenous chicken and ducks.

The aim of the research was to evaluate the performance of laying hens fed different calcium source. The calcium sources were limestone, mixed of limestone and eggshell waste and eggshell waste. Utilization of eggshell waste as a calcium source of feed is expected to reduce environmental pollution.

**MATERIALS AND METHODS**

Eggs were collected from several bakeries and then soaked in the water at temperature of 80°C for 15 minutes, drained and then soaked in H3PO4 5% for 15 minutes. After that the eggshell were dried in the sun for 3 days, ground and then used as a calcium source of feed.

Two hundred of laying hens (Isa Brown strain) aged 25 weeks were raised in battery cages (single cage) during 12 weeks. The hens were distributed in a completely randomized design with 4 treatments and 5 replications. Each experimental unit consisted of 10 laying hens were subjected in the following each treatments: T1: 7.5% limestone as control, T2: 5% limestone + 2.5% eggshell waste, T3 : 2.5 limestone + 5% eggshell waste and T4 : 7.5% eggshell waste as a source of calcium. The composition of feedstuffs and nutrients content of feed used are presented in Table 1.

The parameters measured were feed intake, protein intake, calcium intake, phosphorus intake, egg production, egg weight and feed conversion ratio. Feed intake, protein intake, calcium intake, phosphorus intake were recorded weekly, egg production was recorded daily. Egg weight and feed conversion ratio were measured using all eggs produced during 3 days at the end of week 4, 8 and 12. The data were analyzed by ANOVA. Duncan’s Multiple Range Test was used in mean comparison when found effect of treatment.

**RESULTS AND DISCUSSION**

Table 2 shows that the use of different calcium source in feed had significant effect
(P<0.05) on all the performance parameters (feed intake, protein intake, calcium intake, phosphorus intake, egg production and egg weight) of laying hens, except for feed conversion ratio.

**Feed Intake**

The use of 7.5% limestone (T0), 5% limestone + 2.5% eggshell (T1) and 2.5% limestone + 5% eggshells (T2) in the feed of laying hens had no significant effect (P>0.05) on feed intake, but the use of 7.5% eggshells (T3) increased feed intake significantly (P<0.05). The use of 5% limestone + 2.5% eggshell increased protein content of the feed by 0.14%, the use 2.5% limestone + 5% eggshell increased protein content of the feed by 0.28% and the use of 7.5% eggshell increased protein content of feed by 0.42% (Table 1). The results of this research showed that the increasing of 0.14 and 0.28% protein from eggshell had not been able to increase feed intake, while the increasing of 0.42% protein increased feed intake. Gunawardana et al. (2008) reported that the increasing of protein level caused the increase of feed intake.

Nakano et al. (2003) reported that the decalcified eggshell and shell membrane contained threonine, serine, galactosamine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, proline, and hydroxyproline. This study used eggshell without decalcification. The study of Corzo and Kidd (2004) showed that the addition of lysine increase feed intake and glycine supplementation increase feed intake. Faria et al. (2002) stated that amino acids increased feed intake of laying hens. The research of Bonekamp et al. (2010) showed that lysine, methionine, cystine, threonine, tryptophan, isoleucine, arginine and valine increased feed consumption.

Eggshell contains 4.5 mg/g Mg, 5.13 µg/g Zn, 22.4 µg/g Fe, 7.7 µg/g Cu, and 23.5 ng/g Se (Schaafsma et al., 2000). According to NRC (1994), Mg, Cu, Se, and Zn are the essential minerals for poultry. Maciel et al. (2010) stated that organic Zn, Cu, Mn increased feed intake of laying hens. The addition of 0.3 ppm organic Se and 60 ppm organic Zn in feed increased feed intake.

### Table 1. The Ingredients and Calculated Composition of the Experimental Diet

| Ingredients               | T1  | T2  | T3  | T4  |
|---------------------------|-----|-----|-----|-----|
| 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 |
| Dicalcium Phosphate       | 1.00  | 1.00  | 1.00  | 1.00  |
| Limestone                 | 7.50  | 5.00  | 2.50  | 0.00  |
| Eggshell waste            | 0.00  | 2.50  | 5.00  | 7.50  |
| Topmix*                   | 0.25  | 0.25  | 0.25  | 0.25  |
| 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 (%)         | 16.17  | 16.31  | 16.45  | 16.59  |
| Ca (%)                    | 3.44   | 3.44   | 3.44   | 3.44   |
| P available (%)           | 0.46   | 0.50   | 0.55   | 0.59   |

* Methionine, lysine, vitamin A, D3,E , K, B1, B2, B6, B12, C, Ca-pantothenat, Niacin, Cholin Chloride Mn, Fe, I, Zn ,Co, Cu, Santoquin and Zinc Bacitracin.
intake of quail (Cruz and Fernandez, 2011). The increasing of Se content in the feed (from 0.55 to 0.57 mg/kg) increased feed intake (Scheideler et al., 2010).

Protein Intake
Table 2 presented that protein intake increased due to the increase of feed intake. Result of this research in agreement with Novak et al. (2006) that protein intake linearly increased as feed intake increased. The other factor of protein intake is protein content of the feed. The use of eggshell waste increased protein content of the feed (Table 1). Hudson et al. (2000) stated that protein intake is influenced by the protein content of feed. Gunawardana et al. (2008) reported that dietary protein level had a significant effect on protein intake.

Calcium and Phosphorus Intake
Calcium and phosphorus intake were not significantly different in the use of limestone 7.5% (T0), 5% limestone + 0.25% eggshell (T1) and 2.5% limestone + 5% eggshell (T2), but increased significantly in the use of 7.5% eggshell (T4). The increasing of calcium intake was caused by the increase of feed intake, whereas the increase of phosphorus intake was caused by the increase of feed intake (Table 2) and phosphorus content of the feed (Table 1). Increasing phosphorus from 0.044 to 0.088% (T0 to T2) in feed had no effect on the phosphorus intake, but the increase of phosphorus by 0.13% (T0 to T3) increased phosphorus intake significantly (Table 2). Pelicia et al. (2009) reported that calcium and phosphorus intake is influenced by the calcium and phosphorus level of feed. Increasing calcium of the feed by 3-5% increased calcium intake 1.77 g/hen/day and increasing phosphorus of feed from 0.25 to 0.4% increase phosphorus intake to 0.19 mg/hen/day.

Egg Production and Egg Weight
Eggs production and egg weight in the feed containing of 7.5% limestone, 5% limestone + 0.25 eggshell and 2.5% limestone + 5% eggshell (T0, T1, T2) were not different significantly, while the use of 7.5% eggshell (T3) increased egg production and egg weight significantly (P<0.05). The increasing of egg production and egg weight was caused by the protein, calcium, and phosphorus intakes. This is in agreement with the study of de Beer and Coon (2006) that hens fed high protein had higher egg production. Research of Adeyemo et al. (2012) indicated that there were a direct relationship between protein intake and performance of laying hen at the first production. Joseph et al. (2000) suggested that the high protein level in feed result high egg production. Novak et al. (2006) and Gunawardana et al. (2008) reported that protein had a significant effect on egg production and egg weight. Increasing protein intake significantly increased egg production and egg weight. Kingori et al. (2010) reported that feed protein affects on egg production. Increased protein by 100-120 g/kg dry matter increases egg production, but the increase of 120 and 140 g/kg dry matter does not increase.

Tabel 2. The Effect of Different Calcium Source on the Performance

| Parameters          | Treatments |
|---------------------|------------|
|                     | T0         | T1         | T2         | T3         |
| Feed intake (g/hen/day) | 104.76b    | 107.13b    | 109.10b    | 116.81a    |
| Protein intake (g/ hen /day) | 16.94c     | 17.46bc    | 17.91b     | 19.37a     |
| Calcium intake (g/ hen /day) | 3.60b      | 3.69b      | 3.75b      | 4.02a      |
| Phosphorus intake (g/ hen /day) | 0.48b      | 0.54b      | 0.55b      | 0.64a      |
| Egg production (%)   | 86.83b     | 87.95b     | 87.07b     | 91.42a     |
| Egg weight (g)       | 53.30b     | 52.90b     | 54.99ab    | 55.73a     |
| Feed conversion ratio| 2.26       | 2.30       | 2.28       | 2.29       |

Different superscript at the same raw indicates differ significantly (P<0.05)
the egg production, even 170 g/kg dry matter of egg production is lower than 100 g/kg dry matter. Nahashon et al. (2007) stated that hens on 22 and 24% crude protein diets exhibited higher egg production than those on 20% crude protein diets. Eggshell contains various amino acids (Nakano et al., 2003). Egg production and egg weigh influenced by amino acids content of feed (Faria et al., 2002). Corzo and Kidd (2004) stated that glycine supplementation increases egg production and egg weight. The research results of Bonekamp et al. (2010) showed that lysine, methionine, cystine, threonine, triptophan, isoleusine, arginine and valine increase feed consumption, egg production and egg weight. Glycine supplementation increases egg production (Han and Thacker, 2011).

The use of 7.5% eggshell increases calcium and phosphorus intake. Squires (2003), Leeson and Summers (2005) stated that calcium and phosphorus are very important for the egg formation especially eggshell. Calcium metabolism requires proteins to Ca BP (calcium binding protein) formation. Furthermore, Leeson and Summers (2005) stated that if poultry has calcium deficiency so the egg production will decrease. Egg production will return to normal in 6 to 8 days after hens receive a diet adequate in calcium. Phosphorus is a mineral that is always paired with calcium. The ratio of Ca and P in feed should be balanced, the high P content will reduce the availability of Ca, because Ca is easily digested in acid. Roland et al. (2003) stated that the decrease in phosphorus content of feed from 0.4-0.1% decreased egg production. Phosphorus is an element to form energy and has an important role in the metabolism of carbohydrates, amino acids and fat, metabolism of nerve tissue, normal blood chemistry, the growth of the skeleton and fat transport of other lipids. All chemical reactions in the cell require energy, and this can be established by means of an overhaul of Adenosine Tri Phosphate (ATP) (Muray, 2003). Phosphorus has interaction with other minerals i.e. Mg and vitamin D (Higdon, 2003). Linder (1984) stated that approximately 60% of Mg is in bone and the rest mainly in the soft tissue cells with the main function to stabilize the structure of ATP in enzyme reactions that require ATP. The use of 7.5% eggshell in feed replaces total limestone. The result of this research showed that the quality of calcium eggshell was better then limestone. Similarly, eggshell phosphorus had high availability. Rovenský et al. (2003) stated that the bioavailability of calcium from eggshell is better than purified calcium carbonate. Phosphorus is major inorganic constituents of the avian eggshell (Cusack, et al., 2003). Supplementation of inorganic phosphorus supported optimal egg production (Snow et al., 2003). Kerstetter et al. (2003) suggested that the increase of protein intake increases calcium absorption in small intestine. Calcium, phosphorus and protein intake increased in applied eggshell 7.5% (feed 4), therefore obtained the highest egg production.

Eggshell also contains Mg, Fe, Zn, Se, Cu and Mn, so the use of 7.5% eggshell in feed (T3) increased egg production. According to Gaal et al. (2004), Mg is involved in many biochemical processes, including as the phosphate activator and the metabolism of carbohydrates. Mg intake can increase the digestibility of feed and increase egg production. Ogawa et al. (2004) stated that Mg in eggshell is bound with carbonate to form MgCO₃. According to Leeson and Summers et al. (2005), MgCO₃ is an available Mg. Zinc is a very important mineral in biological processes in the body of poultry and Zn is an essential component of several enzymes, and it has both structural and catalytic function in metalloenzymes. Zn binds protein and acts as the coenzyme (Sahin et al., 2009). Se binds proteins in cells are associated with glutathione, thiamine and biotin (Georgievskh et al., 1982). According to Murray et al. (2003) enzyme plays an important role in physiological processes. Many enzymes requiring coenzyme that acts to increase the catalytic ability to become bigger. Metal ions participate in increasing the chemical reaction speed of enzymes. Metal ions most involved in enzymatic catalysis are Fe, Mg, Co (in coenzyme B12) and Mn. Increasing Se in feed (0.55 to 0.75 mg/kg) had a positive effect on egg production and egg weight. Se source had no significant effect on egg production and egg weight (Scheideler et al., 2010). Se has the role for the health, growth and the body’s physiological processes. Organic Se has higher bioavailability than inorganic Se (Utterback et al., 2005). Se is an essential part of glutathione peroxides (GPx), which controls the activity of metabolism. Egg production was affected by Se level of feed. Egg production was greater in hens feed 0.3 mg/kg than 0.1 mg/kg of Se (Leeson et al., 2008).

**Feed Conversion Ratio**

The use of different calcium source had no
effect on feed conversion ratio. Feed conversion ratio was not significantly different due to the increase of feed intake, protein, calcium and phosphorus intake and is followed by an increase in egg production and egg weight significantly. Kingori et al. (2010) stated that protein level of feed had not significantly effect on feed conversion ratio although it increased egg production. According to Pelcia et al. (2009), the increased Ca intake of 0.60 g/hen/day did not improve feed conversion ratio, however, increased intake of Ca from 3.78 to 5.55 g/hen/day increased feed conversion ratio. Increased phosphorus intake from 0.13 to 0.5 mg/hen/day did not affect feed conversion ratio. In this study, increased Ca intake from 3.60 to 4.02 g/hen/day and phosphorus intake of 0.48 to 0.64 mg/hen/day did not result in a change of feed conversion ratio. The results showed that the use of 7.5% eggshell was efficient in terms of feed conversion ratio.

CONCLUSION

The results of this study indicate that the eggshell waste would be suitable sources of calcium for laying hens. The use of 7.5% eggshell waste increase feed intake, protein intake, calcium and phosphorus intake, egg production and egg weight but not increase feed conversion ratio.

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