ABSTRACT

A feeding trial was conducted to study the use of natural limestone meal originated from Bukit Kamang located at Agam district of West Sumatra to substitute fresh water oyster shell as the main source of calcium in diet of laying hens. The mineral feed formula, as treatments, composed of limestone in combination with freshwater oyster shell in five different percent ratios: 100:0; 75:25; 50:50; 25:75 and 0:100. The minerals were mixed in the level of 6 % into basal diet composed mainly corn, rice bran and concentrates. The five experimental diets were then fed to 150 laying hens. They were divided into 5 groups; each group was subdivided into 3 replicates groups containing 10 hens. Parameters measured included: feed intake, egg production, FCR, eggshell quality, Ca and P retention, tibia bone mineralization. Results showed the nutritive values of limestone of Bukit Kamang as mineral source of laying hen diet were found not significantly different from those of oysters shell. The hens fed with diet supplemented with Bukit Kamang’s limestone tended to give better laying performances than those of supplemented with fresh water oyster shell. The laying performances, egg shell quality and Ca and P retentions did not significantly improved, when limestone was mixed by oyster shell.

Keywords: Mineral Feed, Limestone, Freshwater Oyster Shell, Layer Nutrition.

INTRODUCTION

Calcium (Ca) is the most important mineral in the diet of laying hens. During laying period, laying chicken need 3-4% Ca in the diet or about 6-7 times of phosphor requirement which is 0.5-0.8% in the diet (Scholtyssen, 1987). Calcium is mainly used for eggshell formation. Deficient of calcium causes disturbance of eggshell formation which resulted to thin and fragile eggshell. It was estimated that 13-20% of total egg production was cracked or lost before reaching their final destination (Roland, 1988).

Calcium requirement of laying hens could not be fulfilled by sources of energy, protein and premix in the diet. The diet must be supplemented by high Ca sources. The most common sources of calcium for layer feeds are oyster shell and limestone (Roland, 1989).

In West Sumatra, most of farmers use fresh water oyster shell as calcium source for layer diet. Fresh water oyster (Corbicula sp) with local named “pensi” was abundantly found in fresh water bodies in West Sumatera, especially in three prominent lakes of Maninjau, Singkarak and Kembar. They were collected by fishermen mainly to obtain edible flesh bodies for protein-rich food. The shell parts which were locally called “kulit pensi” represent about 41-50% of total weight. The shell used as feed in coarse ground form contain about 26-30% calcium (Khalil, 2003).

In accordance with increasing awareness of the consumers for healthy food from animal products, the use of fresh water oyster shell should be stopped or limited for feed of laying hens. Oyster shell in fresh form were usually kept by stacking up in lake sides without properly protection measures or spread them out on the road for drying and grinding by vehicle wheels. Because of such poor handling and processing practices, fresh oyster shells are contaminated by sand and other dirty materials. In addition, the products...
both in raw and meal forms often give sting odor most likely due to bacterial contaminations (Khalil, 2004).

Limestone is an alternative feed material for substitution of fresh water oyster shell. West Sumatra abounds with natural limestone deposits in the form of mountains and hills, which are distributed in several cities and districts of Agam, Lima Puluh Kota, Pesisir Selatan, Pasaman, Sawahlunto, Sijunjung and Padang Panjang. One of the most intensively exploited limestone hill deposit is named Bukit Kamang, located at Kamang Mudik villages, Kamang Magek subdistrict, Agam district. Limestone is firstly mined in large split form and then ground into meal form by crushing, grinding and milling processes. The meal products which are mainly utilized or sold as lime for soil fertilizer were potentially used for mineral feed (Khalil and Anwar, 2007).

Bukit Kamang’s limestone contains about 38-40\% calcium. They are also rich on micro minerals of iron (Fe), manganese (Mn) and selenium (Se) which are essential for laying hens (Khalil and Anwar, 2007). In addition to nutrient sources, the use of limestone in the laying diet has several advantages. Limestone is much cheaper than oyster shell. They are also not easily contaminated by microorganisms during storage and processing. Moreover, limestone with particular physical properties might give positively effect on digestion process in gizzard, so that it might improve feed utilization efficiency (Schotyssek, 1987).

The present study was aimed to define the effect of limestone originated from Bukit Kamang for substitution of fresh water oyster shell as main calcium source in the diet on the performances of laying hens.

**MATERIALS AND METHODS**

**Experimental Diets and Treatments**

Limestone meal used in this study was obtained from a limestone milling company of CV. Bukit Raya, located in Durian village, Kamang Mudik, Kamang Magek subdistrict, Agam district, West Sumatra. Raw fresh water oyster shells were bought from a fisherman near Singkarak Lake, Tanah Datar district. The oyster shell were then dried and ground by using the same milling machine for limestone.

A basal diet was prepared by using three main component of commercial layer concentrate, corn and rice bran in the level of 30, 40 and 22 \%, respectively. This formula was referred to the diet used by layer farmers in West Sumatra. The basal diet was supplemented by 2\% bone ash to adjust P level in accordance with standard P requirement of laying hens.

The experiment consisted of five treatments in the form of limestone and fresh oyster shell mixtures in five different percentage ratios of 100:0; 75:25; 50:50; 25:75 and 0:100. The mixture ratios were defined mainly based on practical implementation by farmers. Each formula was then mixed by basal diet in the level of 6\%, which was in compliance with mineral level practiced by farmers in West Sumatra. Moreover, Bukit Kamang’s limestone contained toxic mineral of cadmium (Cd) of about 7 ppm (Khalil and Anwar, 2007). According to NRC (1980) maximum allowable level of Cd in poultry diet is 0.5 ppm. The use of limestone of Bukit Kamang is therefore limited maximum to 7 \% in the diet in order to meet the limited tolerance level recommended by NRC (1980).

| Treatment | Limestone % | Oyster Shell % |
|-----------|-------------|----------------|
| 100:0     | 100         | 0              |
| 75:25     | 75          | 25             |
| 50:50     | 50          | 50             |
| 25:75     | 25          | 75             |
| 0:100     | 0           | 100            |

Table 1 shows the formula of the experimental diets and their nutrient and energy contents. The nutrient and energy compositions which were calculated based on chemical analysis of feed components were justified to the standard requirements of laying hens during production period recommended by NRC (1994) and Scholtyssek (1987).

**Experimental Animals**

The research was conducted at the Poultry Farm of Faculty of Animal Husbandry, Andalas University, located at Limau Manis, Padang, by using 150 laying hens of Isa Brown strain, aged 4.5-5.0 months. The hens were divided into three groups, each group of 50 birds, based on body weight: light (1200-1399 g/bird), medium (1400-1599 g/bird) and heavy (1600-1769 g/bird). Each group was then subdivided into 5 subgroups, each 10 birds, in accordance with the number of treatments, so that each treatment consisted of 3 groups as replicates. Each replicate consisted of 10 birds. They were randomly placed in individual battery cages. Each cage was equipped with feed and drinking water troughs. The birds were vaccinated for Newcastle (ND) and Pullorum diseases about two weeks after starting of feeding trial.
Shell membrane was removed carefully, eggshell part was separated with interior content. Sample egg was weighed and then broken, so that weight and eggshell quality measurements. Each eggs per week. They were brought to laboratory for 7 days period, so that there were in total 30 sampling each treatment were randomly collected from each

Data on mineral retention were obtained through collection of excreta of the birds for 7 days at the k 19th weeks. One bird was randomly selected in each replication or 3 birds per treatment, so that there were 15 birds used for excreta collection. Collected feces in fresh form were daily weighed and then dried. The dried feces were then ground and chemically analyzed for ash, Ca, P and moisture contents. Mineral retention was calculated by using the following formula: Ca retention = \( \frac{(Ca \text{ intake} - Ca \text{ excreta})}{Ca \text{ intake}} \) x 100. At the end of the experiment, eggshell in the same replication were mixed. The total numbers of 15 samples of eggshell were then ground for chemically analysis of Ca, P, ash and moisture contents.

**Table 1. Composition of Experimental Diets**

| Feed components: (%) | 100:0 | 75:25 | 50:50 | 25:75 | 0:100 |
|----------------------|-------|-------|-------|-------|-------|
| Concentrate          | 29.9  | 29.9  | 29.9  | 29.9  | 29.9  |
| Corn                 | 39.9  | 39.9  | 39.9  | 39.9  | 39.9  |
| Rice bran            | 21.9  | 21.9  | 21.9  | 21.9  | 21.9  |
| Bone ash             | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   |
| Limestone meal       | 6.0   | 4.5   | 3.0   | 1.5   | 0.0   |
| Fresh water oyster shell meal | 0.0   | 1.5   | 3.0   | 4.5   | 6.0   |
| Grit                 | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   |
| Total                | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

Calculated nutrient and energy compositions:

|                | 100:0 | 75:25 | 50:50 | 25:75 | 0:100 |
|----------------|-------|-------|-------|-------|-------|
| Crude protein, % | 17.69 | 17.69 | 17.69 | 17.69 | 17.69 |
| Crude fiber, %   | 5.28  | 5.28  | 5.28  | 5.28  | 5.28  |
| Ca, %            | 3.71  | 3.69  | 3.66  | 3.64  | 3.61  |
| P total, %       | 0.48  | 0.48  | 0.48  | 0.48  | 0.48  |
| ME, kkal/kg      | 2607  | 2607  | 2607  | 2607  | 2607  |

**Parameters Measured**

The research was lasted for 24 weeks and started by about 20% of hen-day egg production. Parameters measured included: body weight, feed intake, feed conversion ratio (FCR), hen-day egg production, number and weight of egg production, mortality, eggshell quality (weight and percentage of eggshell, percentage of broken or cracked egg), mineral retention and weight and mineral composition of tibia bone.

Experimental diets were prepared weekly and stored in plastic containers. At the end of each week, the feed rest both in plastic containers and in feed trough were weighed to find out weekly feed intake data. Feed and water offered ad libitum. Egg were collected twice daily at 9.30 am and 3.30 pm. The number of eggs laid by birds in each replication were recorded daily and expressed weekly hen day egg production per cent.

Two eggs from each replication or six eggs from each treatment were randomly collected from each 7 days period, so that there were in total 30 sampling eggs per week. They were brought to laboratory for weight and eggshell quality measurements. Each sample egg was weighed and then broken, so that eggshell part was separated with interior content. Shell membrane was removed carefully, eggshell was measured for thickness. After then dried in the air, the dried shell weight was taken by using electric balance. The shell percentage was calculated by using the following formula: Shell percentage = (Weight of eggshell/Egg weight) x 100. At the end of the experiment, eggshell in the same replication were mixed. The total numbers of 15 samples of eggshell were then ground for chemically analysis of Ca, P, ash and moisture contents.

Experimental diets supplemented with limestone and fresh water oyster shell mixtures in percentage ratios:

The research was lasted for 24 weeks and started by about 20% of hen-day egg production. Parameters measured included: body weight, feed intake, feed conversion ratio (FCR), hen-day egg production, number and weight of egg production, mortality, eggshell quality (weight and percentage of eggshell, percentage of broken or cracked egg), mineral retention and weight and mineral composition of tibia bone.

Experimental diets were prepared weekly and stored in plastic containers. At the end of each week, the feed rest both in plastic containers and in feed trough were weighed to find out weekly feed intake data. Feed and water offered ad libitum. Egg were collected twice daily at 9.30 am and 3.30 pm. The number of eggs laid by birds in each replication were recorded daily and expressed weekly hen day egg production per cent.

Two eggs from each replication or six eggs from each treatment were randomly collected from each 7 days period, so that there were in total 30 sampling eggs per week. They were brought to laboratory for weight and eggshell quality measurements. Each sample egg was weighed and then broken, so that eggshell part was separated with interior content. Shell membrane was removed carefully, eggshell was measured for thickness. After then dried in the air, the dried shell weight was taken by using electric balance. The shell percentage was calculated by using the following formula: Shell percentage = (Weight of eggshell/Egg weight) x 100. At the end of the experiment, eggshell in the same replication were mixed. The total numbers of 15 samples of eggshell were then ground for chemically analysis of Ca, P, ash and moisture contents.

Data on mineral retention were obtained through collection of excreta of the birds for 7 days at the k 19th weeks. One bird was randomly selected in each replication or 3 birds per treatment, so that there were 15 birds used for excreta collection. Collected feces in fresh form were daily weighed and then dried. The dried feces were then ground and chemically analyzed for ash, Ca, P and moisture contents. Mineral retention was calculated by using the following formula: Ca retention = \( \frac{(Ca \text{ intake} - Ca \text{ excreta})}{Ca \text{ intake}} \) x 100. At the end of the experiment, the 15 birds collected their feces were slaughtered and link tibia bone were taken. The tibia bones were the weighed and dried. The dry bone were then ground and analyzed for ash and Ca, P and moisture contents.

**Statistical Analysis**

All data were subjected to statistical analysis using variance analysis in a completely block design with 5
treatments and 3 blocks as replicates. Duncan’s Multiple Range Test (DMRT) was applied to separate means. Differences were considered significant at P<0.05 (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

Laying Performance

The data presented in Table 2 show that the use of Bukit Kamang’s limestone and fresh water oyster shell both in single form and its mixtures in different ratios did not significantly influence (P>0.05) body weight, feed intake, egg production, feed conversion ratio and mortality. The average body weight at initial stage of trial was 1481 g/bird. At the end of the experiment, body weight increased for every treatment irrespective of different sources of calcium of about 169 g/bird, so that the average final body weight was about 1650 g/bird. Total feed intake for 24 weeks ranged 20,224 - 20,730 g/bird, while daily feed intake about 1650 g/bird. Total feed intake for 24 weeks ranged 121.4 g – 123.4 g/bird.

Table 2. Body Weight, Feed Intake, Feed Conversion Ratio, Mortality and Egg Production of Laying Hens Fed Diets Containing Limestone and Fresh Water Oyster Shell For 24 Weeks

| Experimental diets supplemented with limestone and fresh water oyster shell mixtures in percentage ratios: | 100:0 | 75:25 | 50:50 | 25:75 | 0:100 |
|---|---|---|---|---|---|
| P1 | P2 | P3 | P4 | P5 |
| Initial body weight, g/bird | 1,505.0 | 1,468.0 | 1,473.3 | 1,518.5 | 1,466.0 |
| Final body weight, g/bird | 1,659.3 | 1,616.0 | 1,642.0 | 1,674.7 | 1,670.3 |
| Total feed intake, g/bird | 20,659.2 | 20,424.2 | 20,500.8 | 20,221.9 | 20,223.8 |
| Daily feed intake, g/bird | 123.0 | 121.6 | 122.0 | 120.4 | 120.4 |
| Egg production, eggs/bird | 131.0 | 121.5 | 126.2 | 127.7 | 114.7 |
| Egg production, g/bird | 7,391.3 | 6,971.9 | 7,159.0 | 7,303.8 | 6,444.9 |
| Hen-day egg production, % | 77.9 | 72.1 | 75.1 | 76.0 | 68.3 |
| Feed conversion ratio | 2.80 | 2.93 | 2.86 | 2.77 | 3.14 |
| Mortality, % | 0.0 | 10.0 | 0.0 | 6.7 | 10.0 |

Although the results were not significantly different, birds fed on the diets containing only limestone (P1) showed higher egg production in term of number, total weight and hen-day egg production (average: 131 eggs/bird; 7391 g/bird; 78%, respectively) than those fed on diet supplemented only fresh water oyster shell meal (P5) (115 egg/bird; 6445 g/bird; 68%). Figure 1 shows that birds fed on diet supplemented only with fresh water oyster shell (P5) produced lowest hen-day egg production during 24 weeks observation.

Considering the effect of limestone meal mixed with fresh water oyster shell meal in different ratios results indicated that there was no significant (P>0.05) effect of different ratios of mixture of Bukit Kamangs’ limestone meal and fresh water oyster shell meal (P1 vs. P2, P3 and P4) on the egg production and feed utilization efficiency. These results were in agreement with some previous research findings. Cheng and Coon (1990) supplied dietary calcium from various sources and found no significant different in body weight and egg production. Oliveira et al (1997) supplied dietary calcium from different sources and found no significant difference in egg production and body weight.

Eggshell Quality

The results of egg weight and quality of eggshell are presented in Table 3. Figure 2 shows development of egg weight for 24 weeks observation. Average egg weight ranged 55.8 to 56.8 g/egg. Birds fed on
difference in egg weight. Scheideler (1998) and Rabon et al. (1991) also observed that egg weight did not significantly differ due to various calcium sources.

The data presented in Table 3 also show that substitution of fresh water oyster shell meal with Bukit Kamangs’ limestone meal did not significantly (P>0.05) influence shell weight, shell thickness, shell per cent, cracked egg per cent and mineral and ash content of shell. The lowest cracked eggs (3%) were found by birds fed on diets supplemented only Bukit Kamangs’ limestone meal (P1), while birds fed on diet containing mixtures of limestone and fresh water oyster shell, especially by mixture ratios of 50:50 (P3: 5.7%) and 25:75 (P4: 6.5%) tended to give higher percentage cracked eggs. However, these results were statistically not significant difference. These finding coincided with the results Cheng and Coon (1990). They observed no significant difference on shell weight, shell percentage and shell thickness, when oyster shell was replaced by limestone. Richter et al. (1999) used calcium from various sources and found no significant difference in shell weight.

Mineral Retention and Bone Parameter

Data on mineral retention, tibia bone weight and bone mineral composition is summarized in Table 4. The highest bone weight (39 g) was found by birds fed on diet containing only Bukit Kamangs’ limestone meal (P1), while the lowest (32 g) was by birds offered diet containing only fresh water oyster shell meal (P5), but there were statistically no significant difference. Moreover, substitution of fresh water oyster shell meal with Bukit Kamangs’ limestone

| Table 3. Average Egg Weight, Shell Weight, Shell Thickness, Per Cent of Shell Weight, Cracked Egg and Mineral Composition of Shell |
|---------------------------------------------------------------|
| **Experimental diets supplemented with limestone and fresh water oyster shell mixtures in percentage ratios:** |
| 100:0 | 75:25 | 50:50 | 25:75 | 0:100 |
|---|---|---|---|---|
| **P1** | **P2** | **P3** | **P4** | **P5** |
| Egg weight, g/egg | 56.4 | 56.7 | 56.8 | 56.6 | 55.8 |
| Eggshell weight, g/egg | 5.53 | 6.00 | 5.45 | 5.43 | 5.22 |
| Per cent egg shell, % | 9.8 | 10.5 | 9.6 | 9.7 | 9.9 |
| Tebal kerabang, mm | 0.54 | 0.54 | 0.54 | 0.54 | 0.54 |
| Per cent cracked egg, % | 3.0 | 3.6 | 5.7 | 6.5 | 4.0 |
| Mineral and ash composition of shell (% DM): |
| - Ca | 32.8 | 35.9 | 36.7 | 36.9 | 35.8 |
| - P | 0.26 | 0.31 | 0.36 | 0.37 | 0.39 |
| - Ash | 73.9 | 79.5 | 74.2 | 76.5 | 71.2 |

Figure 1. Hen-Day Egg Production of Laying Hens Fed Diets Containing Limestone and Fresh Water Oyster Shell for 24 Weeks.

Figure 2. Average Egg Weight of Laying Hens Fed Diets Containing Limestone and Fresh Water Oyster Shell for 24 Weeks.
meal did not significantly (P>0.05) influence mineral retention and bone mineral composition. Sounders-Blades et al (2009) also reported that the use of limestone and oyster shell as the main calcium sources in the diets of laying hens gave no significant different effect on tibia bone mineralization.

The presented results indicated that Bukit Kamangs’ limestone meal tended to have better nutritive value than that of fresh water oyster shell as calcium sources in the diet of laying hens. The use of fresh water oyster shell meal in different mixture ratios with limestone gave no significant effect on laying performance, eggshell quality and bone mineralization. The positive effect of limestone as calcium sources for laying hens was noted by Guinotte and Nys (1991), Fleming et al. (1998), Richter et al. (1999) and Roland (2000). This could be explained as the effect of particulate limestone on constant measurement of Ca in gastrointestinal tract. It seems also that the positive effect of Bukit Kamangs’ limestone was due to their beneficial physical properties, particularly with respect to hardness and larger particle size. Richter et al. (1999) found that optimal particle size of limestone for laying hen was 0.5-2.0 mm, while about 18% of Bukit Kamangs’ limestone meal had particle size of 0.5-2.0 mm (Nurleni, 2005). Moreover, Roland (2000) mentioned that any particle of calcium sources exceeding about 1 mm in size will be retained in the gizzard and the calcium will be released slowly into the blood stream. In contrast, the smaller particles moved quickly through the digestive tract and were only partially dissolved.

**CONCLUSION**

Considering the above results it may be concluded that limestone originated from Bukit Kamang could be used to substitute fresh water oyster shell as calcium sources in the diet of laying hens. Mixture of limestone with fresh water oyster shell gave no significant improvement on laying performances, eggshell quality and bone mineralization. Fresh oyster shell could be therefore completely replaced by Bukit Kamangs’ limestone as calcium source in the diet of laying hens.

**REFERENCES**

Cheng, T.K. and C.N. Coon, 1990. Effect on layer performance and shell quality of switching limestones with different solubilities. Poult. Sci., 69:2199-2203

Fleming, R.H., H.A. McCormack and C.C. Whitehead, 1998. Bone structure and strength at different ages laying hens and effect of dietary particulate limestone, vitamin K and ascorbic acid. British Poult. Sci., 39:434-440

Guinotte, F. and Y. Nys, 1991. Effect of particle size and origin of calcium sources on eggshell quality and bone mineralization in egg laying hens. Poult. Sci., 70:583-592

Khalil. 2003. Analisa rendemen dan kandungan mineral cangkang pensi dan siput dari berbagai habitat air tawar di Sumatera Barat. J. Peternakan dan Lingkungan. 09(3): 35-41
Khalil. 2004. Pengaruh penggilingan dan pembakaran terhadap nilai nutrisi kulit pensi sebagai sumber utama mineral kalsium dalam ransum ayam broiler. Jurnal Peternakan dan Lingkungan. 10(1):35-42
Khalil dan S. Anwar. 2007. Studi komposisi mineral tepung batu Bukit Kamang sebagai bahan pakan mineral. Media Peternakan. 30(1): 18-25
Nurleni. 2005. Analisa kandungan mineral dan sifat fisik tepung batu yang berasal dari Bukit Kamang. Skripsi, Fakultas Peternakan Universitas Andalas, Padang.
NRC (National Research Council), 1980. Mineral tolerance of domestic animals. Washington D.C., USA.
NRC. 1994. Nutrient requirement of poultry. National Academic Press, Washington, DC.
Olveira, J.D.F., D.L. Oliveira and A.G. Bertechni, 1997. Calcium level, particle size and feeding form of limestone on the performance and egg quality of laying hens in the second laying cycle. Ciencia-E-Agrotechnologia. 21:502-510
Rabon, H.W., D.A. Roland, M. Bryant, D.G. Barnes and S.M. Laurent, 1991. Influence of sodium zeolite A with and without pullet-sized limestone or oyster shell on eggshell quality. Poult. Sci. 70:1943-1947
Richter, G., G. Kiessling, W.I. Ochrimenko und H. Luedke. 1999. Einfluss der Partikelgroesse und der Calciumquelle auf die In-vitro-Loeslichkeit des Calciums, die Leistungen und die Eischalenqualitaet bei Legehennen. Arch. Gefleugelk. 5:208-213
Rolland, D.A. 1988. Research note: egg shell problems estimates of incidence and economic impact. Poult. Sci. 67:801-1803
Rolland, D.A. 1989. Egg shell quality IV. Oyster shells versus limestone and importance of particle size of solubility of calcium source. World Poult. Sci. J. 42:166-171
Richter, G., G. Kiessling, W.I. Ochrimenko und H. Luedke. 1999. Einfluss der Partikelgroesse und der Calciumquelle auf die In-vitro-Loeslichkeit des Calciums, die Leistungen und die Eischalenqualitaet bei Legehennen. Arch. Gefleugelk. 5:208-213
Richter, G., G. Kiessling, W.I. Ochrimenko und H. Luedke. 1999. Einfluss der Partikelgroesse und der Calciumquelle auf die In-vitro-Loeslichkeit des Calciums, die Leistungen und die Eischalenqualitaet bei Legehennen. Arch. Gefleugelk. 5:208-213
Scholtyssek, S. 1987. Geflügel. Eugen Ulmer Verlag, Ulm, Germany.
Scheideler, S.E., 1998. Eggshell calcium effect on egg quality and Ca digestibility in first- or third-cycle laying hens. J. Appl. Poult. Res. 7:69-74
Scheideler, S.E., 1998. Eggshell calcium effect on egg quality and Ca digestibility in first- or third-cycle laying hens. J. Appl. Poult. Res. 7:69-74
Steel, R.G.D. and J.H. Torrie. 1981. Principles and Procedures of Statistics. McGraw-Hill International Book Company, Auckland.
