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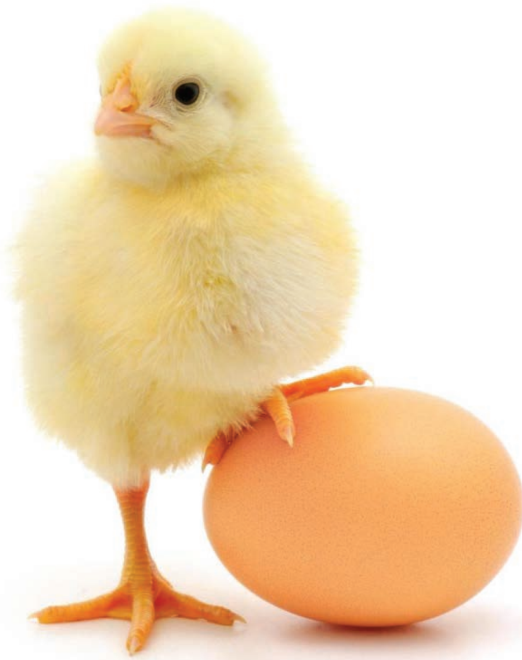

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Research Article
Effect of Different Levels of Egg Shell in Diet on the Production Performance, Egg Quality and Heavy Metal Contents in Laying Hens

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Abstract
Background and Objective: In Bangladesh, huge amount of eggshell-waste is produced every day. Most of them are discarded into the environment. However, it can be a resource upon proper management. As eggshell contains around 40% calcium which is beneficial as poultry feed. Therefore, the present experiment was conducted to convert eggshell-waste as a dietary source of laying hen. **Materials and Methods:** In this experiment, a total of 50 kg of eggshell was collected from farm and local food vendors. At 30 weeks of age, a total of 100 laying hens were randomly selected and were divided into 5 groups with 4 replications. The treatments were: T_0: Control group-100% limestone, T_1: 75% limestone and 25% eggshell based-feed, T_2: 50% limestone and 50% eggshell based-feed, T_3: 25% limestone and 75% eggshell based-feed and T_4: 100% eggshell-feed. The hens were fed with 120 g/hen/day and water was provided *ad-libitum* during the experiment. The standard management system was followed. **Results:** A significantly higher egg weight and eggshell weight was found by feeding of 100% eggshell based-feed treatment ($p<0.05$). No significant differences were observed in the laying performance, egg production, body weight and egg quality among the treatments but the eggshell breaking strength was improved by using eggshell as poultry feed. Therefore, 3.80 kg/cm^2 egg-shell breaking strength was found in 100% eggshell-feed which was numerically higher among the treatments. Inclusion of eggshell to the laying hen diet resulted in similar production performance and egg qualities compared to those of control. On the other hand, heavy metals (Pb, Cr, Cd) concentration followed in decreasing trend. Therefore, Egg shell would be a good source of poultry feed. **Conclusion:** Based on the experiment it can be concluded that eggshell may be used as an alternative source of calcium rather than throwing as waste.

Key words: Eggshell, poultry feed, laying hen, production performance, calcium

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As global awareness of organic waste materials and environmental problems has grown, hen eggshell, a waste material from domestic sources such as hatcheries, poultry farms, fast food industries, egg product manufacturers, residences and restaurants, has become a severe issue[1,2]. In Bangladesh, the egg demand is increasing and the demand level is per capita 104 number[3]. Increasing number of bakery industry is a tremendous producer of egg-shells e.g. The PRAN use 2.5 lakh egg per day which generates 1800 kg day^{-1} eggshell. Therefore, it generates a big problem in food industry and usually turned into landfill. Recycling of eggshell is being done worldwide to solve landfill problem but it has become a challenge in Bangladesh. But there is a scope to use eggshell as food additive, animal feed, soil amendment, purified $CaCO_3$, cosmetics, biomaterial composites, heavy metal immobilization, water and wastewater pollutant removal, pharmaceutical products etc[4].

In Bangladesh, the recycle of eggshell was not introduced, therefore, the eggshell waste usually goes to the landfilling zone. This generates the environmental problem such as bad odour, calcium carbonate leaching to water body etc. With bacterial contamination it can also produce food-borne diseases. The poultry population increased from 2626.28 lakh in 2007-08 to 3379.98 lakh in 2017-18. Around 20 and 50% people are directly and partially involved with livestock and poultry sector, respectively. The production of eggs also increases during the last 10 years. The per year egg production in 2017-18 was 1552.00 crore[3]. The high amount of eggs also generate huge amount of waste but eggshell is a pure source of $CaCO_3$[5]. It fulfills the calcium requirement of hens through feed and every kg of poultry feed must contain 37 g of calcium[6]. So, for eggshell formation a hen needs to take 2.5-3.5 g Ca as a calcite form. For this reason, it needs to take 3.4-3.8% of $CaCO_3$[7]. Poultry feed industry mostly depends on limestone, oyster shell as a source of calcium. Oyster shell contains toxic element such as Aluminium (Al), Cadmium (Cd), Mercury (Hg) etc[5]. Furthermore, high levels of magnesium, sand and silica are common problems in limestone[8]. As a result, finding an alternate source of calcium at a lower cost is a pressing requirement[8]. Eggshell is cheaper than oyster and limestone[8] and did not have any toxic element[9]. It can increase resistance of chickens to endotoxin[10]. In addition, it reduces the cost of eggshell disposal and also avoids the emission of foul odors and $CaCO_3$ mining.

In Bangladesh, the poultry feed ingredients are imported from different countries. However, using eggshells in poultry feed can reduce the import cost which is a sustainable, beneficial and cost-effective solution of eggshell waste. Therefore, this experiment was conducted to convert eggshells as a value-added product. The objectives of this experiment were to determine the properties of eggshell as a source of calcium for laying hens and compare the effectiveness of eggshell as a feed and its effect on production performance and egg qualities of laying hens.

MATERIALS AND METHODS

Eggshell collection and preparation for poultry feed: The eggshells were collected from PRAN (Kallyanpur Branch, bakery industry) and from the Canteen and Bottola of Jahangirnagar University, Savar, Dhaka. Total 50 kg of eggshells were collected from the study area.

The eggshells were washed with tap water[9] and sun dried for several days. After that it was ground to form 1-2 mm powder using feed mill. After grinding, eggshell was autoclaved to remove the pathogenic microorganisms (bacteria, virus) from the feed. The autoclave (model: WiseClave) temperature was 121 °C and 121 lb pressure for 15 min. The autoclave was done with a specialized bag which could tolerate up to 121 °C. Then the ground eggshell was stored at room temperature for feed formulation.

The composition of the eggshell was analyzed with a flame photometer for Ca, atomic absorption spectroscopy for other trace elements, digestion and distillation for crude protein and accounts of proximate analysis for other ingredients (Table 1).

Poultry feed formulation of experimental diet: A total of 100 White Leghorn laying hens (30 weeks of age) were distributed in completely randomized design with 4 replicates and 5 treatments: T_0: Control group -100% limestone, T_1: 75% limestone and 25 % eggshell-feed, T_2: 50% limestone and 50% eggshell-feed, T_3: 25% limestone and 75% eggshell-feed and T_4: 100% eggshell-feed.

Table 1: Composition of the ground eggshell analyzed by proximate analysis

Sample	Ca (%)	DM (%)	CP (%)	Ash (%)	Moisture (%)	Pb (ppm)	Cr (ppm)	Cd (ppm)
Grounded eggshell (before autoclave)	50	98.6	4.21	93.06	1.4	1.11	0.18	0.03
Grounded eggshell (after autoclave)	40	98.8	5.13	90.18	1.2	1.04	0.22	0.02

Ca: Calcium, DM: Dry matter, CP: Crude protein, Pb, lead, Cr: Chromium and Cd: Cadmium

Table 2: Experimental diet composition (per 100 kg)

Ingredients	T_0	T_1	T_2	T_3	T_4
Maize	58.05	58.18	58.21	58.16	57.91
Soyabean	26.34	26.14	26.07	25.78	25.6
Rice polish	3	3	2.6	2.5	2.40
Protein concentrate	1	1	1	1	1
Soyabean oil	1	1	1.13	1.26	1.43
L lysine	0.1	0.1	0.1	0.1	0.1
DL methionine	0.1	0.1	0.1	0.1	0.1
Limestone dust	4.7	0.13	0	0	0
Limestone grit	4.5	6.65	4.6	2.43	0
Eggshell	0	2.5	5	7.5	10.3
DCP	0.87	0.86	0.85	0.84	0.83
Sodium chloride salt	0.34	0.34	0.34	0.33	0.33
Poultry vitamin LC**	0.05	0.05	0.05	0.05	0.05
Poultry mineral**	0.05	0.05	0.05	0.05	0.05
Nutrient analysis (%)					
Dry matter (%)	89.4	90.2	90.6	90.7	90.4
ME kcal kg^{-1}	2750	2750	2750	2750	2750
CP (%)	18.51	19.42	19.82	17.27	19.39
CF (%)	3.096	3.096	3.096	3.096	3.096
ASH (%)	14.61	11.22	15.72	13.59	12.93
EE (%)	3.671	3.671	3.671	3.671	3.671
Calcium (%)	3.75	3.75	3.75	3.75	4
Total phosphorus (%)	0.544	0.544	0.544	0.544	0.544
Available phosphorus (%)	0.3	0.3	0.3	0.3	0.3

*T_0: Control feed (100% limestone), T_1: 75% limestone and 25% eggshell-feed, T_2: 50% limestone and 50% eggshell-feed, T_3: 75% limestone and 25% eggshell-feed, T_4: 100% eggshell, **The vitamin/mineral premix provided per kg of diet: Retinyl acetate: 3.0 mg, Vitamin D_3: 3000 IU, Vitamin E: 30 mg, Niacin: 25 mg, Ca pantothenate: 8 mg, Thiamine: 2.0 mg, Riboflavin: 5 mg, Pyridoxine: 4 mg, Folic acid: 0.5 mg, Biotin: 0.075 mg, Cobalamin: 0.01 mg, Choline chloride: 250 mg, Menadione: 2.0 mg, Betaine: 100 mg but ylated hydroxytoluene: 7.5 mg, Ethoxyquin: 5.6 mg butylhydroxyanisole: 1 mg, Mn: 70 mg, Zn: 50 mg, Fe: 40 mg, Cu: 6 mg, I: 1 mg, Co: 0.3 mg and Se: 0.2 mg

Bird and management: The experimental diet was formulated according to the standard procedure[11] (Table 2). The experiment was conducted at the poultry research farm, Poultry Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka. The birds were kept first three days for the adjustment with the diet. During the experiment the average temperature and relative humidity was 28-36.1°C and 71-85%, respectively. The feed intake, feed refusal and egg production was recorded on daily basis and body weight, egg weight and egg quality was determined once in a week.

Determination of calcium content in egg shell: The calcium content of the eggshells and treated feed were calculated using the Flame Photometer (JENWAY PFP-7) at the Centre for Advanced Research in Sciences (CARS), Dhaka University, Dhaka. For that measurement 2 g sample was taken from eggshell and a shed the sample using muffle furnace at 550 for 2 hrs. Then the sample was mixed with catalysts (0.3 g $CuSO_4$ and 0.7 g K_2SO_4) and 5 mL HNO_3. After that, the sample was heated for digestion. Then the sample was filtered using filter paper and 100 mL sample was prepared in the volumetric flask and analyzed in the flame photometer.

Determination of heavy metal content in egg shell: The heavy metals [lead (Pb), chromium (Cr) and cadmium (Cd)] were determined both in feed samples and eggshell. The samples were prepared with 1 g sample from each treatment and eggshell. The samples were digested using 10 mL HNO_3 and 5 mL $HClO_4$. Then the samples were filtered and filled with 100 ml solution[12]. After that, the samples were detected using Atomic Absorption Spectroscopy (AAS-SHIMADZU AA-7000 series) at Wazed Miah Science Research Centre (WMSRC), Jahangirnagar University, Savar, Dhaka.

Egg quality determination: To assess egg quality egg weight, egg length, egg width, eggshell breaking strength, albumen height, albumen width, albumen length, yolk height, yolk width, yolk colour and eggshell weight was determined at Bangladesh Livestock Research Institute (BLRI) laboratory Savar, Dhaka. Egg quality was determined once in a week. The length and width was determined using Digital Vernier Calipers (Mitutoyo, Japan). The eggshell breaking strength was measured using Eggshell Strength Tester (Fujihira Industry Co., Ltd., Japan) machine which gave the pressure to break the shell. The colour of the yolk was determined using Roche yolk color fan meter. The yolk and albumen height was measured with tripod height meter. Different measurements were taken as per standard procedures[13]. The egg quality also determined using the shape index, albumen index, yolk index and Haugh unit. Albumen and yolk index were determined as described by Doyon *et al.*[14] and Haugh unit was determined according to the method of Haugh[15] and Rabbani *et al.*[16].

Statistical analysis: Data were subjected to a one way analysis of variance (ANOVA) with the help of the General Linear Model procedure (GLM[17]) of the Statistical Analysis System (SAS) with a completely randomized design. The difference among treatments was compared using Duncan's multiple range test[18]. The treatment effect was considered significant at $p<0.05$. The standard error of mean (SEM) was also calculated for overall treatment.

RESULTS AND DISCUSSION

Production performance: Table 3 shows the body weight, production level, their egg mass, feed intake and feed conversion ratio. The results showed that there was no significant differences in the body weight, egg production, egg mass, feed intake and feed conversion ratio. But egg weight among the treatment was significantly different. T_4(100% eggshell-feed) had higher egg weight than that of T_2 and T_3. The lowest egg weight was observed in treatment group T_0 and T_1. Vandepopuliere[19] found a comparable egg weight in birds fed with eggshell meal and ground limestone as a source of calcium[8]. There was no significant difference in body weight among the treatment. Gongruttananun[20] reported that the eggshell mixed with other calcium sources had not any effect on body weight[9]. Makkar[21] did not find any significant difference in body weight of birds fed diet supplemented with eggshell membrane. Results of the present study agree with Vandepopuliere[22] who mixed eggshell in feed and reported non-significant difference in hen performance and feed conversion ratio (FCR). According to Scheideler[23] eggshell as a sole source of calcium had no significant effects on egg production as compared to other dietary sources of calcium for laying hens.

Egg quality determination: Table 4 shows the egg quality determination of experimental laying hens. The diet containing 100% eggshell (T_4) significantly improved egg

Table 3: Production performance of experimental laying hens (30-34 weeks)

Parameter	Treatments T_0	T_1	T_2	T_3	T_4	SEM	p-value
Body weight (g)	1458.45	1452.20	1457.70	1426.75	1462.65	21.46	0.649
Egg production (%)	75.54	71.79	77.50	67.50	73.22	1.62	0.37
Egg weight (g)	56.46^{b}	58.24^{b}	59.18^{ab}	59.80^{ab}	62.44^{a}	0.63	0.02
Egg mass (g)	42.59	41.86	45.99	40.50	45.79	1.11	0.46
Feed intake (g)	91.98	92.23	90.89	89.36	89.92	1.24	0.95
FCR	2.19	2.26	2.20	2.31	2.12	0.07	0.95

^{a,b}Values in the rows with different letters differ significantly ($p\leq 0.05$), FCR: Feed conversion ratio

Table 4: Egg quality determination of experimental laying hens (30-34 weeks)

Parameter	Treatments T_0	T_1	T_2	T_3	T_4	SEM	p-value
Egg weight (g)	56.08^{c}	58.59^{bc}	60.72^{ab}	61.33^{ab}	62.04^{a}	0.64	0.0052
Egg length (mm)	51.77	52.72	53.90	53.99	54.84	1.01	0.9118
Egg width (mm)	40.13	40.76	40.99	41.09	41.01	0.97	0.9985
ESBS* (kg/cm^2)	3.55	3.90	3.14	3.58	3.80	0.10	0.0868
Albumen height (mm)	9.18^{b}	9.04^{b}	10.33^{a}	9.95^{a}	10.26^{a}	0.15	0.0024
Albumen width (mm)	62.60	64.06	63.72	63.87	63.85	0.41	0.8436
Albumen length (mm)	74.59^{b}	75.77^{ab}	76.21^{ab}	78.37^{a}	78.89^{a}	0.58	0.0787
Color	6.19^{b}	6.49^{ab}	6.53^{ab}	6.49^{ab}	6.79^{a}	0.06	0.0319
Yolk height (mm)	17.86	18.35	18.41	18.40	18.48	0.09	0.2551
Yolk width (mm)	39.83^{b}	41.14^{a}	42.08^{a}	41.88^{a}	41.69^{a}	0.24	0.0110
Shell weight (g)	7.20^{b}	7.46^{b}	8.72^{a}	8.39^{a}	8.61^{a}	0.16	<.0001
Shape index (%)	77.53	77.31	76.55	76.19	74.79	0.50	0.4789
Albumen index (%)	45.12	44.71	43.91	40.52	44.95	0.86	0.4441
Yolk index (%)	13.29	12.89	14.77	13.61	14.40	0.27	0.1310
Haugh unit	95.83	94.81	100.14	95.12	99.72	1.09	0.3652

$^{a-c}$The values in the rows with different letters differ significantly ($p\leq 0.05$), *ESBS: Eggshell breaking strength weight, albumen height, yolk colour, yolk width and shell weight. Though the eggshell breaking strength (ESBS) was not significantly influenced by treated diet but numerically increased by T_4 treatment and which corresponds with previous findings[19,24]. It might be due to the digestibility of calcium of hens. According to Scheideler[23] ground eggshell has a significant greater digestibility than other calcium sources. On the other hand, shape index, albumen index, yolk index and Haugh unit were not influenced by the treatment ($p>0.05$). Similarly, Lichovnikova[24] reported no significant difference in albumen index of different calcium sources. Moreover, Lichovnikova[24] also found no difference in eggshell formation among different calcium treatments, because hens digest the calcium slowly from large particle but constantly and it is utilized immediately for eggshell formation.

The T_4 group showed the highest egg weight and shell weight and also better eggshell breaking strength. Vandepopuliere[22] found that inclusion of eggshell in layer diet had comparable breaking strength to limestone feed group. Guinotte and Nys[25] also found that there were no significant differences in breaking strength between calcium sources[8]. The shell weight ratio was significantly lower in limestone group[8,24]. On the other hand, the quality of albumen height and length was better than other treatments but the difference were not reached to the significant level.

The T_4 and T_1 showed better eggshell breaking strength than T_2 and T_0. Yolk height was lower in the T_0 than other treatments. There was no significant difference in egg length, egg width, albumen width and yolk height among the treatments.

Heavy metal concentration: In recent studies, it was found that heavy metal accumulation was increased in poultry feed in Bangladesh[26]. The maximum acceptable limit in feed for lead (Pb) and chromium (Cr) are 5 mg kg^{-1} and cadmium (Cd) is 2 mg/kg[27]. In this experiment eggshell contained lower amount of lead, chromium and cadmium than the permissible limit (Fig. 1-3).

The Pb concentration was significantly different among control (T_0) and other treatment groups (T_1, T_3, T_4) (Fig. 1). In T_4, the Pb concentration level was lower than that of other treatments. There was a decreasing trend of Pb in treated feed. According to Rashid[28], in Bangladesh, the loose feed contained 0.86-0.69 ppm of lead (Pb) which was very lower than that of the permissible limit. On the other hand, the permissible limit of Pb is <1 mg kg^{-1} in the United Kingdom[29]. However, according to the current official regulations, the accepted level of Pb content in feed ingredients is 10 ppm[30]. T_4 contained lower level of lead (Pb) than T_0 (control feed).

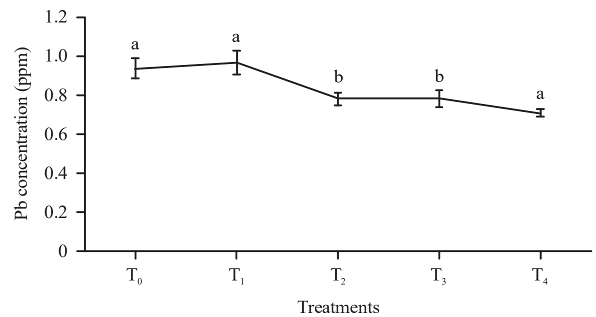

Fig. 1: Lead concentration (ppm) in different treatment groups

Error bar indicates Mean±SD (n = 20) and asterisk 'a,b' denotes significant difference ($p<0.05$) from the control (T_0) group

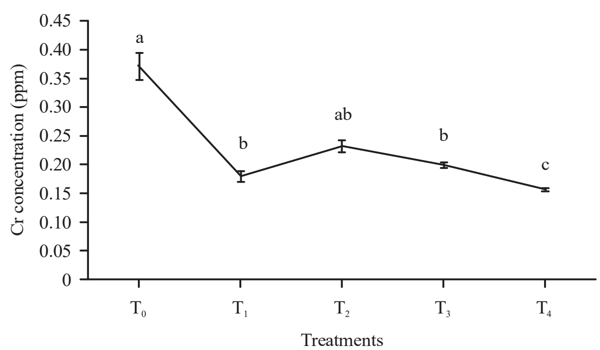

Fig. 2: Chromium concentration of different treatment

Error bar indicates Mean±SD where n = 20. Error bar indicates Mean±SD (n = 20) and asterisk a, b, c denotes significant difference ($p<0.05$) from the control (T_0) group

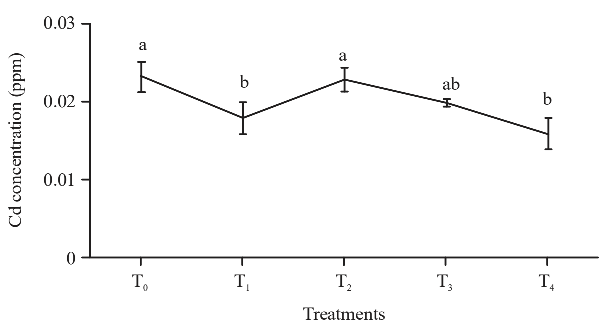

Fig. 3: Cadmium concentration of different treatment

Error bar indicates Mean±SD (n = 20) and asterisk a, b denotes significant difference ($p<0.05$) from the control (T_0) group

Moreover, eggshell contain 0.18-0.22 ppm chromium which is lower than that of the permissible limit and feed contained eggshell have less amount of chromium. It was found that the chromium level of control feed was higher than those of other feeds (Fig. 2). The chromium level of control group was significantly different from all other treatments. The chromium level was lower in the feed treated with eggshell. In the present experiment, Cr level was decreased with increasing level of egg shell in the diet. On the other hand, in T_2 feed, the level of Cr had increased due to the absence of the limestone dust, increasing level of limestone grit and insufficient eggshell. Because limestone grit or oyster shell had higher levels of metals than eggshell[5]. On the other hand, the Cr level decreased due to the increasing level of eggshell. In, T_4, the feed contained lower amount of Cr.

In poultry feed the amount of cadmium (Cd) present as a trace element. In this study, the Cd level of the eggshell was 0.02-0.03 ppm that is very much lower than that of the permissible limit (Fig. 3). Therefore, eggshell might be used as an adsorbent of Cd. Figure 3 shows that use of eggshell in poultry feed is highly beneficial to reduce the amount of Cd. The results of the present study showed that, significantly higher level of Cd was found in control group than that of treated group, Cd level decreased with increasing level of egg shell in the diet. It was also found that the level of Cd in control feed was higher than others. It was also observed that the use of higher level of limestone grit in T_2 increased the concentration of Cd. Finally the increasing level of eggshell could reduce the level of Cd from feed. It might be due to the presence of Carbonate Hydroxylapatite (CHAP) obtained from eggshell which is highly efficient for removal of Pb and Cr. The crushed eggshell had double adsorption capacity (21-160 mg g^{-1}) than activated carbon and animal bones[31]. On the other hand, eggshell is used as an adsorbent material for Cd. The Cd is a highly toxic metal with little exposure limit. In a recent report, it was found that ground eggshell had maximum adsorption capacity (329 mg g^{-1}) of Cd[31,32]. So, eggshell feed reduced the heavy metal concentration.

CONCLUSION

An increasing trend was observed in egg weight and shell weight when eggshell was used in poultry feed. About 100% eggshell-feed has higher egg weight and shell weight. It was also found that T_4 (100% eggshell-feed) can improve the eggshell breaking strength. During this experiment, we did not observe any side effect of eggshell as feed ingredient. Therefore, it is an alternative source of calcium for the laying hens. Further study is needed to determine the microbial load during the collection, before and after processing of eggshell.

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