10.21608/avmj.2025.3321 99.1452Assiut University web-site: www.aun.edu.eg

UTILIZATION OF SEASHELL-BASED CALCIUM CARBONATE AS A FEED ADDITIVE FOR ENHANCING THE GROWTH PERFORMANCE OF BROILERS

Running title: Seashell-based Calcium Carbonate Enhancing the Growth Performance of Broiler

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Received: 9 December 2024; **Accepted:** 19 March 2025

ABSTRACT

Different calcium sources influence the production performance of the broiler chicken. The study was conducted to use shell-based calcium carbonate as a feed additive in broiler ration to increase broiler production. A total of 99 unsexed Day Old Chick (DOC) broiler was divided into three dietary treatment (T) groups, 33 chicks per group or treatment. They are established as Control (Commercial broiler feed), T_1 (Commercial broiler feed and 1gm/bird/day seashell-based powder), and T_2 (Commercial broiler feed and 1gm/bird/day commercial calcium carbonate powder). The three groups of chicks were reared separately in the cage of an open-sided housing condition. Each group was fed ad libitum on feed and water. The weight was measured on the 1st, 9th, 15th, 20th, 24th, 32nd, and 35th day of age of the birds. The final mean body weight (gm) of the broiler in the dietary control, T1 and T2 were 2156.0±197.9, 2302.9± 224.4, and 2195.24± 330.2 respectively. The T1 group of broilers had the highest body weight, while the control group had the lowest. However, the final live weight of the broiler-fed seashell-based diet (T1) was significantly higher ($P<0.05$) compared with the control group. The live weight of the T1 dietary group of birds on the 9th day ($P<0.05$), 16th ($P<0.05$), and 32nd ($P<0.001$) was significantly higher than the control group. The body weight in the commercial calcium carbonate supplement group was apparently improved, compared to the control group throughout the experiment period, but statistically insignificant ($P>0.05$). It can be concluded that seashell powder 1gm/bird/day of feed was better concerning the growth performance, and profitability of broiler production. Seashell-based calcium carbonate was a potential calcium source for poultry as a feed additive.

Keywords: seashell, calcium carbonate, broiler, growth, and production.

INTRODUCTION

The poultry industry has been part and parcel of the world food industry because it provides very nutritious food items (i.e., meat and egg) to the majority of people of the world. For this reason, poultry production is increasing daily due to the enormous protein gap in the world. In recent years, Bangladesh's enhanced poultry production channel has resulted in substantial protein supplies. Modern technology, raising high-yielding poultry varieties, and expanding the number of commercial farms have all been used to significantly increase productivity in recent years (Prank *et al.,* 2024). As a result, the production of chicken in Bangladesh hence

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increased significantly, rising from 261.47 million in 2014–15 to 311.9 million in 2022–23. (DLS, 2023). Even though the entire country has consumed a lot more poultry over the last 30 years, the average person now consumes between 6.3 and 8.5 kg annually (World's Poultry Science Association, 2020). Chicken, duck, quail, pigeons, and turkey are the primary poultry products of Bangladesh. Four further varieties of chicken are available: broiler, layer, Sonali, and local. Particularly, broiler production has shown exponential growth in worldwide meat consumption and economic profit, which will be higher in the next century. This may be due to its comparative advantages, including excellent nutritional value, delicious taste, reduced fat content, quick production time, quick economic growth, and reasonably priced prices, especially for lower socioeconomic backgrounds (Petracci *et al.,* 2015). Feed cost plays a crucial role in the production of poultry, particularly broiler chickens, since its expenses account for roughly 60–70% of total production costs (Wongnaa *et al.,* 2023). Poultry feed costs go up because of the high cost of these substances. Safety margins have become an issue of concern recently, and their applications are being limited across the globe. Consequently, there is a lot of interest in creating natural substitute supplements to preserve the health and performance of animals (Chattopadhyay *et al.,* 2006). Feed additives are typically used to increase production, decrease stress, and increase appetite. Feed additives increase the efficiency of feed utilization, growth, and improved survivability of broilers (Sheikh *et al.,* 2020). For profitable broiler production, the feed cost has to be reduced or the performance of the birds has to be enhanced by adding some additives to obtain the maximum benefit (Sahin *et al.,* 2003). The morphology, intestinal microbiota, serum components, immunological organ indices, and growth performance of broiler chickens are all influenced by the calcium supply (Xing *et al.,* 2020). For chickens, calcium carbonate (CaCO3) is a vital mineral, since it is a major component of bone structure, contributes to the formation of eggshells, and is utilized in enzyme systems and acid-base balance. The supply of calcium may also have an impact on bone development and mineralization (Keshavarz *et al.,* 1993). In broiler nutrition, a variety of calcium sources are used, including oyster shells, limestone, and others (Manangi *et al.,* 2018). The cheapest Ca source is seashell-based calcium carbonate, because seashells are available in the Chattogram district of Bangladesh. Since sea shells are a novel approach to chicken nutrition supplements, growing interest in this addition to the ration could open up new opportunities for the poultry sector to improve the quality of poultry production. A seashell (cockle) is an organic or natural source of calcium carbonate. The sea shells (cockle) are a rich source of calcium carbonate (98.7%), along with other sources of many minerals, P, Mg, Zn, Cu, Na, K, B, Si, and Fe (Awang-Hazmi *et al.,* 2007).

Calcium carbonate ($CaCO_3$) is among the common minerals found in nature and has three polymorphs: calcite, aragonite, and vaterite are available (Manoli & Dalas, 2000). $CaCO_3$ has a wide range of industrial uses, such as in rubber, paints, inks, plastics, paper, feedstuff, medications, and adhesives (Xiang *et al.,* 2004). Numerous methods have been devised to regulate the morphologies and phases of calcium carbonate. Earlier methodologies primarily investigated the study of organic additives, such as the development of sponge-like vaterite in the presence of sodium dodecyl sulfate (Walsh *et al.,* 1999); calcium carbonate is applied to decalcified eggshell membranes using acidic polymers like polyaspartic acid (Ajikumar *et al.,* 2004); development of vaterite by solvothermal means when glycol, 1, 2-propanediol, and glycerin are present (Li *et al.,* 2002); Aragonite is one of the least frequent crystalline polymorphs forms of calcium carbonate produced in laboratories. Numerous mineralized organisms selectively establish calcium carbonate in this crystal structure, because natural aragonite is derived from biogenic sources (Mann *et al.,* 1989). The cockle shells contain valuable calcium carbonate with aragonite polymorph (Islam *et al.,* 2011), and this calcium carbonate can be integrated and replaced with the bone tissue (Stupp & Braun, 1997).

This study aimed to use calcium carbonate from Bay of Bengal-based cockle shells for the mobilization of great resources for the national income of Bangladesh. These shells are available on Chattogram's sea beach. These shells contain valuable calcium carbonate with aragonite polymorph (Islam *et al.,* 2011) which is integrated and replaced with the bone tissue (Stupp & Braun, 1997). Although research has been done about Ca source feed additives, little attention has been paid to the seashell's base calcium carbonate used in the broiler feed ration. However, this study's main objective is to use this shell-based calcium carbonate as a feed additive in broiler ration to increase broiler production by increasing the growth and development of bone and muscle.

MATERIALS AND METHODS

Study area and duration

The present study was conducted from January 2024 to March 2024 in a poultry shed at the Research and Farm-Based Campus, Chattogram Veterinary and Animal Sciences University Hathazari, Chattogram.

Experimental chicks

A total of ninety-nine (99) day-old unsexed broiler chicks were purchased from the Provita Company by a local feed and chick dealer, Hathazari, Chattogram, Bangladesh. Every chick was inspected to make sure they were all the same size and didn’t have any abnormalities. Then, using normal chick cartoons, the chicks were carefully transported to the poultry shed.

Housing and management

The experimental shed was a concrete-floored, open-sided construction. During the duration of the trial, the birds were raised in the cage. In this shed, birds from various treatment groups were raised in distinct cages. Paper was used on the floor of the cages to prevent entry into the legs and comfort the birds. During the brooding period, hard papers were provided surrounding the cages for temperature maintenance. Throughout the trial, the birds were exposed to natural light during the day and artificial light (incandescent bulbs) at night, with the exception of the brooding period, when continuous artificial light was added to the daylight to keep the brooder warm. In order to keep ammonia gas from the shed building, proper ventilation was maintained by utilizing ceiling fans inside and drapes on the open sides. The birds were given a standard floor, feeder, drinker, and sufficient area for the duration of the experiment.

Cleaning and Sanitation

The shed was completely cleaned and washed using normal tap water, wheel washing powder and a hard brush. For disinfection, a 1% v/v phenol solution was sprayed over the ceiling, fan, corners, wall, and floor. Cleaning was done with a brush and clean water after the spray. The same method was used to clean drinkers, feeders, and rearing cages. After cleaning and disinfection, the house was opened for three days for proper drying. After drying, the curtains were closed. After fumigating the space using 35 milliliters of formalin to 10 grams of potassium permanganate per cubic meter, the room was sealed off for four days. A footbath was washed and cleaned with wheel washing powder. After that, this bath with potassium permanganate (1% w/v) was placed at the broiler shed's entrance and was replaced every day. Wheel cleaning powder was used to clean and wash feeders and drinkers, and they were dried every morning. Any gaps or cracks around the shed were investigated and sealed to keep unwanted entry animals, including foxes, cats, rats, mice, etc. The shed door was closed to prevent visitors and limit unwanted entry. The maintenance of biosecurity was strict.

Experimental Units and Diets

A total of unsexed broiler DOC was divided into three dietary treatment (T) groups designated as Control (Commercial ready-made broiler feed), T1 (Commercial readymade broiler feed and handmade seashell-based powder), and T2 (Commercial readymade broiler feed and commercial calcium carbonate powder), having 33 chicks per group or treatment. Throughout the trial period, the birds were supplied with feed and clean drinking water ad libitum. Commercial readymade pellet feed was purchased from the feed and chick dealer in the local market. During purchases, check the date of expiry. T1 group of birds has supplied seashell powder for 1 gm per bird per day mixed with feed. Similarly, the T2 group of birds has supplied commercial calcium carbonate powder for 1 gm per bird per day mixed with feed. However, the control group of birds has provided only commercial feed.

Vaccination and medication

In the first three days, all birds were supplied with water containing the immuno-stimulant (Powder Liso vit®, Reneta PLC). The vaccines were collected from a local veterinary medicine shop and administered to the trial birds according to the vaccination schedule shown in Table 1.

Data collection and analysis

The live weight of the birds was recorded on the 1st, 9th, 15th, 20th, 24th, 32nd, and 35th day of age of the birds. Raw data was recorded in Microsoft Excel Professional 2020 (Microsoft Corporation, USA). Then data were transferred to statistical software, STATA-16 (STATA Corp., Texas, USA) to perform statistical analysis. An unpaired sample t-test is to be done to compare the means of different variables between the two groups. The control group (T) was the baseline parameter. A p-value equal to or less than 0.05 ($P \leq 0.05$) was considered significant for this test. Results were expressed as arithmetic mean ± standard deviation (Mean ± SD).

RESULTS

Final live weight

The final live weight of broiler (g/bird) fed different types of calcium carbonate sources compared to the commercial feed-based diets (control group) was present in Table (2). The mean live weights of the broiler in the dietary control, T1 and T2 were 2156.0±197.9, 2302.9±224.4, and 2195.24±330.2, respectively (Table 2). The highest body weight was observed in the T1 group, whereas the lowest was in the control group of the broiler (Figure 1). The final live weight of the broiler-fed seashell-based calcium carbonate diet (T1) was significantly ($P<0.05$) higher than the control group (Table 2).

Weekly body weight gain

The mean body weight gain (gm) of broiler on the 9th day in different dietary groups control, T1, and T2 were 228.7±20.1, 254.6±7.6, and 254.6±10.2, respectively (Table ٢). The live weight of the T1 dietary group of birds on the 9th day was significantly ($P<0.05$) higher than the control group. On the 16th day, the significantly ($P<0.05$) highest live weight was found in the T1 dietary group, whereas the lowest was in the control group. On the 25th day, the statistically highest body weight was found in the T1 dietary group, but not significant compared with the control group of birds. The average live weight gain of the experimental broiler on the 32nd day in various dietary units' control, T1, and T2 were 1922.7±80.5, 2158.6±166.4, and 2110.0±199.4, respectively. The live weight of the T1 dietary group was significantly ($P<0.001$) higher compared to the control group of broilers.

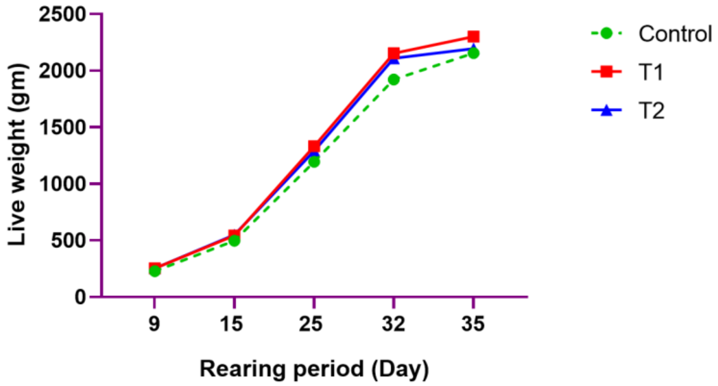

Figure 1: Growth curve (mean) of the three dietary groups of the experimental broiler chickens.

Table 1: Vaccination schedule of the experimental birds.

Age of the birds	Name of the disease	Route of administration
3 days	IB+ND	One drop in each eye
9 days	IBD	One drop in each eye
17 days	IBD	One drop in each eye
21 days	IB+ND	One drop in each eye

Note: IB = Infectious Bronchitis, ND = Newcastle disease, and IBD = Infectious Bursal Disease.

Table 2: Body weight of broiler chickens during the experimental period.

Age	Group	Weight (Mean± SD)	P value
9 days	Control	228.7± 20.1	
	Treatment I	254.6± 7.6	**0.0459**
	Treatment II	254.6± 10.2	0.1496
16 days	Control	496.3± 35.5	
	Treatment I	548.2± 40.2	**0.0142**
	Treatment II	543.3± 58.6	0.1881
25 days	Control	1194.0± 60.1	
	Treatment I	1333.2± 187.9	0.1559
	Treatment II	1291.3± 329.0	0.5312
32 days	Control	1922.7± 80.5	
	Treatment I	2158.6± 166.4	**0.0027**
	Treatment II	2110.0± 199.4	0.106
35 days	Control	2156.0±197.9	
	Treatment I	2302.9± 224.4	**0.0109**
	Treatment II	2195.24± 330.2	0.5996

DISCUSSION

In the current study, the live weight in the seashell- based calcium carbonate supplement group was significantly improved, compared to the control group throughout the experiment period. The body weight in the commercial calcium carbonate supplement group was apparently improved, compared to the control group throughout the experiment period, but statistically insignificant. The results were supported by Wilkinson *et al.* (2014); Xing *et al.* (2020), who reported the effect of the calcium sources on the growth performance of the broiler. Further reported that the calcium phosphate-prepared scallop shell can significantly improve growth performance, carcass characteristics, and meat quality. Scallop shells contain activated calcium phosphate, which has been shown to boost the activity of digestive enzymes, enhance feed nutrient absorption and digestibility, and enhance broiler chicken growth performance (Xing *et al.,* 2020). Another study, (Talpur *et al.,* 2012) observed the impact of calcium supplementation on broiler chicken growth performance. Similar results have also been reported by Bintvihok & Kositcharoenkul, (2006), who found the impact of calcium on broiler performance and showed that dietary calcium supplementation had positive effects on body weight gain. Calcium (Ca) is a vital chemical element, essential for the mineralization of bones in poultry, and a higher Ca level is required, particularly in the finisher periods (Zyla *et al.,* 2000). In broilers, the majority of absorbed calcium is kept in the bone. Ca is necessary for the development of bones, the formation of blood clots, the contraction of muscles, and the production of high-quality eggshells, and dietary Ca performs effectively for leg problems, bone ash, and feed efficiency (Scheideler *et al.,* 1995). An essential purpose of feed additives is to enhance feed utilization effectively. When feed additives are added to broilers, they begin to affect how easily the ingested nutrients are digested, which improves the birds' ability to use feed. The efficient utilization of ingested feed by the broilers might give rise to a better growth response of the broiler chickens (Masudul Alam *et al.,* 2022). Supplementation of calcium can significantly affect digestive enzymes, such as the protease, amylase, and lipase activity of broiler chickens, and enhance them to complete use and digest respective protein, carbohydrates, and fat in feed, and then improve the apparent digestibility of feed nutrients. Calcium can improve the activity of digestive enzymes, increase the digestibility and absorption efficiency of feed nutrients, and enhance the growth performance of broiler chickens (Xing *et al.,* 2020). In the present study, the highest body weight was observed in the seashell-based calcium carbonate supplement group among the three dietary groups, whereas the lowest was in the control group of the broiler (Figure 1). These results concur with Leytem *et al.* (2008), who examined the effects of different dietary Ca levels. There was a linear relationship between dietary Ca and production performance. A previous study observed that lower Ca diets led to identical bone mineralization, improved feed efficiency, and greater weight gain, which could be due to different calcium sources (Abdollahi *et al.,* 2015). Providing Ca as an oyster shell has been found to have a highly improved digestibility and retention than the calcium carbonate fed as powdered limestone (Roland SR, 1988). In an isolated cell investigation, the calcium absorption from shell powder was up to 64% more than from pure calcium carbonate (Daengprok *et al.,* 2003). Proteins, polysaccharides, and lipids have been identified as the constituents of the organic matrix found in molluscan shells. Numerous investigations have shed light on the nature and purposes of the organic matrix, particularly concerning the shell matrix proteins (SMPs) (Marin *et al.,* 2007). In this regard, the Asprich (Aspein) protein is a highly acidic SMP found in the colorful layer of Pterioida, and this protein is characterized by huge aspartic acid, which contributes to calcium binding affinity and promotes calcite formation (Politi *et al.,* 2007; Weiner, 1979). In addition to calcium and protein, sea shells also contain small amounts of other minerals, including sulfur, silicon, potassium, iron, aluminum, magnesium oxide, strontium, and selenium

(Abutu *et al.,* 2019). Just like calcium, these minerals may play a role in bone health (Schaafsma *et al.,* 2000). Aquaculture-derived seashells are an abundant source of biogenic calcium carbonate (bCC), that can be a potential substitute for ground calcium carbonate and precipitated calcium carbonate (Basile *et al.,* 2024). However, the sea shell-based calcium carbonate was available, easily preparable, cheapest, or cost-effective, and supplementary dietary growth performance was comparatively higher than commercial calcium carbonate supplements dietary group of broiler chickens.

CONCLUSION

The effect of dietary calcium carbonate supplements on poultry production was suggested to improve the live weight performance of commercial broiler production. The highest live weight was observed in the sea shell-based calcium carbonate supplementary group among the commercial calcium carbonate supplement and control group of broiler chickens. Overall, for the rearing periods of broiler chicken, the sea shell-based calcium carbonate supplementary group gained comparatively higher weight. Sea shell-based calcium carbonate was a potential calcium source for poultry feed additives.

ACKNOWLEDGMENTS

The author wishes to thank a feed and chick dealer for their help in collecting the chicks, feed, and vaccine. The author would like to express gratefulness to the staff of the poultry shed at the Research and Farm-Based Campus, Chattogram Veterinary and Animal Sciences University Hathazari, Chattogram, Bangladesh, for their support in conducting this research work.

Funding
A fund was received from the Research and Extension division of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

Conflict of Interest
The authors declare that there is no conflict of interest.

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