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Improvement in bioavailability of ascorbic acid and calcium using eggshell powder

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Abstract

Introduction: Eggshells, often treated as waste, are rich in calcium carbonate and can serve as an alternative calcium source for nutritional and pharmaceutical applications.

Methods: This study evaluates the bioavailability of calcium from eggshell powder and its effect on ascorbic acid bioavailability. Eggshell powder was sourced, processed, and formulated with animal feed in varying ratios (3:1 and 6:1 feed-to-eggshell). Experimental groups of Swiss albino mice were treated with these formulations, vitamin C, and control diets over four weeks. Serum calcium and ascorbic acid levels were analyzed weekly.

Results: Results demonstrated that eggshell supplementation significantly increased serum calcium levels, particularly in the 3:1 formulation combined with vitamin C. Serum ascorbic acid levels were also positively influenced, with the 6:1 formulation showing cumulative benefits over time. These findings highlight the potential of eggshell powder as a cost-effective, bioavailable calcium source, enhancing nutrient absorption and overall health outcomes.

Conclusion: This study underscores the importance of waste utilization for sustainable development and dietary supplementation.

Keywords: Calcium; Ascorbic acid; Eggshell; Blood; Formulation

1. Introduction

Eggshells are agricultural waste that is primarily considered useless and are often discarded because they contribute to pollution (1). In many countries worldwide, egg product companies and food manufacturers that use eggs in their products generate tons of waste eggshells, causing significant environmental problems. Over $100,000 is spent annually to dispose of these eggshells in landfills (2). Despite the substantial costs, additional issues arise, such as the rejection of eggshells by landfill owners due to capacity constraints and the attraction of rodents and vermin. In Taiwan, food processors generate over 1.3×10^4 tons of eggshell waste annually, based on 7.1×10^9 pieces of hen eggshell (3). Globally, several tons of eggshell waste are generated daily without further processing, incurring considerable disposal costs (4). Therefore, effective treatment and utilization of biowaste are essential for environmental and economic concerns. For sustainable development, waste should be recycled, reused, and repurposed into value-added products (1). This approach offers both economic benefits and environmental protection.

Eggshells, as one of these biowastes, require more adequate consideration for their usefulness. Experimental and clinical studies have demonstrated several positive properties of chicken eggshells, including antirachitic effects in rats

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and humans. Positive effects on bone density have also been observed in animal models of postmenopausal osteoporosis in ovariectomized female rats (5). Studies further indicate that chicken eggshell powder supports bone and cartilage health and is effective in preventing osteoporosis (5). Additionally, *in vitro* studies show that chicken eggshell powder stimulates chondrocyte differentiation and cartilage growth. Clinical studies involving postmenopausal women and those with senile osteoporosis reveal that eggshell powder reduces pain and osteoporosis while increasing mobility and bone density (5).

Calcium deficiency is a widespread dietary issue. While dairy products are a suitable source of calcium (Ca), many people do not consume the recommended amounts. Calcium supplementation through tablets can be costly and challenging to adhere to. Chicken eggshells, a natural source of calcium, offer an alternative (6). Calcium is vital for various physiological functions, including the maintenance of bone tissues throughout life (7). It serves as a structural and metabolic component, combining with phosphorus to form the mineral portion of bone and participating in numerous biochemical processes (8). Calcium supplementation, particularly calcium carbonate ($CaCO_3$), is central to managing osteoporosis, the most common metabolic bone disorder associated with aging and menopause (9). Chicken eggshell powder, a natural source of calcium and other elements like strontium and fluoride, positively affects bone metabolism (5). A high-quality eggshell contains an average of 2.2 g of calcium, primarily calcium carbonate, with approximately 94 % of its dry weight composed of calcium carbonate. Eggshell calcium is about 90 % absorbable and is a superior natural source compared with limestone and coral calcium available in the market (10).

The benefits of chicken eggshell calcium for bone health are well-documented. A positive effect has been reported on femoral neck bone mineral density in healthy, late postmenopausal Dutch women (11). According to Veda MV et al., peak bone mass achievement is influenced by factors such as adequate calcium intake, with studies indicating a correlation between dietary calcium during growth and bone mass later in life (12). Additionally, the role of vitamin C or carotene, either in its authentic form or from natural sources like oranges, parsley, and pepper juices, in enhancing calcium absorption has been studied. Results suggest that ascorbic acid, orange, and pepper juices improve intestinal calcium absorption, while carotene and parsley show no effect (13). Whether these findings apply to chicken eggshell calcium remains to be clarified in further studies.

Calcium and ascorbic acid are necessary supplements in life and both are needed by all ages in everyday nutrition. These supplements are more often consumed together without much knowledge of the influence each has on the bioavailability of the other. Most calcium sources as supplements are available as pharmaceutical preparations from elemental calcium salt, raw materials, or the food we eat. The former is very expensive, while the latter is not quantified and cannot be relied on. Calcium, however, is disposed of in our homes or near our homes daily without knowing so, as chicken eggshells. These calcium-rich eggshells could be used at homes as calcium sources in food and by pharmaceutical industries to produce cheaper products. But that is only if its actual elemental contents and proper preparation for safe consumption in the most enhanced form are known. The study aimed to evaluate the bioavailability of calcium in eggshell powder and its effect on ascorbic acid bioavailability.

2. Material

Weighing Balance (Scout pro u401, China), incubator (GentLab UK), autoclave, hot air oven (Ohaus, England), UV-Visible spectrophotometer (Jenway 6300 UV, England), Double-beam spectrophotometer (Jasco Model 7800, Japan), spectrometer (model 210, VGP, USA) pH meter (Metrohm model, 694 Swiss), test tube racks, syringes and needle, Micropipette, glass Petri dish, MacCartney bottle, Dropping pipette, Mortar and pestle, sieve, spatula, Tableting machine, stopwatch

2.1. Chemicals and reagents

All reagents and solvents were of analytical reagent grade. The reagents used include: Nutrient Agar, Mueller-Hinton agar and Sabouraud dextrose agar (Biolab India), Sterile normal Saline (Juhel Pharm), Ascorbic acid (Thode Scobel Hamburg Germany), 2,4- Dinitrophenylhydrazine (BuGuCH, Germany), Sulphuric acid (Surya, India), sodium hypochlorite (Zed Chem, India), TCA, sodium hydroxide (Vynova, UK) and hydrochloric acid (Thomas, UAS) phenanthroline molybdate (Ensince Industry co China), Hydroxylamine (Boshan hengjia Fine chem co Ltd China), Ammonium acetate buffer (Future Chem China), potassium hydroxide, EDTA, 2-[(2-Mercaptophenylimino) methyl]phenol Doubly distilled deionized water, methanol, chloroform, ammonium chloride, sodium bicarbonate (Merck Germany) Acacia gum, sweetener, paraben preservatives, and colourant.

2.2. Experimental Animals

Healthy male Swiss Albino mice (8–12 weeks, weighing 25–33 grams) chosen to enable accurate judgment on the effect of treatment; bred and maintained at Animal House of Faculty of Pharmaceutical Science, Nnamdi Azikiwe University were used. The animals were kept in cages and housed in a standard animal house under a natural 12-light and dark cycle at room temperature. They were maintained on a standard pelleted diet and water ad libitum. All mice were acclimatized for one week before the study. All experiments were conducted according to internationally accepted laboratory animal use, care, and handling guidelines.

2.3. Collection and preparation of eggshell powder

Egg shells used for this work were sourced from commercial Indomie spots in Sokoto town of Sokoto state, Nigeria. The egg shells were immersed in 1 % sodium hypochlorite for 12 hours and then were washed thoroughly and adequately rinsed using distilled water. They were allowed to drip and air dry for 24 hours and then further dried and disinfected with hot air oven (Ohaus, England) by subjecting it at 200 ^{o}C for 1 hour. The very dried egg shells were allowed to air cool and thereafter comminuted into rough powder using a Mortar and pestle first and then using an electric blender. The finer powder obtained was sieved using a 0.18mm sieve to get a very fine powdered eggshell.

2.4. Formulation of Eggshell Powder with Animal Feed

Initially, a trial formula was produced by mixing the eggshell with animal feed in the ratios of 2:1 and 3:1 (animal feed: eggshell), respectively, and feeding it to the animals in three different groups. The third group was fed only animal feed without eggshells. It was discovered that animals consumed most of the nil eggshells, more of 3:1 and least of 2:1. Hence, formulas of 3:1 and 6:1 were employed.

A 3:1 batch of Animal feed and eggshell was formulated by weighing 900g and 300g of each. The two were adequately mixed using 394.8 ml of distilled water to aid the mixing. The mixture was air-dried until adequate dryness was achieved. The 6:1 batch was formulated by mixing 900g of feed with 150g of eggshell powder, and the same treatment was given to it as in the 3:1 formulation.

2.5. Experimental design

A total of 20 male albino rats were used for the test. They were grouped into 5 groups of 4 animals and adequately marked, caged in groups, and collected pre-treatment blood samples and weights before being treated for 1 month. Each group member was weighed, and the average weight was calculated. Average weight was used to calculate the appropriate Dose of vitamin C for each group using 1g/kg as the general dose. The first group was treated with vitamin C, 3:1 formula, and water. The second group was treated with Vitamin C, 6:1 formula, and water. The third group was treated with Vitamin C, Normal feed without eggshell powder and water. The fourth group received regular feed with water, and the last group 3:1 formula and water. Those receiving vitamin C received a dose daily for four weeks and were fed as above for the same period. Weight was taken, and blood samples were collected after each week for all groups. Blood samples collected were immediately analyzed for vitamin C and calcium components.

2.6. Determination of serum calcium concentration

Serum calcium concentration was analyzed using the Spectrophotometric method. Using a pipette, 25 µl of sample (serum) was taken into the sample test tube, and 1.0ml of working reagent was added. In the blank test tube, 25 µl of distilled water was added with 1.0ml of working reagent, while the standard test tube had 25 µl of standard solution with 1.0ml of working reagent. Each of the standard and sample test tube contents were mixed, and their absorbances were read against the blank, giving 20 minutes. Ensuring the same time between sample addition and reading for the sample and the standard. Thereafter, one drop of EDTA was added to both the sample blank and the reagent blank and allowed to turn colourless. Their absorbances were read against each other. This yielded corrected absorbance (Asample (corrected) = Asample –Asample/EDTA). The reagents include **Buffer:** 2-amino-2-methyl-propan-l-ol, **Chromogen**- O-Cresolphthalein complexone, 8- hydroxyquinoline and hydrochloric acid

Calculation

$$\text{Concentration of Calcium (mg/dl)} = \frac{Absorbance\ of\ Smple}{Absorbance\ of\ Standard} \times \text{Standard concentration (mg/dl)}$$

2.7. Determination of serum vitamin C concentration

Ascorbic acid in the serum was analyzed using a spectrophotometric method, as described by Roe and Keuther (1943). The ascorbate in the sample was extracted from 1 ml of animal serum using 4 % TCA, and the extract volume was 10 ml with the same 4 % TCA. The supernatant was obtained after centrifuging using benchtop centrifuge model JW-2018H (Ht Instrument Ltd, China) for 10 minutes at 200 rpm and was shaken vigorously using a cyclomixer. It was then kept for 5 minutes. A standard ascorbate solution was prepared, and a volume range of 0.2 ml to 1.0 ml was measured into test tubes. 0.5 ml and 1.0 ml of supernatant were also measured. All tubes were diluted by making them up to 2.0 ml with 4 % TCA. A 0.5 ml volume of 2, 4-Dinitrophenylhyrazine (DNPH) was added to all the tubes, followed by 2 drops of Thiourea Solution. The contents were mixed and incubated at 37 ^{o}C for 3 hours, forming osazone crystals. The crystals were dissolved in 2.5ml of 85 % H_2SO_4 in cold. In the blank, DNPH reagent and Thiourea were added after the addition of sulphuric acid. All tubes were cooled in ice, and their absorbance was read at 540nm with a spectrophotometer (Genesys 10-s, USA).

3. Results and discussion

3.1. Serum calcium level

The effect of eggshell powder on serum calcium level is presented in Figure 1. The administration of formulated feed containing eggshell powder increased serum calcium levels across all groups of animals after the first week,, as shown in Figure 1. Notably, the group receiving a 3:1 ratio of feed to eggshell powder (full dose ES) combined with vitamin C exhibited significantly higher serum calcium levels compared to other groups during this initial period ($p<0.05$). This observation underscores the potential of eggshell powder as a bioavailable source of calcium, which is crucial for various physiological functions, including bone health and metabolic processes.

A shift in serum calcium levels was noted as the study progressed to days 14 and 21. The group receiving a 6:1 ratio of feed to eggshell powder (Half Dose ES) alongside vitamin C showed significantly elevated calcium levels on these days compared to other groups, as shown in Figure 1, except for the full Dose ES group, where no significant difference was observed ($p<0.05$). This suggests that while the full dose may provide immediate benefits, the half Dose could be more effective in maintaining higher calcium levels over time.

On day 21, groups one and two demonstrated significantly higher serum calcium levels than the remaining groups, indicating a sustained effect of the dietary intervention (14). By the fourth week, group one showed superior calcium levels compared to all other groups ($p<0.05$), reinforcing the notion that ongoing supplementation is beneficial for maintaining optimal calcium concentrations.

The study also revealed that by day 14, the serum calcium levels in group four, which received feed only without any eggshell supplementation, began to decline significantly throughout the remaining study period ($p<0.05$). This decline emphasizes the importance of dietary calcium sources for maintaining serum calcium levels and suggests that without supplementation, animals may be at risk for deficiencies.

The groups receiving eggshell powder consistently exhibited increases in calcium concentration throughout the study duration. This finding aligns with existing literature that highlights eggshell powder as a rich source of natural calcium, beneficial for bone health and overall vitality in various species.

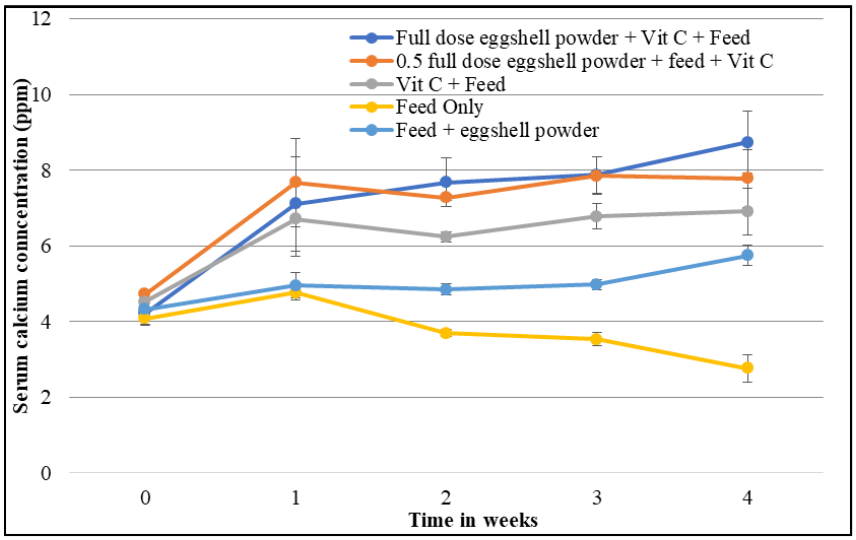

Figure 1 Serum calcium concentration

3.2. Effect on serum ascorbic acid levels

The result of the effect of the eggshell powder on serum ascorbic acid level is presented in Figure 2. In the first week of the study, all groups except those treated with half-dose eggshell powder (ES) combined with feed and vitamin C experienced a significant decrease in serum ascorbic acid levels, as shown in Figure 2. This suggests that the half-dose ES formulation may protect against the decline in ascorbic acid, potentially due to its synergistic interaction with vitamin C and other dietary components. Previous research indicates that ascorbic acid is crucial in enhancing animal health and performance, mainly when included in diets rich in different nutrients like calcium from eggshells (15).

By the second and third weeks, the group receiving complete Dose ES along with feed and vitamin C exhibited significantly higher serum ascorbic acid levels than those receiving only feed and vitamin C ($p<0.05$). However, there was no significant difference between the full Dose ES group and the half-dose ES group during these weeks, as shown in Figure 2. This finding highlight that while higher doses of eggshell powder may enhance nutrient absorption initially, lower doses can still provide comparable benefits over time, emphasizing the importance of dosage in dietary formulations.

On the last week of observation, the half-dose ES + feed + vitamin C group showed significantly elevated serum ascorbic acid levels compared to all other groups ($p<0.05$). This suggests a potential cumulative effect of continued supplementation with lower doses of eggshell powder, which may enhance nutrient retention and absorption over time. The observed general decrease in serum calcium levels among other groups after the third week further supports this notion, indicating that without adequate supplementation, animals may experience deficiencies that could impair overall health (16).

These results underscore the critical role of ascorbic acid in animal nutrition, particularly when combined with other dietary supplements like eggshell powder. Ascorbic acid is known for its antioxidant properties and ability to enhance immune function, whichvital for maintaining health during periods of stress or disease (15).

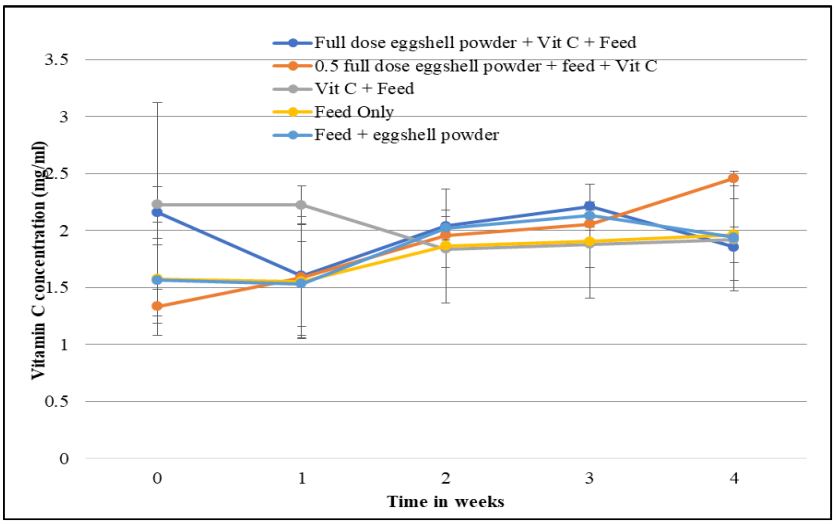

Figure 2 Serum Ascorbic Acid concentration

4. Conclusion

This study demonstrates that incorporating eggshell powder can significantly enhance serum calcium levels over time. The results advocate further exploration into dietary formulations that optimize calcium intake through natural supplements like eggshell powder, particularly in situations requiring additional support for bone health and metabolic functions. This study proves that dietary formulations combining eggshell powder and vitamin C can significantly influence serum ascorbic acid levels. The findings suggest that both full and half doses of eggshell powder can be beneficial, with implications for optimization to improve health outcomes and performance. Further research is needed to explore the mechanisms underlying these interactions and their long-term effects on animal health.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest as regards this research

Statement of ethical approval

Ethical approval was taken from the institutional ethics committee before the start of the study.

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