Combined vitamins, minerals and amino acid supplement improves eggshell quality but not egg weight in Japanese quails (*Coturnix coturnix japonica*)

Christopher Jay M. Tanduyan and Santiago* T. Peña Jr.
College of Veterinary Medicine, Visayas State University, Visca, Baybay City, Leyte, Philippines, 6521-A
*Corresponding author email: santiago.penajr@vsu.edu.ph
Received: 14 November 2018. Accepted: 3 December 2018.

Abstract
The Japanese quail (*Coturnix coturnix japonica*) is remarkable in terms of production potential for meat and eggs and as an alternative model for poultry research. Just like the layer chickens, the quail industry can also suffer from poor eggshell quality resulting in serious economic losses. While there are commercially available supplements manufactured to promote egg performance for chicken layers, these are often difficult to find specific for quails. Hence, this study was conducted to determine the effect of combined vitamins, minerals, and amino acid supplement on the external egg quality of Japanese quail eggs which can be used as baseline information to formulate supplements tailored for quail layers. Twenty birds per treatment were given different levels of supplement *via* the drinking water and external egg quality parameters were measured weekly and after supplementation, respectively. Our study clearly demonstrated an interaction between the levels of supplementation and the amount of time (in wk) the birds were on supplement on egg shell thickness ($p \leq 0.05$). Egg shell thickness was highest between 1.5 g/L and 2 g/L at the fourth wk of supplementation and decreased after two wks the supplementation was withdrawn. Similar results were observed on the egg shell weight except for the overall egg weight which remained relatively unchanged.

Keywords: egg shell weight, egg shell ratio, egg shell thickness, supplementation

Introduction

Research on quails (*Coturnix coturnix japonica*) lags behind chickens due to the limited number of researchers and laboratories investing into quail research (Minvielle, 2004). However, quail has an extensive history as a popular alternative to common poultry species due to its potential to convert limited feed into human food (meat and egg), as specimen for biomedical research, rapid turnover of generations, and early sexual maturity among others (Wilson et al., 1961; Baumgartner, 1994; Ayasan et al., 2005). Moreover, quail production is becoming increasingly popular in some countries due to its lower capital requirements, ease of production, adaptability to local environment temperature and as supplementary income (Nasar et al., 2016). In fact, quail egg can be prepared in some special local delicacies thus promoting market demands (Priti and Satish, 2014).

The quail egg industry is currently facing the challenge to improve productivity in order to remain competitive in today's cost-driven environment. One of the most important challenges facing quail raisers is the cost associated with poor eggshell quality (Roland, 1988; Butcher and Miles, 1990; Vercese et al., 2012). On average, an estimate of 13 to 20% of eggs in chicken layers were cracked or lost before reaching their final destination, i.e., store (Roland, 1977; Roland, 1988) possibly due to thin eggshell resulting to serious economic problems affecting breeders and dealers (Altan et al., 1998). In a much earlier study by Roland (1977), the reduction in the profitability in layer production appears to be affected more by eggshell quality problems rather than by the age of hens where up to 4.77% of all eggs laid were not collected due to problems associated with poor eggshell.

To address this issue, broken oyster-shell, limestone grits or ground dried whole mussels are added to layer diet as a source of calcium (McNaughton et al., 1974; Gerry, 1980; Sultana et al., 2007) and/or Vitamin D3 (Vohra et al., 1979), Vitamins C and E (Kucuk et al., 2003) are added in drinking water to supplement vitamin and mineral requirements of laying hens. In addition, Vitamin A (Parrish and Al Hasani, 1983), prebiotics and probiotics (Guclu, 2011) and animal protein products such as fish, meat and bone meal are commonly used to balance vitamins and amino acid content and maintain egg production (Osti et al., 2002).

While there are readily available vitamin and mineral supplements designed to prevent thin and fragile eggs in chicken layers (Chakoma et al., 2016), these are normally absent or difficult to find for quails especially in remote regions. Hence, this study was aimed to determine the effect of combined vitamins, minerals, and amino acid supplement on the eggshell quality and weight of eggs of Japanese quails. This study could provide useful information to both small and large quail raising enterprises regarding the use of commercially available vitamins, minerals and amino supplements for chicken layers. Moreover, results from this study can be used as baseline information in formulating vitamins, minerals and amino acid supplements tailored for quail layers.

Materials and Methods

Birds, cage construction and experimental design

One hundred clinically healthy and ready-to-lay Japanese quail hens (approx. 56 d old) purchased from a local breeder were used in this study. The experiment was laid out in a completely randomized design composed of one control and four treatment groups replicated four times with five birds per replicate.

The birds according to each treatment group were placed in individual cages measuring 70 x 53 x 24 cm constructed using chicken wire mesh (1.27 x 1.27 cm size). Each cage was divided into four equal compartments to accommodate five birds per compartment. The flooring of the cage was sloped at 7º to allow eggs to roll out in front and facilitate egg collection. All cages were thoroughly washed using detergent soap and rinsed thoroughly with water three d before birds were placed inside the cages. The study was conducted at the animal facilities of the College of Veterinary Medicine, Visayas State University, Baybay City, Leyte, Philippines as duly approved by the Student Research Committee of the college.

Feeding, supplementation and lighting management

The birds underwent a period of acclimatization for a period of three wk prior to the start of supplementation. The birds were given a constant supply of commercial laying mash (B-Meg®, Philippines) using feeders made of polyvinyl chloride (PVC) pipes cut lengthwise (70 cm long). Birds had access to clean potable water at all times supplied *via* improvised PVC pipes cut in half (60 cm long) and placed on the sides of the cages above the area from where eggs were collected. Supplementation was started after three wk using a single multivitamins and amino acid supplement typically used for layer chickens and purchased commercially (Table 1) at the following rate: Control (without supplement) and from Treatments 1-4 given at 1 g, 1.5 g, 2 g and 2.5 g per liter of water, respectively for four wk. About 16.5 h of artificial lighting was provided using an 18-watt fluorescent bulb regularly turned on at 0530 and turned off at 2200 (Seker, 2004).*External egg quality determination* External egg quality measurements were taken at day 1, 1st, 2nd 3rd, 4th wk of supplementation and two wk post-supplementation. Among the parameters for external egg quality measurements taken were egg weight, egg shell thickness, egg shell weight and egg shell ratio. Egg weight per treatment was taken by directly weighing eggs using a triple beam balance. Soon after, the eggs were broken and emptied of contents to separate and collect the egg shells. The egg shells were dried at room temperature for 24 h before measurements of egg shell thickness, egg shell weight and egg shell ratio were taken. Egg shell thickness was determined at different locations (blunt, sharp and equatorial regions) using a calliper. The average value (mm) was then calculated to be the egg shell thickness. Shell weight (g) was determined using a triple beam balance while the egg shell ratio computed by dividing the shell weight over the egg weight, multiplied by one hundred.

Statistical analysis

All data were collected and consolidated using the Microsoft Excel 2016. Analysis was done using the SPSS Statistics version 22 (IBM Corporation, NY, USA). Statistical differences between treatment groups were computed using mixed factorial ANOVA to determine if values were significantly different ($p \leq 0.05$). Tukey test of significance was used to determine significant difference between treatment groups tested by ANOVA.

Table 1: Nutrient composition of commercial vitamins, mineral and amino acid supplement

Ingredient	Amount (per kg supplement)
Ca-D-pantothenate	5.00 g
Methionine	400 g
Lysine	100 g
Nicotinic acid	15 g
Vitamin A	5,000,000 IU
Vitamin D3	500,000 IU
Vitamin E	2,500 IU
Vitamin K3	1.00 g
Vitamin B1	2.00 g
Vitamin B2	4.00 g
Vitamin B6	1.00 g
Vitamin 12	1.00 mg
Vitamin C	20.0 g

Results and Discussion

Interestingly, there was an interaction observed between the levels of supplementation and the amount of time (in wk) the birds were on supplement ($p \leq 0.05$). Eggshell thickness was highest between 1.5g/L and 2g/L at the fourth wk of supplementation but dropped after two wk of no supplementation (Figure 1).

Similarly, significant interaction was also observed between the time and levels of supplementation on the eggshell weight ($p \leq 0.05$; Figure 2). The eggshell weight initially increased on the first week of supplementation particularly with 1.5 g/L, dropped to about 0.90± 0.1 mm on the second and third week and bounced back to 0.95± 0.01 mm on the fourth week. Similar to the eggshell thickness, the eggshell weight decreased two wks after supplementation was withdrawn.

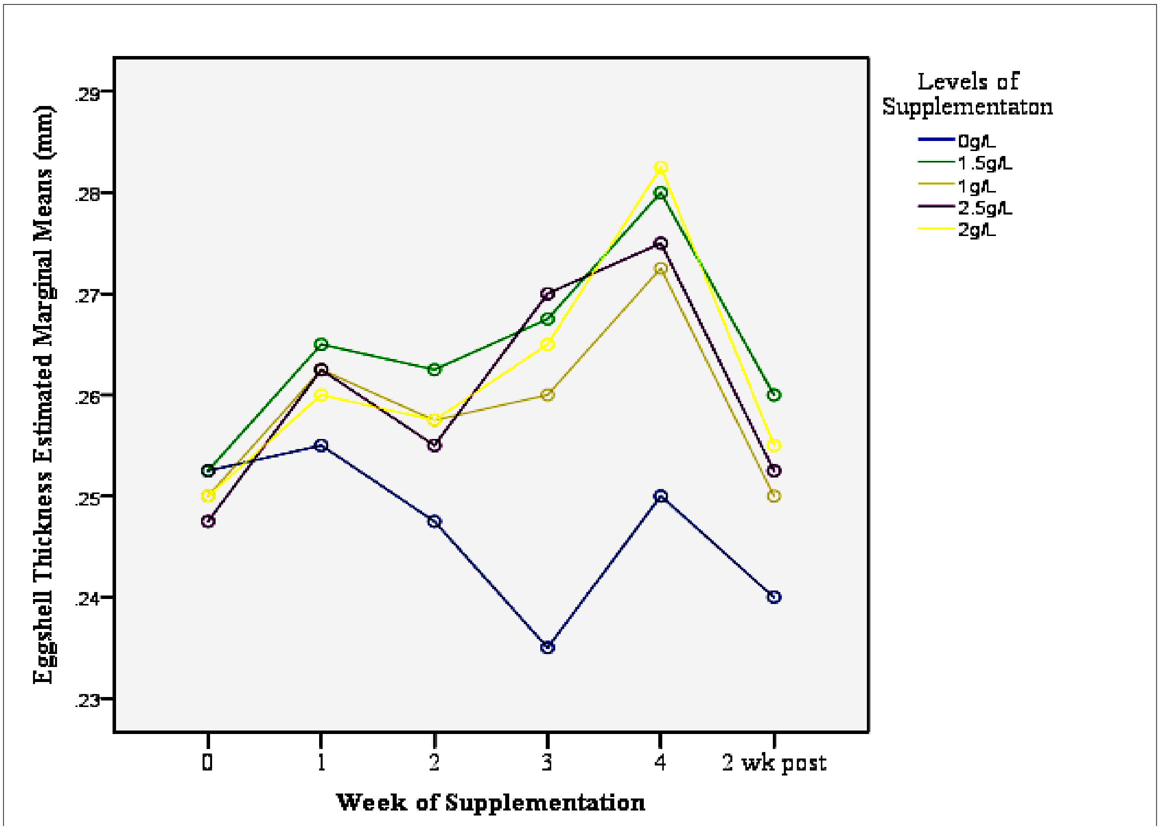

Figure 1: Profile plot showing significant interaction between time and levels of vitamins, minerals and amino acid supplementation on the egg shell thickness of Japanese quail eggs ($p \leq 0.05$); n= 20 birds/treatment.

There was no significant interaction observed between the levels of supplement given and the number of wk the birds were given the supplement on egg weight ($p \geq 0.05$). However, the number of weeks alone appeared to have influenced egg weight ($p \leq 0.05$). Initial egg (wk 0) weight was the lowest while the maximum egg weight was achieved at the fourth week of supplementation particularly with 1.5g/L and

2g/L. Moreover, the effect of supplementation appeared to have been sustained even two wks post-supplementation ($p \leq 0.05$).

While the egg shell ratio was at the lowest for the control (wk 0; $p \leq 0.05$), there appeared to have some varying effects across treatments, and the control didn't differ with 1 g/L and 2 g/L, respectively. The highest egg shell ratio was achieved at 2.5 g/L but this didn't differ to 1 g/L and 1.5 g/L ($p \geq 0.05$; Figure 3).

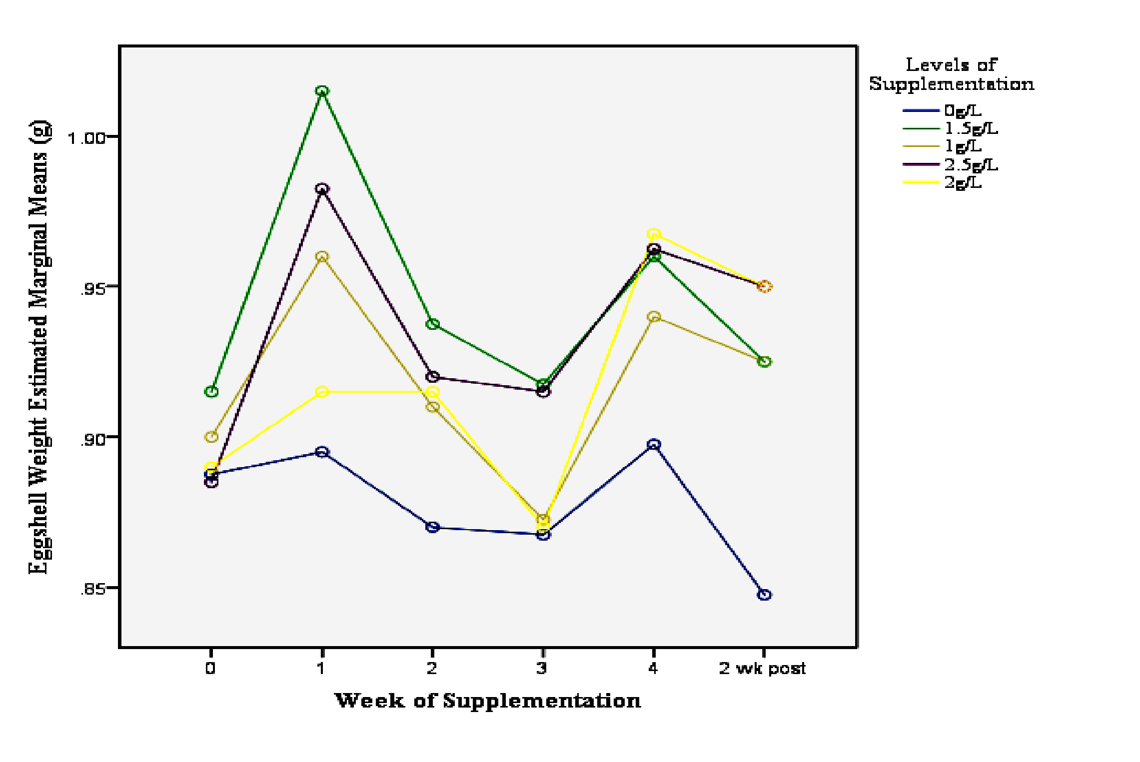

Figure 2: Profile plot showing significant interaction between time and levels of vitamins, minerals and amino acid supplementation on the egg shell weight of Japanese quail eggs ($p \leq 0.05$); n= 20 birds/treatment

The remarkable potential of quails for egg production relies on its capacity to produce up to 200-250 eggs/year under suitable conditions with full laying potential attainable as early as 50 d (Randall and Bolla, 2008; Priti and Satish, 2014). On average, quail eggs of approximately 10 g is equivalent to about 8% of the quail's body weight (Woodward et al., 1973) implying significant nutritional demands on the part of quail layers. Our study clearly demonstrated the improvement of egg shell quality over the course of vitamins, mineral and amino acid supplementation without necessarily affecting egg weight. Improvement of egg shell quality is a practical means by which egg producers can increase their return on investment by reducing the percentage of cracked or lost eggs (Hamilton et al., 1979).

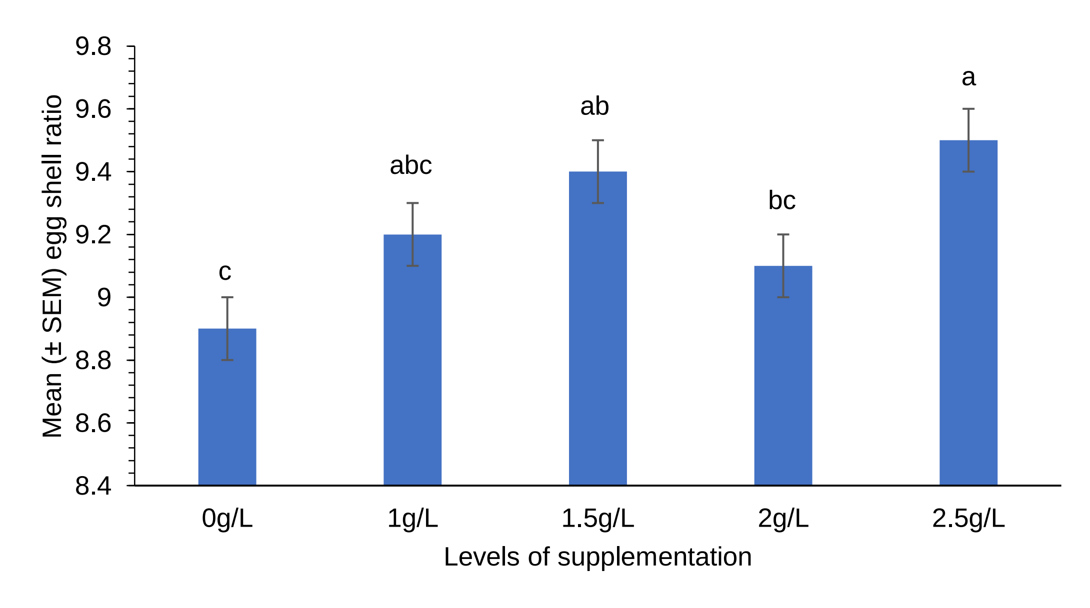

Figure 3: Mean (± SEM) egg shell ratio of Japanese quail eggs after supplementation with different levels of vitamins, minerals and amino acid supplement. Different letters indicate significant difference between treatments ($p \leq 0.05$); n= 20 birds/treatment.

Breakage or cracking of eggs essentially results when egg shell strength is not sufficient to overcome environmental insult to which the egg is exposed (Carter, 1970). Given the potentially stressful handling conditions associated with collection, sorting and transport of eggs to its final destination, thin and fragile eggs are likely to be rejected. Moreover, as the external quality traits influence the internal quality traits of the egg (Alkan et al., 2010) including those of the overall egg quality, chick weight and hatching performance (Farooq et al., 2003), there is a need to find solutions on how to improve external egg quality specifically in quails where egg production is a primary commodity.

The literature provides extensive evidence on the positive impact different vitamins and minerals have on improving egg shell quality on overall egg performance. However, limited studies appear to support potential synergistic effect of a combined vitamins, minerals and amino acids supplement. Moreover, while commercial poultry supplements have become commonly available, these are difficult to find specific for quail layers. Nevertheless, many of these nutrients are integral to the bird's nutritional requirements and in most cases, it is during deficiency or high demands particularly during peak egg production that supplementation becomes paramount.

Quail egg is surrounded by two membranes and an external covering called an egg shell. The egg shell composed primarily of calcium carbonate (Jacob et al., 1998) plays a crucial role in protecting the contents of the egg from microbial contamination while conserving the valuable contents/nutrients within the egg (Gupta, 2008). While egg shell quality can be affected by various factors such as genetic make-up, flock age, flock health, housing and storage (Altan et al., 1998), improper nutrition appears to be the major cause of shell defects and to some extent may eventually cause the hen to stop laying.

Inadequate levels of protein, vitamins and minerals can cause significant drop in egg production and egg shell quality (Jacob et al., 1998). Therefore, it is important to provide constant supply of nutritionally balanced layer diet. Calcium is a primary component of egg shell as inadequate calcium levels could result in lower egg shell quality (Vohra et al., 1979). In one study, about 2.5% calcium was found to increase egg weight and shell thickness, 2.75% increased egg weight and shell weight and 3% resulted in the improvement of egg production and shell percentage (Sultana et al., 2007). Similar results have been demonstrated earlier by Ong and Shim (1972) and Kadam et al. (2006).

Supplementation of vitamins in the diet or drinking water not only promotes healthy birds but also boosts egg laying performance by the layers. While birds can synthesize Vitamin C, several factors can lead to deficiency including stressful conditions, high egg production and inclement environment temperatures among others (Sykes, 1978) which may lead to decreased protein biosynthesis and significant demands for Vitamin C. The supplementation of Vitamins C and E in the diet of laying Japanese quails appeared to have improved the antioxidant status of the quail layers and ultimately improved egg performance despite stressful conditions (Sahin et al., 2003; Ipek et al., 2007). At 200 mg/kg Vitamin C and 250 mg/kg Vitamin E supplementation in the broiler breeder hens, Chung et al. (2005) was able to prevent reduction in egg shell quality and tibia bone strength under highly stressful environmental temperatures. Vitamin D is required for normal absorption of calcium and phosphorus from the intestinal tract and the deposition of calcium on egg shell. In one study, deprivation of supplementary Vitamin D3 did not affect body weight of male and female Japanese quails despite a reduction in feed intake (Vohra et al., 1979) however, the production of eggs was reduced from 74 to 20% and the incidence of soft-shelled eggs was also increased. Vitamin B is required for efficient nutrient utilization along with Vitamin A which was found to support immune functions in commercial layers particularly under heat stress (Lin et al., 2002). In a separate study by (Parrish and Al Hasani, 1983), addition of 2,500 IU and 3,200 IU of Vitamin A/kg of feed improved egg production and fertility in female Japanese quails, respectively.

There are two limiting amino acids (methionine and lysine) that poultry layer ration seems to be often deficient. Supplemental methionine and lysine have been found to be beneficial to increase egg production and hatchability (Jacob et al., 1998; Shim, 2005) as well as higher body weight gain, better feed efficiency and higher gross income (Osti et al., 2002). In a separate study, quail diets supplemented with selenium-methionine combination was able to reduce the negative effects of oxidative stress during heat stress in quails (Sahin et al., 2008). These studies simply proved the positive effects of proper amino acids supplementation on egg quality and production.

Conclusion

External egg quality can be improved by supplementation using commercial vitamins, minerals and amino acid supplement originally designed for chicken layers. Vitamins, minerals and amino acid supplementation improved the egg shell thickness which was influenced by the time (in wk) the birds were on supplement. Egg shell thickness was highest between 1.5g/L and 2g/L at the fourth week of supplementation but dropped two weeks after supplementation. Similar results were observed with the eggshell weight and the eggshell ratio but not with the overall weight of the egg. Commercial vitamins, mineral and amino acid supplements should be made available specific to the needs of quail layers.

Acknowledgements

The authors would like to acknowledge the assistance of Prof. T.J. Fernandez for his ideas and contributions in developing the experimental design of the study.

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