The Effect of Using Exoskeleton of Blue Crab (*Callinectes sapidus*) as a Dietary Calcium Source on the Egg Characteristics of Layer Hens (*Gallus gallus domesticus*)

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Abstract: The study focuses on the ability to use the exoskeleton of blue crab as alternative calcium and carotenoid supplement for layer hens and its effects on egg characteristics. Three groups of eighty layer hens each were fed a base feed formulation following the standards of the University of the Philippines Los Baños. The feeds were base feed as negative control (NG), a base feed with natural egg yolk colorants (capsorubin and lutein) as positive control (PG), and a base feed with 1% ground blue crab exoskeletons, as experimental (EG). The feeding lasted four weeks before egg collection. The eggs were weighed for their albumen, yolk, shell, and egg weight. The egg yolk color was determined using a DSM Fan and a digital Chroma meter, and the shell thickness using digital calipers. Statistical treatment was done through Kruskal Wallis test using SPSS software. Results showed that eggs of EG had significantly heavier shells with a mean of 5.93±0.11g, compared to 5.83±0.08g of the NG and 5.55±0.08g of the PG. Eggshell thickness was not significantly different among the three groups. Egg yolk color was significantly different in PG with a mean DSM gradient value of 11.96±0.11, compared to 5.92±0.14 of the NG and 6.48±0.20 of EG. Ground blue crab exoskeleton as a calcium supplement may increase the weight of the eggshells but may not intensify the egg yolk color.

Key Words: Layer hens; calcium; carotenoid; crab; egg

1. INTRODUCTION

According to the Philippine Statistics Authority (2019), egg production has grown, at the highest rate of increase, by 43.4% from January 2019 to March 2019. The industry has produced 142.01 metric tons of eggs from January to March of 2019, as opposed to the 130.55 metric tons of eggs produced in the same months of the previous year.

Layer hens (*Gallus gallus domesticus*) are domesticated chickens bred specifically for the production of eggs. According to the Philippine Statistics Authority (2019), as of July 2019, there are 40.4 million-layer hens in inventory. The feeds consist of raw ingredients which contain the nutritional requirements of laying hens. These include sources of energy and carbohydrates, protein, vitamins, minerals, and oils (PHILSAN, 2003).

For layer hens, a key component of their feeds is the mineral source, specifically calcium. Calcium increases the quality of the egg in terms of its shell. With its increasing demand for feeds, the layer hen industry varies in sources of calcium, such as limestone grits and limestone fines. The average commercial feed supplemented with calcium carbonate grit usually has sufficient calcium, phosphorus, manganese, and vitamin D to produce sound shells (Ahmadi et al., 2011).

Egg yolk color is also an essential parameter of egg quality. There are egg yolk colorants, which act as a feed component to intensify or modify the color of egg yolks from chickens that consume them. The number of egg colorants added on feeds is vital because, according to Zaheer (2017), the yolk color is "largely dependent on hen's feed composition." The yolk color is important to consumers' acceptability because a richer-colored yolk meant a healthier and more nutritious egg since the hen's diet came from natural pigments (Severson, 2020). Some egg colorants are capsorubin and lutein, making the egg yolk slightly darker and lighter, respectively. Capsorubin is a natural pigment found in chili peppers, which coexists with capsaicin to provide dark red colors. It is an excellent source of vitamin A and contains dietary fiber, vitamin E, B6, and folate (Hassan et al., 2019). On the other hand, Lutein is a natural pigment in vegetables, which gives them their bright, yellow pigment (Zaheer, 2017).

This study used *Callinectes sapidus*, commonly known as blue crabs, widely used for human consumption. The study utilized the exoskeleton of *C. sapidus* since this is being discarded and considered as factory waste after the meat from this crab is extracted and manufactured into other food products.

The exoskeleton of a blue crab covers almost all parts of its body and consists of multiple components, including calcium carbonate ($CaCO_3$), estimated to comprise 27.5% of the total exoskeleton. This may make it a viable source of dietary calcium for layer hens. In line with this, the study aimed to assess if using crab exoskeleton as a dietary calcium source may increase the hardness and thickness of layer hen eggshells. The crab exoskeleton also contains carotenoids, which may modify the egg yolk color.

Proximate analysis is a standardized series of tests to feed to determine crude protein, crude fiber, crude fat, crude ash, dry matter, and moisture content (Mæhre, 2018). It is done using various tests using different apparatuses for each substance measured and is a standardized test for chicken and pig feeds. This was used to determine the calcium content of the crab shells.

Following the statements above, this study aims to investigate the effect of using crab exoskeletons as a dietary calcium source on the egg quality of layer hens in terms of egg characteristics, specifically, egg weight, egg yolk weight, albumen weight, shell thickness, and egg yolk color.

2. METHODOLOGY

2.1 Site and duration of the study

The study was conducted from January 2020 to February 2020 at the University Animal Farm, Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna.

2.2 Experimental design

A total of two-hundred forty (240) laying hens, provided by the UPLB-IAS, were housed and fed at the University Animal Farm and were at post-peak of production (H&N Super Nick).

The hens were randomly allotted to three different dietary treatments of ten replicates, each with eight chickens. The total number of laid eggs was recorded daily. Egg characteristics were recorded during the last week of feeding.

2.3 Feed formulation and feeding

Three different experimental layer diets had been formulated in crumble form. The first diet was the control diet with a formulation given by UPLB-IAS. The second diet was the same formulation as the first diet added with one kilogram per ton of capsorubin and one kilogram per ton of lutein, both provided by UPLB-IAS. The third diet was also the same formulation as the first diet added with 1% crab shell meal. The base feed was milled with respective components using a mill. Each pen was offered with their diet and water ad libitum for four weeks. The diet composition used for feeding the hens is shown in Table 2.1.

Table 2.1 Composition of Experimental Diets of Layer Hens

Ingredient	Amount (%)		
	Negative Control Diet	Positive Control Diet	Experimental Diet
Corn	46.22	46.22	46.22
Soya	29.52	29.52	29.52
Limestone	9.46	9.46	9.46
RBD 1	7.78	7.78	7.78
Coco oil	2.65	2.65	2.65
Molasses	2	2	2
MDCP	1.53	1.53	1.53
Iodized Salt	0.25	0.25	0.25
Vitamins	0.12	0.12	0.12
DL-meth	0.12	0.12	0.12
Choline Powder	0.1	0.1	0.1
Minerals	0.1	0.1	0.1
Toxin binder	0.05	0.05	0.05
Mold inhibitor	0.05	0.05	0.05
Antioxidant	0.05	0.05	0.05
Crab meal	0	0	1
Capsorubin (per ton)	0	0.1	0
Lutein (per ton)	0	0.1	0

2.4 Proximate analysis

The crab exoskeleton had undergone proximate analysis at LQCC. Proximate analysis was done to determine the components of the exoskeleton of the crab. The guidelines given by LQCC were followed for the preparation of crab shell meals for analysis. The components of the base diet are gathered through the indicated values in the sack of the feed.

2.5 Data collection

Eggs were collected for analysis at the end of the 28-day experimental period with 139 eggs. Individual eggs were weighed using a standard digital weighing scale. The eggs were broken manually, and the egg yolk was separated from the egg white using a manual egg separator. The color of the egg yolks was measured in two different methods. The first method used a DSM Yolk fan and was performed by one researcher only. The second method used a Konica Minolta Chroma Meter CR-410, provided by UPLB-IAS Director. The Chroma meter was calibrated first before the start of collection using the proper procedure instructed by the manufacturer. The shell thickness was measured using a digital caliper and was measured in three different parts of an egg, the tip, middle, and butt. Shell weight was recorded after eight (8) days of drying at room temperature.

2.5 Statistical Treatment

The data collected from the eggs were analyzed statistically using the Kruskal-Wallis test at 95% confidence interval through SPSS statistical software to observe the differences between the values of the variables, which are the egg qualities, among the three groups. The researchers originally planned to use One-way ANOVA by comparing the mean of the variables from each treatment since this is the most suitable statistical analysis for the data because this can determine if there is a significant difference in the effect of the independent variables (treatments) to the dependent variables (egg qualities). However, the data violated multiple assumptions of tests, including the normality using Kolmogorov-Smirnov test and homogeneity of variance using Levene's test returning results unsuitable for One-way ANOVA. The data also underwent screening using box plotting to determine extreme outliers. The groups were compared to each other to determine if there is a significant difference among the groups. They were further compared by pair to decide which groups contained substantial differences.

Table 3.1 *Summary of Means and Kruskal Wallis Analysis of the egg characteristics at 95% confidence interval (**α** = 0.05).*

Treatment	Weight (g)				Shell thickness (mm)	Chroma meter			(DSM yolk Fan)
	Egg	Egg White	Yolk	(Shell)		(L*)	(a*)	(b*)	
Negative	63.65±0.82	36.41±0.67	17.61±0.26	5.84±0.09b	0.320±0.01a	82.66±0.15b	10.18±0.21b	56.46±0.81b	5.91±0.14ac
Experimental	63.37±0.91	35.71±0.70	17.60±0.23	5.95±0.11a	0.316±0.01	82.35±0.17a	10.92±0.30a	56.00±0.61a	6.51±0.20ab
Positive	62.10±0.77	34.97±0.57	17.33±0.23	5.57±0.09ab	0.296±0.01a	74.61±0.25ab	23.75±0.28ab	51.33±0.64ab	11.95±0.12bc

Results were presented as mean ± S.E. of 10 replicates observation. groups with significant difference ($p<0.05$) have parenthesis in their row heading.
L * is a measurement of black to white on a scale of 0 to 100.
a * is a measurement of green (-) to red (+).
b * is a measurement of blue (-) to yellow (+).
Values with the same letter (a,b) in the same column are significantly different from each other.

3. RESULTS

3.1 Weight

In Table 4.1, the negative control group (NCG) exhibits the highest values in egg yolk, egg white, and the whole egg weight among the other groups.

The experimental group (EG) has the highest value in shell weight, which is significantly higher than the positive control group (PCG) but not with the NCG. The difference between the three groups is significantly different.

3.2 Shell Thickness

In Table 4.1, the negative control group exhibits the highest eggshell thickness value compared to the other group. It is only significantly higher in the positive control group and not in the experimental group.

3.3 Yolk color

In terms of Chroma meter measure measurement, PCG has the highest a* value which means it is the reddest in color among the feeds. Still, it also has the lowest value in L* and b*, which indicates that it has a darker color and the least yellow. It also exhibited the highest yolk color rating in terms of the DSM yolk Fan, with a mean of 11.95

In comparing NCG and EG, NCG has a greater L* value which means it is more light-colored than EG; however, its a* value is less than the experimental, which indicates that the experimental is redder in color. The highest b* value is shown by the negative control group, which means it is the most yellow.

4. DISCUSSION

From the results, it can be said that there is no significant difference (α=0.05) in the effect of C. sapidus-based feeds on egg weight when compared among the groups.

NCG has the heaviest albumen weight values, whereas EG may have lighter weight but has values that can be attributed to calcium content. This was supported by data in the study of Ribeiro et al. (2016), where they also observed that increasing the amount of calcium in the diet decreases the amount of albumen in the egg.

In terms of shell weight, the EG has the highest value. It supports the proximate analysis of experimental feed and crab meal that it has higher calcium content than the other group. This was in line with the study of Ribeiro, et al. (2016) since their research observed that an increase in calcium content also increases the shell weight. There is a significant difference (α=0.05) in C. sapidus-based feeds on shell weight compared to the groups.

The NCG has the highest shell thickness value, but its feed calcium content does not contain the highest calcium. There is a significant difference (α=0.05) in the effect of C. sapidus-based feeds on shell thickness when compared to the PCG but has no significant difference when compared to the NCG. There may be other factors that affect the eggshell thickness, including the physical characteristics of the layer hen.

For the yolk color based on the Chroma meter, the NCG and the EG have values that are not significantly different, but the PCG has a significantly different value than the two groups. There is a significant difference (α=0.05) in the effect of C. sapidus-based feeds on yolk color L*, a*, and b* when compared among the groups. There is a significant difference in C. sapidus-based feeds on yolk color L*, a*, and b* when compared to the PCG but has no significant difference compared to the NCG.

There is a significant difference (α=0.05) in C. sapidus-based feeds on DSM fan yolk color compared to the groups. The PCG also exhibited the highest yolk color rating in terms of the DSM yolk Fan, with a mean of 11.96 among the varied group. Since the PCG is the basal feed diet supplemented with capsorubin and lutein as egg yolk colorants, the egg yolk color would appear to become much brighter among the three groups. This is supported by Grashorn (2016) study, where it was found that the addition of red and yellow colorants in the egg affects the color of the yolk, which makes it more golden-orange.

5. CONCLUSION

In the study, three groups of layer hens were subjected to three different treatments, NCG, PCG, and EG.

The EG had the eggshells with the most positive significant weight difference but did not significantly change the egg yolk color compared to the NCG. The PCG had the most different egg yolk color yield among the treatments. It could be said from here that ground crab exoskeleton may be a viable calcium supplement for layer hen feeds but may not be a viable egg yolk colorant.

The proximate analysis results further support the effectiveness of the crab exoskeletons as a calcium supplement due to the calcium composition of the crab meal being higher than that of the basal feed diet at 23.59%.

However, compared to limestone grits, the most commonly used calcium supplement, ground crab exoskeleton, has a considerably lower calcium content. Thus, it could be said that limestone grits may still be more effective as a calcium supplement on a basal feed diet. With these mentioned, it can be concluded that ground crab exoskeletons have the potential to be used as a commercially available calcium supplement for layer hens but not as a viable source of egg yolk colorant. However, it may work in conjunction with the egg colorants used in the PCG, namely capsorubin and lutein, as supplements for calcium and egg yolk colorants.

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