Original Research Article

Main and Interactive Effects of Calcium and Xanthophyll Supplementation on the Laying Performance, Internal and External Qualities of Isa Brown Hen Eggs

ABSTRACT

The study investigated the main and interactive effects of calcium and xanthophyll supplementation on the laying performance, internal and external qualities of Isa brown hen eggs. One hundred and forty-four point of lay hens were used in a (2x3) factorial experiment. They had calcium and xanthophyll as the factors each with three (3) qualitative levels. For calcium, the levels were, no calcium, calcium from egg shell and calcium from snail shell. For xanthophyll, the levels were no xanthophyll, xanthophyll from pepper, xanthophyll from carrot. There were nine (9) treatment combinations each replicated four times, making 36 experimental units with four hens per battery cage unit. Data collected were analyzed using genstat package. Significant means were separated using least significant difference (LSD). Results showed that neither calcium supplementation nor xanthophyll supplementation had any significant effect on feed intake, hen day production, egg weight and feed per dozen egg ($P>0.05$). However, there was significant interaction between calcium and xanthophyll supplementation for egg weight and feed per dozen egg laid ($P<0.05$). The largest eggs were laid by supplementation with calcium from snail shell and xanthophyll from pepper (59.31 g). Also the lowest feed were consumed by hens that received calcium supplementation but supplemented with xanthophyll from carrots (2.09 kg) ($P<0.05$). The haugh unit was significantly affected by either calcium supplementation or xanthophyll supplementation. Yolk colour was only affected by xanthophylls supplementation with the deepest colour (13.58) recorded when hens were offered xanthophyll from pepper ($P<0.05$). Significant interaction ($P<0.05$) were recorded for both haugh unit and yolk colour. The highest haugh unit (93.70) was recorded among hens not supplemented with ether calcium or xanthophyll, while the lowest (76.40) was recorded among hens supplemented with xanthophyll from carrot and calcium from snail shell. Yolk colour was consistently and significantly deeper among hens that received xanthophylls from pepper whether supplemented with no calcium or supplemented with calcium from either egg shell or snail shell ($P<0.05$). It was concluded that egg weight can be improved by offering extra calcium from snail shell supplemented with pepper to hens outside feed. Again supplementing hens diet with pepper alone or in addition with either calcium from snail or egg shell supplementation would result in deeper yolk colour that is always the choice of consumers.

Keyword: Isa brown, Xanthophyll, Supplementation, Haugh unit, Hen day production, Feed per dozen egg, Yolk colour, Egg quality.

INTRODUCTION

Over the years, eggs from birds in general and hens in particular, have provided part of the animal proteins that are necessary for human health (Atteh, 2004; Olomu, 2011). An egg is vehicle for reproduction and it also serves as a source of food for human consumption. The size and shape of eggs differs among the various species of birds, but all eggs have three main parts like yolk, albumen and shell. These three parts of the egg are separated from each other by membranes. The shell is separated from the albumen (egg white) by the shell membranes and the yolk is separated from the albumen by the yolk membrane (Habtamu, et, al, 2019). Hens eggs indeed contain the nine essential amino acids that the human body cannot synthesize (Olomu, 2011). It has thus been preferred by World Health Organization (W.H.O.) as the reference protein source for the child (reference 100, which is slightly higher than women's milk) (Olomu, 2011). Beyond proteins, eggs are also a valuable and easily renewable source of lipids, minerals, and vitamins. The importance of eggs in industries other than agro-alimentary is also growing. Its antioxidant, cryoprotective, antiviral, antibacterial, emulsifying, and coagulating properties are indeed valorized in the pharmaceutical or cosmetic sectors (Mine, 2002;Atteh, 2004;Mine and Kovacs- Nolan, 2004; Olomu, 2011).Moreover, as eggs are accepted by most cultures and religions, it constitutes an interesting tool in solving the world nutrition problem. Egg quality is determined by its consumers acceptance concerning several characteristics including cleanliness, freshness, surface area, mass, volume and coefficient of packaging, egg weight, shell quality, yolk index, albumen index, Haugh unit, and chemical composition (Narushin, 1997).The egg quality composition comprises some aspects related to the shell (10%), albumen (60%), and yolk (30%) and can be divided intoexternal and internal quality. All egg quality characteristics are affected by several factors including age and genotype ofhen, nutrition, type of rearing system, and the time of oviposition (Ahmadi and Rahimi, 2011; Yang, *et al.*, 2014).

The consumer demands highly colored (fancy) yolks in eggs throughout the year and the eggs with pale-coloured yolks are the object of many complaints particularly in cities. The use of plants as source of carotenoids in layer diets is a natural way of obtaining egg yolk with a pleasant (golden yellow or orange yolk) colour and high in carotenoids. This type of plants usually have antioxidant properties, improve feed efficiency and have favourable effects on the poultry because they influence the taste and flavour of the diet.(Hammershoj, et, al, 2010; Leeson and Summers, 2005; Leeson, and Caston , 2004). Researches had demonstrated that carotenoids, especially lutein and zea xanthin, play an important role in human health for preventing certain eye disorders or other diseases. The most effective prevention to date is increasing our intake of lutein, which accumulates in the muscular region of the eye and seems to aid in the prevention of such blindness. Consumers tend to associate golden yellow to orange yolk with good health (Caliscar and Uygur, 2010). The yolk color depends not only on the levels of xanthophylls present in the feed but also on the type and ratio of these compounds (Galobart, *et, al, 2004*).

Many researchers have indicated that the primary compounds with a colouring role in poultry products are carotenoids, namely, xanthophyll, the concentrated extracts from the marigold meal (natural xanthophyll), capsanthin, and canthaxanthin (Perez, *et al,* 2001). Animals, including poultry, absorb carotenoids from their diets and store them after having modified their structure by oxidative metabolism (Blanch, *et, al.,* 2000). For this reason, high levels of natural or synthetic pigments are usually added to the diet of commercial layers as yellow or red xanthophylls to achieve the desired yolk color and make eggs more appealing for consumers and more suitable for the egg-products industry (Sirri, *et, al,* 2007). The use of pigments has gradually changed from synthetic colorants to natural ones (Niu, *et, al,* 2008). Some leaf powders or extracts from plants can be used to increase the pigmentation of broiler skin and egg yolks, producing a more desirable level of pigmentation (Karadas, *et, al*, 2006) as well as health benefits to the animals (McGraw, *et, al,* 2005). In recent years, the use of natural colorants has been actively exploited in many countries, with xanthophylls and carotenoids of plant origin becoming the main sources of natural feed colorants because of their many advantages, such as safety, strong biological activity, and greater bioavailability.

In Nigeria, local hens raised on free-range or extreme systems scavenge on vegetable material, some of which are known to contain carotenoid substrate; Observation has shown that eggs laid by such hens have deeper yellow and are more appealing yolk colour.

Calcium is a very important nutrient needed for egg production. A laying hen requires about 3.45% calcium in its feed for egg production. However, when birds are fed in a group, the amount of calcium required will be available for all of the hens at the same time. Presenting an allowance for extra calcium in-situ in form of cafeteria feeding may enable hens to get more calcium on a need basis, and that may help to enhance better production and egg quality. In the calcium deposition model, calcium is deposited constantly throughout the eggshell formation period. The eggshell consists mainly of calcium carbonate, hence calcium plays an important role in the eggshell formation (Shen and Chen, 2003).The integrity of the egg shell depends on the calcium availability to the hens. To enhance laying performance and the internal quality of eggs laid, offering extra xanthophyll from vegetable nutrients and calcium from feed ingredients may be a plausible idea worth experimenting with. The focus of this study was to investigate the main and interactive effects of calcium and xanthophyll supplementation on the laying performance, internal and external qualities of Isa Brown hens eggs.

MATERIAL AND METHODS

Experimental Site

This experiment was carried out at the Teaching and Research Farms, Animal Unit of Benson Idahosa University, Benin City, Edo State, Nigeria. The farm lies within the rain forest vegetation belt in the South-South geopolitical zone of Nigeria and is characterized by two district seasons which runs from November to March for dry and April to October for wet each year. The area is located between Latitude 05^0 44' North and 07^0 34' North of the Equator and Longitude 05^0 04' East and 06^0 43' East of the Greenwich Meridian on an elevation of 73m above sea level

Management of Birds and Experimental Design

A total of one hundred and forty-four (144) point of lay Isa Brown hens reared under similar management practices were used for the experiment. They were randomly subdivided into nine (9) treatment combinations and four replicates each with four (4) birds in each replicate using a completely randomized design. The experiment was set on a 2x3 factorial design, with two factors, calcium and xanthophyll having three qualitative levels each. The qualitative levels for calcium were; No calcium, calcium from egg shell and calcium from snail shell, while the qualitative levels for xanthophyll were: No xanthophyll, xanthophylls from carrot (*Daucus carota*) and xanthophylls from pepper (*Capsicum frutescens*). The birds were administered anti-stress orally at the recommended dosage after randomization before the commencement of the experiment. The birds were reared in a constructed open sided battery cages wire mesh measuring 48 cm x 35 cm x 42 cm to allow ventilation in the poultry house. The hens were administered medication, vaccination and other standard routine management practices were strictly followed.

RESULT

A commercial layer diet with the proximate analysis presented in table 1 was used throughout the experiment for all the treatment combinations. Hens were fed *ad libitum* and water was presented to satisfaction, throughout data collection that lasted for 9weeks covering phase 1 of the hens laying cycle. Grated carrot and pepper that are served as sources of xanthophyll and grounded eggshell and snail shell which served as sources of calcium were given directly to the hens through separate containers attached to the individual cage unit. Hens had unrestrained access to the tin containers.

Table 1: Proximate Composition of the Commercial Layers Feed (As Seen in the bag label)

Ingredients	Percentage
Moisture Content (%)	12.00
Crude Protein (min) %	16.50
Crude Fats/ Oil (max) %	5.00
Crude Fiber (max) %	6.00
Calcium %	3.60
Available Phosphorus %	0.45
Ash (max) %	12.00
Lysine	0.80
Methionine	0.34
Salt	0.30
Metabolisable energy (kcal/kg)	2500

Data Collection Procedure

Performance parameters taken were feed intake, body weight and body weight gain.

Feed intake was determined by the difference in the quantity of feed offered to the birds in each replicate at the beginning of each week and the leftover at the end of the week.

Body weight of the birds was taken at the beginning of the experiment and subsequently on weekly basis using the digital weighing scale.

Body weight gained was calculated by subtracting the initial weight at the beginning the week from the final weight at the end of the week.

The laying records taken were egg number, egg weight, hen day production, and feed per dozen egg. Eggs laid were collected regularly on daily basis at 5 pm each day and recorded for each replicate to obtain the egg number. Egg weight was obtained by weighing individual eggs using sensitive weighing scale to the nearest 0.1gm.

Hen day production was calculated by dividing the total egg laid by the number of hen days and the mean expressed as percentage. The hen day production (HDP) was calculated with the formula;

$$HDP(\%) = \frac{Total\ eggs\ produced}{Total\ Hen\ Days} \times \frac{100}{1}$$

Where Hen Days = No of hens X No of days in lay.

Feed per dozen eggs refers to the amount of feed consumed by the hen to lay twelve (12) eggs (a dozen). It was calculated by dividing total feed consumed per hen, by total eggs laid multiplied by 12. Feed by dozen eggs was calculated using the formula;

$$Feed\ per\ dozen\ eggs(\text{kg}) = \frac{Total\ feed\ consumed\ per\ hen}{Total\ egg\ laid} \times \frac{12}{1}$$

The egg internal and external parameters taken were yolk colour, yolk index, yolk height, yolk width, Albumen height, haugh unit, egg diameter, shell thickness and shell weight.

Yolk Colour was determined using the Roche fan colour chart.

Yolk height and yolk width were measured using a ruler.

Yolk index (YI) was calculated by dividing the yolk height by the yolk diameter (width).

$$Yolk\ index\ = \frac{Yolk\ height}{Yolk\ diameter(width)}$$

Albumen height was measured with the use of tripod micrometer gauge.

Haugh unit was determined by measuring the height of the thick albumen 1 cm from the yolk edge. The height of the thick albumen surrounding the yolk, combined with the egg weight, determines the haugh unit score.

Haugh unit (HU) was calculated using the equation of Haugh (1937);

$$HU = 100 \times \log_{10} (\text{Albumen height} + 7.57 - 1.7 \times \text{Egg weight}^{0.37})$$

Egg diameter was determined using the veneer caliper (mm)

Shell thickness was determined by measuring individual egg shell (plus membrane) at three different locations of the shell (narrow, middle and broad portions) using the micrometer gauge to the nearest 0.01mm and the mean shell thickness was then calculated.

Shell weight was determined by carefully breaking the eggs and their content emptied. The shells were washed to remove adhering albumen leaving the shell membranes intact. The shells were dried at 105 C for about 24 hours and then weighed individually to the nearest .01 g.

Statistical Analysis

Data collected were analyzed using GenStat. Statistical package Release 12.1 as applicable to factorial designs in a completely randomized design. Significant different means were separated using Least Significant Difference (LSD) with probability set at $P<0.05$.

RESULTS AND DISCUSSION

4.1 RESULTS

The analyzed data presented in Table 2 shows the growth and laying performance of Isa Brown hens fed calcium and xanthophyll supplementation. The result showed that there were no significant differences in feed intake, body weight, body weight gain, egg weight, feed per dozen, and hen day production ($P>0.05$) with regards to calcium supplementation. However, though not significant, there was a slight incremental trend in HDP when additional calcium was supplied either through eggshells or snail shells. Also xanthophyll supplementation did not result in any significant effect on the aforementioned parameters ($P>0.05$). Although slight incremental trend was observed with HDP when xanthophylls was supplemented with carrot and pepper.

The interactive effect of calcium and xanthophyll supplementation given to Isa brown hens is shown in Table 3. Compared with hens that received neither calcium nor xanthophyll supplementation, feed per dozen eggs laid was significantly reduced either when hens were offered xanthophyll from carrot without calcium or when they were offered calcium from snail shell without xanthophyll ($P<0.05$). The lowest feed per dozen eggs were recorded with xanthophyll supplementation from carrot (2.09 kg) and pepper (2.14 kg) without calcium as well as calcium supplementation using snail shell without xanthophyll (2.15 kg) ($P<0.05$). Egg weight was significantly ($P<0.05$) highest with no calcium and no xanthophyll (59.70 g) and calcium supplementation using snail shell with pepper (59.30 g). The lowest egg weight was recorded with calcium supplementation using egg shell with pepper (54.62 g)

Table 2: Effects of calcium and Xanthophyll supplementation on the growth and laying performance of ISA Brown Hens (9weeks)

Parameter	Calcium				Xanthophyll			
	No Calcium	Egg Shell	Snail Shell	SEM	No Xanth	Carrot	Pepper	SEM
FI (kg)	14.95	15.18	14.38	0.51	14.93	14.81	14.77	0.51
Bw(kg)	1.48	1.49	1.49	0.03	1.48	1.46	1.52	0.03
BwG(kg)	0.85	0.06	0.04	0.03	0.02	0.06	0.06	0.03
Egg weight (g)	57.07	55.82	56.91	0.88	57.09	56.42	56.30	0.88
F/ dE(kg)	2.25	2.23	2.16	0.07	2.29	2.17	2.18	0.07
HDP(%)	70.31	72.69	71.36	1.98	70.46	72.62	71.29	1.98

**There was no significant difference at p>0.05

FI =Feed Intake Bw = Body weight BwG = Body weight gain F/De = Feed/ dozen egg HDP = Hen day production

SEM = Standard Error of Mean No Xanth = No Xanthophyll.

Table 3: Interaction of calcium and Xanthophyll supplementation on the growth and laying performance of ISA Brown Hens (9weeks)

Parameter	No Calcium			Egg Shell			Snail Shell			SEM
	No Xanth	Carrot	Pepper	No Xanth	Carrot	Pepper	No Xanth	Carrot	Pepper	
FI (kg)	15.31	14.56	14.99	15.10	15.62	14.81	14.38	14.24	14.1	0.62
Bw(kg)	1.47	1.47	1.50	1.45	1.46	1.56	1.53	1.44	1.49	0.48
BwG(kg)	0.05	0.08	0.01	0.00	0.06	0.12	0.02	0.04	0.04	0.05
Egg weight (g)	59.70^{a}	56.55^{b}	54.96^{b}	56.48^{b}	56.35^{b}	54.62^{b}	55.08^{b}	56.35^{b}	59.31^{a}	1.52
F/ dE(kg)	2.51^{a}	2.09^{b}	2.14^{b}	2.21^{ab}	2.27^{ab}	2.24^{ab}	2.15^{b}	2.27^{ab}	2.23^{ab}	0.12
HDP(%)	66.74	73.22	70.98	73.44	73.22	71.43	71.20	71.43	71.45	2.42

**There were no significant difference at p>0.05

**^{ab}Means in the same row with different superscript are significantly different at p>0.05

SEM = Standard Error of Mean No Xanth = No Xanthophyll FI =Feed Intake

Bw = Body weight BwG = Body weight gain F/De = Feed/ dozen egg HDP = Hen day production

The effect of calcium and xanthophyll supplementation on the internal and external quality of Isa brown hens eggs is shown in Table 4. For calcium supplementation, no significant difference in Haugh unit, yolk index, yolk colour, shell weight, shell thickness and shell diameter (P>0.05). However, the best haugh unit was recorded without calcium and xanthophyll. Both haugh unit and yolk colour were significantly affected by xanthophyll supplementation (P<0.05). The best haugh units were reached in experimental hens that did not receive xanthophyll (89.3) and hens that were fed on pepper supplementation as a source of xanthophyll (85.2) (P<0.05). Also significantly higher yolk colour balance of 13.58 was recorded for hens fed pepper as a source of xanthophyll (P<0.05).

The interactive effect of calcium and xanthophyll on the internal and external qualities of eggs is shown in Table 5. There was no significant interaction (P>0.05) between calcium and xanthophyll for the yolk index, shell weight, shell thickness and egg diameter. However, the best (P<0.05) haugh units were recorded for hens fed without calcium and xanthophylls (93.70) and those fed with snail shell without xanthophyll (89.30) (P<0.05). A significant interaction occurred between calcium and pepper as a source of xanthophyll for yolk colour (P<0.05). Yolk colour value reached highest where pepper was fed either without calcium or calcium from eggshell or calcium from snail shell with the value of 14, 13.25 and 13.50 respectively. There were no significant interaction for egg shell weight, shell thickness and shell diameter (P<0.05)

Table 4: Effects of calcium and Xanthophyll supplementation on the internal and external quality of ISA Brown egg (9 weeks)

Parameter	Calcium				Xanthophyll			
	No Calcium	Egg Shell	Snail Shell	SEM	No Xanth	Carrot	Pepper	SEM
Haugh Unit	89.3^{a}	83.9^{b}	83.0^{b}	2.32	89.3^{a}	81.6^{c}	85.2^{b}	2.32
Yolk Index	0.41	0.40	0.44	0.03	0.40	0.41	0.44	0.03
Yolk Colour	12.00	11.75	11.92	0.29	10.92^{b}	11.17^{b}	13.58^{a}	0.29
Shell Weight (g)	7.16	7.14	7.09	0.13	7.04	7.39	7.06	0.22
Shell Thickness (mm)	49.83	51.67	51.08	0.85	49.00	52.02	50.67	0.86
Egg Diameter (mm)	43.18	42.81	42.81	0.36	42.81	43.31	43.31	0.36

** Means within column means carrying the same superscript are not significantly different (p< 0.05)

SEM = Standard Error Mean No Xanth= No Xanthophyll

Table 5: Interaction of calcium and Xanthophyll supplementation on the internal and external quality of ISA Brown eggs (9weeks)

Parameter	No Calcium			Egg Shell			Snail Shell			SEM
	No Xanth	Carrot	Pepper	No Xanth	Carrot	Pepper	No Xanth	Carrot	Pepper	
Haugh Unit	93.70[a]	85.80[b]	88.50[ab]	85.4[b]	82.7[b]	83.6[b]	89.3[a]	76.40[c]	83.6[b]	4.02
Yolk Index (mm)	0.45	0.37	0.43	0.41	0.36	0.43	0.35	0.50	0.47	0.06
Yolk Colour	10.75[b]	11.25[b]	14.00[a]	11.50[b]	10.50[b]	13.25[a]	10.50[b]	11.75[b]	13.50[a]	0.50
Shell Weight (g)	7.22	7.24	6.97	7.23	6.8	7.33	7.35	7.06	6.87	0.22
Shell Thickness (mm)	49.25	50.75	49.50	50.75	54.25	50.00	49.25	50.75	49.50	1.46
Egg Diameter (mm)	43.64	42.99	42.90	42.34	42.23	43.87	42.81	42.31	43.31	0.62

**[ab]Means in the same row with different superscript are significantly different at $p<0.05$

SEM = Standard Error of Mean   No Xanth = No Xanthophyll

DISCUSSION

The growth performance of Isa brown hens fed calcium (eggshell and snail shell) and xanthophyll (carrot and pepper) supplementation *a la carte* from 1-9 weeks showed a non-significant difference (p>0.05) in feed intake, weight gain, and body weight gain. Feed per dozen egg and hen day production were not significantly different. These results are in agreement with those of Mansoori, *et, al*.,(2008), who reported that adding crescent levels of dry tomato pomace (0 to 100 g/kg) in laying hens diets did not affect egg production, feed intake, or egg mass. The significant interactive effect of pepper supplementation when offered with eggshell *a la carte* on the bodyweight of the hen showed that the various interactions did not have a significant difference on the feed intake, body weight, body weight gain, and hen day production however the control interaction of no calcium and no xanthophyll showed significant (p>0.05) difference with the control interaction of no calcium and carrot on the feed per dozen egg. The Haugh unit was significantly (p>0.05) affected by the absence of any supplementation of xanthophyll and also significantly (p>0.05) affected by carrot supplementation even though less than that of no xanthophyll supplementation but pepper had no such significance and also with calcium supplementation. The yolk index was not affected significantly by any of the supplementations. The yolk colour was significantly different (p<0.05) highest with the supplementation of pepper. It was also significant with pepper and no xanthophyll supplementation. However, egg weight was not affected by any of the supplementations. This result is in agreement with Niu *et al*, (2008) and Mansoori *et al.*,(2008), who reported that egg weight was not affected by pigments. In the interaction of the various supplementation, the Haugh unit was significant ($p<0.05$) at no calcium and no xanthophyll while it was least significant at the interaction between snail shell and carrot while the others had no interaction. The yolk index had no significant effect on it with the various interactions. In the interaction of supplementation of calcium and xanthophyll, showed that whether calcium is present or absent, pepper will still produce the highest significance ($P<0.05$) on the yolk colour followed by carrot before the least at no xanthophyll supplementation. This result is in agreement with Hamilton (1992) that hens were fed pepper and it showed an increased redness in the yolk colour. The egg weight was significant at the interaction between no calcium and pepper. It was also significant at eggshell and pepper, snail shell, and no xanthophyll ($p<0.05$).

Conclusion and Recommendations

The results from this study have shown that supplementing vegetables that are sources of xanthophyll on Isa brown hens for nine (9) weeks had no significant effect ($P>0.05$) on feed intake, body weight, body weight gain, feed per dozen egg, and hen day production. For higher feed per dozen eggs, the control treatment of no calcium and no xanthophyll was seen to have the highest effect, followed by the supplementation of carrot with no calcium. However, none of these interactions had any effect on the growth and laying performance of Isa brown hens. Calcium supplementation did not affect the internal quality of the egg, however, xanthophyll supplementation reduced in its significance ($p>0.05$) on haugh unit with the presence of vegetable sources of xanthophyll. Yolk colour was affected by xanthophyll supplementation with the highest with pepper. The control treatment in the interaction had the highest effect on the haugh unit, followed by snail shell and carrot interaction. Yolk index was not affected by the interaction. However, the yolk colour was affected the most by the interaction between snail shell and pepper. Based on the results from the experiment the following are the recommendations: For better haugh units, no supplementation of calcium and xanthophyll should be used. It is recommended that for better egg weight, no calcium or xanthophyll supplementation should be used. It is recommended that for better feed per dozen eggs, no supplementation of pepper should be used. It is also recommended that for better yolk colour, carrot and pepper should be supplemented.

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