EFFECTS OF STRAIN AND AGE OF LAYER CHICKENS ON MINERAL CONTENTS OF EGG YOLK AND ALBUMEN

Kruenti[1], F., Hagan[2], J. K., Ofori[2*], S. A. and Lamptey[1], V. K.
[1]Farm Animal Technology Development Division, CSIR-Animal Research Institute, P.O. Box AH 20, Achimota, Ghana
[2]Department of Animal Science, School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana
[*]Corresponding author's email: samuel.ofori@ucc.edu.gh

ABSTRACT
This study evaluated the effects of strain (genotype) and age on the mineral composition of egg yolk and albumen of the domestic chicken. A total of 810 eggs were used in a 3 X 3 factorial arrangement of treatments involving the Lohmann White, Lohmann Brown and White Leghorn which were 31, 40 and 53 weeks old. Data obtained were subjected to the analysis of variance (ANOVA) using the General Linear Model procedure in Minitab (version 18). Phosphorous was highest in yolks from the White Leghorn (4433.4 µg/g) than in yolks from the Lohmann Brown (4402.4 µg/g) and Lohmann White (4397.5 µg/g) strains. Iron content of egg yolk was significantly ($p < 0.05$) different among all the layers with the highest in the Lohmann Brown (77.0 µg/g) followed by the Lohmann White (37.2 µg/g) and lowest in the White Leghorn (21.7 µg/g). There was significant ($p < 0.05$) effect of layers' age on all the mineral elements of the chicken yolk except calcium, while all but phosphorous could be substantially affected by hen-age in the albumen. Copper and zinc concentrations of the yolk significantly increased from week 31 (0.7 and 21.2 µg/g) to week 40 (1.2 and 28.9.2 µg/g) to week 53 (1.9 and 39.5 µg/g) correspondingly. Chicken strain and age interactions were detected for all minerals in the egg parts with the exception of calcium in the yolk. The results denote that yolk from White Leghorn at week 40 had about 5% more potassium than at weeks 31 and 53, comparable to the observation made for the same element where the yolk from the Lohmann White at week 40 had about 11% more potassium than at weeks 31 and 53. In summary, there are variations in the mineral contents of egg yolk and albumen of layer chickens by virtue of their genetic constitution and ages. Consequently, farmers must produce from the best strains (genotypes) at the appropriate hen-ages to make mineral contents of eggs meet the needs of specific consumers and products. The outcomes will therefore be beneficial for dieticians to recommend quality eggs for persons with specific mineral challenges and also assist the manufacturing industry to choose eggs for particular products.

Keywords: *Genetic constitution, Lohmann White, Lohmann Brown, White Leghorn, variations*

INTRODUCTION

The world's population is projected to grow from 8 billion in 2022 to around 8.5 in 2030 and to about 9.7 billion by 2050 (United Nations Department of Economic and Social Affairs, Population Division, [2022]) and standard of living has been projected to rise with about 70% increase in the demand for agricultural products around the same period (International Egg Foundation-IEF, 2014; FAO, 2017). Animal products contribute nearly 17% and 33% of global kilocalorie and protein consumption respectively (Rosegrant *et al*., 2009). The chicken egg has formed significant part of human foods globally for many ages (Forson *et al*., 2011); providing high quality but relatively cheaper nutrition to many people (Pascoal *et al*., 2008; Menezes *et al*., 2009; IEF, 2014). Eggs provide a balanced amount of minerals (macronutrients and micronutrients) required for good health. Minerals, such as iron, calcium, phosphorus, potassium, copper, zinc, etc. are important elements present in the chicken eggs (Stadelman and Cotterill, 2013). In particular, eggs contain good amount of phosphorus, which is needed for bone and teeth health, normal nervous system function and energy metabolism. Again, it is reported that, the iron in egg yolk, is highly bioavailable; hence eggs may be a vital source of iron in diet of individuals who are deficient in iron and are lacto-ovo vegetarians (Ebegbulem and Asukwo, 2018). Eggs are also a rich source of naturally available zinc needed for proper functioning of the immune system (Sahin *et al*., 2009; Kucukyilmaz *et al*., 2012). Calcium, a critical mineral necessary for bone growth and maintenance, muscular contraction, and nervous system functioning, is largely concentrated in eggs. The mineral composition of eggs may be affected by several factors including genotype (genetic make-up) (Rizzi and Marangon, 2012; Youssef *et al*., 2014) and age (Kucukyilmaz *et al*., 2012) of layer chickens. In most countries such as Ghana, greater part of table eggs is obtained from the commercial or improved layer breeds of the domestic chicken, kept under intensive management system by farmers (Aning, 2006). The Lohmann Brown, Lohmann White and White Leghorn genotypes (strains) are the most common layer chicken strains on Ghanaian farms (Kruenti *et al*., 2022) although the mineral compositions of their eggs are not known. The objective of this research was to examine the effects of genotype (strain) and age of layer chickens on the mineral content of their eggs. Such information could help farmers to select better strains for egg production and to collect eggs at appropriate bird ages for specific mineral requirements. The findings will also be useful by dieticians to prescribe quality eggs for people with peculiar mineral challenges as well as enable industry to select eggs for specific products. Moreover, since no information is available regarding the effect of genotype and age of layer chickens on egg mineral concentration in Ghana, the results of this study may serve as a baseline data for the egg or layer industry in the country.

MATERIALS AND METHODS

Study areas

Eggs used for the study were obtained from a farm in Abokobi located on latitude 5° 44′ N and longitude 0° 12′ W in the Greater Accra Region of Ghana. The investigation was carried out at the Nutrition Laboratory of the School of Agriculture, University of Cape Coast (UCC) which is situated on latitude 5.1036° N and longitude 1.2825° W (Kruenti *et al*., 2022). The Central Region where UCC is found, has annual average temperature and precipitation of 26°C and 1300 mm correspondgly (Kruenti *et al*., 2022).

Experimental design, data collection and analysis

Eggs were collected from a flock of layer chicken strains (genotypes) kept in the same deep litter houses with the same management practices. The birds were all fed *ad libitum* the same commercial layer mash of 18% crude protein and 3200 kcal/kg ME throughout the experimental period. This was to ensure that there is absence of dietary and housing differences. All essential vaccinations and medications were duly followed. Eggs were randomly collected on the same day between 8:00 – 8:30 am (when the layer chickens were at ages of 31, 40 and 53 weeks old) from the farm, examined, cleaned with a dry cloth, packed onto paper crates in a carton and transported to the laboratory by road for an average of four hours. A 3 X 3 factorial experimental design involving three strains/ genotypes (Lohmann White [S1], Lohmann

Brown [S2] and White Leghorn [S3]), which were of the same ages of 31 weeks old (A1), 40 weeks old (A2) and 53 weeks old (A3) was used. Eight hundred and ten (810) eggs were randomly selected and divided into nine treatment groups, with 90 eggs in each treatment; each treatment was replicated 3 times of 30 eggs. Thus, 270 eggs (90 from each strain) were collected and analysed within 24 hours when the three strains were at the same ages of 31, 40 and 53 weeks old. Before analysis was carried out, the eggs were broken onto a petri dish using a scalpel and the yolk entirely separated from the white (albumen) using a plastic yolk separator. All yolks as well as albumen per treatment were poured into a clean and sterilised beaker each, labelled and centrifuged to homogenise. The mineral elements (phosphorous, calcium, potassium, sodium, iron, copper and zinc) were determined by methods of the AOAC (2012). Data collected were subjected to ANOVA using the General Linear Model procedure in Minitab (version 18) (Minitab LLC, 2011). Differences between means were separated using the Tukey Pairwise Comparisons Method at 5% level of significance. The model used for analysis was:

$$Y_{ijk} = \mu + S_i + A_j + (SA)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ij} = the dependent variable,
μ = the general mean,
S_i = fixed effect of the ith strain of bird,
A_j = fixed effect of the jth age of bird,
$(SA)_{ij}$ = interaction effect between strain and age and
ε_{ij} = the random error associated with the dependent variable.

RESULTS AND DISCUSSION

Effect of strain (genotype) of layers on mineral composition of egg

Results presented in Table 1 show the effect of strain of layers on mineral composition of egg. The outcome indicates substantial strain effect

Table 1: Effect of strain of layers on mineral composition of egg

Strain of Layers Nutrient (µg/g)	Lohmann White	Lohmann Brown	White Leg- horn Egg Yolk	SEM	p-value
Phosphorous (P)	4397.5^{a}	4402.4^{b}	4433.1^{b}	95.0	0.044
Calcium (Ca)	1455.1	1445.4	1430.3	66.0	0.065
Potassium (K)	1318.5	1329.3	1342.1	76.0	0.094
Sodium (Na)	533.2	531.8	538.8	35.0	0.128
Iron (Fe)	37.2^{b}	77.0^{c}	21.7^{a}	4.09	0.001
Copper (Cu)	1.9^{b}	1.3^{a}	1.6^{a}	0.56	0.051
Zinc (Zn)	27.1^{a}	35.2^{b}	36.6^{b}	1.49	0.001
Nutrient (µg/g)			**Albumen (Egg white)**		
Phosphorous (P)	146.7	149.2	151.6	15.0	0.081
Calcium (Ca)	74.4	72.3	73.0	3.9	0.227
Potassium (K)	2023.1^{a}	1961.6^{a}	2269.3^{b}	66.4	0.003
Sodium (Na)	1930.3^{a}	1963.3^{a}	2218.0^{b}	62.8	0.001
Iron (Fe)	1.0^{b}	0.9^{b}	0.3^{a}	0.13	0.001
Copper (Cu)	0.5^{b}	0.2^{a}	0.1^{a}	0.9	0.001
Zinc (Zn)	0.4^{b}	0.7^{c}	0.2^{a}	0.17	0.001

Means that carry different superscripts are significantly different;
SEM: Standard Error of Means; µg/g: microgram per gram; p-value: probability value ($p < 0.05$)

on phosphorous, iron and zinc contents of egg yolk. Phosphorous was highest in yolks from the White Leghorn (4433.4 µg/g) than in yolks from the Lohmann Brown (4402.4 µg/g) and Lohmann White (4397.5 µg/g) egg; possibly due to variations in the genetic constitution of genes controlling phosphorous levels in yolks of the three genotypes. Iron content of egg yolk was significantly different among all the layers with the highest in the Lohmann Brown (77.0 µg/g) followed by the Lohmann White (37.2 µg/g) and lowest in the White Leghorn (21.7 µg/g); demonstrating that the Lohmann strains may have genetic (allelic) dominance for the trait than the White Leghorn.

The similarity of calcium, potassium and sodium levels in egg yolk among the three strains signifies that, genotype does not affect the content of these mineral elements in the egg yolk of domestic chickens. This implies that modifier genes or regulatory elements responsible for these minerals in the yolk may be similar in the three genotypes.

Sodium and potassium contents of the albumen were largely lower ($p < 0.05$) in Lohmann commercial layers than in the White Leghorn birds while iron, copper and zinc contents of the same egg part could be higher in the former than the latter. In concomitant, significant variation in calcium content was reported between the indigenous (149.1mg/100g) and a commercial (100.3mg/100g) chicken eggs (Chepkemoi *et al*., 2017). Meanwhile, high levels of calcium in the yolk and albumen of the eggs make the eggs of layer chicken strains of our contemporary study a good source of the element for regulation of muscle contraction during the foetal and early stages of human life for bones and teeth formation (Vickery and Vickery, 1997). Although significant strain effect on yolk and albumen potassium concentrations have been reported in the domestic guinea fowl (321.50mg/100g; 119.50mg/100g), the domestic chicken (197.50 mg/100g; 32.50mg/100g) and a hybrid chicken (162.00mg/100g; 82.00mg/100g) accordingly (Bashir *et al*., 2015), the level of potassium in the yolks of this work recorded no significant strain effect inferring that gene regulation of potassium in the yolk is comparable among the three genotypes. The significant variation in the concentration of potassium in the albumen between the Lohmann strains and the White Leghorn shows important strain effect. Meanwhile, the levels of potassium in both egg parts may be good to augment the maintenance of osmotic pressure and acid-base equilibrium in the body (Odoemena and Ekanem, 2006). Iron, copper and zinc contents of both yolk and albumen are genetically influenced and are within the human requirement of 6 – 40 mg/kg (Stadelman and Cotterill, 2013) and could be more in improved chickens, which is in agreement with Bashir *et al*. (2015). Moreover, the high concentration of iron in the yolk of the commercial layers used in this work renders their eggs superior in enhancing the nutritional standing of people – specifically the iron deficiency or anaemia group (Demmouche *et al*., 2011).

Although the current findings do not show significant strain effect on phosphorous and calcium contents of the albumen, phosphorous content of the yolk has noticeable strain effect. The data also suggest that, phosphorous content of the yolk and albumen could be substantially higher in eggs laid by white than brown layer strains; implying that the former has superior genetic potential for the trait than the latter. Generally, it was noticed that most mineral elements (basic nutrients) examined, which are vital for human and chicken nourishment (Attia *et al*., 2014) are abundant in yolk from white-feathered chickens. It has been reported that, one notable variation between the eggs of white and brown chicken strains is that some white eggs have approximately 3% more yolk than brown eggs and consequently, have lower percentage albumen (Anderson, 2013). And because the majority of egg minerals are sequestered in the yolk (Giannenas *et al*., 2009), eggs with a higher yolk -to-albumen ratio may have slightly higher concentrations of minerals (Heflin *et al*., 2018). Therefore, yolk from white-feathered layer chicken strains may be more suitable for making food supplements than those from brown-feathered chickens. The variations in the mineral contents of the three chicken genotypes observed in the current work support the results reported by Heflin *et al*. (2018) who found significant differences in the concentrations of some minerals (calcium, copper, iron, magnesium and manganese) in eggs of TA Tetra White (TW) and Hy-Line Brown (HB) chicken strains.

Effect of age of layer chickens on mineral composition of egg

The influence of age of layers on mineral composition of eggs is given in Table 2. The recorded values show significant effect of layers' age on all the elemental constituents of the chicken yolk except calcium, while all but phosphorous were substantially affected by hen-age in terms of the minerals in the albumen. This outcome is consistent with the findings of Heflin *et al*. (2018) who noticed non-significant difference in the calcium and phosphorus contents of eggs laid by chickens at 44, 68 and 88 weeks of age. On the other hand, mineral elements including phosphorous, potassium, iron, copper and zinc in the egg yolk, as well as the iron and zinc in the albumen would increase as chickens grow older, which is partly supported by the report of Pambuwa and Tanganyika (2017) who observed total ash (mineral) content of whole eggs consistently increasing from 1.288% to 1.296% to 1.394% at weeks 20, 24 and 28 of rearing the Malawian normal-feathered indigenous chicken respectively. These findings may be further explained by the fact that, in the absence of dietary disparities, differences in egg mineral nutrients due to differences in hen age may be ascribed to variations in egg size or weight, which affects yolk-to-albumen ratio with relatively higher yolk proportion (Rossi and Pompei, 1995; Anderson 2013). Egg size/weight is expected to increase with increasing age of layer chickens

Table 2: Effect of age of layers on mineral composition of egg

Age (Weeks)	**31**	**40**	**53**	**SEM**	**p-value**
Nutrient (µg/g)			**Yolk**		
Phosphorous (P)	4433.7[b]	4406.4[ab]	4392.9[a]	100.0	0.029
Calcium (Ca)	2021.7	2018.3	1990.0	66.0	0.841
Potassium (K)	1286.1[b]	1222.4[a]	1388.7[ab]	276.0	0.026
Sodium (Na)	490.2[ab]	683.8[a]	425.0[b]	65.0	0.015
Iron (Fe)	52.0[a]	58.9[b]	79.8[c]	3.09	0.001
Copper (Cu)	0.7[a]	1.2[b]	1.9[c]	0.56	0.001
Zinc (Zn)	21.2[a]	28.9[b]	39.5[c]	3.43	0.001
Nutrient (µg/g)			**Albumen (Egg white)**		
Phosphorous (P)	144.5	140.6	150.6	18.0	0.175
Calcium (Ca)	77.0[ab]	82.3[a]	68.5[b]	11.9	0.053
Potassium (K)	1708.2[a]	1682.8[ab]	1633.2[b]	66.4	0.035
Sodium (Na)	1532.5[a]	1514.1[ab]	1504.0[b]	32.8	0.008
Iron (Fe)	0.4[b]	0.5[b]	0.9[a]	0.23	0.001
Copper (Cu)	0.1[b]	0.2[ab]	0.4[a]	0.13	0.001
Zinc (Zn)	0.5[a]	0.3[b]	0.1[c]	0.14	0.001

Means within rows with different superscripts are significantly different; ***SEM:*** *Standard Error of Means;* ***µg/g:*** *microgram per gram;* ***p-value:*** *probability value ($p < 0.05$)*

Table 3: Effect of strain by age interaction on mineral compositions of commercial layer eggs

Nutrient (µg/g)		P	Ca	K	Na	Fe	Cu	Zn	P	Ca	K	Na	Fe	Cu	Zn
Strain of Layers	Age of Layers	**Egg Yolk**							**Albumen (Egg white)**						
Loh. White	Week 31	4377.7^{ab}	1426.2	1409.0^{b}	518.5^{ab}	67.3^{a}	20.8^{a}	36.3^{a}	166.7^{ab}	48.4^{abc}	1632.1^{c}	2199.9^{abc}	0.6^{c}	0.8^{bc}	1.6^{a}
	Week 40	4363.6^{b}	1410.9	1561.5^{ab}	576.2^{ab}	48.6^{b}	22.9^{a}	34.6^{a}	142.1^{b}	41.3^{ab}	2275.9^{ab}	1833.7^{bc}	1.3^{ab}	0.7^{a}	0.3^{b}
	Week 53	4453.4^{a}	1428.3	1585.1^{ab}	504.7^{b}	45.8^{b}	13.6^{b}	30.5^{b}	151.3^{ab}	33.5^{abc}	2161.3^{ab}	2057.3^{abc}	1.4^{a}	0.4^{a}	0.3^{b}
Loh. Brown	Week 31	4402.3^{ab}	1435.4	1385.0^{b}	462.3^{b}	60.1^{a}	18.7^{ab}	35.1^{a}	156.9^{ab}	68.9^{a}	2404.9^{ab}	2270.4^{abc}	1.1^{bc}	0.2^{cd}	1.1^{a}
	Week 40	4414.6^{ab}	1420.7	1408.0^{b}	430.3^{b}	68.2^{a}	14.2^{b}	34.3^{a}	158.7^{ab}	80.6^{abc}	1960.0^{bc}	2127.9^{abc}	0.7^{c}	0.9^{d}	1.2^{a}
	Week 53	4453.4^{a}	1440.2	1434.9^{b}	402.8^{b}	42.6^{b}	08.7^{c}	30.2^{b}	174.2^{a}	67.4^{c}	1520.1^{c}	1791.7^{c}	1.7^{a}	0.5^{a}	0.6^{b}
White Leg-horn	Week 31	4396.4^{ab}	1429.3	1447.1^{b}	514.5^{ab}	42.9^{b}	25.8^{a}	24.7^{c}	165.0^{ab}	71.8^{bc}	2587.5^{a}	2497.2^{a}	1.1^{bc}	0.3^{b}	0.5^{b}
	Week 40	4410.9^{ab}	1424.2	1530.5^{a}	509.6^{a}	43.2^{b}	14.1^{b}	23.2^{c}	156.8^{ab}	50.0^{a}	2012.5^{bc}	2461.9^{a}	0.7^{c}	0.2^{bc}	0.2^{b}
	Week 53	4398.9^{ab}	1447.4	1478.7^{b}	392.2^{b}	43.1^{b}	09.4^{bc}	23.1^{c}	146.1^{b}	42.4^{ab}	2080.0^{ab}	2294.9^{ab}	0.8^{c}	0.2^{cd}	0.4^{b}
SEM		19.0	88.0	478.0	52.0	8.8	3.2	6.1	38.0	11.0	115.0	109.0	0.14	0.11	0.12
p-value		0.052	0.349	0.050	0.050	0.001	0.014	0.001	0.023	0.001	0.001	0.049	0.001	0.001	0.001

Means within columns with different superscripts are significantly different; **Loh.:** Lohmann; **SEM:** Standard Error of Means; **P:** phosphorous; **Ca:** calcium; **K:** potassium; **Na:** sodium; **Fe:** iron; **Cu:** copper; **Zn:** zinc; **µg/g:** microgram per gram; **p-value:** probability value ($p<0.05$)

(Heflin *et al*., 2018; Réhault-Godbert *et al*., 2019). Thus, as egg size and percentage yolk solids increase with increasing age, older birds may be expected to produce eggs with higher mineral content (Fletcher *et al*., 1981; Heflin *et al*., 2018). However, the potassium, sodium and zinc in the albumen did not follow this trend as shown (Table 2). Meanwhile, sodium in the yolk and calcium in the albumen recorded significantly higher values when the layer chickens were at 40 weeks old. The general increase in the mineral elements as the birds aged from week 31 to week 53 shows that, the domestic layer chicken may be housed into lay up to or beyond 53 weeks if eggs high in minerals are targeted and provided the rate of lay makes economic sense. It is recommended from the current data that, eggs from older chickens may be appropriate for manufacturing food supplements for humans as they may contain more mineral elements than eggs laid by younger hens.

Effect of strain (genotype) by age interaction on mineral composition of eggs

Table 3 shows the effect of strain by age interaction on the mineral composition of egg yolk and albumen. Strain (genotype) by age interaction can be defined as the change in relative performance of a trait expressed in two or more strains (genotypes), when measured in two or more ages (Falconer and Mackay, 1996). Hammami *et al.* (2009) reported that, the existence of strain (genotype) by age interaction could be explained by the fact that some alleles may only be expressed (switched on) at some specific ages; consequently, gene regulation may change depending on the age. Favourable genes at certain ages may become unfavourable at other ages. In the presence of significant genotype by age interactions, the relative advantages of genotypes may differ from one age to the other with respect to certain traits; leading to re-ranking of the strains (genotypes). In the current work, interaction effects between chicken strain and age were observed for all minerals in the egg parts except calcium in the yolk. Yolk from Lohmann White at week 31 had about 32% iron than at week 53.

Similar trend was observed for the same trait where the yolk from Lohmann Brown at week 31 had about 29% iron more than at week 53. Contrariwise, albumen from Lohmann White at week 53 had 57% more iron than at week 31, analogous to the albumen iron, which was found to be 35% more in Lohmann Brown at week 53 than at week 31. In addition, the results indicate that yolk from White Leghorn at week 40 had about 5% more potassium than at weeks 31 and 53 similar to the observation made for the same trait where the yolk from the Lohmann White at week 40 had about 11% more potassium than at week 31 and 53. These strain-age interaction effects suggest that gene regulation of these mineral elements (traits) is different at different ages. Subsequently, these layer chicken strains (genotypes) will rank differently for different traits such that a strain may rank highest for a given mineral at particular age but will rank lowest for a different trait at the same age. This gives layer poultry farmers and breeders the opportunity to select or choose strains (genotypes) at certain ages for a targeted mineral content as may be needed or required by industry for the production of food supplements for humans. The findings of the current research confirm in part the only available related work done by Heflin *et al*. (2018) who noted significant effect of strain by age interaction on zinc concentration in whole dried egg. Nonetheless, the results of the present investigation are comparable to global standard reference values of mineral elements in the yolk and albumen of chicken eggs (Roe *et al*., 2013; Stadelman and Cotterill, 2013; USDA, 2016; Réhault-Godbert *et al*., 2019; McCance and Widdowson, 2021) and therefore the outcome of the contemporary study may serve as a baseline data for the egg industry in Ghana.

CONCLUSION

Strain (genotype) and age of layer chickens can significantly affect the mineral compositions of their egg parts (yolk and albumen). In summary, eggs must be produced from the best chicken strains and collected at the appropriate hen-ages to meet the elemental mineral needs of specific consumers and products. Consequently, dieticians who prescribe quality eggs for people with peculiar mineral challenges as well as industry, which select eggs for specific products, can apply the findings of the current research. The results of the study may serve as a baseline data for the egg industry in Ghana.

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