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Age and Cage Tiers: Effects on productive performance, blood profile and egg quality traits of egg-type chickens

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***Target Audience:** Resource-poor poultry farmers, Poultry Scientist, Poultry cage fabricators, Policy makers*

Abstract

Age of birds is critical for laying performance and egg quality traits of birds; however, the effect of cage tiers in laying performance is rarely investigated. This study determined the effects of age and cage tier on laying performance and egg quality traits of Isa Brown® chickens. A total of 576 birds of commercial layers (Isa Brown®) of average body weight 1.4 kg were used for the experiment. They were housed in three-tier conventional battery cages (top, middle and bottom) with spacing of 610 cm²/bird. The experiment was conducted on Isa Brown® chickens from the age of 21-30 weeks, 31- 40 weeks and 41-50 weeks. Birds were randomly distributed into 9 treatments, consisting of 4 replicates and 16 birds per replicate. Data were collected on the average weight gain (AWG), feed intake, net feed efficiency index (NFEI), hen-day egg production (HEDP) and feed per dozen eggs, blood parameters (haematological and serum biochemical indices) and egg quality traits (external and internal). The experiment was laid out in a 3×3 factorial arrangement and data collected were subjected to two-way Analysis of Variance in a Completely Randomised Design. Results showed significant variations (p<0.05) in the AWG, HDEP and NFEI from ages 21-30, 31- 40 and 41-50 weeks in birds housed at top-tier, respectively. The interaction effects between age and cage tier significantly (p<0.05) influenced total protein and globulin across the ages with highest values (5.55 g/dl and 2.15 g/dl) between ages 41-50 weeks and 21-30 weeks, respectively in birds housed on top-tier. Interaction effects between age and cage tier on external and internal egg quality traits showed significant influence (p<0.05) on the egg weight and yolk weight across the treatments. Birds within ages 31- 40 weeks had the highest (p<0.05) egg weight (68.30 g) in birds kept at mid-tier and yolk weight within ages 31- 40 weeks (16.65 g) at top-tier. The study concluded that egg production was better within ages 21-30 and 31- 40 weeks on top-tier while external and internal quality traits of eggs are best within ages 31 -40 weeks in birds kept in mid-tier and top-tier. However, any of the cage tiers is applicable in maintaining the serum biochemical parameters of the Isa Brown® chickens.

***Keywords:** Age of birds, Cage tiers, Isa Brown® pullets, Egg quality traits*

Description of Problem

Welfare and egg quality traits of layers are greatly affected by production systems including indoor environment of the poultry house (1). Rearing of layers in cages is widely accepted over floor rearing to enhance egg production and achieve a cleaner and disease-free environment (2). However, in recent times, the increased emphasis on regulation has driven changes on how animals are housed, fed and managed with much pressure on reducing cost to increase supply through the use of larger and more integrated facilities (3).

Poultry eggs constitute one of the most valuable sources of animal protein recommended for human consumption in many African countries (4). In the tropics, egg production has remained between 180-200 eggs per cycle on the average though a higher level of performance, no doubt, is the aim of most enterprise involved in egg production (5). Egg shell colour is reported to be influenced by a number of variables such as disease, age (6; 7), sunlight (8), and stress, including stocking density (9). Protoporphyrin is the main eggshell pigment in brown-shelled eggs, located primarily in the calcareous portion of the eggshell rather than the cuticle (10).

External egg quality including egg weight, poorer and variable egg shell quality, shape index, and internal egg qualities, such as proportion of yolk, albumen and lipid depends on the age of laying hens (11; 12; 13). Egg weight, egg shell content, albumen height and albumen pH are affected by interactions between the age of laying hens, oviposition time and housing system (14; 15).

Bird's age has an effect on egg size and composition traits. Most of these trends can be reversed by forced moulting, but resumes afterwards. Eggs increase in weight over a production period. This increase is associated with development of both yolk and albumen constituents, although such changes occur disproportionately, with the percentage of albumen increasing progressively while the percentage of yolk decreases (16). It was reported that the environments of rearing of the birds such as the housing systems, the feed they consume and the climatic factors affect the performance of the birds (3; 17). In addition, rearing condition is an important management input in poultry production (2). This affects growth, egg production and qualities as well as health of the laying birds (3; 17).

Battery cage systems are commonly used in poultry houses to provide some commercial and health benefits (18; 19). Cages are still the most economic housing to produce eggs and the best system for disease prevention (2; 20) as well as protection of birds from pecking and other social vices. Cages also minimize egg losses and the number of rejects (cracks and soiled eggs). Cages are usually constructed in varying tiers. However, due to unavoidable variation in light intensity and ammonia (from the birds' droppings) among tiers in multi-tier cage systems, a balance is needed between providing sufficient light at the bottom tier and avoiding excessive light intensity at the top tier (21; 22; 23). Provision of homogeneous illumination to each tier is an inevitable challenge in semi-confined laying hen houses. Therefore, optimal environmental conditions should be provided in caged houses with sufficient quality and quantity of equipment (24).

The stress exerted by cages on production and animal welfare has been investigated (25), but the impacts of varying micro-environments on performance have seldom been investigated. In addition, previous studies (26; 27) determined the effects of nutrition, strains and housing systems on laying performance of egg-type chicken with less emphasis on the effect of the cage tier and age of the birds. This study thereby investigated the laying performance and egg quality traits of Isa Brown® chickens with respect to age and cage tiers.

Materials and Methods

Experimental site

The experiment was carried out at Debowale Farms Limited, "Igbo Olomi" along Alabata village road, Odeda local government, Abeokuta, Ogun State, Nigeria. It is located on latitude 7^{o} 19'N, longitude 3^{0} 30'E (28). The egg quality characteristics analysis was carried out at the Animal Products Processing Laboratory, Department of Animal Production and Health, College of

Animal Science and Livestock Production, Federal University of Agriculture Abeokuta, Ogun State.

Experimental birds and management

Five hundred and seventy-six commercial layers (Isa Brown®) were purchased from a reputable commercial farm in Abeokuta, Ogun State. Formulated feed (Table 1) and water were supplied *ad libitum* with automatic watering system and front trough feeders throughout the experimental period. All necessary medications and vaccinations were observed, daily routine and occasional management practices in poultry production were observed as at when due. Birds with weight ranged between 0.9 - 1.8 kg and of ages 21 - 30 weeks, 31- 40 weeks and 41-50 weeks were used for the experiment.

Table 1: Percentage composition of experimental diet

Ingredients	Layers mash (%)
Yellow maize	50.00
Wheat offal	4.00
Corn bran	10.00
Soya beans	23.00
Bone meal	3.00
Salt (NaCl)	0.30
Lysine	0.10
Methionine	0.20
Limestone	9.00
*Layer Premix (Vitamin /Mineral)	0.30
Toxin binder	0.10
Total	100
Calculated Analysis	
Metabolisable energy (kCal/kg)	2500
Crude protein (%)	16.50
Crude fibre (%)	6.00
Ether extract (%)	5.00
Ash (%)	2.80
Calcium (%)	3.50
Phosphorus (%)	0.41

**Layer premix supplied mgkg-1 diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4*

The vitamin premix supplied per kg diet: Vitamin A, 8250 IU; Vitamin D3, 1200 ICU; Vitamin K, 1 mg; Vitamin, 40 IU; Vitamin B1, 2 mg; Vitamin B2 4 mg; Vitamin B12, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg.

Housing of experimental birds

The experimental birds were reared in a poultry house which contains conventional battery cages of a 3-tier design (top, middle and bottom) with 610 cm^2/bird. The long axis of the poultry house was oriented east to west. The size of the house was 40 m × 9 m (Length × Width) with surface area of 360 m^2. The building was automated watering, feeding and litter removal systems. There were two double-sided cage blocks, and each block has three tiers. The base dimensions of the galvanized wire cages 60 × 60 cm, 45.5 cm front height and 41 cm rear height, having 4 cells per unit.

Data Collection

Data were collected on the laying performance, blood parameters (haematological and serum) and egg quality traits (external and internal) at the ages (21-30, 31- 40 and 41-50 weeks) in the cage tiers (bottom, middle and top). The experiment lasted for 10 weeks. However, blood parameters were collected at 30th, 40th and 50th week, body weight gain was determined at the initial and final stages of the experiment and feed intake records were taken daily.

Laying performance of lsa Brown® chickens

Data were collected from 21st to 50th week of age. Daily record of feed consumption, egg production and mortality from 21st week till the end of the experiment were recorded and used for the determination of age and cage tier effects on laying performance and egg qualities of Isa Brown® chickens.

- Hen-day production (HDEP): This is the ratio of eggs laid in a day divided by the number of birds alive multiplied by 100.

$$\text{HDEP}= \frac{\text{Eggs laid per day}}{\text{Number of birds alive}} \times 100$$

- Laying house mortality: This is a measure of viability, which gives an indication of the survivability of a laying stock. It was determined by the formula:

  $$\text{Mortality}= \frac{\text{Number of layers that died}}{\text{Total number of layers housed}} \times 100$$

- Net Feed Efficiency Index**:** It is based on egg production, egg weight, feed intake and body weight gain. NFEI= (EM+ BW) × 100÷ FCs

  Where,

  EM = Mean egg mass in g during a specific period

  BW = Mean body weight gain or loss in g during a particular period

  FC = Mean Feed consumption/hen in g during a particular period

  NFEI value of 45 and above is desirable.
- Feed efficiency per dozen eggs: This takes into consideration of the feed intake and egg production. It is the ratio between the feed consumed and the number of eggs produced.
- Feed per dozen eggs = (Kg of feed consumed × 12) ÷ Total eggs produced

Blood sample collection

On the 30^{th}, 40^{th} and 50^{th} week, 4 birds were selected randomly from each age group and cage tier of each replicate for haematological and serum biochemical parameters analysis. With a 3 ml syringe fitted with a 24-gauge sterile hypodermic needle, blood samples of about 3 ml each were carefully drawn from the brachial vein. Two ml of blood was collected into bottles containing Ethylene Diamine Tetra-Acetic Acid (EDTA) as anticoagulant, and mixed gently to prevent coagulation for haematological assessment, while the rest of the blood was put into plain sterile bottle for serum biochemical analysis. All blood samples collected were put inside ice pack and taken to the laboratory for analysis using standard laboratory procedures (29).

Determination of haematological parameters

The percentage of packed red cells in the blood was determined using the haematocrit centrifuge method as described (30). The Haemoglobin concentration of each blood sample was determined using cyanomethaemoglobin method as described (29). From each blood sample of experimental bird, 20 ml of blood was mixed with 4 ml of modified Drabkin's solution. The mixture of blood sample in experimental animal and Drabkin's solution was allowed to stand for 3 minutes before the haemoglobin concentration was read using a spectrophotometer. The actual value of haemoglobin was extrapolated from a standard curve. The total red blood count was determined from an anticoagulant blood sample which was diluted with 0.99% NaCl and was mixed thoroughly (31). The diluted blood was mounted on a haemacytometer and the number of erythrocytes in a circumscribed volume of 0.01 m^3 was counted micropically (32). The total number of white blood cells was carried out immediately after collection of blood sample from experimental animals using Neubauerhaemocytometer counting chamber (29). The sample was then be put into the haemocytometer and cell counted and express as 10^9 WBC per litre of blood.

- Basophil was analysed through blood stain, likewise neutrophils, lymphocyte, eosinophils, monocytes were analysed through blood stain.
- Mean Corpuscular Volume (MCV) was calculated as

  MCV = (Hct × 10) ÷ RBC (range: 84 – 96 fl)

- Mean Corpuscular Haemoglobin (MCH) was calculated using the formula
  MCH = (Hb × 10) ÷ RBC (range: 26 – 36 pg)
- Mean Corpuscular Haemoglobin Concentration (MCHC) was calculated using the formula:
  MCHC = (Hb × 10) ÷ Hct (range: 32 - 36%)
  Hb = Haemoglobin concentration; Hct = Haematocrit; RBC = Red Blood Cell

Determination of serum biochemical parameters

The total protein and albumin were determined spectro-photometrically according to the method (33) as described in Randox® diagnostic kit manual. The bromocresol purple method was used to determine the serum albumin. The bromocresol purple is a stable complex with abundance maximum at 600 nm. Four ml of bromocresol was added to 0.02 ml of each of the serum samples. Bromocresol purple solution (4 ml) was used as blank. The content of each tube was mixed and left at room temperature for 10 minutes at pH 4.2 ± 0.05. After 10 minutes, the test solution was read at a wavelength of 640 nm in a spectrophotometer set to zero with the blank solution.

Total protein (TP) of each sample was calculated using the formula:

TP (g/100 ml) =

$$\frac{Optical\ density of test \times concentration\ of\ standard}{Optical\ density\ of\ standard \quad 1}$$

The globulin was calculated using the formula:

Globulin = Total Protein – Albumin

Egg quality parameters

Thirty-six eggs were selected at 2 weeks interval for the evaluation of egg quality characteristics based on age and cage tier of Isa Brown® chickens for the period of 10 weeks. Two eggs were collected per replicate across the nine treatments (with the use of a clean egg crate) in the morning and transported to the laboratory for external and internal egg qualities analysis.

External egg qualities

i. Egg weight: Each egg sample collected from the treatment was weighed using a sensitive weighing scale.
ii. Egg length and width: The length and width of each egg were measured using Vernier callipers. The width was measured at the distance between two ends of the egg at the widest cross sectional region using Vernier callipers. The length was measured as the distance between the broad and narrow ends of the eggs.
iii. Egg Shape Index (ESI): This was measured as the percentage of the breadth (width) to the egg length according to the formula (34). The formula is given below:
   Egg Shape Index = $\frac{Width\ of Egg (mm)}{Length\ of Egg\ (mm)} \times 100$
iv Shell weight: Egg shells were air-dried in the crates and weighed with the use of sensitive weighing scale.
v Shell thickness: The thickness of individual air-dried shell was measured to the nearest 0.01mm using micrometre screw gauge.

Internal egg qualities

i. Albumen height: The eggs were gently broken and the maximum albumen height was measured with tripod micrometre (35).
ii. Percentage Albumen: This was calculated as the percentage of the albumen weight (g) to the egg

weight (g) or as the difference between the egg weight and the sum of weight of yolk and dry egg shell expressed as a percentage of the whole egg.

iii. Percentage Yolk: This was calculated as the percentage of the yolk weight (g) to the egg weight.

iv. Yolk colour: This was determined for individual yolk by comparing the yolk with colour chips of a Hoffman-LaRoche yolk colour fan. The yolk colour fan has different scores for different colours.

v. Haugh Unit: This was calculated using the values obtained for the egg weight and albumen height as expressed (36) and enunciated (37) in the formula shown below:

$HU: \quad 100 \log (H+7.57 - 1.7W^{0.37})$

Where, H = Albumen height in mm

W = Egg weight in gram.

Statistical design

Data collected were subjected to two - ways Analysis of Variance (ANOVA) in a 3×3 factorial arrangement. Significantly ($p<0.05$) different means among variables were separated using Tukey test as contained in the same statistical package (38).

Model;

$Y_{ijk} = \mu + Hi + S_j + (HS)_{ij} + \mathcal{E}_{ijk}$

Where:

Y_{ijk} = Observed values of dependent variables;

μ = Population mean

H_i = Fixed effect of the i^{th} Cage tiers (i =, 1, 2, 3 (tiers)

S_j= Effect of the j^{th} age (j = 21-30 weeks, 31-40 weeks and 41-50 weeks)

$(HS)_{ij}$ = Effect of the interaction between age and tier

$\mathcal{E}_{ijk}$ = Random error associated with each observation.

Results

Effects of age and cage tier on laying performance of Isa Brown® chickens

The effect of age and cage tier on laying performance of Isa Brown® chickens are presented in Table 2. In the main effect, age of laying chickens had significant ($p<0.05$) influence on average weight gain, hen-day egg production and net feed efficiency index. Effect of age shows highest average weight gain (55.45 g) in birds of age 21-30 weeks compared to other treatments of the same group (17.52 g and 23.49 g). Hen-day egg production (HDEP) was highest ($p<0.05$) in ages 31 - 40 weeks (76.08%) and decreased in age 41 - 50 weeks. Highest ($p<0.05$) net feed efficiency index (104.69) was observed in birds at age 21 - 30 weeks. In cage tier, birds housed at the top tier had significantly ($p<0.05$) highest HDEP (76.71%).

The interaction shows significant ($p<0.05$) influence on average weight gain (AWG), hen-day egg production (HDEP) and net feed efficiency index (NFEI) in the laying performance. Highest average weight gain (67.56 g/d) was obtained in birds in the age range of 21- 30 weeks and the on top tier while the least (6.07 g/d) was obtained from birds in the age range of 31- 40 weeks at the bottom of the cage tier. Hen-day egg production (HDEP) was significantly ($p<0.05$) highest (96.40) in birds in the age range of 31- 40 weeks on top tier. Highest NFEI (121.64) was obtained in birds in the age range of 21-30 weeks on top tier which differed significantly ($p<0.05$) from what was obtained in birds in the age range of 31-40 weeks at the bottom tier.

Table 2: Main effects of age and cage tier on egg laying performance of Isa Brown® chickens

Parameters	Age (Weeks) 21-30	31- 40	41-50	SEM	P-value	Cage tier Bottom	Mid	Top	SEM	P-value
Weight gain (g/d)	55.45^{a}	17.52^{b}	23.49^{b}	5.64	0.00	29.16	25.35	41.95	5.64	0.11
Feed intake (g/b/d)	109.55	129.17	119.62	5.89	0.08	116.28	117.07	125.00	5.89	0.52
HDEP	67.05^{b}	76.08^{a}	64.05^{b}	1.62	0.00	65.51^{b}	65.90^{b}	76.71^{a}	1.62	0.00
Net feed efficiency Index	104.69^{a}	63.79^{b}	69.75^{b}	4.66	0.00	76.65	75.69	85.89	4.66	0.25
Feed/dozen eggs (kg)	1.75	1.85	2.00	0.09	0.14	1.91	1.91	1.79	0.09	0.53

[a,b]Means on the same row having different superscript are significantly ($p<0.05$) different

SEM = Standard error of mean, HEDP = Hen-Day Egg Production

Table 3: Effects of interaction between age and cage tier on laying performance of Isa Brown® chickens

Age (Weeks)	21-30			31- 40			41-50				
Cage tiers / Parameters	Bottom	Mid	Top	Bottom	Mid	Top	Bottom	Mid	Top	SEM	P-value
Average weight gain (g/d)	54.18^{ab}	44.62^{abc}	67.56^{a}	6.07^{c}	14.94^{bc}	31.55^{abc}	27.23^{abc}	16.49^{bc}	26.74^{abc}	9.77	0.04
Feed intake (g/b/d)	108.57	112.90	107.19	116.09	126.81	144.63	124.19	111.49	123.18	10.2	0.45
HDEP	65.73^{b}	67.02^{b}	68.40^{b}	66.30^{b}	65.54^{b}	96.40^{a}	64.49^{b}	65.16^{b}	63.72^{b}	2.80	0.00
Net feed Efficiency index (g)	102.55^{ab}	89.89^{abc}	121.64^{a}	61.39^{c}	65.38^{bc}	64.59^{bc}	66.01^{bc}	71.81^{bc}	71.43^{bc}	8.07	0.03
Feed/dozen eggs (kg)	1.77	1.80	1.68	1.88	2.08	1.61	2.09	1.89	2.08	0.15	0.28

[a,b,c] Means on the same row having different superscript are significantly ($p<0.05$) different

SEM = Standard error of mean, HEDP = Hen Day Egg Production

Table 4: Main effects of age and cage tier on blood profile of Isa Brown® chickens

Parameters	Age (weeks) 21-30	31- 40	41-50	SEM	P-value	Cage tier Bottom	Mid	Top	SEM	P-value
Haematological parameters										
Packed cell volume (%)	32.00	36.00	35.33	1.01	0.09	36.17	34.83	32.33	1.01	0.07
Haemoglobin concentration (g/dl)	10.78	12.02	11.83	0.35	0.07	12.07	11.72	10.85	0.35	0.09
Red blood cell ($\times10^{6}/mm^{3}$)	2.72	2.98	2.95	0.10	0.17	3.03	2.93	2.63	0.10	0.08
White blood cell ($\times10^{6}/mm^{3}$)	10.75	11.57	11.10	0.55	0.59	10.62	11.03	11.77	0.55	0.37
Heterophils (%)	28.50	28.83	26.83	1.42	0.59	27.67	28.00	28.50	1.42	0.92
Lymphocytes (%)	70.50	69.33	71.83	1.44	0.50	70.83	70.00	70.83	1.44	0.90
Eosinophil (%	0.33	0.67	0.50	0.17	0.41	0.67	0.50	0.33	0.17	0.41
Basophil (%)	0.33	0.33	0.33	0.14	1.00	0.17	0.83	0.18	0.14	0.16
Monocytes (%)	0.33	0.68	0.35	0.23	0.50	0.52	0.52	0.33	0.23	0.81
Mean corpuscular volume (fl)	118.02	120.78	119.83	1.10	0.25	119.25	118.90	120.48	1.10	0.59
Mean corpuscular haemoglobin (g/dl)	39.75^{b}	40.32^{a}	40.12^{ab}	0.36	0.03	39.78	39.97	40.43	0.36	0.45
Mean corpuscular haemoglobin concentration (g/dl)	33.55	33.37	33.47	0.14	0.67	33.35	33.63	33.40	0.14	0.36
Serum biochemical Parameters										
Total protein (g/dl)	5.50	5.57	5.93	0.17	0.25	5.80	5.60	5.80	0.17	0.64
Albumin (g/dl)	3.30	3.45	3.28	0.12	0.59	3.20	3.48	3.35	0.12	0.31
Globulin (g/l)	2.22	2.30	2.65	0.16	0.18	2.58	2.12	2.47	0.16	0.15
Glucose (mg/dl)	125.53	103.88	112.73	8.74	0.26	131.98	97.97	112.20	8.74	0.06

SEM= Standard error of mean

Effects of age and cage tier on blood profile of Isa Brown® chickens

Table 4 shows the main effects of age and cage tier on blood profile of Isa Brown® chickens. The results revealed that mean corpuscular haemoglobin (MCH) of the birds was significantly ($p<0.05$) influenced across treatments. In the age, significantly highest value was observed in birds in the age range of 31- 40 weeks across the treatment groups. In the cage tier, there were no significant ($p>0.05$) differences in the blood profile. In the interaction, none of the blood profile measured was significantly ($P>0.05$) influenced.

Table 5: Effects of interaction between age and cage tier on blood profile of Isa Brown® chickens

Age (Weeks)	21-30			31- 40			41-50				
Cage tier	Bottom	Mid	Top	Bottom	Mid	Top	Bottom	Mid	Top	SEM	P-value
Parameters											
Haematological parameters											
Packed cell volume (%)	31.50	33.00	31.50	40.50	34.50	33.00	36.50	37.00	32.50	1.75	0.21
Haemoglobin concentration (g/dl)	10.50	11.15	10.70	13.50	11.55	11.00	12.20	12.45	10.85	0.61	0.20
Red blood cell ($\times 10^6/mm^3$)	2.65	2.85	2.65	3.40	2.85	2.70	3.05	3.10	2.70	0.17	0.23
White blood cell ($\times 10^6/mm^3$)	10.30	10.40	11.55	11.65	10.60	12.45	9.90	12.10	11.30	0.95	0.52
Heterophils (%)	28.00	27.00	30.50	28.00	30.50	28.00	27.00	26.50	27.00	2.47	0.80
Lymphocytes (%)	71.50	70.50	69.50	69.50	68.00	70.50	71.50	71.50	72.50	2.49	0.94
Eosinophil (%	1.00	1.00	0.50	1.50	2.50	1.00	3.00	1.00	1.50	0.29	0.06
Basophil (%)	0.50	0.50	0.55	1.00	0.50	0.50	1.50	1.00	0.50	0.24	0.28
Monocyte (%)	0.50	0.50	0.50	1.00	0.50	1.00	1.50	1.00	2.50	0.40	0.61
Mean corpuscular volume (fl)	118.85	116.25	118.95	119.10	121.05	122.20	119.80	119.40	120.30	1.91	0.80
Mean corpuscular haemoglobin (g/dl)	39.60	39.25	40.40	39.70	40.50	40.75	40.05	40.15	40.15	0.62	0.80
Mean corpuscular haemoglobin concentration (g/dl)	33.30	33.80	33.55	33.35	33.45	33.30	33.40	33.65	33.35	0.25	0.93
Serum biochemical parameters											
Total protein (g/dl)	5.30	5.00	6.20	6.00	5.75	5.55	6.10	6.05	5.56	0.29	0.10
Albumin	3.00	3.30	3.60	3.25	3.85	3.25	3.35	3.30	3.20	0.21	0.23
Globulin (g/dl)	2.30	1.70	2.65	2.70	1.90	2.30	2.75	2.75	2.45	0.27	0.25
Glucose (mg/dl)	161.60	101.20	113.70	109.90	83.60	118.90	124.30	109.00	104.80	15.10	0.36

SEM = Standard error of mean

Effects of age and cage tier on external and internal egg quality traits of Isa Brown® chickens

Table 6 shows the effects of age and cage tier on external and internal egg quality traits of Isa Brown® chickens. In the external quality traits, egg weight, egg length and egg width were significantly ($P<0.05$) influenced by the age with the highest values of 65.27 g, 57.06 mm and 45.02 mm, respectively in eggs from birds in the age range of 31-40 weeks. In the internal quality traits, the yolk weight was significantly influenced by the age effect. Cage tier had no significant ($P>0.05$) influence on egg external and internal quality traits.

In the interaction, the egg weight was significantly ($p<0.05$) influenced by the age and cage tier. The highest weight gain (68.30 g) was obtained in eggs from birds in the age range of 31-40 weeks in the mid-tier but comparable with the values obtained in the other age ranges and cage tiers except in eggs from birds in the weight range of 41-50 weeks at the bottom tier which was lowest.

Discussion

Age of birds is one of the factors that determine laying performance and egg quality traits of laying birds. Also, the health status of the chickens is paramount to the performance of chickens (39). The study revealed better hen-day egg production and net feed efficiency index as the hen ages unlike in the cage tier. These results are in agreement with the findings that the egg weight increases with age of hens (12; 40). However, these results are not consistent with the findings (41; 42), where positive effects were observed on average egg weight of Isa Brown and Lohmamn layers with respect to cage tier. In the cage tier, net feed efficiency index of the birds was not affected. The results contradicted the findings (43) that found significant differences in the net feed efficiency index of laying hens on different cage tiers. This study showed that increase feed intake in layers indicates the production of more eggs until the peak period when further increase in feed intake may not necessarily correspond to increase egg production. In this study, cage tier did not influence feed intake thereby corroborating earlier report (44) that feed intake was not significantly different with respect to cage tiers in the denizli genotype. The significant effect of age in hen-day egg production (HDEP) is consistent with earlier reports (45; 46) that egg production was highly influenced by age of birds.

Table 6: Main effects of age and cage tier on external and internal egg quality traits of Isa Brown® chickens

	Age (Weeks)					Cage Tier				
Parameters	21-30	31- 40	41-50	SEM	P-value	Bottom	Mid	Top	SEM	P-value
External egg quality										
Egg weight (g)	57.28^{b}	65.27^{a}	59.38^{b}	1.52	0.02	59.26	62.17	60.89	1.52	0.41
Egg length (mm)	52.58^{b}	57.06^{a}	54.33^{ab}	0.98	0.01	55.12	55.06	53.80	0.977	0.56
Egg width(mm)	43.12^{b}	45.02^{a}	43.78^{ab}	0.03	0.01	43.76	44.16	43.99	0.32	0.78
Egg shape index	82.56	78.94	80.79	1.69	0.34	79.44	80.57	82.28	1.69	0.50
Shell thickness (mm)	0.51	0.52	0.49	0.01	0.40	0.50	0.50	0.523	0.01	0.28
% Shell	9.94	9.40	9.98	0.38	0.50	9.91	9.59	9.73	0.38	0.75
Internal egg quality										
Albumen height (mm)	9.93	10.58	10.30	0.71	0.81	10.18	10.46	10.16	0.71	0.95
% Albumen	60.42	59.11	59.77	1.17	0.73	59.59	60.52	59.20	1.17	0.72
% Yolk	23.04	23.68	23.10	0.85	0.84	23.29	22.69	23.83	0.85	0.64
% Membrane	6.60	7.81	7.15	0.95	0.67	7.21	7.20	7.24	0.95	0.99
Haugh unit	97.92	99.85	98.62	3.33	0.92	98.33	99.40	102.76	3.33	0.97
Yolk colour	5.29	5.83	5.47	0.40	0.48	5.55	5.67	5.37	0.40	0.80

^{ab} Means on the same row having different superscript are significantly ($p<0.05$) different
SEM = Standard error of mean

The effect of cage tier level on hen-day egg production is supported by the observation (41) of increased egg production by hens in the upper tier with better light illumination than hens in the bottom tier. This result however did not agree with (25; 42) that cage tier level did not influence hen-day egg production. The non-significant effect of the treatments on feed/dozen eggs in laying birds in this study is not consistent with the reports (47; 14) that egg weight usually increases as hen's advances in age. Natural illumination is another factor that can influence the appetite and feeding of birds kept at the top tier. Increasing the amount of light during the rearing period can encourage feed intake and growth. These findings were not in consonance with earlier report (25) where no differences were obtained in average weight gain of birds on different cage tiers at the end of the experiment. Significant effects of interaction between age and cage tiers on hen-day egg production was not similar to the findings (41; 42) that cage tier position did not affect hen-day egg production.

Table 7: Effects of interaction between age and cage tier on external and internal egg quality traits of Isa Brown® chickens

Age (weeks)	21-30			31- 40			41-50				
Cage tier	Bottom	Mid	Top	Bottom	Mid	Top	Bottom	Mid	Top	SEM	P-value
Parameters											
External egg quality											
Egg weight (g)	57.03[ab]	55.90[ab]	58.93[ab]	66.08[ab]	68.30[a]	62.63[ab]	54.68[b]	62.33[ab]	61.13[ab]	2.63	0.002
Egg length (mm)	54.28	51.34	52.13	56.81	58.40	55.98	54.27	55.44	53.29	0.98	0.68
Egg width (mm)	43.34	42.54	43.50	45.38	45.35	44.32	42.57	44.59	44.18	0.68	0.16
Egg shell index	79.94	83.56	84.17	79.87	77.69	79.26	78.52	80.44	83.41	1.28	0.45
Shell thickness (mm)	0.50	0.50	0.53	0.55	0.50	0.51	0.46	0.49	0.54	0.02	0.15
% Shell	10.16	9.87	9.79	9.64	9.28	9.28	10.20	9.63	10.10	0.66	0.99
Internal egg quality											
Albumen height (mm)	9.23	9.65	10.90	11.73	10.13	9.88	9.60	11.60	9.70	1.23	0.44
% Albumen	58.33	59.45	63.49	61.32	61.65	54.38	59.12	60.46	59.73	2.02	0.09
% Yolk	23.95	23.06	22.08	23.16	21.36	26.52	22.76	23.65	22.90	1.47	0.20
% Membrane	7.56	7.62	4.64	5.97	7.71	9.82	7.92	6.08	7.27	1.64	0.26
Haugh unit	94.36	96.64	102.76	104.60	97.13	96.03	104.42	104.42	95.43	5.76	0.47
Yolk colour	5.75	5.25	5.75	5.50	6.25	6.60	5.50	4.75	4.75	0.70	0.80

[ab] Means on the same row having different superscript are significantly ($p<0.05$) different
SEM = Standard error of mean

Haematological parameters are influenced by age, feed, medications, toxic compound, infections and sex of birds. Accordingly, changes in haematological parameters are often used to determine health status of birds and assess the degree of environmental, nutritional and pathological stresses. In the age ranges and cage tiers, none of the birds' haematological parameters was affected. However, the values obtained were within the reference ranges for healthy birds. In laying birds, this could be an indicator that the bone marrow of the birds is properly functioning (48). Mean corpuscular volume (MCV) is used to calculate the average erythrocyte size and identified to perform immune regulatory functions both in health and disease (49) while mean corpuscular haemoglobin (MCH) measures haemoglobin (amount per blood cell) and the mean corpuscular haemoglobin concentration (MCHC) measures the amount of haemoglobin relative to the size of the cell per red blood cell. The observed MCV, MCH and MCHC values did not suggest that the birds were anaemic. This same trend was realised in the effects of the interaction between age and cage tier thereby depicting normal physiological conditions of the laying birds (50; 51).

Accordingly, serum biochemical parameters are used as physiological indicators for nutritional or even pathological changes that occur in the birds or organism (52). They are also indicators of the health of birds housed in cage systems and the amount of available protein in the diets. None of the parameters determined was significantly different. This result was in consonance with the findings (53), where significant differences were observed in the level of serum biochemical indices as a result of coloured light in birds either due to age or in the different cage tiers.

Egg weight had been reported as a function of the laying hen's age and generally it increases with the hen's age (11; 14; 47). Egg weight could be predicted as the sum of yolk, albumen and shell. It is an essential quality parameter for both producers and consumers (54).

In the main effect of age, egg weight was influenced by the age of birds and this corroborates the reports by numerous investigators (11; 47), who observed an increase in egg weight as the age advances. In this study, the highest value was obtained at 31 - 40 weeks of age. On the other hand, cage tier had no significant effect on egg weight. Cage location, but not tier level was reported to affect egg weight (41). The authors reported that hens facing the window produced heavier eggs than those facing the corridor. Hens at the top tier and facing the window produced an average of 2.8 g heavier eggs than hens at the bottom tier and facing the corridor, suggesting that light intensity cum illumination led to increased egg weight. This is in consonance with the findings (34) where consistent increase in mean length with age of laying hens were reported. Egg width of birds at ages 31- 40 weeks showed the highest value at ages 31- 40 weeks but reduced at ages 41-50 weeks of the hen. This may be due to the decrease in calcium deposition for egg shell by the aging hen. Other parameters such as egg shape index, shell thickness, shell weight and % shell were not influenced by the main effects of either age or cage tier. These results did not agree with report (47) that shell thickness declined with advancing age. This could be explained by an initial shell amount to spread over large surface area. Poor shell weight might be due to calcium and phosphorus chelation by phytate and shell weight generally increased with age (55).

Egg shape index which was used to estimate air cell volume was not influenced by age and cage tier in this study. This contradicted the findings (56; 14), where significant differences were obtained in egg shape index as the laying hen's age increases. This indicates that eggs become elongated in shape as the hen ages.

The interaction effects between age and cage tier significantly influenced egg weight at age 31-40 weeks in the birds housed at bottom, mid and top tiers. This result indicated that birds in mid-tier cage at age 31- 40 weeks of produced eggs with the highest weight thereby corroborating the finding (41) that egg generally increased in weight as the hen ages.

In the main effect of age on internal egg quality traits of Isa Brown® chickens, only yolk weight was influenced across the treatment groups. Highest yolk weight was recorded in eggs from birds at ages 31 - 40 weeks. This result is in consonant with the report (57) that as the hen ages there are increases in egg yolk weight. The results obtained on the interaction between age and cage tier on internal egg quality traits of Isa Brown® chickens indicated significant difference only in the yolk weight. Among the internal egg quality parameters, yolk weight and albumen weight are very important for the birds' nutrition (58). Interaction effects between age and cage tier significantly influenced yolk weight of eggs from birds at age 31- 40 weeks in the top tier. Yolk quality is determined by its colour, texture, firmness and smell of the yolk. The primary determinant of yolk colour is the xanthophyll (pigment) content of the diet consumed. It is easy to manipulate the yolk colour of eggs by the addition of xanthophyll to the diet (59). However, this result is not in agreement with the findings (60; 61) where variation in yolk colour at different ages were reported.

Haugh units are hinged on albumen quality and it determines an overall quality of egg content and egg freshness (62). Reduced Haugh units as the ages of hens increased was earlier reported (60). Increase in the albumen weight with increase in age was reported (48; 50) which was contradicted by the findings of this study. The albumen percentage obtained in this study contradicted the report (47) that there was decrease in albumen percentage as the hen age advances. However, cage tier did not significantly affect percentage albumen.

Conclusion and Applications

It could be concluded that:

1. Laying performance of the Isa Brown® chickens were better in terms of average weight gain (21-30 weeks), hen day egg production (31 - 40 weeks). However, the net feed efficiency index was best in the birds at 21 - 30 weeks of age in the top-tier.
2. Isa Brown® chickens from ages 31 - 40 weeks housed at mid-tier had highest egg weight and the best external egg quality trait.
3. Isa Brown® chickens in the age ranges 21- 30 and 31- 40 weeks in the top-tier were found to produce more eggs and of better external and internal egg quality traits than birds in the weight range of 41-50 weeks of age in the other cage tiers.
4. Any of the tiers are applicable in maintaining the birds' blood profile.

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