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Original Research Article

EGG QUALITY PARAMETERS OF ISA BROWN LAYER CHICKENS FED VARYING DIETARY LEVELS OF ACTIVATED CHARCOAL

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ABSTRACT
Assessment of egg quality is very crucial for the egg industry as egg configuration affects consumer preference, grading, and price. This study evaluated the quality of eggs from 120 Isa Brown laying hens reared on deep litter and fed varying dietary levels of activated charcoal (AC). The birds were randomly assigned to four groups of 30 birds per group with three replicates of 10 hens each: G1 (control), G2, G3 and G4 with 0.0 0.5, 1.0 and 1.5kg AC/100kg feed, respectively. The hens were fed 125g/bird/day of basal layer ration and clean drinking water *ad libitum.* Six (6) freshly laid eggs were randomly selected per group per day at 24, 28 and 32 weeks of age (woa) for the evaluation of external and internal egg quality traits namely: egg weight (EW), egg length (EL), egg diameter (ED), egg volume (EV), shell weight (SW), shell thickness (ST), albumen height (AH), yolk height (YH), albumen weight (AW), yolk weight (YW), and Haugh unit (HU). Data obtained were analyzed using Analysis of variance (ANOVA) in a completely randomized design. Results showed that feeding AC in the diet of laying hens significantly improved YW and HU at week 24; EW, EL, ED, SW, and YH at week 28; and ST at week 32 ($p \leq 0.026$). It was concluded that AC could be included at 0.5 - 1.5 kg/100kg of feed without detrimental effect on egg quality of laying hens, and improvement in some external and internal egg quality traits.

Keywords: Activated charcoal, external and internal egg qualities

INTRODUCTION
Eggs are consumed around the world as a good source of calories and animal protein (Malheiros *et al*., 2016). However, the internal and external qualities of eggs affect consumer preference and acceptance (Mertens *et al.*, 2006; Malheiros *et al*., 2016).

Egg qualities are influenced by numerous factors such as hen genetics, age, nutrition, welfare, and disease (Malheiros *et al*., 2016). The size of the egg increases with age, while the actual percentage of eggshell decreases leading to an increased need for calcium in the diet.

Shell strength is important for safe egg transportation and storage (Ledvinka *et al*., 2012). Shell and yolk colour influence consumer perception of egg quality with many believing that darker yolk and shell indicate higher egg quality (Malheiros *et al*., 2016). The egg shell is regarded as a window to a layer flock's health, welfare and productivity (Pedro *et al.*, 2024). It also serves as an important diagnostic aid. Pale shell colour (discoloured shell) in brown hens has been attributed to diseases such as infectious bronchitis, poor gut health, adverse effects of medication, poor nutrition, aging, parasitic infection, stress and immunosuppression (Faithi *et al.*, 2019; Hamilton & Bryden, 2021). Presence of shell pimples was also attributed to infectious bronchitis due

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to damage to the oviduct (Faithi *et al.*, 2019; Hamilton & Bryden, 2021).

Newcastle disease was shown to cause abnormal shell shape such as pointy and misshaped eggs while Egg Drop Syndrome led to pale and weak shelled eggs accompanying a significant drop in egg production (Mertens *et al.*, 2006). Shell defects cause financial losses to producers, marketers, egg processors, and hatchery operators. Consumers demand uniform, and aesthetically pleasing eggs (Fathi *et al.*, 2019).Eggshell quality can be improved through nutritional interventions such as optimizing calcium, phosphorus, and vitamin D metabolism (Swiatkiewicz *et al.*, 2010; Fathi *et al.*, 2019; Rattanawut *et al.*, 2021).

The egg white or albumen is a critical indicator of egg quality (egg freshness) (Haugh, 1937). Albumen quality is expressed as Haugh units where higher values indicate better quality. Haugh unit and albumen height are the main indicators of internal egg quality. The albumen makes up about 60% of the egg. The quality of the albumen is also influenced by age of the hen, storage and disease conditions.

Egg yolk consistency and colour are other criteria to judge egg quality (Malheiros *et al.*, 2016). Consumers demand various yolk colours depending on their preferences. Yolk colour correlates with the amount of carotenoids in feed ingredients. High carotenoid content results in deeper golden yolk colour which appeals to consumers (Malheiros *et al.*, 2016). Carotenoid rich feed ingredients include seaweed meal, alfalfa meal, flower petal meal, dried sweet potatoe and dried carrot (Malheiros *et al*., 2016).

Activated charcoal (AC) has been used for medicinal and veterinary purposes as universal poison antidote and for the improvement of gut health as a result of its large surface area, high porosity and adsorption capacity (AACT, 1999; Mabbett, 2005; Hasen & Abdulkadir, 2022; Soonmin *et al.*, 2022). Ayanwale *et al.* (2006) fed activated shea butter charcoal to day-old pullets till 22 weeks of age and observed significantly improved ($P < 0.05$) egg weight and reduced egg cracks from 15.33% to 0.2% but no effect on the internal egg qualities. The beneficial effects on egg production and shell quality were attributed to improved mineral utilization and adsorption of excess dietary fats.

In an earlier study by Kutlu *et al*. (2001), oak charcoal was shown to reduce the incidence of cracked eggs in a dose-related manner in Hy-line layer breed supplemented with 0, 10, 20 and 40g/kg oak charcoal. This was attributed to more efficient digestion and absorption of dietary minerals particularly calcium and phosphorus, brought about by a decrease in intestinal pH as was also suggested by more recent studies (Swiatkiewicz *et al.,* 2010; Rattanawut *et al.*, 2021).

A mixture of bamboo charcoal and vinegar was shown to induce a significant increase in laying performance in early phase egg production by stimulating intestinal functions of laying hens (Yamauchi *et al*., 2010).

Watarai & Tana (2005) reported that AC administration reduced intestinal *Salmonella enteritidis* and minimized the removal of beneficial flora such as *Enterococcus faecium*. Rattanawut *et al.* (2017) reported that bamboo AC improved egg shell quality through several mechanisms including an increase in digestive enzyme secretion, and decrease in gut pH which inhibited the growth of pathogens, and enhanced beneficial intestinal microbiota.

Furthermore, the intestinal villus height in the duodenum and jejunum of broiler chickens were increased when fed with bamboo charcoal powder at 1.0 and 1.5% inclusion levels (Rattanawut *et al.,* 2021).

Besides laying performance and external egg qualities that have been mostly investigated, information on the effect of activated carbon on internal egg qualities such as albumen and yolk traits are still scanty.

The present study therefore, evaluates the effect of varying dietary levels of AC inclusion on external and internal egg qualities of laying hens in a hot and humid production environment to inform decision on AC utilization in laying flocks.

MATERIALS AND METHODS

ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee, College of Veterinary Medicine Michael Okpara University of Agriculture, Umudike with code MOUAU/CVM/REC/2019112 assigned to the study.

LOCATION OF THE STUDY

The study was carried out at the Teaching and Research Farm of Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located within the South East agro-ecological zone of Nigeria with geographical coordinates of 5.4801^{o} N and 7.5437^{o} E.

ACTIVATED CHARCOAL PREPARATION

The physical method of AC preparation which involved thermal decomposition or carbonization of feedstock followed by steam activation (Gunamartha & Widana, 2018) was employed in this study.

The details of the procedure for AC production had been published (Okey *et al*., 2022). Briefly, a blend of palm kernel shell, pig dung, and palm fruit fiber (4:3:3 by weight) was sun-dried to constant weight and then thermally carbonized in earthen pot under limited atmospheric oxygen.

At complete combustion indicated by cessation of smoke emission from material, physical activation was achieved by rapid introduction of water into the red hot char with the pot covered immediately.

On cooling, the activated charcoal was removed from the pot, sun-dried to constant weight and ground to powder using a blender. The material was stored in airtight container until used.

EXPERIMENTAL ANIMALS, DESIGN, MANAGEMENT AND DURATION OF STUDY

This study was carried out using 120 point of lay (16 weeks of age) Isa Brown hens procured from Animal Care Services Konsult Nigeria Limited. The hens were randomly divided into four groups (G1- G4) of 30 hens each. Each group was further replicated into 3 with 10 hens each. Maize-soya based basal grower and layer diets were formulated to conform to the nutrient requirements of growers and layer chickens, respectively according to NRC (1994). Hens in control group (G1) were fed a basal diet with metabolizable energy and crude protein as shown in Table I while those in G2, G3 and G4 were fed basal diet supplemented with AC at inclusion levels of 0.5, 1.0 and 1.5kg per 100kg feed, respectively.

The inclusion levels were based on the recommendations by the Food and Fertilizer Technology Centre, Taiwan (2002) that suggested 1.0 - 1.5kg per 100kg as the best inclusion level of activated charcoal in layer chicken diets. The hens were reared on deep litter and fed grower mash till 22 weeks of age when 10% egg production was attained. This was followed by layer ration offered at 125g/bird/day. The hens had free access to clean drinking water provided *ad libitum* throughout the study duration. Appropriate vaccinations and other preventive medications were administered to ensure optimal health and performance.

The care and management of the birds followed accepted guidelines for layers as recommended by Federation of Animal Science Societies (Savoy, 1999).

DATA COLLECTION

Two freshly laid eggs were collected from each replicate making 6 eggs per group at 24, 28 and 32 weeks of age (woa). The eggs were used for the determination of external and internal egg characteristics for each group.

The external egg parameters determined were egg weight (EW), shell weight (SW) and shell thickness (ST). The EW, and SW were determined using a digital weighing balance (Metler, AutoChem Inc. USA; sensitivity, 0.01g) while ST was determined according to Monira *et al.* (2003) using a digital micro meter screw-guage (sensitivity, 0.01mm).

Egg length (EL), and ED were determined using a vennier calipers (sensitivity, 0.01cm), while EV was determined as 0.51 x Length x maximum diameter.

Determined internal egg characteristics were yolk weight (YW), albumen weight (AW), albumen height (AH), Haugh unit (HU) and yolk/albumen ratio (Y/A). The YW and AW were measured using a digital Metler weighing balance (AutoChem Inc. USA; sensitivity, 0.01g).

The AH was measured using electronic calipers (sensitivity, 0.01cm) while Y/A was calculated by dividing the YW by the AW.

Haugh unit (HU) was calculated using the formular $HU=100\log(H + 7.5-1.7W^{0.37})$ (Haugh, 1937),

where: H = Observed albumen height (mm)
W = Observed weight of egg (g)

RESULTS

The external and internal egg parameters of the experimental hens at 24, 28 and 32 woa are presented in Table I. At 24 woa, EW, EL, ED, EV, SW, ST, AH, YH, and AW did not differ significantly ($p > 0.05$) between the experimental groups. Significant differences were however, observed in YW and HU values. Dietary supplementation of AC caused a dose dependent increase in the value of YW across experimental groups with hens fed 1.5 kg AC per 100kg feed (G4) having the highest YW which was statistically similar to the value for eggs from hens in G3 (1.0 kg/100 kg) but significantly ($p = 0.007$) higher compared to hens in G1 and G2 (0.0, and 0.5 kg/100kg, respectively).

Haugh unit was highest in eggs of hens belonging to G4 and G2 (103.73 ± 2.80 and 103.56 ± 2.45, respectively) and these were significantly ($p = 0.026$) higher compared to the values observed in eggs of hens belonging to G1 and G3 (100.72 ± 1.73 and 100.56 ± 2.45, respectively).

At 28 woa, EW, EL, ED, EV, SW, and YH differed significantly between experimental groups while non-significant differences were observed in ST, AH, AW, YW, and HU. Hens fed 0.5 kg AC in 100kg feed (G2) laid eggs of statistically similar values for EW, EL, ED, SW, and YH with counterparts fed 1.5 kg AC in 100kg feed but significantly ($p \leq 0.026$) higher values compared to eggs of counterparts in the control and G3 groups. Egg volume (EV) was also significantly ($p = 0.002$) higher in hens belonging to G2 (44.33 ± 1.37 m^3) compared to G3 (42.33 ± 1.03 m^3), and G4 (41.83 ± 0.75 m^3) but was non-significantly different from the value for G1 (43.00 ± 0.63 m^3).

From the results observed at 32 woa, there were non-significant differences between experimental groups for all the egg quality indices except shell thickness (ST) which was significantly ($p = 0.000$) higher in the AC supplemented groups compared to the control group.

DISCUSSION

The observed significantly higher YW and HU at 24 woa, EW, EL, ED, SW, and YH at 28 woa, and ST at 32 woa in AC supplemented groups suggest enhanced egg quality at these growth stages.

TABLE I: INTERNAL AND EXTERNAL EGG PARAMETERS AT 24 WOA IN HENS FED VARYING DIETARY LEVEL OF ACTIVATED CHARCOAL

	Experimental groups				
Parameters	**G1**	**G2**	**G3**	**G4**	**p-value**
24 WOA					
Egg weight (g)	36.81±2.89	39.40±3.09	42.10±5.01	41.62±4.73	0.126
Egg length (mm)	49.73±1.44	50.40±1.74	50.97±2.30	51.30±1.68	0.479
Egg diameter (mm)	37.00±0.82	37.82±0.83	38.37±1.42	38.60±1.46	0.119
Egg volume (m^3)	34.50±2.88	38.50±9.50	38.17±7.00	40.83±5.23	0.440
Shell weight (g)	4.89±0.10	4.84±0.11	5.08±0.46	4.71±0.54	0.384
Shell thickness (mm)	0.30±0.02	0.31±0.01	0.31±0.02	0.30±0.02	0.870
Albumen height (mm)	9.02±0.54	9.78±0.64	9.28±0.60	10.10±0.95	0.060
Yolk Height (mm)	17.63±0.12	17.72±0.21	17.65±0.17	17.48±0.20	0.163
Albumen Weight (g)	19.95±1.41	20.71±2.20	22.76±2.88	21.36±2.50	0.222
Yolk Weight (g)	12.45±0.16^{b}	13.11±0.27^{b}	14.22±2.13^{ab}	15.86±2.30^{a}	0.007
Haugh Unit	100.72±1.73^{b}	103.56±1.47^{a}	100.56±2.45^{b}	103.73±2.80^{a}	0.026
28 WOA					
Egg weight (g)	51.80±2.80^{b}	57.49±4.85^{a}	51.67±2.29^{b}	54.08±3.14^{ab}	0.026
Egg length (mm)	53.13±1.18^{b}	56.25±1.37^{a}	53.62±1.58^{b}	55.77±2.39^{ab}	0.009
Egg diameter (mm)	41.53±0.43^{b}	43.08±0.82^{a}	41.70±0.71^{b}	42.23±0.89^{ab}	0.007
Egg volume (m3)	43.00±0.63^{ab}	44.33±1.37^{a}	42.33±1.03^{b}	41.83±0.75^{b}	0.002
Shell weight (g)	5.26±0.09^{b}	5.64±0.24^{a}	5.31±0.11^{b}	5.49±0.29^{ab}	0.017
Shell thickness (mm)	0.24±0.01	0.24±0.01	0.23±0.01	0.24±0.01	0.502
Albumen height (mm)	11.18±0.39	11.72±0.08	11.23±0.21	11.37±0.23	0.411
Yolk height (mm)	17.37±0.87^{b}	18.62±0.75^{a}	17.37±0.28^{b}	17.70±0.37^{ab}	0.007
Albumen weight (g)	33.88±1.58	38.49±4.82	33.40±1.49	35.00±4.02	0.062
Yolk weight (g)	13.12±0.58	14.61±1.47	13.01±0.49	13.71±1.27	0.057
Haugh unit	103.73±2.11	105.89±0.99	103.91±1.29	104.80±1.28	0.073
32 WOA					
Egg weight (g)	51.34 ± 1.48	53.37 ± 3.46	53.86 ± 4.28	53.40 ± 2.61	0.521
Egg length (mm)	52.90 ± 0.90	53.92 ± 1.61	53.72 ± 2.02	53.73 ± 1.83	0.717
Egg diameter (mm)	42.40 ± 0.69	43.03 ± 1.01	42.52 ± 1.24	43.17 ± 0.57	0.406
Egg volume (ml)	41.33 ± 0.82	42.55 ± 0.81	42.50 ± 1.87	42.33 ± 1.03	0.293
Shell weight (g)	5.50 ± 0.14	5.59 ± 0.25	5.69 ± 0.35	5.64 ± 0.32	0.657
Shell thickness (mm)	0.26 ± 0.00^{b}	0.29 ± 0.01^{a}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}	0.000
Albumin height (mm)	11.28 ± 0.10	11.43 ± 0.37	11.58 ± 0.44	11.42 ± 0.22	0.441
Yolk height (mm)	17.07 ± 0.15	17.17 ± 0.24	17.27 ± 0.24	17.27 ± 0.23	0.352
Albumin weight (g)	32.56 ± 1.03	34.29 ± 3.14	34.46 ± 3.33	34.50 ± 2.51	0.543
Yolk weight (g)	12.95 ± 0.60	13.80 ± 1.53	13.87 ± 1.34	13.58 ± 1.31	0.577
Haugh unit	104.09 ± 0.71	104.84 ± 1.68	105.43 ± 1.95	104.97 ± 1.10	0.457

Results are mean ± SD; a, b: means on the same row with different superscripts are significantly different ($P < 0.05$).

The significantly higher YW and YH in AC fed groups suggest enhanced yolk synthesis, and deposition, follicular health, and development in hens fed diets supplemented with AC. Follicle development is influenced by numerous factors such as oxidative stress. Feed additives that reduce hepatic and ovarian oxidative stress would enhance yolk synthesis and deposition, follicular development and maturation.

Dietary supplementation of AC have been shown to reduce hepatic and ovarian oxidative stress through enhanced free radical scavenging, and adsorption of anti-nutritional factors (bacteria toxins, mycotoxins, and noxious gases) in feed, gastro-intestinal tract, and the circulatory system, and to enhance total antioxidant capacity in various animal models (Abd El-hameed *et al.*, 2021; El-Ghalid *et al.*, 2022; Hassan *et al.*, 2023; El-Kelawy *et al.*, 2024).

These effects could ameliorate hepatic and ovarian oxidative stress thereby enhancing yolk synthesis, and follicular (yolk) development. In addition to suggesting increased yolk size, YH also indicates yolk quality.

Therefore, the enhanced YH also suggests better preserved yolk quality in eggs of hens fed AC probably through enhanced antioxidant capacity and reduced yolk oxidative stress. The Haugh unit is the main indicator of internal egg (albumen) quality and egg freshness. The higher the score, the better the albumen (hence egg) quality. The significantly higher Haugh units of eggs from hens fed AC indicate that this additive could enhance the preservation of internal egg quality.

The high adsorption capacity of AC for antinutritional factors, and toxins in feed, noxious gases, and reactive oxygen species (ROS) in the gastrointestinal tract, and ovary could enhance the antioxidant capacity of eggs from hens fed AC thereby enhancing internal egg quality.

The reported non-significant effect of AC on external and internal egg qualities at week 32; EW, EL, ED, SW, ST, AH, YH, and AW at week 24, and ST, AH, AW, YW, and HU at week 28 agree with Rattanawut *et al.* (2021) while the observed significant effect on YW and HU at week 24 disagrees with the authors. Ayanwale *et al.* (2006) had reported non-significant effect of sheabutter charcoal on internal egg qualities but a significant effect on egg weight as was observed at week 28 in the present study.

The positive and significant effect of AC dietary supplementation on EW, EL, ED, SW, and YH at week 28 could be attributed to the beneficial effects of AC on gut health. El-Kelawy *et al.* (2024) reported that AC dietary supplementation in broiler chicks decreased harmful intestinal bacterial count but boosted the count of beneficial bacteria. Improved intestinal health would lead to more efficient digestion, absorption and utilization of feed nutrients particularly calcium and phosphorus required in egg formation. In addition, the organic acids contents of AC also exert beneficial effects on intestinal health (Rattanawut *et al.*, 2021).

Activated charcoal had been shown to contain many compounds including phenols, alkanes, alcohols, aldehydes and organic acids especially acetic acids (Kimura *et al.*, 2002). The acetic acid content may have contributed in shifting the balance of intestinal microflora in favour of beneficial microbiota which most likely led to enhanced intestinal functions, calcium and phosphorus metabolism (Lutz & Scharrer, 1991; Lin *et al.*, 2008).

Even though EW, EL, ED, EV, SW, ST, AH, YH, and AW were not significantly different between AC fed hens and control at 24 and 32 woa, the values observed for these egg traits across the age periods showed an increasing trend with higher levels of AC supplementation.

Egg quality parameters are very crucial for the egg industry as they determine consumer's preference and impact the overall egg business. Egg weight directly influences the revenue from egg sales while shell weight impact the safety of eggs during handling, transportation, and storage. Combined with the significantly improved shell thickness (ST) observed at week 32, these results imply bigger eggs and stronger eggshell for eggs from AC fed hens. Shell thickness and strength are important factors in the preservation and transportation of eggs (Mertens *et al.*, 2006).

Improvement in eggshell quality following AC dietary supplementation would reduce the incidence of cracks and egg breakage. Egg shell breakage remains a source of economic loss and a serious problem in egg production. According to Hamilton and Bryden (2021), about 3% of eggs laid by hens housed in battery cage or deep litter systems have cracked shells.

Estimates from other researchers (Youssef *et al.*, 2013; Fathi *et al.*, 2019) indicate that losses from damaged and broken eggs in commercial layer operations may account for up to 8-11% of total egg production. Besides, eggshell cracks have public health implications as cracks provide entrance into the egg for pathogenic microbes such as *Salmonella* species (Hamilton & Bryden, 2021). Such eggs if consumed become a threat to human health.

Therefore, management interventions to reduce the incidence of cracks and egg breakage such as supplementation of feed additives like AC will reduce economic losses and ensure food safety.

CONCLUSION AND RECOMMENDATION

Yolk weight and Haugh unit were better at week 24; egg weight, egg length, egg width, shell weight and yolk height were better at week 28; while shell thickness was better at week 32 in AC supplemented groups than the control. Activated charcoal supplementation in diet of laying hens at 0.5 to 1.5 kg/100 kg feed could improve egg size, yolk size, and albumen, shell, and yolk qualities.

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