



ASSESSMENTS OF PRODUCTIVE PERFORMANCE, EGG QUALITY TRAITS, STORAGE STATUS INDEX, AND EXCRETA GAS EMISSION ON LAYING HENS FED BLEND OF BIOCHAR, ZEOLITE, AND CHROMOLAENA LEAF MEAL

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ABSTRACT: *Sustainable solutions for intensive poultry production can help farmers, rural communities, consumers, and regulatory agencies. The movement of eggs from farms to end users usually takes a period of time due to bad road, vehicular breakdown, and poor logistics, and the situation could be more apparent during a period of lockdown. Hence, the study assessed supplementation of laying hens' diet with biochar (0.33- 0.50%), zeolite (0.33-0.50%), and chromolaena leaf meal (0.33-1.00) to lower the environmental impact while maintaining egg quality. The effect on feed intake, laying performance, egg quality, ammonia (NH₃) emissions from excreta, and the excreta composition was evaluated. The result showed that those dietary treatments had no significant effect ($p > 0.05$) on parameters such Hen-day production (HEDP), egg mass, feed intake g/bird, total feed intake, feed/dozen egg and total number eggs but chicken on supplemented diet laid highest number of eggs (449) with (HEDP) percentage of 66.81 and feed /dozen egg at 1.93. CH₄ concentration reduced by 59% in the excreta chickens in T3, Reduction in CO₂ value by 50.5% and Hydrogen gas concentration by 18.6% when compared with control. Studies on storage revealed that pH values of the stored eggs changes on a weekly basis during the storage period of 35days while T3 and T4 maintained slightly acidic medium (5.8 and 5.6) respectively. Bacteria load in eggs reduced by (25.3% and 28%) respectively at day 35. It can be concluded that inclusion of zeolite, biochar and leafmeal combination improved laying parameters, reduced gaseous emission and extend shelf-life eggs.*

KEYWORDS: Eggs, biochar, zeolite, leaf meal, gas emission, laying hens.



INTRODUCTION

The surge in the production of poultry meat and eggs worldwide is as a result of the adaptability of poultry farming to different environmental conditions and thereby bridging the protein gap (Eniola *et al.*, 2012). Eggs are common source of protein with high biological value which is necessary by human for the supply of basic nutrients in the body. The composition of these eggs may be influenced by different factors which may include age, diet and environmental factors (Fraeye *et al.*, 2012). Natoli *et al.*, (2007) also reported that egg contain several mineral such as zinc, selenium, vitamin A and vitamin E which has been reported to provide antioxidant properties thereby preventing the development of many degenerative diseases (Natoli *et al.*, 2007). However, Intensive poultry production raises concerns about gaseous emissions and its impact on poultry workers, birds and the environment. Odor nuisance can be a significant problem for nearby communities, mitigation of the gaseous emissions and pen indoor air quality are serious challenges for regulatory agencies and farmers (Grzanic *et al.*, 2023). In quest to manage the fastidious odours and gas emission from poultry farms technologies like manure handling and treatment, bio-filtration of exhaust air, manure additives and others has been attempted. However, not all technologies that successfully lower odor and gas emission at the laboratory scale can be upscale to farm level due to cost and challenges. Other attempts ranging from microbial enzyme, probiotics, antibiotics and nutrition manipulations has been used but most are not readily available to African poultry farmers and are usually expensive, thus there is necessity to look for efficient and cheaper alternatives.

Therefore, the application of entero-sorbents like biochar and zeolite can assist in ameliorating the air quality in and around the farm as well as improving the quality of products from farm. Studies have shown that beechwood biochar and its combination with aluminosilicate–glycerin mixture supplementation reduced ammonia emissions from manure without negatively impacting on production parameters (Babalola *et al.*, 2017). Zeolites are an important class of crystalline, micro porous, aluminosilicate materials with a three dimensional fully cross linked open framework structures that form uniformly sized pores of molecular dimensions (Babalola *et al.*, 2017). Zeolite have various industrial applications which include adsorbents in separations, ion exchange ability, purification processes, environmental pollution control, molecular sieve and acidic catalysts for size and shape selective catalytic reactions (Perez, *et al.*, 2022). Biochar is a fine-grained product of carbonization, characterized by a high content of organic carbon and low susceptibility and high absorbent properties. It is obtained through the pyrolysis of biomass and biodegradable waste (Malinska, 2015). Studies have shown that inclusion of biochar as feed additive reduce ammonia emission manure as well as improvement in mineral intake in laying chickens thereby reducing incidence of cracks (Kalu *et al.*, 2020).

Chromolaena odorata is very rich in protein which could make it an unconventional source of protein for ruminants and non-ruminants (Akinmutimi and Akufo, 2006). The nutritive value assessment of *C. odorata* by (Apori *et al.*, 2000) showed that it is a plant that has good potential for feeding livestock due to its high crude protein which maybe more than 25% when found on a virgin, fallowed farmland, low fibre and low extractable phenolic contents. Its dry matter and crude protein contents are highly degradable, and the protein contains about 56% amino acids (Apori *et al.*, 2000). This has placed a demand on *C. odorata* to be considered as a potential feed supplement for livestock in the tropics where feed for livestock production is always a problem.



Antibiotics have been used a long time ago as growth promoters because of their effectiveness in enhancing growth performance for poultry (Omidwura *et al.*, 2020.) Thus, to ensure satisfactory succession to antibiotics, growth promoters, it is imperative to look for substitute products with similar zootechnical effects such as probiotics, prebiotics, organic acids, clays etc. However, the production of these compounds requires sophisticated equipment; this is not within the reach of pastoralists in developing countries and hence the need to search for natural products, locally available and recoverable (Mongo *et al.*, 2020; Pierrette Ngo *et al.*, 2021). Again, eggs create considerable value for animal husbandry as animal products. Antibiotics as performance enhancers in layer production have primarily been applied to improve utilization of feed and reduce pathogenic bacteria in the gut, thereby improving production performance (Mongo *et al.*, 2020; Pierrette Ngo *et al.*, 2021). However, due to the increase of multiple resistance bacteria and the decrease of consumers acceptance, the use of antibiotics has been gradually prohibited [2]. In recent years, phytogenic feed additives have attracted more and more attention because of their safety and environmental friendliness (Mongo *et al.*, 2020; Pierrette Ngo *et al.*, 2021). This study aimed at evaluating the effect of inclusion of biochar, zeolite with plant extracts on number and size of egg laid, egg qualities characteristics like egg weight, egg shape, shell thickness, yolk colour and haugh unit, egg yolk cholesterol, the pH and microbial load of the stored eggs, air quality of odor emitted from poultry house, CO₂, CH₄, NO₂, H₂S, SO₂ emitted from the excreta of the laying chickens fed the experimental diets.

LITERATURE/THEORETICAL UNDERPINNING

This experiment falls under three theories:

- A. Adsorption Theory
- B. Phytogenic Theory
- C. Synergetic Theory

Adsorption Theory

Zeolite: Zeolite are hydrated, crystalline aluminosilicates which has physicochemical characteristics such as adsorption, ion exchange, size exclusion framework characteristics, and catalytic properties (Moshoeshe *et al.*, 2017; Mahmodi *et al.*, 2020).

Biochar: Biochar facilitates absorption and enhances the productivity of feed, because energy is consumed by feed in particular. According to Eca (2020), biochar's potential to support microbial activity in the digestive system may positively influence nutrient absorption and overall gut health in broilers.

Phytogenic Theory

Siam Weed-*Chromolaena odorata* belonging to the family *Asteraceae* possess anticancer, antidiabetic, anti-hepatotoxic and most importantly for this study, anti-inflammatory, antimicrobial, and antioxidant properties. Its phytochemical components are alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids (Sirinthiporn and Jiraungkoorskul, 2017).



Synergetic Theory

Siam Weed-*Chromolaena odorata* provides nutrient and antioxidants while biochar and zeolite (enterosorbents) stabilizes (reduces) the toxic metabolites thereby allowing absorption of *C.odorata* nutrients which reduces microbial decomposition and greenhouse emission.

MATERIALS AND METHODS

Location of the Experiment: The study will be carried out at the Teaching and Research Farm of the School of Agricultural Technology, Yaba College of Technology, Epe. The site is on latitude 6.58 °N, Longitude 3.98 °E. It lies in the low land rain forest within the savannah agro-ecological zones with annual rainfall of 1694 mm and temperature of 27.1⁰C (Google Earth, 2023).

Preparation of the biochar feed additive

Corn cob was sourced from Maize processing mill and arranged into a small size pyrolysis kiln and tightly seal (drum -in drum) and moved into a larger one that has pores to aid movement of little amount of oxygen and exit for flames during pyrolysis. Wood materials were filled up into the larger kiln to serve as means of generating fire and heat during combustion. The kiln was then covered and allowed to undergo pyrolysis for a period of about 2hours with a temperature ranging about 300-600°C (monitored using an infrared digital thermometer) after which a resultant biochar was formed. The pyrolyzed material was grinded into powder form and stored in an air tight plastic container.

Preparation of zeolite

The starting material (Kaolin) was sampled from geo-referenced location at Oke-Ako village in Ijebu–Ode local government area of Ogun State, Nigeria. The sampled kaolin (1.5 kg) was soaked with water for duration of six days, in order to remove impurities such as soluble salts, grits and metallic oxides of Fe₂O₃, MgO, Na₂O, CaO and CaCO₃. The solid clay cake was split into smaller sizes and open dried in the laboratory for 4 days and further dried at 150°C in an electric oven for 4 h, after which it was milled. The milled sample was followed calcinations, dealumination, gel formation, gel aging and crystallization to derive zeolite according to the methods of (Macivers *et al.*, (2020); Ayele, (2018); (Olaremu *et al.*, (2018); Babalola *et al.*, 2015).

Equation1: $Al_2O_3 (s) + 3H_2SO_4 (aq) \leftrightarrow Al_2 (SO_4)_3 (aq) + 3H_2O \dots$ Dealumination.

Equation2: $6Al_2Si_2O_7$ (metakaolinite) + 12NaOH → Na₁₂ (AlO₂)₁₂ (SiO₂)₁₂ + 6H₂O (zeolite-X gel formation).

Preparation of leaf-meal

Chromolaena odorata leaves were sourced around college farm. The leaves were harvested before flowering, chopped and air-dried. The air-dried samples were milled at 0.2 mm diameter sieve using Kenwood blender mode and stored in plastic containers.



Experimental bird's management and design

A total of One hundred and eighty (180) points of lay birds with body weight between 1470-1550g were used. The birds were allotted on a weight equalization basis in a completely randomized design (CRD) to four dietary treatments with 45 birds per treatment consisting of 3 replicates of 15 birds per replicate. The four dietary treatments consist of diet 1 (control diet), diet 2 (1% biochar plus 1% leaf-meal), diet 3 (1% zeolite plus 1% leaf-meal) diet 4 (0.67% biochar, 0.67% zeolite and 0.67% leaf-meal). The diets were formulated to meet the nutrient requirement of the laying chicken in line with NRC (1994) recommendations. The birds were offered growers mash till 10% production was recorded before introducing the experimental diets. Medications and vaccinations were done as when due.

Table1: Gross composition of Experimental diets

Ingredients	T1	T 2	T 3	T4
Maize	50.00	50.00	50.00	50.00
Soybean meal	21.50	21.50	21.50	21.50
Wheat offal	14.00	14.00	14.00	14.00
Fish meal (72%)	1.00	1.00	1.00	1.00
Limestone	8.00	8.00	8.00	8.00
DCP	4.50	4.50	4.50	4.50
Salt	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Biochar	0.00	0.50	0.00	0.33
Zeolite	0.00	0.00	0.50	0.33
Leaf meal	0.00	1.00	1.00	0.33
Total	100	100	100	100
Determined Analysis (%)				
Crude protein	16.23	16.23	16.23	16.23
Crude fibre	3.08	4.50	4.50	4.08
Calcium	3.70	3.70	3.70	3.70
Phosphorus	0.81	0.81	0.81	0.81
Energy(kcal/Kg)	2904	2904	2904	2904
Ash	8.16	8.16	8.16	8.16
Moisture	1.26	1.26	1.26	1.26
Fat	2.65	2.65	2.65	2.65
NFE	68.58	68.58	68.58	68.58

Vitamin/mineral premix: vitamin A, 10,000,000 I.U.; vitamin D3, 2,000,000 I.U.; vitamin E, 16.0g; vitamin K, 1.0g; vitamin B1, 0.509 mg; Riboflavin, 2-4 mg; pyridoxine, 0.35 mg; niacin, 3.5 mg; biotin, 0.005 mg; choline chloride 30.0 mg; folic acid 0.1 mg; vitamin B12, 0.002 mg; vitamin C, 2.50 mg; manganese, 10.0 mg; zinc, 4.5 mg; Copper 0.20 mg; iron 5.0 mg; methionine 2.0 mg; calcium panthothenate 1.0 mg; antioxidant 120,000 mg; selenium, 120mg. T1 =control, T2= biochar+ leaf-meal, T3= zeolite +leaf-meal and T4= biochar+zeolite +leaf-meal



Laying performance

The initial weights of the birds were taken at the onset to the nearest 0.01g. Records of daily feed consumption and egg production on a replicate basis were recorded from two weeks in lay to 8 weeks; Weekly egg production per replicate were pooled and expressed as a percentage Hen-day egg production (NAPRI, 2000).

$$\% \text{ HDEP} = \frac{\text{Number of eggs laid}}{\text{Number of hen-days}} \times \frac{100}{1}$$

Feed per dozen Egg (FDE)

Feed per dozen eggs was calculated by using a simple efficiency formula commonly applied in poultry production. Feed consumed in relation to egg lay, were calculated for each of the treatment by first measuring the total feed consumed by the birds over specific period of time in kilogram (kg) or (g) and then count the number eggs produced over a specific period of time. This is followed by converting the eggs to dozen and then calculate the feed per dozen egg using the formula given below:

$$\text{Feed per dozen eggs} = \frac{\text{Total feed consumed}}{\text{Total egg produced} \div 12}$$

Determination of external egg qualities

Fifteen eggs (5per replicate) from each treatment were selected on a weekly basis for eight weeks. Egg quality assessment was done same day of lay. The weight of each egg sampled was determined with a sensitive weighing scale to the nearest 0.01g. The linear measurements were taken with vernier calipers to the nearest 0.01cm and the length of the egg was taken as the longitudinal distance between the narrow and the broad ends. The egg width was taken as the diameter of the widest cross-sectional region. Egg shape index (ESI) was calculated using the measured values of egg width and length from individual eggs sampled and multiplied by 100 as expressed below:

$$\text{ESI} = \frac{\text{Width of egg}}{\text{Length of egg}} \times \frac{100}{1}$$

Eggshell weight (ESW) was determined after air-drying shells for 72 hr in egg trays at room temperature and the individual eggshells were weighed using an electronic weighing balance (sensitivity of 0.01g). The eggshell thickness (EST) was measured with a digital micrometer screw gauge to the nearest 0.01mm.

Determination of internal egg qualities

The internal egg traits were measured by cracking the egg and pouring the contents into a flat plate. The albumen height (AH) was measured off the chalazae at a point above mid-way between the inner and circumference of the thick white with a spherometer having an accuracy of 0.01mm. Thereafter, the albumen was separated from the yolk using a smooth plastic egg separation funnel to take yolk weight (YW) and the albumen weight (AW) was calculated as the difference between the egg weight and the combined weight of the yolk and dry eggshell. The yolk colour was evaluated and scored using Hoffman-La Roche yolk colour fan rated 1-



15 with colour intensity ranging from pale yellow to deep orange (Hoffman-La Roche, 1984) and the Haugh unit was calculated using the formula of Haugh (1973).

$$\text{Haugh Unit (Hu)} = 100 \text{ Log (H + 7.57 - 1.7W}^{0.37})$$

where;

H= Albumen height (mm),

W= Weight of the egg (g)

and the values 7.57 and 1.7 remain constant

Egg pH determination

Ninety-six fresh eggs were sampled with twenty-four(24) eggs per treatment and labeled on treatment and replicate basis, arranged in plastic crates and stored for 35days at room temperature ranged (25.1-28.0°C). The egg pH were taken on Day 1, 7, 14, 21, 28 and 35 respectively. The room temperature was taken using digital thermometer and recorded every other day. The eggs were cracked carefully and the mixture of yolk and white were homogenized in a piston-gap homogenizer at 100 bar. Calibrated pH meter probe {(TESTO 206) was inserted into the sample and record the reading once stabilized, the probe was rinsed thoroughly between measurements to avoid contamination.

Total heterotrophic count of bacteria in the egg samples

One ml of each homogenized sample (1 – 4) per treatment was added to 9ml of sterile distilled water in a tube. 1ml of this was transferred to a second tube also containing 9ml of sterile distilled water. The procedure was repeated up to the 8th tube to give 10⁻⁸ dilution. One ml each from dilutions 10⁻⁶ and 10⁻⁸ was placed in sterile petri dish and about 10ml of plate count agar (PCA) added. The plates was swirled gently for even distribution of the organisms and allowed to set. The plates were incubated at 35°C for 24 – 48 hr in an inverted position as stated by Olayemi *et al.*, (2021).

Excreta gas estimation.

On the last six days of the experiment, flat plastics were arranged under battery cage for ease of faecal collection. Fresh excreta sample was collected from each replicate and stored in refrigerator for three days. 300g of faecal sample from the pooled was mixed into 2 litres of water to form slurry and stored in a sealed plastic keg according Lei *et al.* (2017) on treatment basis. Each plastic keg had a hose with clip, sealed with an adhesive to prevent gas leakage. The samples were kept at room temperature and allowed to ferment for a period of 2weeks. At the fourteenth day gas produced from excreta samples was measured using Portable syngas analyzer (model IR-GAS -600P). Values obtained will be compared with WHO, NESREA and FMEnv stipulated standard. Evaluation of effectiveness in gases emission mitigation was made on relative reduction value (RRV, %) calculated as the ratio of the difference between the control and treatments mean (of 3 replicates) CH₄, O₂, CO₂, H₂, CO, C_nH_n concentrations.



Statistical analysis

All data collected were subjected to a one-way analysis of variance using SAS (2009). The significant means were separated using Turkey's test of the same software. A significant difference was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Result

Performance traits of laying chickens

Table 2 shows the performance traits of laying chickens fed the diets supplemented with biochar, zeolite, chromolaena leaf-meal and its combinations. From the results, the dietary treatments has no significant effect ($p > 0.05$) on parameters such Hen-day production (HEDP), egg mass, feed intake g/bird, total feed intake, feed/dozen egg and total number eggs produced during the feeding trials. It was observed that chicken on diet supplemented with combination of biochar, zeolite and leaf-meal laid highest number of eggs (449) with (HEDP) percentage of 66.81 and feed /dozen egg at 1.93.

Table 2: Performance traits of laying chickens diets supplemented with biochar, zeolite, chromolaena leaf-meal and its combinations

Parameters	T1	T2	T3	T4	SEM	P-value
Hen-day production %	62.98	63.78	64.78	66.81	2.60	0.97
Egg mass	3355.34	3387.48	3425.97	3482.10	145.98	0.99
Feed intake g/bird	107.97	109.22	108.86	108.76	0.22	0.25
Total feed intake	6046.13	6116.51	6096.16	6090.54	12.44	0.25
Feed/dozen egg	2.03	2.13	2.07	1.93	0.09	0.92
Total production	428.33	428.67	435.33	449.00	17.28	0.98

T1 =control, T2= biochar+ leaf-meal, T3= zeolite +leaf-meal and T4= biochar + zeolite +leaf-meal

Effect of dietary supplemented of biochar, zeolite, chromolaena leaf-meal on external and internal qualities of egg

Dietary supplementation of biochar, zeolite, chromolaena leaf-meal and its combinations on external and internal qualities of egg laid is shown in Table 3. Results showed that inclusion of the additive has no significant effect on egg quality traits. Hen on diet 4 has the highest Haugh unit compare to hen on control diet. The diet has no influence on yolk colour, shell thickness as well as egg shape.



Table 3: Egg quality traits of chicken diets supplemented with biochar, zeolite, chromolaena leaf-meal and its combinations

Parameter	T1	T2	T3	T4	SEM	P-value
Egg weight (g)	53.31	53.34	52.70	52.13	0.32	0.54
Egg breath (cm)	35.77	34.82	35.99	32.51	0.67	0.26
Egg length(cm)	43.87	46.02	44.17	45.34	0.63	0.66
Albumen height(mm)	9.13	9.18	9.12	9.12	0.02	0.63
Albumen weighta (g)	32.64	33.21	32.53	31.84	0.39	0.73
Yolk colour	10.00	9.00	10.00	10.00	0.22	0.29
Yolk weight (g)	12.43	11.89	12.39	12.20	0.13	0.46
Shell thickness (mm)	0.22	0.23	0.23	0.23	0.00	0.73
Shell weight (g)	5.20	5.54	5.40	5.36	0.09	0.63
Shape index	81.70	75.66	81.78	71.64	1.98	0.20
Haugh unit (%)	93.52	93.31	93.28	93.53	0.07	0.53

T1 =Control, T2= biochar+ leaf-meal, T3= zeolite +leaf-meal, T4= biochar + zeolite +leaf-meal

Diet supplemented of biochar, zeolite, chromolaena leaf-meal and its combinations on external and internal qualities of egg laid is shown in Table 3. Results showed that inclusion of the additive has no significant effect on egg quality traits. Hen on diet 4 has the highest Haugh unit compare to hen on control diet. The diet has no influence on yolk colour, shell thickness as well as egg shape.

Table 4: Egg storage status index (pH, total bacteria count)

Parameters	T1	T2	T3	T4	SEM	P-value
pH values						
DAY 1	6.50 ^b	7.20 ^a	6.50 ^b	6.40 ^b	0.10	0.00
DAY 7	7.27 ^b	8.47 ^a	6.70 ^c	6.50 ^d	0.23	0.00
DAY 14	7.40 ^a	6.50 ^b	6.10 ^c	6.20 ^c	0.16	0.00
DAY 21	6.80	6.10	6.00	6.00	0.10	.
DAY 28	6.30 ^a	6.00 ^b	5.60 ^c	6.00 ^b	0.08	0.00
DAY 35	6.00 ^a	6.00 ^a	5.80 ^b	5.60 ^c	0.05	0.00
Total bacteria counts						
DAY 1	1.27 ^a	0.73 ^b	0.90 ^{ab}	0.97 ^{ab}	0.07	0.05
DAY 7	0.73	0.70	0.60	0.47	0.05	0.20
DAY 14	1.07 ^a	0.40 ^b	0.20 ^b	0.33 ^b	0.10	0.00
DAY 21	1.53 ^a	1.37 ^a	0.60 ^b	0.73 ^b	0.12	0.00
DAY 28	2.53 ^a	1.50 ^b	1.80 ^{ab}	2.17 ^{ab}	0.15	0.04
DAY 35	4.03 ^b	5.93 ^a	4.43 ^b	4.27 ^b	0.26	0.01

a, b, c, d Means on the same row having different superscript are significantly different ($P < 0.05$). T1 =control, T2= biochar+ leaf-meal, T3= zeolite +leaf-meal and T4= biochar +zeolite +leaf-meal



Table 4 revealed that pH values of the stored on a weekly basis, the results showed that at fresh state the eggs are slightly acid except treatment T2 that is slightly alkaline with ph value of 7.2). It was observed that the dietary treatment significantly ($P < 0.05$) affected the pH values of the eggs during storage. T2 values ranged from slightly alkaline to slightly acidic as the eggs aging. T3 and T4 maintained slightly acidic medium while eggs in T1 were slightly acidic at day 1, slightly alkaline at day 7 and 14 and pH scale dropped to acidic medium as the eggs aging. Effect of the dietary treatment on Total bacteria counts (TBC) in eggs during storage, It can be observed that there was changes in total bacteria values from day 1 through storage interval. Within 24 hours of laying, there was significant effect ($P < 0.05$) of the dietary treatment on total bacteria counts with eggs laid by hen on diet 1 with highest total bacteria count while the east value was recorded in eggs laid in hen on diet 2. At Day 7 and 14, significant effect ($P < 0.05$) of the diet on (TBC) were still stable. However, from Day 21, there was increase in the values (TBC) across the dietary treatments. The eggs from control diet had the highest (TBC) at expiration (Day 35) storage interval with least in values recorded in eggs from diet 2.

Table 5: Methane, Carbon monoxide, oxygen, Carbon dioxide and hydrogen concentrations in fermented excreta.

Parameters	1	2	3	4	SEM	P-value
CH ₄	1.1 ^a	0.68 ^b	0.45 ^c	0.66 ^b	0.07	0.00
CO ₂	23.58 ^a	18.86 ^b	11.67 ^d	14.77 ^c	1.35	0.00
O ₂	0.03	0	0	0.09	0.01	.
CO	0	0	0	0	0	.
H ₂	0.43 ^a	0.42 ^a	0.35 ^b	0.37 ^b	0.01	0.00
C _n H _m	0	0	0	0	0	.

a, b, c, d Means on the same row having different superscript are significantly different ($P < 0.05$). T1 = control, T2 = biochar + leaf-meal, T3 = zeolite + leaf-meal and T4 = biochar + zeolite + leaf-meal

Mean CH₄, CO₂, O₂, H₂, CO concentrations for all of the treatment groups evaluated 15 days after fermentation are presented in Table 7. Compared with the control group, CH₄ concentration in the treated excreta had reduction values of 38% in T2, 59% in T3 and 40% in T4. Hydrogen gas concentration reduction value ranged between 2.3% -18.6% in comparison with control while carbon dioxide concentration relatively reduced from 20% to 50% in the treated groups. Carbon monoxide gas was not detected across treatments, carbon hydrogen not detected, No or less in laying chickens is all about optimizing egg production in relation to dietary treatments and management during the feeding trial. The oxygen was detected in the samples.



DISCUSSION

Laying performance.

This study investigated the effects of inclusion of biochar, zeolite and chromolaena leaf meal blend in the diets of laying chickens in assessing laying performance parameters, in-depth egg quality traits, shelf-life status index over a storage period, odour and gas emission from the poultry house. The results obtained, showed that performance characteristics like HEDP, Egg mass and Feed/dozen egg in laying hens on T4 was 66% and highest when compared with other treatments. The elevated egg production observed suggests improved quality of the diet. Olayemi *et al.*, (2024); Adeyemo *et al.* (2012) stated that there is a direct relationship between the performance of laying hens and protein intake. Earlier studies by (Prasai *et al.*, 2018; Prasai *et al.*, 2016) observed enhanced laying production parameters and improve feed conversion ratio in hen diet supplemented with biochar, bentolite and zeolite. This could be attributed to inclusion of biochar, zeolite and leafmeal blend. Precious studies has revealed that inclusion of biochar in diet promotes the intestinal integrity, improve gut health and thus increase nutrient availability and absorption which result to increased laying performance. Yamauchi *et al.*, (2010) reported a significant increase in egg production in laying hen diet supplemented with mixture of bamboo charcoal powder and bamboo vinegar. Several experiments were conducted using natural zeolite on egg production and egg quality in different production periods, with varied results in parameters measured. Improvement in laying in the present study could be linked to ability of zeolite to selectively exchange its own cation for ions from the environment which brands it (zeolite) as a unique selective adsorber in gastrointestinal tract thereby improving gut health, nutrient absorption and overall performance. This result aligns with the report of Okey *et al.*, (2021) and Amad, (2021) concluding that natural zeolite can be used as additives in layer hens diet with positive effect on the egg production and egg shell quality of laying hens.

Egg quality traits; The key traits in egg qualities assessment include shell quality, yolk quality, albumen quality, egg size, egg weight and other interior qualities. Data from present study shown in Table 3 revealed that inclusion of biochar, zeolite and chromolaena leafmeal blend in hen diet linearly improved albumen height, Haugh unit, yolk colour, and eggshell thickness, which agrees with the studies of Prasai *et al.*, (2018) reporting increased feed conversion ratio, better egg weight in laying hens fed by wood biochar with improvement on egg quality traits such as shell strength, thickness and better egg production. Studies (Kajetan *et al.*, 2020; Prasai *et al.*, 2018; Kutlu, *et al.*, 2001) have shown that dietary addition of biochar improved FCR, Egg weight, yolk colour, egg shell weight, shell thickness, breaking strength and number of cracks.

Although, this study showed no significant difference in egg quality parameters measured, however, results obtained are consistent with previous studies (Lixi *et al.*, 2018; Kim *et al.*, 2008) indicating no alteration in most of the egg quality traits in hens fed *S. chinensis* and pine needles. Eggs are graded based on their HU values: AA, 72 or more; A, 71 to 60; and B, <60 (Jonnes, 2012). Herein, HU in all the dietary treatments were above 72, suggesting that the eggs produced in this study are of good quality. These improvements in HU values recorded may be attributed to the antioxidant property of bioactive constituents in the blend which might have reduced the lipid and protein oxidation in eggs. Radwan *et al* (2008) has earlier reported that bio-active constituents of plants were shown to protect magnum and uterus, as well as enhance the albumen secretion in laying birds. Improvement in productive performance may



attribute to zeolites supplementation by having a number of beneficial effects they provide including better utilization of feed nutrients and positive effects on intestinal microflora and the mechanism of digestion and protection of animals against harmful effects of mycotoxin, stimulation of liver detoxification processes, elimination of heavy metals and radioactive elements (Macháček *et al.*, 2010). The findings of Rattanawut *et al.* (2017) reported no significant differences between the groups with respect to egg shell weight, yolk colour and Haugh unit (an index of albumen quality) in diet supplemented with the commercial bamboo vinegar as charcoal supplementation induced a non-significant reduction in quality traits.

Egg pH: Eggs are perishable food and storage temperature is an important factor that affects the shelf life of table egg pH can be used as an indicator of egg freshness, as pH increases with age of eggs other factors such as Haugh units etc. (Ayoola *et al.*, 2016). Fresh eggs are slightly acidic with pH around 7.6-7.9 for albumen and 6.0-6.2 in the yolk.

These values changes as eggs ages due to the loss of carbon dioxide through the shell's pores. The pH values increases in older eggs and this affects protein structure, making the albumen less viscous. Dietary modification like inclusion of additives can influence egg pH values during storage. It has been shown that when biochar are used as an additive it maintain and stabilizes pH values and internal quality traits reduced odour and contamination during storage (Acta Ref),

The result of the present studies show that a stabilized pH values as the egg ages. This is attributed to ability of biochar or zeolite to alter fundamental chemical process of egg pH rising during storage (Kajetan *et al.*, 2020). Earlier studies (Stadelmen and Cotterill, 1995) have reported that whole eggs are relatively pH neutral, egg white with an initial pH value that can be as low as 7.6 at time of lay, but with increasing alkalinity as the egg ages, and can reach pH of 9.2. Supplementing diets of laying hen with biochar, zeolite assist in reducing loss in carbon dioxide and moisture thus making the content stable as against the natural condition where pH increases with both time of storage. This statement agreed that reduction in pH values recorded in the present studies was as a result of dietary treatment (Brown, 2011).

Total bacteria counts in stored eggs: Inclusion of biochar, zeolite and chromolaena leafmeal alters bacterial contamination and extends shelf life by controlling moisture and proliferation of bacteria due to adsorbent properties in biochar and zeolite. Reduction in Total bacteria counts in the present study could due to pH values of the eggs. The initial near-neutral pH gives egg's initial protective mechanisms against bacterial growth also egg shell acts as a barrier, and the slightly acidic environment within eggs inhibit the proliferation of microorganisms. At lay the pH of both the albumen and the yolk is approximately 6.5-6.7, once egg is laid, carbon dioxide is released from the albumen and this increases albumen pH which prevents penetration of bacteria. Olayemi *et al.*, 2021) in a feeding trial with leafy vegetables observed a relative neutral pH at time of lay but the value increases as the egg ages due increase water loss, carbon dioxide, and subsequent increase in the pH and weaken vitelline which could be due to phenolic compounds in the herbal plants which reduced the microbial load of eggs with their antimicrobial properties and antioxidant compounds. The mechanisms of action of biochar, zeolite and chromolaena leaf meal are likely to be multifactorial; zeolites have been associated with bactericidal effects on pathogenic organisms in the guts of birds and reduced viable counts of *Salmonella enteritidis* and *Escherichia coli* in the proximal and distal gut (Oliver, 1991) while biochar and the leaf-meal reduced the abundance of microorganism as found in the current study.



Gas Emission

The observed reduction in methane gas emission in excreta of hen supplemented with biochar, zeolite and combination of leaf meal. The reduction in methane production can be linked to previous studies that inclusion of zeolite, biochar reduced enteric methane production by preventing the activity of methanogen bacteria as gaseous emissions from excreta are associated with microbial activity in the excreta, the reduction of total N content resulted likely due to the loss of nitrogen from the transformation of ammonium ions (NH_4^+) into gaseous NH_3 that is associated with pH increase. A decrease in methane production in a growing steer and finishing cattle given biochar at 8g/kg DM decreased methane generation by 9.5 and 18.4%, respectively (Winder *et al.*, 2019). The adsorption of methane production can be linked to porous structure of biochar and zeolite with a large surface area, when mixed in the feed absorb methane gas within its pores and also with electron-mediating characteristics (Yu *et al.*, 2015). Inclusion of biochar, zeolite in diet greatly influence the microbiota dynamics in the digestive system with improved gut microbiota to synthesis of beneficial ones to improvement in digestion efficiency led to reduction of residues in faecal resulting in less methane-production when compared to non –biochar diet. Studies have shown that buffering capacity of biochar and zeolite alter pH values resulting in decreased excreta Ph hence reduction in ammonia release from excreta (Romero *et al.* (2012) and Schneider *et al.* (2017). Thus diet fortified with either biochar or zeolite reduced

excreta pH due to the protonation of volatile ammonia (NH_3) to the less volatile ammonium ion (NH_4^+) as its structural arrangement, zeolite can absorb ammonia, reducing the potential for volatilization of ammonia compounds (Pavelić *et al.*, 2018) hydrogen ions (H^+) as desirable.

It should be noted that evaluation of excreta pH is an important factor in poultry production, as it is decisive in the process of ammonia emission in the sheds, since the acidification ($\text{pH}<7.0$) of the excreta can minimize the release and the impacts caused by ammonia (Schneider *et al.*, 2017). The reduction in the values of methane gas emitted (38%, 59%) in the excreta of hen fed diets supplemented with biochar, zeolite and leafmeal aligned with (Romero *et al.*, 2012).

IMPLICATION TO RESEARCH AND PRACTICE

This work interacts carbon-based and mineral-based sorbents and its effects on layers performance, gut health and immunity and also assess the emission factors like methane (CH_4) carbon monoxide and hydrogen gas concentration (H_2) for global livestock environmental impact.



CONCLUSION

Dietary supplementation of zeolite, biochar and *Chromolaena* leafmeal blend offers a promising dietary strategy for enhancing laying hen performance and egg quality traits. Calculating feed per dozen eggs is important in poultry farming because it measures how efficiently birds convert feed into egg production. Feed is the highest cost in egg production, so this indicator helps farmers monitor expenses, control waste, and improve profitability. A higher feed per dozen eggs value recorded was an indication of better feed utilization and healthier, well-managed birds.

The blend also contribute to improved shell quality, which helps in maintaining internal quality and reduce breakages during transportation and slowing moisture loss and microbial load during storage. As nutritional strategies, the blend mitigates the negative environmental impact of poultry production by reducing harmful gas emissions from manure leading to improvement in air quality for health concern of birds and humans in the environment.

FUTURE RESEARCH

Future Research has to be done on biochemical research into isolating its specific bioactive compounds in *Chromolaena odorata* for standardized poultry supplements.

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Conflict of Interest

The authors have declared no conflicts of interest for this article.

Informed Consent

Written informed consent was obtained from all study participants.

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