Nutrición Animal Tropical 19 (2): 38-62. Julio-Diciembre, 2025
ISSN: 2215-3527 / DOI: 10.15517/6ebwzj88

Technical note

Impact of selected phytogenic feed additives on performance, haematological, biochemical, and antioxidant enzyme responses in 64-week-old laying hens

Akinlolu Oluwafemi-Ayeni [1], *Oluwatobiloba Emmanuel-Popoola* [2], *Johnson Oluwasola-Agbede* [3]

ABSTRACT

The effects of turmeric, ginger, and garlic powders on the performance, blood profile, serum biochemistry, and antioxidant properties of 64-week-old laying hens were evaluated. The study was carried out at the Teaching and Research Farm of the Department of Animal Production and Health, The Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. A 12-week feeding trial was conducted with a total of 135 Isa Brown laying hens, arranged in a completely randomized design. Five diets were formulated for the prosecution: Diet 1 (control); Diet 2 (3.00% addition of turmeric); Diet 3 (3.00% addition of ginger); Diet 4 (turmeric and garlic at 2.25% and 0.75%, respectively); and Diet 5 (ginger and garlic at 2.25% and 0.75%, respectively). The proximate compositions of the phytogenic feed additives (PFAs) were determined as follows: moisture (5.03 – 7.50%); ash (3.26 – 6.11%); ether extract (7.39 – 14.58%); crude fiber (10.28 – 13.79%); crude protein (3.07 – 7.23%); and nitrogen-free extract (58.88 – 61.54%). The performance indices were not significantly influenced by the test diets ($p > 0.05$), except for hen-day production (HDP). The lowest HDP (57.50%) and feed conversion ratio (FCR) (2.37%) were observed in Diet 4. No significant differences were recorded among

[1]Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria. Email address: aoayeni@futa.edu.ng (https://orcid.org/0000-0001-7558-0781).
[2]Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria. Corresponding author: popoolaaph171714@futa.edu.ng (https://orcid.org/0009-0005-0551-008X).
[3]Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria. Email address: joagbede@futa.edu.ng (https://orcid.org/0000-0001-7297-6350).
Recibido: 17 julio 2024 Aceptado: 01 octubre 2025

the haematological parameters and biochemical indices ($p > 0.05$). Glutathione peroxidase (GSH) activity was significantly highest (2.02 μM/mL) in birds fed Diet 2 ($p < 0.05$). The activities of catalase (0.80 μM/mg protein) and superoxide dismutase (60%) were significantly ($p < 0.05$) highest in birds on Diet 5. The results of this study indicate that PFAs do not exert adverse effects on the health of older laying hens; on the contrary, they help maintain productive performance and improve serum and antioxidant profiles.

Keywords: Old-laying hens, turmeric, ginger, garlic, phytogenic feed additives.

RESUMEN

Impacto de aditivos fitogénicos seleccionados sobre el rendimiento, la respuesta hematológica, bioquímica y antioxidante en gallinas ponedoras de 64 semanas de edad. Se evaluaron los efectos de la cúrcuma, el jengibre y el ajo en polvo sobre el rendimiento, el perfil sanguíneo, el suero y las propiedades antioxidantes de gallinas ponedoras de 64 semanas de edad. El estudio se llevó a cabo en la Granja de Enseñanza e Investigación del Departamento de Producción y Salud Animal de la Universidad Federal de Tecnología, Akure (FUTA), Estado de Ondo, Nigeria. Se realizó un ensayo de alimentación de 12 semanas con un total de 135 gallinas ponedoras de la cepa Isa Brown, dispuestas en un diseño completamente aleatorio. Se formularon cinco dietas para el ensayo: Dieta 1 (control); Dieta 2 (3% de cúrcuma); Dieta 3 (3% de jengibre); Dieta 4 (cúrcuma y ajo al 2,25% y 0,75%, respectivamente); y Dieta 5 (jengibre y ajo al 2,25% y 0,75%, respectivamente). La composición proximal de los aditivos fitogénicos para piensos (AFP) se determinó de la siguiente manera: humedad (5,03 – 7,50%); cenizas (3,26 – 6,11%); extracto etéreo (7,39 – 14,58%); fibra cruda (10,28 – 13,79%); proteína cruda (3,07 – 7,23%); y extracto libre de nitrógeno (58,88 – 61,54%). Los índices de rendimiento no fueron significativamente influenciados por las dietas de prueba ($p > 0.05$), excepto la producción diaria por gallina (HDP). Los valores más bajos de HDP (57,50%) y de relación de conversión alimentaria (FCR) (2,37%) se obtuvieron en la Dieta 4. No se observaron diferencias significativas entre los parámetros hematológicos ni entre los índices bioquímicos evaluados ($p > 0.05$). La actividad de la glutatión peroxidasa (GSH) fue significativamente mayor (2,02 uM/mL) en las aves alimentadas con la Dieta 2 ($p < 0.05$). Las actividades de catalasa (0,80 uM/mg proteína) y superóxido dismutasa (60%) fueron significativamente más altas ($p < 0.05$) en las aves alimentadas con la Dieta 5. Los resultados de este estudio indican que los AFP no generan efectos adversos sobre la salud de las gallinas ponedoras de mayor edad; por el contrario, contribuyen a mantener el rendimiento productivo y a mejorar los perfiles séricos y antioxidantes.

Palabras clave: gallinas ponedoras senescentes, cúrcuma, jengibre, ajo, aditivos alimentarios fitogénicos.

INTRODUCTION

Livestock production plays a vital role in the agricultural economies of developing countries, providing not only a direct source of food but also essential raw materials such as hides, wool, fertilizer, and fuel (Okoro, 2016). Within this sector, poultry production is particularly significant but faces a major challenge in nutrition, as feed accounts for approximately 70% of total production costs (Mmadubuike and Ekenyem, 2001). Therefore, improving feed efficiency while reducing costs is crucial to ensure the sustainability of poultry farming (Alhotan, 2021).

In the past, growth-promoting antibiotics were commonly used as feed additives. However, concerns about issues such as disruption of the natural gut microbiota and the development of antibiotic resistance in both bacteria and humans have led to their restriction or ban in many countries (Borazjanizadeh et al., 2011; Diarra et al., 2011). As a result, the use of herbs and medicinal plants, such as turmeric, ginger, and garlic, as natural alternatives to antibiotics has gained momentum in the last decade, owing to their wide range of potential health benefits (Ali et al., 2008; Mahesh and Prabhakar, 2018).

Turmeric rhizome (*Curcuma longa*), a member of the ginger family (Zingiberaceae), is a widely recognized medicinal herb (Barazesh et al., 2013). According to Rajesh and Devvrat (2018), turmeric contains several active compounds, including curcuminoids, aromatic turmerones (32.5%), α-turmerones (15.6%), β-turmerones (17.1%), and curlone. Curcuminoids have been shown to exhibit a broad spectrum of biological activities, including antioxidant, antibacterial, antifungal, antiprotozoal, antiviral, anticoccidial, and anti-inflammatory effects (Rajesh and Devvrat, 2018).

Ginger, the rhizome of *Zingiber officinale*, is widely used as a delicacy, spice, and in traditional medicine (Okoro, 2016). Previous research has identified nine compounds in ginger capable of binding to serotonin receptors, potentially influencing gastrointestinal function. Ginger oleoresins have been associated with diverse pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, anti-hypercholesterolemic, anti-hyperglycemic, and antispasmodic effects (Banerjee, 2018). In addition, ginger extract may regulate free radical levels and lipid peroxidation, and it has demonstrated antidiabetic potential (Al-Amin et al., 2006; Morakinyo et al., 2011).

Garlic (*Allium sativum*) is widely used both as a food flavoring agent and as a traditional remedy for various ailments (Sallam et al., 2004; Hanieh et al., 2010). Animal studies have demonstrated that garlic exhibits hypolipidemic, hypotensive, hypoglycemic, antithrombotic, and antiatherogenic effects. Its bioactive compounds, primarily flavonoids and organosulfur derivatives, have also shown therapeutic and antioxidant properties in laying birds (Navidshad et al., 2018).

Blood biochemical parameters and antioxidant activity are critical indicators of an organism's health status and nutrient metabolism (Lokesh et al., 2012). Recent studies investigating the use of turmeric, ginger, and garlic as additives have reported promising outcomes, including improved weight gain, enhanced feed efficiency, reduced mortality, and increased survivability in poultry (Issa and Omar, 2012; Oleforuh-Okoleh, 2014). Therefore, the present study aimed to evaluate the beneficial effects of turmeric, ginger, and garlic powder on the performance, hematological profile, serum biochemistry, and antioxidant capacity of aging laying hens.

MATERIALS AND METHODS

Experimental Site

The field study was conducted at the Teaching and Research Farm of the Department of Animal Production and Health at The Federal University of Technology, Akure (FUTA), Ondo State, Nigeria.

Collection, processing of garlic, ginger, and turmeric, and proximate composition analysis

Fresh turmeric, garlic, and ginger were purchased, washed to remove dirt, and peeled before being chopped into smaller pieces. The turmeric and ginger rhizomes were air-dried at room temperature (28 °C) for five days, milled into a fine powder, and stored in airtight containers. Garlic bulbs were peeled and oven-dried at 125 °C for the first two hours, then adjusted to 102 °C for four hours before milling. The additives were incorporated into the experimental diets in their dehydrated powdered form at the specified inclusion levels (3.00% turmeric, 3.00% ginger, 2.25% turmeric + 0.75% garlic, and 2.25% ginger + 0.75% garlic). No extracts or essential oils were used in this study.

The proximate composition (moisture, ash, crude fiber, and ether extract) was determined using AOAC (2009) methods. Crude protein content was determined using the micro-Kjeldahl method as described by Agbede et al. (2009), while nitrogen-free extract (NFE) was calculated algebraically using the formula provided by AOAC (2009):

%NFE = [100 – (%Moisture + %Ash + %Crude Protein + %Ether Extract + %Crude Fiber)]

Experimental layout and hens' management

A 12-week feeding trial arranged in a completely randomized design was conducted to evaluate the performance of hens fed diets containing garlic, ginger, and turmeric. One hundred thirty-five, 64-week-old, Isa Brown laying birds were obtained from the poultry section of the Teaching and Research Farm of the Department of Animal Production and Health at the University. The birds were housed in deep-litter cages in an open-sided house and divided into five dietary groups, each consisting of three replicates of nine birds. The control diet was formulated to meet the NRC (1994) requirements for laying hens, while the other diets were duplicates of the control with various phytogenic feed additives (PFAs). Accordingly, the five diets used for the trial, as presented in Table 1, were: Diet 1, control diet with no inclusion of PFAs; Diet 2, 3% turmeric; Diet 3, 3% ginger; Diet 4, 2.25% turmeric + 0.75% garlic; and Diet 5, 2.25% ginger + 0.75% garlic. The birds were fed their respective experimental diets and provided water *ad libitum* throughout the experiment.

The response criteria included feed intake (FI), feed conversion ratio (FCR), and hen-day production (HDP). Daily FI was calculated as the difference between the weight (g) of the feed administered and the weight of the leftover feed. The individual body weight of all hens was recorded at the start of the first phase and at the end of the last phase, using a Kenny Camry electronic price platform scale, to determine the average weight gain. Egg production was recorded daily for each treatment, and the average egg production rate (hen-day percentage) was calculated for each phase.

Table 1. Gross composition of diets fed at laying phase.

Ingredients	Diet 1 (kg)	Diet 2 (kg)	Diet 3 (kg)	Diet 4 (kg)	Diet 5 (kg)
Turmeric	0.00	3.00	0.00	2.50	0.00
Garlic	0.00	0.00	0.00	0.50	0.50
Ginger	0.00	0.00	3.00	0.00	2.50
Corn	59.50	56.50	56.50	56.50	56.50
GNC*	14.00	14.00	14.00	14.00	14.00
Soybean meal	8.00	8.00	8.00	8.00	8.00
Wheat offal	6.00	6.00	6.00	6.00	6.00
DCP*	2.00	2.00	2.00	2.00	2.00
Limestone	7.00	7.00	7.00	7.00	7.00
Premix	0.30	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Vegetable oil	2.50	2.50	2.50	2.50	2.50

*GNC = Groundnut Cake; DCP = Dicalcium phosphate.

Data Collection

Haematological And Serum Biochemical Studies

Three birds were randomly selected from each replicate, and 10 mL of blood was collected through their jugular veins. Of this, 3 mL of blood was drawn into an EDTA plastic tube for hematological analysis. Packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), lymphocytes, and monocytes were measured following methods described by Dacie and Lewis (1991). BIOBASE automatic biochemistry analyser was used to assess serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, albumin, globulin, and glucose.

Antioxidant assay

Serum glutathione peroxidase (GSH-Px) activity was measured according to the method of Paglia and Valentine (1967), using a Randox/RS-504 kit (Ransel, Randox Laboratories, UK). Superoxide dismutase (SOD) activity was determined using the Ransod enzyme kit (RANDOX/SD-125) following the procedure by Nishikimi et al. (1972). Malondialdehyde (MDA) concentration was measured with the Elabscience MDA colorimetric kit in accordance with the method by Ohkawa et al. (1979). Total antioxidant capacity (T-AOC) was assessed using auto-analyzers from BioMed Diagnostics (Egypt) following the method described by Koracevic et al. (2001).

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics version 23.0 (IBM Corp, 2015). When significant differences were detected, Duncan´s multiple range test (DMRT) was applied to separate the means.

Below is the statistical model:

$$Y_{ij} = U + T_i + E_{ij}$$

Where:

Yij is the individual observation of all treatments.

U is the universal means.

Ti is the effect of the diets, i.e., treatment.

Eij is the experimental error.

RESULTS

Proximate composition

The proximate composition of the phytogenic feed additives (PFAs) used in the experimental diets varied across treatments, as shown in Table 2. Diet 4 exhibited the highest values for moisture, ash, and crude fiber, whereas Diet 2 showed the highest fat and extract contents. Crude protein levels were highest in Diet 3 and lowest in Diet 5. Significant differences ($p < 0.05$) were observed among the diets for all measured parameters, indicating distinct compositional profiles among the PFAs.

Table 2. Proximate composition of phytogenic feed additives

Nutrients (%)	Diet 2	Diet 3	Diet 4	Diet 5	±SEM*	P value
Moisture	5.03^{d}	7.08^{b}	7.50^{a}	5.89^{c}	0.01	0.001
Ash	3.26^{d}	5.69^{b}	6.11^{a}	4.16^{c}	0.01	0.001
EE*	14.98^{a}	9.61^{c}	7.39^{d}	12.50^{b}	0.06	0.001
CF*	11.52^{c}	10.28^{d}	13.79^{a}	13.49^{b}	0.03	0.001
CP*	3.67^{c}	7.23^{a}	6.33^{b}	3.07^{d}	0.07	0.001
NFE*	61.54^{a}	60.11^{b}	58.88^{c}	60.89^{a}	0.12	0.001

^{a,b,c} – Means on the same row having different superscripts are significantly ($p < 0.001$) different. *SEM = standard error of the mean; EE = ether extract; CF = crude fiber; CP = crude protein; NFE = nitrogen free extract.

Performance indices

The performance indices (Table 3) were not significantly ($p > 0.05$) influenced by the experimental diets, with the exception of HDP. The highest HDP was observed in birds fed Diet 3 and 5; whereas the lowest HDP was recorded in birds fed Diet 4.

Table 3. Performance indices of laying birds fed diet containing phytogenic feed additives.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM*	P-value
Initial weight (kg)	1.84	1.97	1.77	1.87	1.81	0.02	0.090
Final weight (kg)	1.99	2.02	1.93	1.92	1.95	0.02	0.590
Weight gained (kg)	0.15	0.05	0.16	0.04	0.14	0.02	0.100
FI/hen/day (g)	112.53	113.07	112.66	113.35	112.59	0.12	0.220
% HDP*	58.25^b	58.47^b	62.92^a	57.50^b	62.04^a	0.98	0.040
FCR*	2.34	2.33	2.15	2.37	2.18	0.04	0.350

^{a,b,c} – Means on the same row having different superscripts are significantly ($p < 0.05$) different.

*SEM = standard error of the mean; FI = feed intake; %HDP = percentage hen day production; FCR = feed conversion ratio.

Haematological parameters

The haematological profiles of laying hens were not significantly affected by the inclusion of phytogenic feed additives, with no differences observed across treatments ($p > 0.05$). Despite the lack of statistical significance, some numerical variations were noted. Birds fed Diet 2 exhibited higher erythrocyte sedimentation rate (ESR) and lymphocyte (LYM) values compared to the other groups, while the lowest ESR and LYM were recorded in birds receiving Diets 4 and 1, respectively. Diet 4 resulted in the highest values of packed cell volume (PCV), red blood cells (RBC), haemoglobin (Hb), and eosinophils (EOS), whereas Diet 2 showed the lowest values for PCV, RBC, and Hb. The Monocyte (MON) percentage was slightly higher in Diet 1 and lowest in Diet 4. Detailed values are presented in Table 4.

Table 4. Effect of phytogenic feed additives on haematology of laying hens.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	±SEM*	P value
ESR* (mm/h)	3.78	4.33	3.78	3.67	3.89	0.12	0.410
PCV* (%)	26.11	25.00	26.00	26.22	25.78	0.25	0.550
RBC* ($\times 10^6$ mm^3)	2.12	1.87	2.09	2.19	2.06	0.07	0.620
Hb* (g/100mL)	8.68	8.30	8.62	8.71	8.54	0.08	0.540
LYM* (%)	59.11	60.22	59.56	59.56	59.89	0.16	0.270
MON* (%)	12.56	12.33	12.00	11.56	11.67	0.28	0.770
EOS* (%)	1.33	1.22	1.11	1.56	1.33	0.11	0.790

*SEM = standard error of the mean; ESR = erythrocyte sedimentation rate; PCV = packed cell volume; RBC = red blood cell; Hb = haemoglobin; LYM = lymphocyte; MON = monocytes; EOS = eosinophil.

Serum biochemical indices

No significant differences ($p > 0.05$) were observed in the serum biochemical parameters of aged laying hens fed diets containing phytogenic feed additives. However, some numerical trends emerged across treatments. Birds receiving Diet 1 exhibited higher values of alkaline phosphatase (ALP), total protein, and globulin compared to those on the supplemented diets. The highest aspartate aminotransferase (AST) and cholesterol concentrations were recorded in birds fed Diet 5, whereas the lowest values for both parameters were observed in birds fed Diet 3. Complete results are presented in Table 5.

Table 5. Effect of phytogenic feed additives on blood serum parameters.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	±SEM*	P value
AST* (IU/L)	48.50	39.75	36.00	47.00	70.75	7.70	0.660
ALT* (IU/L)	20.75	60.75	29.25	28.25	10.25	5.75	0.120
PO_3* (IU/L)	284.65	151.28	224.58	204.00	254.43	16.34	0.160
Protein (g/L)	50.80	47.08	47.08	39.50	44.55	3.28	0.860
Albumin (g/L)	18.75	27.00	20.50	20.00	21.00	1.16	0.240
Globulin (g/L)	32.05	17.58	26.58	19.60	23.05	2.66	0.470
Urease (mmol/L)	1.75	3.28	2.33	2.33	3.63	0.25	0.150
Creatinine (umol/L)	55.00	74.50	59.50	69.75	77.50	3.93	0.350
Cholesterol (mmol/L)	2.70	2.73	2.65	3.08	3.43	0.18	0.610
HDL* (mmol/L)	2.28	2.73	1.95	2.85	2.88	0.15	0.240
LDL* (mmol/L)	2.73	2.78	2.43	3.10	3.28	0.21	0.720
Triglycerides (mmol/L)	6.05	7.85	4.63	7.08	7.30	0.55	0.400

*SEM = standard error of the mean; AST = Aspartate aminotransferase; ALT = alanine aminotransferase; PO_3 = alkaline phosphate (ALP); HDL = high density lipoprotein; LDL = low density lipoprotein.

Antioxidant properties

Dietary inclusion of phytogenic feed additives significantly ($p < 0.05$) influenced the antioxidant parameters in aged laying hens. Clear differences were observed across treatments. Diet 2 exhibited the highest glutathione (GSH) concentrations, while Diet 1 showed the lowest.

Catalase activity peaked in hens receiving Diet 5 and was lowest in those fed Diet 3. Similarly, superoxide dismutase (SOD) activity was substantially higher in birds fed Diet 5, while birds on Diets 1, 3, and 4 exhibited comparably lower values. Detailed results are presented in Table 6.

Table 6. Antioxidant effect of phytogenic feed additives of laying hens.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	±SEM*	P value
GSH* (uM/mL)	0.21^{d}	2.02^{a}	1.49^{b}	0.53^{c}	0.21^{d}	0.03	0.001
Catalase (uM/mg protein)	0.32^{c}	0.32^{c}	0.19^{d}	0.33^{b}	0.80^{a}	0.01	0.001
SOD* (%)	10.00^{b}	20.00^{b}	10.00^{b}	10.00^{b}	60.00^{a}	2.31	0.001

^{a,b,c} – Means on the same row having different superscripts are significantly ($p < 0.001$) different. *SEM = standard error of the mean, GSH = glutathione peroxidase, SOD = superoxide dismutase.

DISCUSSION

In evaluating the nutritional properties of the experimental ingredients, the proximate analysis of the phytogenic feed additives (PFAs) highlights the natural compositional variability inherent to plant-based ingredients, driven by factors such as maturity stage, environment, and processing methods (Ikpeama et al., 2014; Ahaotu and Lawal, 2019). While not intended as primary protein sources, their crude protein content suggests a supplementary role in monogastric diets, contributing to overall amino acid availability and dietary balance (Ogbuewu et al., 2014).

Regarding mineral composition, ash content —an important factor influencing bone mineralization and eggshell strength— ranged from 3.26% to 6.11% in the tested PFAs, indicating a meaningful mineral contribution (Javadi et al., 2021). Notably, Diet 4, with the highest ash value, may enhance dietary mineral availability. This finding is consistent with Kostić et al. (2023), who reported that the combination of turmeric and garlic yields synergistic effects on mineral composition.

In the context of dietary fiber, crude fiber (CF) is essential in poultry nutrition for maintaining gut health and promoting the digestion and absorption of nutrients (Jiménez-Moreno et al., 2016). According to NRC (2012), feedstuffs with CF levels above 18% are considered roughages and are poorly digested by monogastric animals. The CF levels observed in the PFAs studied (10.28–13.79%) fall within a tolerable range, supporting their use as dietary supplements without impairing nutrient digestibility in laying hens.

The ether extract values, particularly the highest level in Diet 2 (14.98%), are consistent with previous reports on turmeric's rich content of beta-carotene, polyphenols, essential fatty acids, and volatile oils (Ikpeama et al., 2014). These bioactive compounds contribute to the elevated energy value of PFA-based diets. As noted by Babayemi and Bamikole (2006), such phytochemicals can enhance energy availability and overall efficiency in poultry systems.

Nitrogen-free extract (NFE), representing the carbohydrate portion of feed, serves as a vital energy source for poultry (Moniruzzaman and Fatema, 2022; Ndelekwute et al., 2019). The NFE values reported support the notion that PFAs contribute to growth performance, body composition, and glucose metabolism (Ge et al., 2023). Furthermore, the presence of plant-derived bioactive compounds may enhance metabolic pathways and improve energy utilization, thereby helping maintain overall nutritional balance (Murugesan et al., 2015).

Although no significant effects were observed on performance parameters and serum biochemical indices, notable improvements were seen in hen-day production (HDP) and antioxidant enzyme activities. These findings indicate that PFAs contributed to improve production efficiency and oxidative balance. While feed intake (FI) and feed conversion ratio (FCR) remained unaffected, HDP was significantly higher in birds receiving Diets 3 and 5. This enhancement may be linked to improved nutrient utilization and metabolic activity, potentially mediated by compounds such as gingerols and shogaols, which are known to enhance gastrointestinal function and enzymatic activity, thereby facilitating more efficient nutrient absorption (El-Kashef, 2022; Soliman et al., 2021; Olaniyi et al., 2020; Ayeni et al., 2020; Banerjee, 2018; Oleforuh-Okoleh et al., 2014).

Haematological indices remained stable across all treatment groups, indicating that PFA supplementation had no adverse effects on blood health or physiological status in laying hens. This finding is consistent with previous studies showing that turmeric and ginger supplementation does not significantly alter hemoglobin concentration, erythrocyte sedimentation rate, or packed cell volume (Bounous and Stedman, 2000; Dacie and Lewis, 1991; Hosseini-Vashan et al., 2012; Basavaraj et al., 2011; Sugiharto et al., 2011).

Similarly, serum biochemical parameters—including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, and cholesterol—remained within physiological reference ranges. Specifically, the ranges for AST (36.00–70.75 IU/L), ALT (10.25–60.75 IU/L), ALP (151.28–284.65 IU/L), total protein (39.50–50.80 g/L), albumin (18.75–27.00 g/L), and cholesterol (2.65–3.43 mmol/L) were all within or near the normal limits for laying hens, as reported by Kaiser et al. (2022) and Kraus et al. (2021).

Although ALT in Diet 2 (60.75 IU/L) slightly exceeded the typical upper limit, previous research has documented ALT values up to 60 IU/L in healthy birds (Lumeij, 2008). Furthermore, in avian species, ALT is not the primary indicator of liver function, whereas AST and ALP are more reliable markers (Kaneko et al., 2008). Given that both AST and ALP remained within normal ranges and that no clinical signs of hepatic dysfunction were observed, the elevated ALT level does not suggest liver impairment. Additionally, the trend of reduced ALP values in the treatment groups may indicate hepatoprotective effects, though further investigations, including metabolomic profiling, histological evaluation, and longitudinal studies, are needed to confirm this hypothesis (Onu, 2010).

Antioxidants are essential for protecting against oxidative stress, cellular damage, and chronic degenerative diseases (Sreelatha and Padma, 2009). A key outcome of this study was the significant increase in antioxidant enzyme activities, particularly glutathione peroxidase (GSH-

Px) and catalase, in hens receiving PFA-enriched diets. The highest levels of these enzymes were observed in birds fed Diets 2 and 5, suggesting that the bioactive compounds in these additives strengthen the birds' antioxidant defense mechanisms.

Turmeric, for instance, is rich in curcuminoids (especially curcumin), which possess potent antioxidant and anti-inflammatory properties through free radical scavenging and modulation of inflammatory pathways (Rajesh and Devvrat, 2018). Ginger contains gingerols and shogaols, which enhance gastrointestinal health and stimulate antioxidant enzyme activity, thereby improving nutrient absorption and metabolic performance (El-Kashef, 2022; Banerjee, 2018; Morakinyo et al., 2011). Garlic provides allicin and other organosulfur compounds that support lipid metabolism and reinforce antioxidant responses, thus mitigating oxidative stress (Navidshad et al., 2018; Sallam et al., 2004).

The synergistic interaction of these phytochemicals likely explains the observed improvements in antioxidant status and overall physiological condition of the hens. Consistent with these findings, Hosseini-Vashan et al. (2012) also reported enhanced GSH-Px activity in chicks fed turmeric-supplemented diets. Therefore, PFA supplementation appears promising for boosting glutathione levels and maintaining a robust antioxidant system, which is essential for sustaining health and optimizing productivity in laying hens (Mohammed et al., 2022).

FINAL CONSIDERATIONS

The findings of this study indicate that the inclusion of phytogenic feed additives (PFAs), notably ginger, turmeric, and garlic, can positively influence both productive performance and oxidative balance in 64-week-old laying hens. Diets supplemented with ginger, either alone or in combination with garlic, significantly enhanced hen-day production (HDP) without negatively affecting feed intake or feed conversion ratio, suggesting improved nutrient utilization and metabolic efficiency. These outcomes support the practical application of ginger-based additives in commercial egg production systems, particularly in aged layers where performance tends to decline.

The effects in antioxidant enzyme activities, especially glutathione peroxidase and catalase, detected in hens fed diets containing turmeric or the ginger–garlic blend, are likely attributable to bioactive phytochemicals such as gingerols, curcuminoids, and allicin, known for their capacity to modulate oxidative stress responses. The observed enhancement in antioxidant defense highlights the potential of these additives to improve physiological resilience under oxidative or metabolic challenges.

Importantly, haematological and serum biochemical profiles indicated the absence of adverse effects and supporting the safety of these PFAs in layer nutrition. Additionally, subtle trends suggesting improved liver function in supplemented groups warrant further investigation to clarify underlying mechanisms. Overall, the use of ginger, alone or synergistically with garlic, emerges as a promising strategy to improve both performance and health in laying hens. Future research should focus on elucidating the molecular pathways through which these compounds exert their effects and refine optimal inclusion rates for application in various production settings.

LITERATURE CITED

Agbede, J.O., Kluth, H. and Rodehutscord, M. (2009). Studies on the effects of microbial phytase on amino acid digestibility and energy metabolizability in caecectomized laying hens and the interaction with the dietary phosphorus level. *British Poultry Science*, 50 (5), 583-591. https://doi.org/10.1080/00071660903196900

Ahaotu, E.O. and Lawal, M. (2019). Determination of Proximate and Minerals Content of Turmeric (*Curcuma longa* Linn) Leaves and Rhizomes. *Journal of food, nutrition and packaging*, 6, 1-4.

Al-Amin, Z.M., Thomson, M., Al-Qattan, K.K., Peltonen-Shalaby, R. and Ali, M. (2006). Anti-diabetic and hypolipidemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *British Journal of Nutrition*, 96, 660-666. https://doi.org/10.1079/BJN20061849

Alhotan, R.A. (2021). Commercial poultry feed formulation: Current status, challenges, and future expectations. *World's Poultry Science Journal, 77* (2), 279-299.

Ali, B.H., Blunden, G., Tanira, M.O. and Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): a review of recent research. *Food and Chemical Toxicology*, 46 (2), 409–420. https://doi.org/10.1016/j.fct.2007.09.085

AOAC (Association of Official Analytical Chemists). (2009). *Official Methods of Analysis, 17th edition*. Association of Official Analytical Chemists, Washington, DC, USA.

Ayeni, A.O., Oladedun, A.E. and Agbede, J.O. (2020). Performance and egg qualities of old-laying hens fed with diets containing selected phytogenic feed additives. *Journal of Food*, 3, 19-25. https://doi.org/10.21839/jfna.2020.v3.330

Babayemi, O.J. and Bamikole, M.A. (2006). Effects of *Tephrosia candida* DC leaf and its mixtures with Guinea grass on *in vitro* fermentation changes as feed for ruminants in Nigeria. *Pakistan Journal of Nutrition*, 5 (1), 14-18. https://doi.org/10.3923/pjn.2006.14.18

Banerjee, G.C. (2018). *A textbook of animal husbandry*. Oxford and IBH publishing.

Barazesh, H., Pour, M.B., Salari, S. and Abadi, T.M. (2013). The effect of ginger powder on performance, carcass characteristics and blood parameters of broilers. *International Journal of Advanced Biological and Biomedical Research*, 1 (12), 1645-1651.

Basavaraj, M., Nagabhushana, V., Prakash, N., Appannavar, M.M., Wagmare, P. and Mallikarjunappa, S. (2011). Effect of dietary supplementation of *Curcuma longa* on the biochemical profile and meat characteristics of broiler rabbits under summer stress. *Veterinary World*, 4 (1), 15-18. https://doi.org/10.5455/vetworld.2011.15-18

Borazjanizadeh, M., Eslami, M., Bojarpour, M., Chaji, M., and Fayazi, J. (2011). The effect of clove and oregano on economic value of broiler. *Journal of Animal and Veterinary Advances*, 10, 169-173. http://dx.doi/org10.3923/javaa.2011.169.173

Bounous, D. and Stedman, N. (2000). *Normal avian hematology: chicken and turkey*. In: Feldman BF, Zinkl JG, Jain NC, editors. Schalm's veterinary hematology. New York: Wiley, p.1147-1154.

Dacie, J.V. and Lewis, S.M. (1991). *Practical haematology*. ELBS with Churchill Livingston. Churchil Livingstone.

Diarra, S.S., Kwari, I.D., Girgiri, Y.A., Saleh, B. and Igwebuike, J.U. (2011). The use of sorrel (*Hibiscuss sabdariffa*) seed as a feed ingredient for poultry: A review. *Research Opinions in Animal and Veterinary Sciences*, 1 (9), 573-577.

El-Kashef, M.M. (2022). Impact Of Using Ginger (*Zingiber Officinale*) In Laying Quail Diets on Egg Production, Egg Quality and Blood Parameters. *Egyptian Poultry Science Journal*, 42 (4), 419-435. https://doi.org/10.1080/09712119.2011.558612

Ge, Y., Zhang, L., Chen, W., Sun, M., Liu, W. and Li, X. (2023). Resveratrol Modulates the Redox Response and Bile Acid Metabolism to Maintain the Cholesterol Homeostasis in Fish Megalobrama amblycephala Offered a High-Carbohydrate Diet. *Antioxidants*, 12 (1), 121. https://doi.org/10.3390/antiox12010121

Hanieh, H., Narabar, K., Piao, M., Gerile, C., Abe, A. and Kondo, Y. (2010). Modulatory effects of two levels of dietary Alliums on immune response and certain immunological variables, following immunization, in White Leghorn chicken. *Animal Science Journal*, 81, 673-680. https://doi.org/10.1111/j.1740-0929.2010.00798.x

Hosseini-Vashan, S.J., Golan, A., Yaghobfar, A., Zarban, A., Afzali, N. and Esmaeilinasab, P. (2012). Antioxidant status, immune system, blood metabolites and carcass characteristic of broiler chickens fed turmeric rhizome powder under heat stress. *African Journal of Biotechnology*, 11: 16118 -16125. https://doi.org/10.5897/AJB12.1986

IBM Corp (International Business Machines Corporation). (2015). IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

Ikpeama, A., Onwuka, G.I. and Nwankwo, C. (2014). Nutritional Composition of Turmeric (*Curcuma longa*) and its Antimicrobial Properties. *International Journal of Scientific and Engineering Research*, 5, 2229-5518.

Issa, K. and Omar, J. (2012). Effect of garlic powder on performance and lipid profile of broilers. *Open Journal of Animal Science*, 2, 62-68. http://dx.doi.org/10.4236/ojas.2012.22010

Javadi, M., Pascual, J.J., Cambra-López, M., Macías-Vidal, J., Donadeu, A., Dupuy, J., Carpintero, L., Ferrer, P. and Cerisuelo, A. (2021). Effect of Dietary Mineral Content and Phytase Dose on Nutrient Utilization, Performance, Egg Traits and Bone Mineralization in Laying Hens from 22 to 31 Weeks of Age. *Animals*, 11. https://doi.org/10.3390/ani11061495

Jiménez-Moreno, E., de Coca-Sinova, A., González-Alvarado, J.M. and Mateos, G.G. (2016). Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. Effects on growth performance and water intake. *Poultry science*, 95 (1), 41-52. https://doi.org/10.3382/ps/pev309

Kaiser, J. C., Reider, H., Pabilonia, K. L., and Moore, A. R. (2022). Establishment of biochemical reference values for backyard chickens in Colorado (*Gallus gallus domesticus*). *Veterinary Clinical Pathology*, *51*(4), 577-584.

Kaneko, J. J., Harvey, J. W., and Bruss, M. L. (Eds.). (2008). *Clinical biochemistry of domestic animals*. Academic press.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. and Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *Journal of clinical pathology*, 54 (5), 356. https://doi.org/10.1136/jcp.54.5.356

Kostić, K., Brborić, J., Delogu, G.L., Simic, M., Samardžić, S., Maksimović, Z., Dettori, M.A., Fabbri, D., Kotur-Stevuljević, J. and Saso, L. (2023). Antioxidant Activity of Natural Phenols and Derived Hydroxylated Biphenyls. *Molecules*, 28 (6), 2646. https://doi.org/10.3390/molecules28062646

Kraus, A., Zita, L., Krunt, O., Härtlová, H., and Chmelíková, E. (2021). Determination of selected biochemical parameters in blood serum and egg quality of Czech and Slovak native hens depending on the housing system and hen age. *Poultry Science*, 100 (2), 1142-1153.

Lokesh, G., Ananthanarayan, S.R. and Murthy, V.N. (2012). Changes in the activity of digestive enzymes in response to chemical mutagen diethyl sulfate in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Asian Journal of Applied Science*, 5, 431-437. https://doi.org/10.3923/ajaps.2012.431.437

Lumeij, J. T. (2008). Avian clinical biochemistry. *Clinical biochemistry of domestic animals*, 839-872.

Mahesh, M.G. and Prabhakar, B.Y. (2018). Natural antibiotic effect of turmeric in poultry management*. International Journal of Poultry and Fisheries Science*, 2 (1), 1-3. http://dx.doi.org/10.15226/2578-1898/2/2/00109

Mmadubuike, F.N. and Ekenyem, B.U. (2001). *Non-ruminant livestock production in the tropics*. Gustchucks Graphics Centre, Owerri, Nigeria.

Mohammed, A.B., Abdulwahid, A.S. and Raouf, S.M. (2022). Effect of *Thymus vulgmus* addition to the diet of laying hens on egg production, egg quality, biochemical and antioxidant parameters. *Advances in Animal and Veterinary Science*, 10 (2), 427-433.

Moniruzzaman, M. and Fatema, U.K. (2022). Proximate analysis of local ingredients as a prospective resource for feed production in Bangladesh. *Journal of Multidisciplinary Sciences*, 4 (1), 1-9. https://doi.org/10.33888/jms.2022.411

Morakinyo, A.O., Akindele, A.J. and Ahmed, Z. (2011). Modulation of antioxidant enzymes and inflammatory cytokines: possible mechanism of anti-diabetic effect of ginger extracts. *African Journal of Biomedical Research*, 14 (3), 195-202.

Murugesan, G.R., Syed, B., Haldar, S., and Pender, C. (2015). Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. *Frontiers in Veterinary Science,* 2 (21). doi: 10.3389/fvets.2015.00021.

Navidshad, B., Mostafa, M. and Babak, D. (2018). Garlic: An alternative to antibiotics in poultry production, a review. *Iranian Journal of Applied Animal Science*, 8 (1), 9-17.

Ndelekwute, E.K., Unah, U.L. and Udoh, U.H. (2019). Effect of dietary organic acids on nutrient digestibility, faecal moisture, digesta pH and viscosity of broiler chickens. *MOJ Anatomy and Physiology*, 6, 40-43. https://doi.org/10.15406/mojap.2019.06.00242

Nishikimi, M., Rao, N.A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and biophysical research communications*, 46 (2), 849-854. https://doi.org/10.1016/S0006-291X(72)80218-3

NRC (National Research Council). (1994). *Nutritional Requirements of Poultry*. 9th edition. National Academic Press, Washington, DC.

NRC (National Research Council). (2012). *Nutrient requirements of Swine*. Division on Earth, Life Studies, Board on Agriculture, and Committee on Nutrient Requirements of Swine.

Ogbuewu, I.P., Jiwuba, P.D., Ezeokeke, C.T., Uchegbu, M.C., Okoli, I.C. and Iloeje, M.U. (2014). Evaluation of phytochemical and nutritional composition of ginger rhizome powder. International *Journal of Agriculture and Rural Development*, 17 (1), 1663-1670.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, 95 (2), 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Okoro, M.C. (2016). Effect of Dietary Inclusion of Ginger (*Zingiberofficinale*) on Performance of Broilers and Laying Chickens. Unpublished M.Sc. thesis. Federal University of Technology, Owerri.

Olaniyi, C.O., Atoyebi, M.O., Obafunmiso, H.T. and Salaam, K.A. (2020). Effect of ginger (*Zingiber officinale*) in the nutrition of African catfish-A cholesterol reducer and fertility enhancer. *International Journal of Aquaculture and Fisheries Sciences*, 6 (2), 21-28. https://doi.org/10.17352/2455-8400.000052

Oleforuh-Okoleh, V.U., Chukwu, G.C. and Adeolu, A.I. (2014). Effect of ground ginger and garlic on the growth performance, carcass quality and economics of production of broiler chickens. *Global Journal of Bioscience and Biotechnology*, 3 (3), 225-229

Onu, P.N. (2010). Evaluation of two herbal spices as feed additives for finisher broilers. *Biotechnology in Animal Husbandry*, 26, 383-392. https://doi.org/10.2298/BAH1006383O

Paglia, D.E. and Valentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of laboratory and clinical medicine*, 70 (1), 158-169.

Rajesh, D. and Devvrat, K. (2018). Turmeric powder as feed additive in laying hen A-review. *Journal of Pharmacognosy and Phytochemistry*, 7(3), 2686-2689

Sallam, K.I., Ishioroshi, M. and Samejima, K. (2004). Antioxidant and antimicrobial effect of garlic in chicken sausage. *Lebensmittel-Weissenschaft and Technologie*, 37, 849-855. https://doi.org/10.1016/j.lwt.2004.04.001

Soliman, A., Abdel–Azeem, A.A. and El-Sisi, E.S. (2021). Laying performance, health status and some physiological traits of mandurah laying chickens affected by the addition of different levels of anise and caraway seed powder. *Journal of Productivity and Development*, 26 (3), 531-559.

Sreelatha, S. and Padma, P.R. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant foods for human nutrition*, 64, 303-311. https://doi.org/10.1007/s11130-009-0141-0

Sugiharto, S., Isroli, I., Widiastuti, E. and Prabowo, N.S. (2011). Effect of turmeric extract on blood parameters, feed efficiency and abdominal fat content in broilers. *Journal of the Indonesian Tropical Animal Agriculture*, 36, 21–26. http://dx.doi.org/10.14710/jitaa.36.1.21-26