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Scientific article

Haemato-biochemical indices and antioxidant status of layers-fed diets supplemented with garlic-composite leaf mix

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ABSTRACT

The study aimed to evaluate haemato-biochemical indices and antioxidant status in laying hens fed varying levels of garlic-composite leaf mix (GCM), addressing limited research in this area. A twelve-week feeding trial was conducted at the Poultry unit of the Teaching and Research Farm (TRF) of the Federal University of Technology, Akure (FUTA), in Nigeria. The soursop, avocado, sandpaper, and gliricidia leaves were manually harvested, air-dried, and milled to produce the GCM; and then combined with garlic in a 2:2:2:3:1 ratio, respectively. The mix was added to the basal diet, designated as diet 1 (control, 0 g/kg), diet 2 (5 g/kg), diet 3 (10 g/kg), diet 4 (15 g/kg),

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and diet 5 (20 g/kg). A total of 135 hens were distributed across these 5 dietary treatments with 3 replicates, each containing 9 birds, in a completely randomized design. Packed cell volume, red blood cell count, and haemoglobin concentration showed significant differences ($p < 0.05$), whereas other haematological parameters did not ($p > 0.05$). Mean cell haemoglobin concentration presented higher values observed in the GCM-fed groups (32.45 ± 0.51 - 32.74 ± 0.34 g/dl) compared to the control diet (32.13 ± 0.07 g/dl). White blood cell counts were highest (6.60 ± 1.27×10^{6}/L) in birds fed diet 4. Alanine aminotransferase levels were lowest (10.20 ± 2.31 IU/L) in birds fed diet 3 and highest (14.40 ± 0.35) in those supplemented with diet 5. Superoxide dismutase and glutathione peroxidase levels increased significantly ($p < 0.05$) in birds fed the test diets. Catalase levels were higher (78.56 ± 11.74) in birds on the control diet than those on test diets (20.46 ± 2.13 - 56.86 ± 19.78). The study concluded that the inclusion of GCM had no adverse effects on the health of laying hens but rather helped maintained their haematological, blood serum, and antioxidant parameters.

Keywords: haematology, serum biochemistry, antioxidant, Isa brown, garlic mix, composite leaf mix.

RESUMEN

Índices hemato-bioquímicos y estado antioxidante en aves ponedoras alimentadas con dietas suplementadas con una mezcla de hojas compuestas con ajo. El estudio tuvo como objetivo evaluar los índices hemato-bioquímicos y el estado antioxidante en gallinas ponedoras alimentadas con diferentes niveles de una mezcla de hojas compuestas con ajo (MCA), abordando la investigación limitada en esta área. Se realizó un ensayo de alimentación de 12 semanas en la Unidad Avícola de la Granja de Enseñanza e Investigación (TRF) de la Universidad Federal de Tecnología de Akure (FUTA), Nigeria. Las hojas de guanábana, aguacate, higuera de lija y gliricidia fueron cosechadas manualmente, secadas al aire y molidas para producir la MCA; se combinaron con el ajo en una proporción de 2:2:2:3:1, respectivamente. La mezcla se añadió a la dieta base, designadas como dieta 1 (control, 0 g/kg), dieta 2 (5 g/kg), dieta 3 (10 g/kg), dieta

4 (15 g/kg) y dieta 5 (20 g/kg). Un total de 135 gallinas fueron distribuidas en 5 tratamientos con 3 repeticiones, cada una con 9 aves, en un diseño completamente aleatorizado. El volumen celular empaquetado, eritrocitos y la hemoglobina fueron significativos ($p < 0.05$), mientras que otros parámetros hematológicos no fueron significativos ($p > 0.05$). La concentración media de hemoglobina celular presentó valores más altos observados en grupos alimentados con MCA (32.45 ± 0.51 - 32.74 ± 0.34 g/dL) en comparación con la dieta control (32.13 ± 0.07 g/dL). El conteo de glóbulos blancos fue mayor ($6.60 \pm 1.27 \times 10^6$/L) en las aves alimentadas con la dieta 4. Los niveles de alanina aminotransferasa fueron más bajos (10.20 ± 2.31 IU/L) en las aves alimentadas con la dieta 3 y más altos (14.40 ± 0.35 IU/L) en aquellas suplementadas con la dieta 5. El superóxido dismutasa y la glutatión peroxidasa incrementaron significativamente ($p < 0.05$) en las aves alimentadas con dietas experimentales. Los niveles de catalasa fueron más altos (78.56 ± 11.74) en las aves de la dieta control que en aquellas de las dietas de prueba (20.46 ± 2.13-56.86 ± 19.78). El estudio concluyó que el MCA no tuvo efectos adversos en la salud de las gallinas ponedoras, sino que mantuvo sus parámetros hematológicos, bioquímicos y estado oxidativo.

Palabras clave: hematología, bioquímica sérica, antioxidante, isa brown, mezcla de ajo, mezcla de hojas compuestas.

INTRODUCTION

The increasing prevalence of antibiotic-resistant organisms has prompted extensive research into reliable alternatives, leading to the identification of plant-derived products such as leaves and seeds. In addition to the inherent benefits of natural plant extracts, recent studies have shown that herbal medicines and their derivatives can positively influence laying performance and enhance the antioxidant capacity of poultry (Alagawany et al., 2017).

In this context, phytogenic feed additives, particularly those derived from plants, have emerged as potential alternatives to antibiotic growth promoters in poultry nutrition and other areas of livestock production. Given the growing global concerns over meat and egg safety, the search for effective antibiotic substitutes is more urgent than ever. Among these alternatives, plant leaves and their extracts have recently been recognized as some of the most promising options (Mahrose et al., 2019).

Research has revealed that certain types of plant foliage contain chemical compounds with potent medicinal properties, which can improve reproductive tract function, enhance overall animal welfare, and elevate the quality of animal-derived products such as meat and eggs (Jamshidparvar et al., 2017). Moreover, some bioactive compounds found in plant-based products have demonstrated a significant prophylactic role in preventing various infections (Hashemi and Davoodi, 2012).

The inclusion of herbal medicine in poultry feed has been shown to stimulate feed intake, thereby enhancing and sustaining bird welfare and productivity (Windisch et al., 2008). The use of plant foliage in poultry farming not only contributes to antimicrobial effects against pathogenic microorganisms but also promotes improved nutrient utilization in the gut (Dhama et al., 2015). Based on this evidence, it is proposed that plant leaves possess significant phytogenic activity and can be effectively used in layer management to improve performance and health.

Hematological and serum biochemical parameters are often influenced by factors such as medication, toxic substances, diet, infections, age, and sex of the birds (Schmidt et al., 2009). For instance, dietary supplementation with soursop leaves has been found to reduce intramuscular lipid cell muscle fibers, carcass yield, and carcass components. Avocado leaves have demonstrated therapeutic potential for treating conditions such as anemia and hypertension and exhibit notable antioxidant properties. Similarly, sandpaper leaf supplementation has been shown to reduce free fatty acids, acid value, and peroxide value. Additionally, garlic has gained a longstanding reputation across various cultures as both a prophylactic and therapeutic medicinal plant (Huyghebaert, 2003).

However, research on the use of soursop (*Annona muricata*), avocado (*Persea americana*), sandpaper (*Ficus exasperata*), and gliricidia (*Gliricidia sepium*) leaves—whether individually or in combination—as dietary supplements to enhance the health of laying hens remains limited. This gap in knowledge forms the basis of the present study, which was designed to investigate the hematological and biochemical indices, as well as the antioxidant status, of laying hens fed a composite mixture of soursop leaf, avocado leaf, sandpaper leaf, gliricidia leaf, and garlic powder.

MATERIALS AND METHODS

Experimental Site

This research was conducted at the Poultry Unit of the Teaching and Research Farm (TRF) of the Federal University of Technology, Akure (FUTA), Nigeria. Akure is located at latitude 7.3070°N and longitude 5.1398°E, within the humid rainforest zone of western Nigeria, which is characterized by bimodal rainfall patterns and high humidity during the rainy season (Agbelade and Akindele, 2013). Additional laboratory analyses were performed at the Central Research Laboratory of FUTA.

The birds were housed in a three-tier Californian colony cage system [dimensions: 6 m (length) × 4 m (width) × 3 m (height)], with three birds per cell, three cage units per replicate, and three replicates per treatment. The cages were equipped with open galvanized feeders and plastic pipe drip water troughs. The average annual temperature and relative humidity in the poultry house was 27 °C and 75%, respectively (Olabode and Adeleke, 2017).

Each bird received 110 g of feed per day, divided into two portions administered at 9:00 a.m. and 4:00 p.m. Water was provided *ad libitum*.

Test ingredient preparation

Fresh soursop, avocado, sandpaper, and gliricidia leaves were harvested from mature stems. The leaves were chopped and air-dried at 35 °C until reaching average moisture contents of 11.01% (soursop), 10.45% (avocado), 11.61% (sandpaper), and 13.5% (gliricidia). Once dried, the leaves were ground into a fine powder with a particle size of 0.02 mm using a Granulex® 3 Series hammer mill. Garlic bulbs were obtained from a local vendor and similarly processed into powder.

Subsequently, the leaf meals (soursop, avocado, sandpaper, and gliricidia) and garlic powder were blended in a ratio of 2:2:2:3:1, respectively, to produce a garlic-composite leaf meal (GCM).

Antioxidant concentration and activity were determined using a spectrophotometric method. Four replicates of each test ingredient were analyzed and labeled S1, S2, S3, S4, and S5. Samples were stored in well-covered Petri dishes before analysis.

Experimental diets

The gross composition of the experimental diets is presented in Table 1, and Table 2 provides the calculated nutrient composition.

The basal diet was formulated to contain 17% crude protein (CP) and 2655 kcal/kg metabolizable energy (ME), following the nutritional requirements of laying hens at 19 weeks of age, as recommended by the NRC (1994). Based on this formulation, five experimental diets were developed. Diet 1 served as the control and did not include any supplementation. Diet 2 included 125 g of GCM per 25 kg of feed (5 g/kg), while Diet 3 contained 250 g per 25 kg (10 g/kg). Diets 4 and 5 were supplemented with 375 g (15 g/kg) and 500 g (20 g/kg) of GCM per 25 kg of feed, respectively. In all supplemented treatments, the GCM was thoroughly mixed into the feed offered to the birds.

Table 1. Gross composition of the experimental basal diet.

Ingredients	Quantity (%)
Maize	55.45
Soybean meal	9.00
Groundnut cake	16.50
Wheat offal	6.50
Dicalcium phosphate	1.50
Fishmeal	0.50
Limestone	9.70
Lysine	0.15
Methionine	0.15
Salt	0.30
Premix	0.25
Total	100.00

Table 2. Calculated analysis of the experimental basal diet.

Nutrient	Value
Metabolizable energy (kcal/kg)	2655.00
Crude protein (%)	17.00
Moisture content (%)	13.00
Crude fiber (%)	3.80
Calcium (%)	4.01
Available phosphorus (%)	0.64
Methionine (%)	0.41
Lysine (%)	0.84

Experimental birds and management

A total of 135 Isa Brown laying hens, 19 weeks of age and at the point of lay, were used in this experiment. The birds were randomly assigned to one of five dietary treatment groups corresponding to 0, 5, 10, 15, and 20 g/kg of garlic-composite leaf meal (GCM). The treatments reflected different supplementation levels of the GCM, and the birds were fed their respective diets for a period of 12 weeks. Each treatment was replicated three times, with nine birds per replicate, in a completely randomized design. Replicates were tagged as Diets 1 to 5.

Biochemical analysis and data collection

Three laying birds per treatment were fasted prior to slaughter and exsanguination. Blood samples were collected from the jugular vein into three labeled, heparinized tubes. Additional blood samples were collected into clean, dry centrifuge tubes without anticoagulants and transported to the laboratory for analysis of hematological indices, serum biochemical parameters, and antioxidant enzyme activities. Analyses were performed using an automated hematology analyzer (BIOBASE, BK-6400).

Hematological parameters measured included packed cell volume (PCV), erythrocyte count, hemoglobin concentration, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Serum biochemical indices determined were total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum cholesterol, and creatinine levels.

Antioxidant enzyme concentrations measured included glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT). Serum GSH-Px activity was determined according to the method described by Weiss et al. (1980), using Ransel (RANDOX/RS-504; Randox Laboratories, Crumlin, UK). SOD activity was measured following the procedure of Nishikimi et al. (1972), using a commercial enzyme kit (Ransod, RANDOX/SD-125; Randox Laboratories, Crumlin, UK).

Statistical analysis

The statistical model used to analyze the generated data was an inferential statistical model. All data collected were subjected to analysis of variance (ANOVA) using SPSS software, version 25. Duncan's Multiple Range Test (DMRT, 2002) was employed to separate means when significant differences were observed. Below is the statistical model:

$$Yij=\mu+Ti+Eij$$

Where

Yij is the individual observation of all treatments

μ is the universal means

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

Eij is the experimental error

RESULTS

Antioxidant properties of garlic-leaf meals and the composite mix

Table 3 presents the antioxidant compositions of soursop, avocado, sandpaper, gliricidia leaf meal, garlic mix, and composite leaf mix. The results indicate that GCM exhibited mean values of 7.93 mg/100 g, 18.31%, 51.26 mg/100 g, and 0.23 mg/100g for flavonoids, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP), and phenol content, respectively, with corresponding coefficient variations of 72.07%, 73.54%, 58.91%, and 31.89%.

Soursop leaf meal contained the highest flavonoids content (14.17 mg/100 g) as compared to gliricidia, which recorded the lowest (0.62 mg/100 g). DDPH was, however, higher for avocado leaf meal (33.28%), while the lowest (5.45%) was recorded in sandpaper leaf meal.

The phenol content was highest (0.35 mg/100 g) in avocado leaf meal and lowest (0.14 mg/100 g) in garlic meal.

Table 3. Antioxidant composition of leaf meals and composite leaf mix meal (GCM).

Parameters	Antioxidants								
	SSM	AVM	SPM	GCM	GLM	CLM	Mean	Stdev	CoV
Flavonoids (mg/100g)	14.17	12.30	4.12	0.62	12.49	3.87	7.93	5.71	72.07
DPPH (%)	25.31	33.28	5.45	7.55	5.88	32.35	18.31	13.46	73.54
FRAP (mg/100g)	80.15	80.02	29.11	15.10	28.14	75.01	51.26	30.19	58.91
Phenol (mg/100g)	0.25	0.35	0.21	0.14	0.22	0.18	0.23	0.07	31.89

SSM: Soursop leaf meal; AVM: Avocado leaf; SPM: Sandpaper leaf; GCM: Gliricidia leaf meal; GLM: Garlic meal; CLM: Composite leaf mix; Stdev: Standard error of variation; CoV: Coefficient of variation; DDPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: ferric ion reducing antioxidant power.

The haematological parameters of laying hens fed diets supplemented with GCM

The hematological parameters of laying hens fed diets containing varying levels of garlic-composite leaf meal (GCM) are presented in Table 4. The results showed that among all the hematological parameters evaluated, only packed cell volume (PCV), red blood cell count (RBC), and mean corpuscular volume (MCV) were significantly affected by the dietary treatments ($p < 0.05$), whereas mean corpuscular hemoglobin (MCH), granulocyte count, and lymphocyte count were not significantly influenced ($p > 0.05$).

The highest PCV (38.50 ± 0.29%) was observed in birds fed Diet 3, compared to those on other treatments. Similarly, RBC count was highest in birds fed Diet 3 ($5.05 \pm 0.38 \times 10^6$/L), while the lowest value ($2.95 \pm 0.03 \times 10^6$/L) was recorded in birds fed Diet 5. The highest MCV (93.22 ± 0.07 fL) was recorded in birds fed Diet 5, whereas the lowest (58.11 ± 0.13 fL) was found in birds fed Diet 4.

Although not statistically significant, MCH was highest in birds fed Diet 5 (31.07 ± 0.02 pg/cell) and lowest in those on Diet 4 (19.37 ± 0.04 pg/cell). Hemoglobin concentration peaked in birds fed Diet 3 (12.83 ± 0.10 g/dL) and was lowest in those on Diet 4 (7.17 ± 0.10 g/dL). The highest lymphocyte percentage (67.00 ± 2.89%) was recorded in birds fed Diet 2, while the lowest (52.00 ± 6.93%) was found in birds on Diet 5. Finally, the highest granulocyte percentage (48.00 ± 6.93%) was observed in birds fed Diet 5, and the lowest (31.00 ± 4.04%) in those fed Diet 2.

Table 4. Hematology parameters of laying hens fed diets containing varying levels of garlic-composite mix supplementary.

Treatment	Diet 1 (Control)	Diet 2 (5 g/kg)	Diet 3 (10 g/kg)	Diet 4 (15 g/kg)	Diet 5 (20 g/kg)	P-Value
PCV %	28.00 ± 1.73b	30.50 ± 1.44b	38.50 ± 0.29a	21.50 ± 0.29c	27.50 ± 0.29b	<0.001*
RBC (x106/L)	3.40 ± 0.35bc	4.15 ± 0.49ab	5.05 ± 0.38a	3.70 ± 0.06bc	2.95 ± 0.03c	0.008*
MCHC (g/dL)	32.13 ± 0.07	32.74 ± 0.34	32.45 ± 0.51	32.57 ± 0.44	32.74 ± 0.34	0.762
MCV (fL)	86.61 ± 13.92	75.42 ± 5.44	77.65 ± 6.34	58.11 ± 0.13	93.22 ± 0.07	0.054*
MCH (pg/cell)	28.87 ± 4.64	25.14 ± 1.81	25.88 ± 2.12	19.37 ± 0.04	31.07 ± 0.02	0.054*
Haemoglobin (Hb g/dL)	9.33 ± 0.58b	10.17 ± 0.48b	12.83 ± 0.10a	7.17 ± 0.10c	9.17 ± 0.10b	<0.001*
WBC (x 106/L)	3.20 ± 0.58	5.90 ± 2.25	2.90 ± 0.06	6.60 ± 1.27	4.20 ± 0.35	0.192
Granulocyte (%)	36.00 ± 2.31	31.00 ± 4.04	42.00 ± 1.15	42.00 ± 0.00	48.00 ± 6.93	0.074
Lymphocyte (%)	64.00 ± 2.31	67.00 ± 2.89	58.00 ± 1.15	57.00 ± 0.58a	52.00 ± 6.93	0.086
Monocytes (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.58	0.00 ± 0.00	0.072

a, b, and c: Means on the rows with different superscripts are significantly different.

PCV: packed cell volume; RBC: red blood cell; MCHC: mean cell haemoglobin concentration; MCV: mean cell volume; MCH: mean cell haemoglobin; WBC: white blood cell.

*Significant at the 0.05 probability level.

Serum biochemical indices of laying hens fed diet supplemented with GCM

The serum biochemical indices of laying hens fed diets containing varying levels of GCM, as presented in Table 5, revealed that among all parameters measured, aspartate aminotransferase, cholesterol, creatinine, total protein, albumin, and globulin were significantly ($p < 0.05$) influenced by the dietary treatments. It was also observed that globulin levels increased in the treated birds, which resulted in an increase in total protein production in these groups. Birds fed diet 3 recorded the highest aspartate aminotransferase and cholesterol levels (78.70 ± 5.95 IU/L and 3.95 ± 0.26 mmol/L, respectively) compared to birds fed diet 1 and diet 5. Creatinine levels were higher (79.85 ± 12.27) in birds fed diet 5 compared to those fed the other treatments.

Table 5. Serum biochemistry indices of laying hens fed diets containing varying levels of garlic-composite mix supplementary.

Treatment	Diet 1 (Control)	Diet 2 (5 g/kg)	Diet 3 (10 g/kg)	Diet 4 (15 g/kg)	Diet 5 (20 g/kg)	P-Value
AST (IU/L)	52.45 ± 0.09c	62.75 ± 3.44b	78.70 ± 5.95a	66.45 ± 0.72b	71.85 ± 2.22a	0.002*
ALT (IU/L)	10.55 ± 0.32	13.35 4± 0.38	10.20 ± 2.31	12.20 ± 1.15	14.40 ± 0.35	0.131
Cholesterol(mmol/L)	3.45 ± 0.03b	3.55 ± 0.09b	3.95 ± 0.26a	3.20 ± 0.06b	3.20 ± 0.00b	0.010*
Creatinine (mmol/L)	39.90 ± 4.62bc	53.20 ± 12.30b	18.65 ± 1.53c	21.30 ± 3.06c	79.85 ± 12.27a	0.002*
Total protein (g/L)	59.75 ± 0.32c	73.65 ± 1.18a	70.40 ± 2.42ab	68.40 ± 1.56b	67.70 ± 0.52b	0.001*
Albumin (g/L)	45.35 ± 1.36a	37.55 ± 1.59bc	39.65 ± 2.68bc	36.65 ± 0.95c	42.55 ± 1.36ab	0.024*
Globulin (g/L)	14.40 ± 1.04d	36.10 ± 0.40a	30.75 ± 0.26b	31.75 ± 2.51ab	25.15 ± 1.88c	<0.001*
Glucose (mg/dL)	217.50 ± 1.44	208.50 ± 15.88	196.50 ± 48.21	219.00 ± 4.04	208.50 ± 2.60	0.956

a, b, and c: Means on the rows with different superscripts are significantly different.

AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase.

*Means significant difference.

Antioxidant properties of garlic-composite leaf supplemented in the diet of laying hens

Table 6 shows the antioxidant properties of the diets evaluated, containing varying levels of GCM. From the results of all the parameters measured, only SOD was significantly ($p < 0.05$) influenced by the dietary treatments. The highest SOD activity (90.73 ± 1.97 U/mL) was observed in laying hens fed diet 4, while the lowest (59.02 ± 2.25 U/mL) was recorded in hens fed diet 1. CAT and GSH levels were not significantly ($p > 0.05$) influenced by the dietary treatments.

Table 6. Antioxidant parameters of the diets evaluated containing varying levels of garlic-composite leaf mix supplementary.

Treatment (U/mL)	Diet 1 (Control)	Diet 2 (5 g/kg)	Diet 3 (10 g/kg)	Diet 4 (15 g/kg)	Diet 5 (20 g/kg)	P-Value
SOD	59.02 ± 2.25d	74.63 ± 4.51bc	87.32 ± 2.82ab	90.73 ± 1.97a	64.39 ± 7.04cd	0.001*
CAT	78.56 ± 11.74	56.86 ± 19.78	20.46 ± 2.13	47.96 ± 23.03	23.53 ± 3.90	0.092
GSH	215.09 ± 16.60	236.84 ± 2.60	223.34 ± 0.29	224.84 ± 5.77	232.84 ± 5.20	0.426

a, b, c, and d: Means on the rows with different superscripts are significantly different.

SOD: superoxide dismutase; CAT: catalase; and GSH: glutathione peroxidase.

*Means significant difference.

DISCUSSION

The results of the haematological indices showed that the packed cell volume (PCV) of birds across the treatments ranged from 21.50% to 38.50%, with no discernible trend. According to Akanbi et al. (2020), the normal PCV range for adult chickens is 30-40%. PCV serves as a useful indicator of nutritional anaemia and general physiological status. Elevated PCV may indicate dehydration or stress, while a low PCV suggests anaemia or nutritional deficiencies. The low PCV values recorded in birds fed Diets 1, 4, and 5 may be attributed to iron or vitamin deficiencies or excessive water intake, possibly due to high ambient temperatures (45 °C). However, as birds fed Diets 2 and 3 presented PCV values within the normal range, it is unlikely that the GCM supplementation had a negative influence on PCV. Therefore, GCM inclusion at the tested levels can be considered physiologically safe for PCV maintenance.

Regarding RBC, the values recorded ranged from 2.95 to 5.05 × 10^6 µL, aligning with the normal range (2.5–3.5 × 10^6 µL) reported by Bounous and Stedman (2000). Birds fed Diets 2 and 3 exhibited higher RBC counts, which may not be directly linked to GCM supplementation, but instead to other physiological responses, such as dehydration, as confirmed during the post-mortem examination of control birds. Given the role of RBCs in oxygen transport, elevated counts may reflect compensatory mechanisms in response to physiological stress.

Haemoglobin concentrations ranged between 7.17 and 12.83 g/dL, mostly within the physiological range of 9–13 g/dL (Akanbi et al., 2020). Although some values were slightly below this threshold, particularly in birds fed Diet 4, the variations remained within acceptable biological limits, suggesting no detrimental effects from GCM (Akanbi et al., 2020).

White blood cell (WBC) counts, and differential indices such as lymphocytes and granulocytes were not significantly influenced by the dietary treatments, indicating that GCM supplementation did not negatively impact the birds' immune status (Schmidt et al., 2009).

Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly affected by dietary GCM levels, while mean corpuscular haemoglobin concentration (MCHC) remained unaffected. The values for MCV (80–100 fL), MCH (27–33 pg/cell), and MCHC (32–36 g/dL) fell within normal physiological ranges (Akanbi et al., 2020). These indices are crucial for assessing red blood cell morphology and function. Elevated MCH and MCV, coupled with normal MCHC, suggest the absence of macrocytic anaemia or dietary toxicity (Roberts et al., 2000; Etim et al., 2014). This reinforces the notion that GCM did not introduce toxic elements into the feed.

In terms of serum biochemistry, GCM appeared to reduce albumin levels at lower inclusion rates but increased it at the highest supplementation level (Diet 5). This contrasts with the findings of El-Katcha et al. (2016), who reported that garlic supplementation at 50 mg/kg improved albumin content. Similarly, other studies found no significant influence of garlic extract on total protein and albumin levels compared to controls (El-Katcha et al., 2016).

Serum cholesterol levels initially rose with GCM inclusion but declined in birds fed Diets 4 and 5. This agrees with Elbaz et al. (2021), who noted that garlic at 20 g/kg significantly reduced serum cholesterol in broilers. Several other studies (Horton et al., 1991, Khan et al., 2008; Rehman et al., 2011; Khan et al., 2012; Omer et al., 2019) have similarly shown that garlic-based supplements improve lipid profiles. In this study, birds fed Diets 4 and 5 exhibited a 7.25% reduction in total cholesterol compared to controls, corroborating previous findings on garlic's hypocholesterolemic properties (El-Katcha et al., 2016).

Total serum protein and globulin levels are critical indicators of immune function. In this study, globulin was elevated in birds fed Diet 2, possibly explaining the lower mortality in this group. Albumin, essential for nutrient transport and tissue repair, also plays a role in binding free radicals, suggesting potential antioxidant benefits of GCM (Piotrowska et al., 2021; Oleforuh-Okoleh et al., 2015). These findings align with Oleforuh-Okoleh et al. (2015), who reported improved serum biochemistry in broilers supplemented with garlic and ginger.

Alanine aminotransferase (ALT) levels were highest in birds fed Diet 3. ALT is primarily a hepatic enzyme involved in amino acid metabolism and detoxification (Kim et al., 2008). Although elevated ALT may suggest liver stress, it is also influenced by factors such as glucose metabolism (Kim et al., 2008). In this study, the increase in ALT may be linked to higher glucose concentrations rather than hepatocellular damage.

The antioxidant enzymes evaluated, glutathione peroxidase (GSH), superoxide dismutase (SOD), and catalase (CAT), provide insight into the birds' oxidative status. SOD levels increased significantly with GCM supplementation, while CAT decreased. SOD neutralizes superoxide radicals, converting them into hydrogen peroxide, which is then broken down by CAT (Ighodaro and Akinloye, 2018; Nandi et al., 2019). The imbalance observed (elevated SOD and reduced CAT) may be a result of environmental stressors such as high ambient temperatures or humidity. Elevated SOD has been associated with neurological disorders and apoptosis (Younus, 2018) and may explain the gliosis observed in the brain cortex of GCM-fed birds. However, the concurrent increase in GSH activity suggests a compensatory antioxidant response (Yoon et al., 2016).

Glutathione plays a crucial role in cellular defense against oxidative stress. Increased GSH activity, as observed in GCM-fed birds, supports the hypothesis that the phytogenic additives in the GCM mixture, soursop, avocado, sandpaper, and gliricidia leaves, contributed to enhanced antioxidant capacity (Sameer, 2013). This aligns with findings from Yoon et al. (2016), who linked elevated GSH levels to improved health and resistance to infection.

In summary, GCM supplementation at levels up to 20 g/kg in the diets of laying hens had no adverse effects on haematological or serum biochemical indices and even conferred potential health benefits, particularly regarding antioxidant status and cholesterol reduction.

CONCLUSION

Based on these findings, the addition or supplementation of garlic composite leaf mix (soursop, avocado, sandpaper and gliricidia) may have a positive effect on the birds' biomarkers, although without a consistent pattern. This supplementation could improve the immune response and cholesterol levels of the laying hens, thereby enhancing their overall performance. Incorporating composite mixtures into the basal diet of laying hens may contribute to healthier Isa Brown chickens and offer an opportunity to improve productivity. Furthermore, it is recommended that future research be conducted to investigate the potential synergistic effects that GCM on the health status and egg production performance of laying hens.

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