

## EFFECT OF PHOTOPERIOD AND DIETARY INNOVATIVE MATERIALS (MONK'S PEPPER AND THYME) ON THE HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF GUINEA FOWL HENS

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### Abstract

A total of 96 guinea fowl hens (28-32 weeks old) were used to study the effect of photoperiod and dietary innovative materials (DIM) (Monk's pepper and Thyme) on the haematological and serum biochemical indices of guinea fowl hens. The birds were weighed and randomly assigned to eight treatment groups in a 2 (two photoperiods) × 4 (DIM) factorial arrangement in a Completely Randomized Design (CRD). The birds were exposed to two photoperiods of 12 hours of normal light (12 L) and 20 hours of extended light (20 L) period using ECKO 100W solar LED lamp with an average light intensity of 5.78 lux. The four diets used contained 6g of monks pepper (M) and thyme (T)/kg diet in different ratios as follows No DIM, 50M50T, 75M25T, and 25M75T. Each treatment was replicated three times having four birds per replicate. Feed and water were provided *ad libitum* and after nine months, blood samples were collected for haematological and serum biochemical evaluation. The results indicated that photoperiod significantly ( $P < 0.05$ ) influenced White blood cell (WBC) count and monocytes content while DIM significantly ( $P < 0.05$ ) improved all haematological parameters measured except lymphocytes, heterophils and eosinophils. For serum parameters, birds on extended photoperiod had significantly ( $p < 0.05$ ) higher total protein, globulin and uric acid content but no significant difference ( $p > 0.05$ ) was recorded for albumin. DIM inclusion significantly ( $p < 0.05$ ) affected total protein, globulin and uric acid content. However, albumin content was not significantly ( $p > 0.05$ ) affected. It can be concluded that 12 hours of light and DIM at ratio 25M75T/kg diet improved the blood parameters and general health of guinea fowl hens.

**Keywords:** Day Length, Guinea Fowl, Haematology, Serum Biochemistry, Thyme, Vitex.

## INTRODUCTION

Guinea fowl, *Numida meleagris* is native to Africa and it's in the order Galliformes, and Numidae family. Its name derives from the coast of Guinea in West Africa, where it is believed to have originated (ICAR 2021; Salgado *et al.*, 2022). Guinea fowl meat is white like chicken meat, drier, and regarded as very lean. It may be considered a high-quality protein source due to being rich in vitamins and containing fewer cholesterol and fats (Issaka *et al.*, 2016; Batkowska *et al.*, 2021). Their eggs are noteworthy for their thick shell, high proportion of yolk, high content of vitamins and trace elements and longer shelf life in comparison to chicken eggs (Batkowska *et al.*, 2021). Despite these attributes, the availability of their meat and eggs is a challenge because of inadequate feeding, poor housing, and lack of broodiness in the female, monogamous mating behaviour in the males and the seasonality in their breeding pattern (Okyere *et al.*, 2020). Kyere *et al.*, (2017) reported that that due to insufficient lighting, the reproductive performance of Guinea fowl in Ghana and Africa is quite poor. Light stimulates the release of reproductive hormones, may accelerate or delay sexual maturation and stimulate egg laying (Hassan *et al.*, 2014). Light duration has widely been used for the improvement of the reproductive performance of layers. The search for least cost rations due to the increasing competition between man and animals for available grains has led to the use of antibiotics and growth promoters to boost growth and productivity. Recently the use of antibiotics has been restricted for the growing concern due to the presence of antibiotic resistant bacteria and the transfer of the residues of antibiotics in meat and eggs which may cause side-effects in human (Ghanem *et al.*, 2021). Therefore, it is important to use alternatives to antibiotic feed additives in diets. Phytogetic feed additives (PFAs), as one choice for the replacement of antibiotics have already been used in livestock feeds due to the presence of some active components like flavonoids, phenolic compounds and essential oils (Ghanem *et al.*, 2021).

Thyme (*Thymus vulgaris*) is an aromatic plant of the Lamiaceae family and has received major attention as both a pharmaceutical and therapeutic agent across the globe. Phenols, thymol, carvacrol, parasimol, linalool and cineol are the main components of thyme which have shown to possess potent anti-oxidant, anti-bacterial, anti-viral, anti-inflammatory and growth promoting properties could be used instead of commercial antibiotics (Chan *et al.*, 2018).

Monk's pepper (*Vitex*) is a genus of flowering plants in the family *Lamiaceae*. Common name includes "chaste tree", traditionally referring to *Vitex agnus-castus* (*Vac*) but often applied to other species as well. The *Vitex agnus-castus* fruit is about the size of a pepper corn. It contains among others, essential oils, diterpenes, flavonoids and iridoid glycosides which have demonstrated to have anti-oxidant, anti-mutagenic, anti-microbial, anti-fungal, opioidergic, anti-epileptic, and anti-inflammatory properties (Feldman *et al.*, 2000; Souto *et al.*, 2020). This study was designed to investigate the effects of photoperiod and dietary innovative materials on the haematological and serum biochemical indices of guinea fowl hens.

## MATERIALS AND METHODS

### Collection and Preparation of Dietary Innovative Materials

Monk's pepper dried fruit were procured from Ikare market in Ondo state with the help of a taxonomist from the Department of Botany, Faculty of sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The fruit were washed and air dried at room temperature. Thyme leaves were sourced from Ogige market in Nsukka, Enugu State, Nigeria. The fruit and leaves were sorted to take out foreign materials and thereafter ground into powder using a locally fabricated commercial grinding machine

### Experimental Animals and Management

The study was carried out at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Nsukka, Enugu State, South Eastern Nigeria. Ninety-six guinea fowl hens (28-32 weeks old) were used for this study which lasted for 9 months. The birds were weighed, wing tagged and randomly assigned to eight treatment groups in a 2 (two photoperiods) × 4 (DIM) factorial arrangement in a Completely Randomized Design (CRD). The birds were exposed to two photoperiods of 12 hours of normal light (12 L) and 20 hours of extended light (20 L) period using ECKO 100W solar LED lamp with an average light intensity of 5.78 lux which was automatically programmed to provide light for 8 hours immediately after sunset. The four diets used contained 6 g of monks pepper (M) and thyme (T)/kg diet in different ratios as follows No DIM, 50M50T, 75M25T, and 25M75T. Each treatment was replicated three times having four birds per replicate. The birds were fed with a commercial layers mash (Hybrid Feed Limited) having a crude protein level of 16% and metabolizable energy of 2700kcal/kg in line with the dietary requirements of laying hens. Fresh experimental diets and clean water were made available *ad libitum* for the birds throughout the experimental period. All the management practices and ethics associated with animal use for experimentation in the University of Nigeria were strictly adhered to.

### METHODOLOGY OF ASSAY

At the end of the study, three birds were randomly selected from each treatment for blood analysis. About 2.5ml of blood was collected from the wing vein of the birds and was emptied directly into bottles containing ethylene diamine tetra-acetate (EDTA) for the determination of haematological parameters. Parameters analysed include; Haemoglobin concentration which was determined by the cyanmethaemoglobin method (Gillet *et al.*, 2009), Red blood cell count (RBC), Packed cell volume (PCV), White blood cell (WBC) and differential leukocytic counts were done according to the routine haematological procedures for avian (Ladokun *et al.*, 2008). Mean corpuscular values were calculated using the following:

$$\text{Mean Corpuscular Volume (MCV) (fl)} = \frac{\text{PCV}}{\text{RBC}} \times 10$$

$$\text{Mean Corpuscular Haemoglobin (MCH) (pg)} = \frac{\text{Hb}}{\text{RBC}} \times 10$$

$$\text{Mean Corpuscular Haemoglobin Concentration (MCHC) (\%)} = \frac{\text{Hb}}{\text{PCV}} \times 100$$

The remaining 2.5ml of sampled blood were put into a well labelled sterile blood sample bottles without anti-coagulant to determine serum biochemical parameters - Total Protein, Albumin, Globulin, and Uric acid.

### Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the Jeffrey's Amazing Statistical Program (JASP) version 17.2.1 (2023). Significantly different means were separated using Tukey's test and accepted at 5% or 1% level of probability.

## RESULTS

**Table 1: Proximate Analysis of Monk's Pepper (*Vitex agnus-castus*) and Thyme (*Thymus vulgaris*)**

Samples	Moisture (%)	Protein (%)	Ash (%)	Fiber (%)	Fats and oil (%)	Nitrogen free extract (%)
Thyme	7.00	12.92	3.00	10.00	0.50	66.58
Monk's pepper	9.50	9.41	13.50	2.00	0.50	65.09

### Proximate Analysis of Test Ingredient

The result of proximate composition of monk's pepper and thyme used in the experiment is presented in Table 1. The result indicated that the moisture, crude protein, ash, crude fiber, fats and nitrogen free extract of thyme were 7.0%, 12.92%, 3.0%, 10.00%, 0.50% and 66.58%, respectively. This results differ from the values reported by El-Refai *et al.*, (2020). Likewise, the result also indicated that the moisture, crude protein, ash, crude fiber, fats and nitrogen free extract of monk's pepper were 9.50%, 9.41%, 13.50%, 2.0%, 0.50% and 65.09%, respectively. These values are not comparable to the values reported by Zhlev *et al.*, (2022). The slight differences in the nutrient composition may be attributed to varietal and geographical differences. Ekpo *et al.*, (2020) reported that geographical and varietal differences are the fundamental causes of differences in nutrient composition of plants.

### The Effects of Photoperiod and Dietary Innovative Materials (thyme and monk's pepper) on the Haematological Indices of Guinea Fowl Hens

The effects of photoperiod and dietary innovative materials (DIM) (monk's pepper and thyme) on the haematological indices of guinea fowl hens is shown in Table 2.

**Table 2: Effect of Photoperiod and Dietary Innovative Materials (Thyme and Monk's Pepper) on the Haematological Indices of Guinea Fowl Hens**

Parameters/ Treatments	Hb. Conc (g/dl)	PCV (%)	RBC count (10 <sup>6</sup> / μl)	TWB count (10 <sup>3</sup> / μl)	Lymph ocytes (%)	Heter ophils (%)	Eosin ophils (%)	Mono cytes (%)	MCV (fL)	MCH (pg)	MCH C (g/dL)
Photo period											
12 L	12.71± 0.52	36.81± 0.92	4.22± 0.14	59.06± 3.80 <sup>b</sup>	59.13±2 .51	32.88± 2.11	6.75±0 .80	0.73± 0.15 <sup>b</sup>	87.53 ±1.68	30.06 ±0.40	34.43 ±0.79
20 L	12.10± 0.39	35.39± 1.23	3.95± 0.17	72.19± 3.76 <sup>a</sup>	57.25±2 .51	34.00± 2.34	6.38±0 .84	1.88± 0.29 <sup>a</sup>	90.15 ±3.04	30.77 ±0.64	34.24 ±0.58
P- value	0.09 <sup>NS</sup>	0.17 <sup>NS</sup>	0.09 <sup>NS</sup> s	0.007* *	0.61 <sup>NS</sup>	0.71 <sup>NS</sup>	0.80 <sup>NS</sup>	0.012*	0.47 <sup>NS</sup> s	0.37 <sup>NS</sup> s	0.81 <sup>NS</sup> s
Diet											
No DIM	10.67± 0.49 <sup>c</sup>	32.98± 0.77 <sup>b</sup>	3.51± 0.22 <sup>b</sup>	54.25± 5.61 <sup>b</sup>	53.50±2 .40	37.75± 2.18	6.75±0 .48	1.17± 0.38	94.70 ±4.00	30.56 ±1.91	34.29 ±1.04
50M50 T	12.22± 0.21 <sup>ab</sup>	35.10± 0.84 <sup>ab</sup>	4.07± 0.15 <sup>a</sup>	71.00± 3.85 <sup>a</sup>	59.50±3 .48	33.75± 3.27	5.25±0 .85	1.49± 0.62	86.31 ±1.24	30.08 ±0.71	34.84 ±0.35
75M25 T	13.16± 0.25 <sup>a</sup>	36.53± 0.53 <sup>ab</sup>	4.36± 0.06 <sup>a</sup>	61.63± 4.85 <sup>a</sup>	64.00±3 .65	27.50± 2.63	6.50±1 .44	1.13± 0.30	83.77 ±1.52	30.07 ±0.42	35.91 ±0.43
25M75 T	13.61± 0.28 <sup>a</sup>	39.80± 1.37 <sup>a</sup>	4.40± 0.06 <sup>a</sup>	75.63± 5.42 <sup>a</sup>	55.75±2 .78	34.75± 2.32	7.75±1 .49	1.42± 0.54	90.57 ±3.96	30.95 ±0.72	34.29 ±1.04
P- value	0.001**	0.006**	0.006 **	0.012*	0.25 <sup>NS</sup>	0.17 <sup>NS</sup>	0.67 <sup>NS</sup>	0.89 NS	0.20 NS	0.80 NS	0.06 NS
Photoperiod×Diet											
12L×N o DIM	10.74± 1.09	33.70± 1.20	3.69± 0.33	57.50± 5.50	57.50±0 .50	34.00± 0.00	7.00±1 .00	0.84± 0.21	91.88 ±4.85	29.11 ±0.37	31.79 ±2.09
12L ×50M5 0T	12.54± 0.24	36.50± 0.50	4.31± 0.11	66.50± 2.50	59.50±6 .50	34.00± 6.00	5.50±0 .5.	0.67± 0.03	84.81 ±0.91	29.14 ±0.15	34.37 ±0.20
12L ×75M2 5T	13.51± 0.21	37.30± 0.30	4.45± 0.04	44.75± 1.70	64.00±8 .00	28.00± 5.00	6.50±3 .50	0.68± 0.25	83.93 ±1.34	30.39 ±0.24	36.22 ±0.86
12L ×25M7 5T	14.04± 0.14	39.75± 1.75	4.44± 0.04	67.50± 5.50	55.50±5 .50	35.50± 5.50	8.00±1 .00	0.73± 0.73	89.50 ±3.14	31.62 ±0.04	35.37 ±1.20
20L×N o DIM	10.60± 0.49	32.25± 1.05	3.34± 0.37	65.75± 8.75	49.50±1 .50	41.50± 0.50	6.50±0 .50	1.51± 0.77	97.53 ±7.53	32.02 ±2.04	32.86 ±0.45
20L ×50M5 0T	11.90± 0.06	33.70± 0.30	3.84± 0.12	75.50± 6.50	59.50±5 .50	33.50± 5.50	5.00±2 .00	2.33± 0.95	87.82 ±1.96	31.02 ±1.13	35.31 ±0.49
20L ×75M2 5T	12.72± 0.08	35.75± 0.65	4.28± 0.10	63.75± 2.25	64.00±4 .00	27.00± 4.00	6.50±0 .50	1.58± 0.26	83.61 ±3.47	29.74 ±0.88	35.60 ±0.44
20L ×25M7 5T	13.18± 0.29	39.85± 2.85	4.36± 0.12	83.75± 3.75	56.00±4 .00	34.00± 1.00	7.50±3 .50	2.11± 0.51	91.65 ±9.06	30.29 ±1.49	33.21 ±1.66
P- value	0.85 <sup>NS</sup>	0.77 <sup>NS</sup>	0.75 <sup>NS</sup>	0.67 <sup>NS</sup>	0.80 <sup>NS</sup>	0.67 <sup>NS</sup>	0.10 <sup>NS</sup>	0.81 <sup>NS</sup>	0.94 <sup>NS</sup>	0.21 <sup>NS</sup>	0.47 <sup>NS</sup>

<sup>a,b,c</sup>: Means on the same column with different superscripts are significantly different at ( $p < 0.05$  or  $P < 0.01$ ). Abbreviations: DIM: Dietary Innovative Materials, M: Monk's pepper, T: Thyme, L: Light, Hb: Haemoglobin, PCV: Packed cell volume, WBC: White blood cell, RBC: Red blood cell, MCV: Mean Corpuscular Volume MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, NS: Not significant table 2 shows the effects of photoperiod on haematological indices of guinea fowl hens. Results showed that there were ( $p < 0.05$ ) significant differences in white blood cell (WBC) count and monocytes values of guinea fowl hens while no significant differences ( $p > 0.05$ ) were observed in the other parameters measured. The WBC contents were higher in the 20 hours light groups ( $72.19 \pm 3.76 \times 10^3/\mu\text{l}$ ) compared to those in the 12 hours light group ( $59.06 \pm 3.80 \times 10^3/\mu\text{l}$ ). Similarly, same trend ( $P < 0.05$ ) was observed in the monocytes contents of the 20 hours light group ( $1.88 \pm 0.29\%$ ) compared to the 12 hours light group ( $0.73 \pm 0.15\%$ ).

The effects of DIM on haematological indices of guinea fowl hens showed significant ( $p < 0.05$ ) differences in haemoglobin concentration (Hbc), packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC) count. Guinea fowl hens in 50M50T, 75M25T and 25M75T groups had similar ( $P > 0.05$ ) Hbc ( $12.22 \pm 0.21$  g/dl,  $13.16 \pm 0.25$  g/dl and  $13.61 \pm 0.28$  g/dl, respectively) which were higher than those of the control group ( $10.67 \pm 0.49$  g/dl). A significant ( $P < 0.01$ ) increase in PCV was observed in 25M75T which had the highest PCV percentage of  $39.80 \pm 1.37$  but was however similar ( $P > 0.05$ ) with those of hens fed 75M25T and 50M50T ( $36.53 \pm 0.53\%$ ,  $35.10 \pm 0.84\%$ ). The control group had the lowest PCV percentage of  $32.98 \pm 0.77$  which was similar ( $P > 0.05$ ) to those of birds in 75M25T and 50M50T. The results also showed that birds fed diet containing DIM recorded a highly significant ( $p < 0.01$ ) difference in RBC count compared with the control group. Birds in 25M75T ( $4.40 \pm 0.06 \times 10^6/\mu\text{l}$ ), 75M25T ( $4.36 \pm 0.06 \times 10^6/\mu\text{l}$ ) and 50M50T ( $4.07 \pm 0.15 \times 10^6/\mu\text{l}$ ) had similar ( $P > 0.05$ ) RBC concentration while those in the No DIM ( $3.51 \pm 0.22 \times 10^6/\mu\text{l}$ ) had the lowest RBC concentration. Similarly, there were progressive increases in WBC concentration across treatment. The WBC concentration of birds in 25M75T ( $75.63 \pm 5.42 \times 10^3/\mu\text{l}$ ) were similar ( $P > 0.05$ ) to those in 75M25T ( $61.63 \pm 4.85 \times 10^3/\mu\text{l}$ ) and 50M50T ( $71.00 \pm 3.85 \times 10^3/\mu\text{l}$ ) while hens on the No DIM ( $54.25 \pm 5.61 \times 10^3/\mu\text{l}$ ) group had the lowest WBC concentration. However, no significant difference ( $P > 0.05$ ) was observed in the lymphocytes, Heterophils, eosinophils, MCV, MCH and MCHC contents of guinea fowl hens among all treatment groups.

**Table 3: Effect of Photoperiod and Dietary Innovative Materials (Thyme and Monk's Pepper) on the Serum Biochemical Indices of Guinea Fowl Hens**

Parameters/ Treatments	Total Protein (g/l)	Albumin (g/l)	Globulin (g/l)	Uric Acid ( $\mu$ /mol/l)
Photoperiod				
12 L	40.01 $\pm$ 2.33 <sup>b</sup>	30.19 $\pm$ 0.89	9.97 $\pm$ 2.14 <sup>b</sup>	236.34 $\pm$ 15.45 <sup>b</sup>
20 L	44.45 $\pm$ 1.57 <sup>a</sup>	30.09 $\pm$ 0.93	14.35 $\pm$ 2.18 <sup>a</sup>	309.05 $\pm$ 29.40 <sup>a</sup>
P-value	0.024*	0.926 <sup>NS</sup>	0.001**	0.005**
Diet				
No DIM	35.98 $\pm$ 2.38 <sup>c</sup>	30.70 $\pm$ 1.56	5.34 $\pm$ 1.26 <sup>c</sup>	341.32 $\pm$ 36.12 <sup>a</sup>
50M50T	43.91 $\pm$ 2.17 <sup>ab</sup>	30.32 $\pm$ 1.56	13.83 $\pm$ 1.92 <sup>b</sup>	296.69 $\pm$ 25.77 <sup>b</sup>
75M25T	40.51 $\pm$ 1.44 <sup>b</sup>	31.31 $\pm$ 0.36	9.20 $\pm$ 1.37 <sup>c</sup>	258.01 $\pm$ 25.65 <sup>b</sup>
25M75T	48.51 $\pm$ 1.65 <sup>a</sup>	28.25 $\pm$ 0.96	20.27 $\pm$ 1.29 <sup>a</sup>	194.78 $\pm$ 20.62 <sup>c</sup>
P-value	0.003**	0.267 <sup>NS</sup>	0.001**	0.004**
PhotoperiodxDiet				
12LxNo DIM	31.90 $\pm$ 0.17	28.55 $\pm$ 0.67	3.45 $\pm$ 0.60	287.05 $\pm$ 7.24
12L x50M50T	42.91 $\pm$ 4.12	32.35 $\pm$ 2.41	11.06 $\pm$ 2.30	252.07 $\pm$ 1.21
12L x75M25T	38.10 $\pm$ 0.25	31.27 $\pm$ 0.02	6.84 $\pm$ 0.27	225.36 $\pm$ 5.86
12L x25M75T	47.14 $\pm$ 2.40	28.61 $\pm$ 2.19	18.54 $\pm$ 1.97	180.91 $\pm$ 24.13
20L xNo DIM	40.07 $\pm$ 0.79	32.85 $\pm$ 2.23	7.22 $\pm$ 1.43	395.59 $\pm$ 43.42
20L x50M50T	44.92 $\pm$ 3.03	28.28 $\pm$ 0.75	16.60 $\pm$ 2.30	341.32 $\pm$ 1.21
20L x75M25T	42.92 $\pm$ 0.91	31.36 $\pm$ 0.88	11.56 $\pm$ 0.03	290.66 $\pm$ 42.21
20L x25M75T	49.88 $\pm$ 2.63	27.87 $\pm$ 0.65	21.99 $\pm$ 1.97	208.65 $\pm$ 39.80
P-value	0.55 <sup>NS</sup>	0.12 <sup>NS</sup>	0.84 <sup>NS</sup>	0.52 <sup>NS</sup>

<sup>a,b,c</sup>: Means on the same column with different superscripts are significantly different at ( $p < 0.05$  or 0.01).

Abbreviations: DIM: Dietary Innovative Materials, M: Monk's pepper, T: Thyme, L: Light, NS: Not significant

The results of the effects of photoperiod on serum biochemical indices of guinea fowl hens showed that there were significant differences ( $p < 0.05$ ) in total protein content of guinea hens with the extended light group (44.45 $\pm$ 1.57g/l) having higher serum protein content than those in the normal light (40.01 $\pm$ 2.33g/l) group. Significant differences ( $p < 0.05$ ) were also observed in Globulin content and uric acid profile of the hens. Hens in the extended light group had higher globulin (14.35 $\pm$ 2.18lg/L) and uric acid (309.05 $\pm$ 29.40 g/L) profile compared to those in the normal light group which had 9.97 $\pm$ 2.14g/L and 236.34 $\pm$ 15.45g/L, respectively for globulin and uric acid. However, no significant differences ( $p > 0.05$ ) in the effect of photoperiod was observed in albumin content of guinea fowl hens among all treatments.

The results of the effects of dietary innovative materials on serum biochemical indices of guinea fowl hens showed that hens fed diet containing DIM recorded a highly significant ( $p < 0.01$ ) difference in serum total protein compared with the control group. Birds in 25M75T (48.51 $\pm$ 1.65g/l) and 50M50T (43.91 $\pm$ 2.17g/l) had similar ( $p > 0.05$ ) total protein contents. Same trend ( $p > 0.05$ ) in the total protein contents were observed for birds on

50M50T (43.91±2.17 g/l) and 75M25T (40.51±1.44 g/l). Guinea fowl in the control group (35.98±2.38) recorded the lowest total protein content. A highly significant ( $p<0.001$ ) difference was observed in the globulin content of guinea fowl hens among all treatment groups. Birds on 25M75T (20.27±1.29g/l) had the highest globulin content which was followed by those in 50M50T (13.83±1.92g/l). Birds in the No DIM (5.34±1.26g/l) group although similar ( $p>0.05$ ) with 75M25T (9.20±1.37g/l) hens, recorded the lowest globulin content. Uric acid was significantly ( $p<0.05$ ) influenced by the DIM treatments. The highest uric acid contents were observed in birds on the control (341.32±36.12  $\mu\text{mol/l}$ ) group while the 25M75T (194.78±20.62  $\mu\text{mol/l}$ ) recorded the lowest uric acid content.

## DISCUSSION

The haematological parameters in our study fall within the range reported by Akpamu *et al.*, (2011) and Ali *et al.*, (2019) for the Guinea fowl. In our study, the WBC counts ranged from 55.26-75.95×10<sup>3</sup>μl and is in agreement with the reports of Straková *et al.*, (2010). Haematological values are indicator of physiological status of animals. These values are important to know the stress level in different physiological stages of animals like, lactation, pregnancy and parturition (Padheriya *et al.*, 2020). Haematological indices play important roles in the productivity and adaptability of birds in their production environments. Coupled with other diagnostic procedures, these indices enable the evaluation of health and nutritional status of animals (Ali *et al.*, 2019). Haematological indices of importance to ornithological studies include Haemoglobin, Red Blood Cells, Packed Cell Volume, White Blood Cells, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration (Reece and Swenson, 2004; Etim *et al.*, 2014). White blood cells are important components of the blood, their function is to fight against foreign substances, and they are also substantial part of immune system. High concentration of WBC count is an indication of immune response fighting against foreign bodies (Akpamu *et al.*, 2011). Thus, the significant increase observed in the 20 hours light group could be as a result of the birds' increased metabolic activity during prolonged photoperiod. With prolong photoperiod in birds, the opportunity for rest and sleep decreases which results in an increase in physical and physiological stress. This result is consistent with the results of Brennan *et al.*, (2002) and Kim *et al.*, (2022). Monocytes and macrophages are critical components of the avian defense system, working in tandem with lysozymes (Adams, 2023). In this study, it was observed that the number of monocytes considerably increased as a result of exposure to longer photoperiods, which implies or suggests that longer photoperiods may be stressful for the birds and may make them more susceptible to disease.

The significant increase in Hbc, PCV, RBC and WBC count observed in the DIM treated groups compared to the control treatment groups is consistent with studies of Al-Kassie (2009) and Boujbiha *et al.*, (2023) who found that supplementing broiler bird's diets with oil derived from thyme dramatically enhanced Hbc, PCV, RBC, and WBC counts when compared to the basal control diet. The Hbc, PCV, RBC and WBC counts in this study are within the range reported by (Akpamu *et al.*, 2011) for guinea fowls. Haemoglobin

(Hb) and packed cell volume (PCV) are among the most useful indicators in the diagnosis of anaemia in humans and animals. Padheriya *et al.*, (2020) reported that higher haemoglobin concentration means there was a more supply of oxygen from the lungs to the body tissues. Haemoglobin also plays an important role in maintaining the shape of the red blood cells. PCV is involved in the transport of oxygen and absorbed nutrients (Soetan *et al.*, 2013). The destruction of Hb or PCV or their decreased production, may lead to anaemia (Adedapo *et al.*, 2007). The significant increase in Hbc and PCV also could be explained by the presence of high iron content in monk's pepper and thyme which is capable of stimulating haemoglobin synthesis (Hammoudi Halat *et al.*, 2022). Also the presence of variety of other important flavonoids in monks pepper with antioxidant activity are responsible for improvement of the complete blood count. The increase in WBC values in the DIM groups is a good indicator of improvement in the immunity of the birds. The improved immunity could be attributed to the immunostimulatory effects of thyme bioactive phytochemicals such as polyphenols present in thyme. These bioactive phytochemicals with interesting properties, like anti-inflammatory, antibacterial and antioxidant, may also play immunostimulatory roles in the body (Ghafarifarsani *et al.*, 2022). However, other blood parameters did not exhibit any significant changes with the dietary inclusion of DIM. This is an indication that DIM supplementation had favourable influences on the birds without any detrimental impact.

In this study, total protein and globulin values were significantly higher in the extended photoperiod group compared to the normal photoperiod group but were within the normal range of 20-49.13g/L and 16.72-22.66g/L, respectively reported by other researchers (Obese *et al.*, 2018; Mayengbam *et al.*, 2022) for guinea fowls. These observations are in agreement with the reports of Yameen *et al.* (2020) who noted that serum total protein, albumin and globulin contents in birds were significantly high in the extended (24h) light group, followed by the 12 h light group and the 24 h dark group. Uric acid levels maintained the same trend, which suggests that 24 h light exposure may lead to more protein catabolism, higher blood protein levels, which may not be good for chick health at below or above normal levels in the blood. The results of this study corroborates the reports of Ibrahim *et al.* (2012). Ibrahim and co-researchers reported that a high level of uric acid (hyperuricemia) is commonly observed in female birds due to ovulatory activities. This was observed in birds under 20h light group (extended photoperiod) probably because they laid more eggs compared to the 12h light group of hens.

Dietary innovative materials significantly influenced all serum biochemical parameters except the albumin. The increase in the protein and globulin contents observed in the 25M75T, 50M50T and 75M25T hen groups could be attributed to the nutritional properties of monk's pepper and thyme. These phytochemicals are rich in protein and loaded with minerals that are essential for general wellbeing of an animal (Hammoudi Halat *et al.*, 2022). Birds that were fed DIM also had a decrease in blood uric acid which is usually due to increased physiological stress. Serum urea level is an index which reflects the status of protein metabolism, renal function and nutrition of the body. Uric acid is mainly generated by protein and nucleic acid degradation and it is the main form of ammonia

excretion in poultry. It directly reflects the level of protein metabolism in animals (Zhu *et al.*, 2014). Ramadan *et al.* (2021) reported that uric acid is a potent scavenger of free radicals in poultry, and increasing levels of Adrenocorticotrophic hormone (ACTH) have been associated with increased concentrations of uric acid. Elevated plasma corticosterone concentrations due to physiological stress have been implicated to cause an increase in serum uric acid level and this in turn increases the uric acid content of blood (Ramadan *et al.*, 2021). The decreased levels of uric acid in birds fed phyto-genic supplements depicts improved protein synthesis, decreased protein degradation speed and an increase in the efficiency of nitrogen use by guinea hens.

## CONCLUSION

The research investigated the effect of photoperiod and dietary innovative materials (DIM) (Monk's pepper and Thyme) on the haematological indices of guinea fowl hens. It was concluded from the above experimental data that photoperiod and dietary innovative materials had marked influence on the haematological indices, serum biochemical indices and general health of guinea fowl hens. The inclusion of monk's pepper and thyme in the diet can easily replace the use of antibiotics, growth promoters, as well as certain conventional sources of vitamins and minerals commonly used in poultry production especially in rural areas where these premixes are not easily accessible and affordable due to their prohibitive cost. It is therefore recommended that guinea hens be reared using 12 hours of light and DIM at ratio 25M75T/kg diet.

### Data Availability

The data that support the findings of this study are listed in the article and are available from the corresponding authors upon reasonable request.

**Source of Support:** Nil

### Declaration of Interest

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. The authors certified that there are no conflicts of interest associated with this publication, and there has been no significant financial support for publishing this work that could have influenced its outcome. As corresponding Author, I conform that the manuscript has been read and approved for submission by all the named authors.

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