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Original Research Article

EFFECTS OF XYLOPIA AETHIOPICA AND TETRAPLEURA TETRAPTERA FRUIT MEALS ON AGE AT FIRST EGG AND HAEMATOLOGY IN LAYER CHICKENS AFTER AN OUTBREAK OF GUMBORO

*[1]Igwe, I. R., [1]Ogbu, C. C., [1]Egwu, L. U., [2]Okonkwo, C. J., [3]Chima, M. U., [3]Ukwueze, J.I., [4]Otuh, P.I., [1]Onyenegecha, C. & [1]Ufomadu, V.

[1]Department of Veterinary Biochemistry and Animal Production, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; [2]Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; [3]Department of Veterinary Physiology and Pharmacology, Michael Okpara University Agriculture Umudike, Umuahia, Abia State Nigeria; Department of veterinary physiology and pharmacology. Michael Okpara university of agriculture, Umudike, Abia State, Nigeria; [4]Department of public health, faculty of allied health sciences, David Umahi federal university of health sciences, Uburu, Nigeria / Institute of infectious diseases, biosafety and biosecurity research, David Umahi federal university of health sciences, Uburu, Nigeria.

Correspondence: omeiri1000@gmail.com; +2348038108426

ABSTRACT

Age at first egg (AFE) and haematological effects of *Xylopia aethiopica* and *Tetrapleura tetraptera* fruit meals following an outbreak of Gumboro were investigated in layer chickens. A total of 450-day-oldpullet chicks were procured for the study, after which 138 birds died following an outbreak of Gumboro, leaving behind a total of 300 birds which were used for the study. The birds were allotted into 10 treatments groups of 3 replicates each containing 10 chicks. Treatment 1 was the control, while 2, 3 and 4 were fed diets supplemented with 0.5, 1.5 and 2.5g of *X. aethiopica*. Treatments 5, 6 and 7 were fed diets supplemented with 0.5, 1.5 and 2.5g of *T. tetraptera*, while 8, 9 and 10 were fed diets supplemented with a combination of 0.5, 1.5 and 2.5g of *X. aethiopica* and *T. tetraptera*. At the end of 10, 50 and73 weeks of age, 3 birds were selected for haematological studies. The results showed no significant difference ($p > 0.05$) for AFE, though it took a long time for the birds to come to lay. This was attributed to immunosuppression caused by gumboro. There were significant differences in haematology especially the ratio of heterophil to lymphoyte. At 10 and 50 WOA T1 recorded the highest values (0.69 and 0.71) while T9 recorded the lowest values (0.54 and 0.49) respectively. It was concluded that *X. aethiopica* and *T. tetraptera* can reduce stress in hens because of their effect on H:L. Gumboro being immunosuppressive can prolong AFE.

Keywords: Haematology, layer chickens, phytochemical, *T. tetraptera, X. aethiopica*

INTRODUCTION

There has been a great deal of interest in the role of complementary and alternative medicines for the treatment of acute and chronic diseases (Chengbo *et al.*, 2015). Of the various classes of phytochemicals, interests have been focused on the anti-inflammatory and antioxidant properties of botanicals. Plants, vegetables and spices used in folk and traditional medicine have gained wide acceptance as sources of prophylactic and chemotherapeutic drug discovery and development (Hannah *et al.*, 2016).

When it comes to feed additives, the livestock industry is inundated with numerous options, such as the use of vitamins, minerals, and antibiotics; these feed additives promote the performance of animals, improve the quality of animal products, and enhance profitability. Optimal combination of various alternatives (probiotics, prebiotics, synbiotics, organic acids, enzymes, phytogenics) together

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with good management and husbandry practices, will be required to maximize performance (Kim & Lillehoj, 2019). Phytogenics, also called phytobiotics or botanicals are compounds of plant origin incorporated into animal feeds to enhance livestock productivity through the improvement of digestion, nutrient absorption and elimination of pathogens resident in the gastrointestinal tract (Huyghebaert *et al.*, 2011; Puvaca *et al.*, 2013). Many of these botanicals have been shown to possess haematopoietic ability partly due to their mineral compositions (Ferri-Lagneau *et al.*, 2012).

Xylopia aethiopica or Ethiopian pepper is an angiosperm belonging to the order Magnoliales and family Annonaceae, and is among the species that thrive in the evergreen rain forests of tropical and subtropical Africa (Isikwenu *et al.*, 2014); while *T. tetraptera* belongs to the *Mimosaceae* family. Members of this family are mainly tropical and subtropical trees or shrubs with bipinnate leaves and flowers that are hermaphroditic (Obeagu *et al.,* 2018). *Xylopia aethiopica* and *T. tetraptera are two phytogenics that have been reported to have* medicinal and nutritional properties (Eze, 2012; Adesina *et al.,* 2016). The fruit of *Xylopia aethiopica* is used as a spice and the aqueous decoction is used to prevent and/or arrest postpartum bleeding, probably due to its coagulant properties (Abaidoo *et al*., 2011; Holy *et al.,* 2016; Oso *et al.*, 2019); while the fruit of *T. tetraptera* is used in the traditional treatment and management of some diseases such as arthritis, hypertension, diabetes mellitus, and asthma (Soladoye *et al.,* 2014; Adesina *et al.,* 2016). Most of the early works on *X. aethiopica* and *T. tetraptera* had centered on their biologic activities, such as, antimicrobial, membrane stabilization, and protective action on the liver and kidneys (Somova *et al.*, 2001; Ezekwesili *et al*., 2010; Onyebuagu, 2012; Adams *et al.*, 2022). Though some works have been done on the haematology of birds, especially broiler chickens (Nwafor *et al.*, 2009), comparably sparse work have been done in layer chickens.

Haematology is the study of blood and blood – forming tissues and it is currently considered an integral part of clinical laboratory diagnostic support in avian medicine (Adams *et al.,* 2022). Blood examinations in general are used as good indicators of the physiological status of animals (Khan & Zafar, 2005). The performance characteristics of animals are reflective of their blood picture (Isaac, *et al*., 2013), because it plays a vital role in physiological, nutritional, and pathological status of organism (Muhammed, *et al.*, 2000).

Egg production is a compound trait determined by age at first lay, clutch size, and pause length (Isa *et al.,* 2020). In hens, the onset of egg laying is a sign of sexual maturity (Jambui *et al.,* 2017), as such, age at first egg is an important reproductive trait in hens (Xu *et al.,* 2011; Tan *et al.,* 2021). The factors that regulate age at first egg are diet, photoperiod, and hen genetics (Lewis *et al.,* 2008). Pullets that laid first between 149 – 153 days had a good number of hierarchical follicles, better hormonal regulation, higher levels of gonadotrophin releasing hormone (GnRH), gonadotrophin releasing hormone receptors (GnRHR), estrogen receptor 1 (ESR1), KIT proto-oncogene ligand (KITLG), and Cytochrome P450 Family 11 Subfamily A Member 1 (CYP11A1) expression levels than late-maturing individuals which indicate high ovulation ability and good egg production capability (Tan *et al*., 2021).

One of the major problems of the poultry industry is immunosuppression, which may be due to infection with pathogens and/or environmental factors (Schat *et al.,* 2014). Gumboro disease is considered to be the Acquired Immune Deficiency Syndrome (AIDS) of chickens because it adversely affects the chicken's immune system (Coster *et al.,* 2022). Following recovery from IBDV infection in neonatal chicks, two types of follicles emerge in the bursa of fabricius: small follicles lacking a distinct cortex and medulla, and large follicles having rapidly proliferating B cells and a normal structure derived from small numbers of surviving bursal stem cells (Withers *et al.,* 2005). The B cells in the large follicles retain the capacity to undergo gene conversion. In contrast, the B cells in the small follicles are derived from more mature B cells that had already undergone gene conversion (Withers *et al.,* 2006). Biro *et al.* (2011) reported that B cell maturation may be negatively affected by IBDV-induced changes in the extracellular matrix of the antigen-trapping regions of the spleen, which may contribute to permanent immunosuppression.

MATERIALS AND METHODS

EXPERIMENTAL SITE

This work was carried out at the Veterinary teaching and research farm of College of Veterinary Medicine, Michael Okpara university of Agriculture, Umudike, Abia State of Nigeria.

EXPERIMENTAL PLANTS

Prior to arrival of experimental birds, ripe fruits of *X. aethiopica* and *T. tetraptera* were purchased from Orie-Ugba local market in Umuahia North Local government area of Abia State, Nigeria, and were identified by Dr. Nwajiobi Benson of Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The fruits were dried to constant weight under shed and thereafter ground and stored in air tight containers at 4^{o}C until used.

EXPERIMENTAL BIRDS AND THEIR MANAGEMENT

A total of four hundred and fifty (450) day old *Isa Brown* pullet chicks procured from 'Agrited' were used for the

study. They were vaccinated against New Castle disease on arrival by the intra ocular route. On day 10, they were given the first dose of infectious bursal disease vaccine (IBDV) through drinking water. On day 18 (8 days post IBDV), high morbidity and a few mortalities were observed in the flock. Clinical signs and post mortem examination revealed infectious bursal disease outbreak. Supportive therapy was instituted and by day 24 (6 days of clinical disease), the birds stopped dying. By then, a total of 138 birds (30.67 %) had died leaving a total of 300 birds which were then used for the study.

EXPERIMENTAL DESIGN

The 300 birds were randomly allocated to 10 treatment groups of 30 birds per group; each group was shared into three replicates containing 10 birds each. The study was a 2 x 3 factorial in completely randomized design. The birds were brooded until 42 days of age when they were shared into experimental groups. The study was carried out in three phases, namely chick (6 to 10weeks), grower (11 weeks to point of lay) and layer (point of lay to end of study). The groups were as follows:

Treatment 1 or Control- Basal diet (No dietary supplementation);
Treatment 2- 0.5 g *X. aethiopica* per kg basal diet;
Treatment 3- 1.5 g *X. aethiopica* per kg basal diet;
Treatment 4- 2.5 g *X. aethiopica* per kg basal diet;
Treatment 5- 0.5 g *T. tetraptera* per kg basal diet;
Treatment 6- 1.5 g *T. tetraptera* per kg basal diet;
Treatment 7- 2.5 g *T. tetraptera* per kg basal diet;
Treatment 8- 0.5 g *X. aethiopica* +0.5 g *T. tetraptera* per kg basal diet;
Treatment 9- 1.5 g *X. aethiopica* +1.5 g *T. tetraptera* per kg basal diet; and
Treatment 10- 2.5 g *X. aethiopica* +2.5 g *T. tetraptera* per kg basal diet.

Chick mash was fed to the birds until ten weeks of age. Grower mash (110g/bird/day) was fed from eleventh weeks of age to point of lay, while layer mash (120g/bird/day) was fed from point of lay to end of the study. Water was given *ad libitum.* From point of lay the birds were housed in individual laying cages. The chick mash contained 20.05 % crude protein and 2653 kcal ME/kg; grower mash contained 16.25 % CP and 2650 kcal ME/kg, while layer mash contained 18.26% CP and 2656 kcal ME/kg (Table 1). *Xylopia aethiopica* and *T. tetraptera* fruit meal were added to the diets after formulation in the correct proportion.

TABLE I: PERCENTAGE AND CALCULATED NUTRIENT COMPOSITION OF BASAL DIET

	Basal diet		
Ingredient	**Chick mash**	**Grower mash**	**Layer mash**
Yellow Maize	51.00	57.00	55.50
Soya bean meal	26.40	15.30	20.00
Wheat offal	18.10	24.00	11.50
Fish meal	-	-	2.70
Bone meal	2.50	1.70	2.50
Oyster shell	-	-	7.00
Limestone	1.30	1.30	-
Common salt	0.35	0.35	0.35
Lysine	0.10	0.10	0.10
Methionine	0.10	0.15	0.20
Premix	0.15	0.10	0.15
Total	**100**	**100**	**100**
Calculated nutrient composition			
Crude protein (%)	20.05	16.25	18.26
Metabolizable energy (Kcal/kg)	2653	2650	2656
Calcium	1.30	1.05	3.39
Phosphorus	0.58	0.70	0.88

At the end of 10, 50, and 73 week of age, three birds were randomly selected from each treatment for the determination of haematological parameters. The birds were monitored daily for age at first egg (AFE) and weight of first egg (WFE).

DATA ANALYSIS

Data collected were subjected to analysis of variance (ANOVA) and separation of means was made using Duncan's Multiple Range Test. Significance was accepted at $p \leq 0.05$.

RESULTS

Table II presents the age at first egg (AFE) and weight of first egg (WFE) of layer chickens fed varying dietary levels of *X. aethiopica and T. tetraptera*.

TABLE II: AGE AT FIRST EGG AND WEIGHT OF FIRST EGG OF LAYER CHICKENS FED VARYING DIETARY LEVELS OF *X. AETHIOPICA* AND *T. TETRAPTERA* FRUIT MEAL

	Parameter	
Treatment	**Age at first egg (days)**	**Weight of first egg (g)**
1	194.3	47.3
2	226.0	50.7
3	220.7	55.3
4	201.3	50.0
5	224.3	52.3
6	214.0	54.7
7	209.3	50.0
8	226.7	54.0
9	225.0	57.7
10	214.0	52.7
SEM	10.32	3.90
P-value	0.36	0.77

AFE: Age at first egg; WFE: Weight of first egg; T1: No dietary supplementation; T2: 0.5 g *X. aethiopica* per kg basal diet; T3: 1.5 g *X. aethiopica*; T4: 2.5 g *X. aethiopica*; T5: 0.5 g *T. tetraptera*; T6: 1.5 g *T. tetraptera*; T7: 2.5 g *T. tetraptera*/ T8: 0.5 g *X. aethiopica* +0.5 g *T. tetraptera;* T9: 1.5 g *X. aethiopica* +1.5 g *T. tetraptera;* T10: 2.5 g *X. aethiopica* + *2*.5 g *T. tetraptera*.

The Table shows that AFE and WFE did not differ significantly between the treatment groups ($p > 0.05$). Birds in the control group (T1) however, laid earlier (194.3±10.32 days) than birds in the treated groups. Among the treated groups, birds in T2 and T8 laid later (226.0 and 226.7±10.32 days, respectively) than those in other treatment groups. For WFE, the highest but non-significantly different value was recorded in T9 while the lowest value was recorded in T1.

Table 111 shows the results of haemoglobin concentration (Hb), packed cell volume (PCV), total red blood cell count (TRBC), total white blood cell count (TWBC), lymphocyte, heterophil and heterophil/lymphocyte ratio at 10, 50, and 73 weeks of age.

At 10 Weeks of Age (WOA), percent lymphocyte and heterophil/lymphocyte ratio (H:L) differed significantly between treatments. Hens in T9 had the highest percent lymphocyte value (61.0±0.93). This was higher ($p < 0.00$) compared to T1 and T8 (56.7±0.93, respectively) but not significantly different ($p > 0.05$) from values observed for other treatments. For H:L, T1 recorded the highest value of 0.69 which was not significantly different from values observed in T3, T7 and T8, but different ($p < 0.03$) from values recorded in T2, T4, T5, T6, T9, and T10. The least values were recorded in T6 and T9 (0.54, respectively) and these were statistically similar ($p > 0.05$) to values recorded in T2, T3, T4, T5, T8, and T10. At 50 WOA, hens in T10 recorded the highest Hb concentration (14.00±0.34g/dl) followed by T4 and T8 which had statistically similar values as T10, while the least values for Hb concentration were recorded in T5 and T7 (11.67±0.34 and 12.00±0.34g/dl, respectively). Packed cell volume (PCV) did not differ significantly ($p > 0.05$) between treatments at this age. Total red blood cell count (TRBC) was highest in T10 with a similar value recorded in T4 but significantly lower values observed in the other treatments. Hens belonging to T9 and T5 recorded the lowest TRBC (3.01±0.12 and $3.13\pm0.12 \times 10^6 mm^3$, respectively). Statistically similar values were recorded for TWBC in T8, T10 and T2. These were non-significantly ($p > 0.05$) different from the value for T9 but higher ($p < 0.00$) compared to values for other treatments. The least value for this variable was observed in T5 ($19.78\pm0.41 \times 10^3 mm^3$). The values for percent lymphocyte at this age were statistically similar between T9, T8, and T10. These values were similar to those of T6 and T5 which in turn did not differ significantly but higher compared to other treatments ($p < 0.00$). Hens in T1 and T2 recorded the highest percent heterophil of 38.32±0.96 and 38.00±0.96%, respectively and these were similar to the values for T4 and T7 but significantly ($p < 0.00$) higher compared to other treatments. The least values for this variable were recorded in T9, T10, T5 and T8. For H:L, T1 and T2 had the highest values (0.71 and 0.68, respectively). They were followed by T7 and T4 while T9 recorded the least value.

At 73 WOA, all blood indices differed significantly between experimental groups. Hens in T5 recorded the highest Hb concentration (14.73±0.41g/dl), followed by T3, T6, T7 and T10, while T8 and T9 recorded the least values. Hens in T5 also had the highest values for PCV and TRBC (34.0±1.27% and $3.79\pm0.13 \times 10^6 mm^3$, respectively). These values were similar to values observed in T2 for these indices but significantly higher ($p < 0.00$) than those of hens in the other treatments. The least value for PCV and TRBC were recorded in T8 and T9. For TWBC, T8 had the highest value ($23.20\pm0.59 \times 10^3 mm^3$), followed by T9, T4, and T6, while T1 and T7 recorded the least values (20.10±0.59 and $20.08\pm0.59 \times 10^3 mm^3$, respectively). Percent lymphocyte was highest in T3 and T5 (61.7±0.89%, respectively) and these did not differ significantly ($p>0.05$) from values recorded in T6, T7, T9, T10, and T4, but were significantly ($p < 0.00$) higher compared to values recorded in T1, T2, and T8. Percent heterophil was highest in T8.

TABLE IIIA: HAEMATOLOGICAL INDICES OF LAYER CHICKENS FED VARYING DIETARY LEVELS OF *X. AETHIOPICA AND T. TETRAPTERA* FRUIT MEAL AT 10 WEEKS OF AGE

Parameter	Hb (g/dl)	PCV (%)	RBC ($X10^6mm^3$)	WBC ($X10^3mm^3$)	Lymphocyte (%)	Heterophil (%)	H:L
1	11.07	28.70	3.24	19.53	54.70^{c}	37.70	0.69^{a}
2	11.53	30.00	3.40	22.50	59.00^{ab}	34.00	0.58^{bc}
3	11.33	30.00	3.43	20.77	58.30^{ab}	36.00	0.62^{abc}
4	11.27	29.30	3.36	20.28	59.70^{ab}	34.00	0.57^{bc}
5	10.80	26.00	2.94	19.98	59.30^{ab}	33.33	0.56^{bc}
6	10.87	26.00	3.15	19.75	59.70^{ab}	32.33	0.54^{c}
7	10.87	26.70	3.07	21.52	56.70^{bc}	37.00	0.65^{ab}
8	10.40	25.30	2.89	20.00	56.70^{bc}	35.33	0.62^{abc}
9	10.47	26.30	2.98	21.00	61.00^{a}	32.70	0.54^{c}
10	10.73	26.70	3.06	20.07	59.30^{ab}	34.00	0.57^{bc}
SEM	0.28	1.43	0.17	0.68	0.93	1.29	0.01
P-value	0.13	0.18	0.30	0.12	0.00	0.10	0.03

a, b, c: means on the same column bearing different superscripts differ significantly (P<0.05); SEM: Standard error of mean; Hb: Haemoglobin; PCV: Packed cell volume; RBC: red blood cell; WBC: white blood cell; H:L: Heterophil - lymphocyte ratio; Treatment 1 or Control (No dietary supplementation); T 2: 0.5 g *X. aethiopica* per kg basal diet; T 3: 1.0 g *X. aethiopica*; T 4: 1.5 g *X. aethiopica*; T 5: 0.5 g *T. tetraptera*; T 6: 1.0 g *T. tetraptera*; T 7: 1.5 g *T. tetraptera*/ T 8: 0.5 g *X. aethiopica* +0.5 g *T. tetraptera;* T 9: 1.0 g *X. aethiopica*+1.0 g *T. tetraptera;* T 10: 1.5 g *X. aethiopica*+1.5 g *T. tetraptera*

TABLE IIIB: HAEMATOLOGICAL INDICES OF LAYER CHICKENS FED VARYING DIETARY LEVELS OF *X. AETHIOPICA AND T. TETRAPTERA* FRUIT MEAL AT 50 WEEKS OF AGE

Parameter	Hb (g/dl)	PCV (%)	RBC ($X10^6mm^3$)	WBC ($X10^3mm^3$)	Lymphocyte (%)	Heterophil (%)	H:L
1	12.60^{bc}	28.0	3.38^{bc}	21.97^{cd}	54.3^{c}	38.3^{a}	0.71^{a}
2	12.53^{bc}	26.7	3.25^{bc}	23.93^{a}	56.0^{c}	38.0^{a}	0.68^{a}
3	12.53^{bc}	27.3	3.30^{bc}	20.77^{de}	57.3^{bc}	33.0^{bc}	0.58^{bcd}
4	13.27^{ab}	30.3	3.58^{ab}	22.58^{bc}	54.7^{c}	35.3^{ab}	0.65^{ab}
5	11.67^{c}	27.3	3.13^{c}	19.78^{e}	59.7^{ab}	31.7^{c}	0.53^{de}
6	12.73^{bc}	27.3	3.32^{bc}	22.23^{bc}	60.0^{ab}	33.3^{bc}	0.56^{cde}
7	12.00^{c}	28.3	3.30^{bc}	22.52^{bc}	56.7^{c}	35.7^{ab}	0.63^{abc}
8	13.13^{ab}	28.3	3.37^{bc}	24.63^{a}	61.3^{a}	32.0^{c}	0.52^{de}
9	11.67^{c}	26.0	3.01^{c}	23.52^{ab}	62.3^{a}	30.3^{c}	0.49^{e}
10	14.00^{a}	31.3	3.78^{a}	23.95^{a}	61.3^{a}	31.3^{c}	0.51^{de}
SEM	0.34	1.12	0.12	0.41	0.96	0.96	0.01
P-value	0.00	0.08	0.01	0.00	0.00	0.00	0.00

a, b, c: means on the same column bearing different superscripts differ significantly (P<0.05); SEM: Standard error of mean; Hb: Haemoglobin; PCV: Packed cell volume; RBC: red blood cell; WBC: white blood cell; H:L: Heterophil -lymphocyte ratio; Treatment 1 or Control (No dietary supplementation); T 2: 0.5 g *X. aethiopica* per kg basal diet; T 3: 1.0 g *X. aethiopica*; T 4: 1.5 g *X. aethiopica*; T 5: 0.5 g *T. tetraptera*; T 6: 1.0 g *T. tetraptera*; T 7: 1.5 g *T. tetraptera*/ T 8: 0.5 g *X. aethiopica*+0.5 g *T. tetraptera;* T 9: 1.0 g *X. aethiopica*+1.0 g *T. tetraptera;* T 10: 1.5 g *X. aethiopica*+1.5 g *T. tetraptera*

TABLE IIIC: HAEMATOLOGICAL INDICES OF LAYER CHICKENS FED VARYING DIETARY LEVELS OF *X. AETHIOPICA AND T. TETRAPTERA* FRUIT MEAL AT 73 WEEKS OF AGE

Parameter	Hb (g/dl)	PCV (%)	RBC ($X10^6mm^3$)	WBC ($X10^3mm^3$)	Lymphocyte (%)	Heterophil (%)	H:L
1	13.07^{bc}	28.3^{bc}	3.18^{bc}	20.10^{d}	54.3^{d}	36.0^{ab}	0.66^{a}
2	14.03^{ab}	30.7^{ab}	3.50^{ab}	20.83^{cd}	58.7^{bc}	33.3^{abcd}	0.57^{bc}
3	13.27^{b}	28.0^{bc}	3.39^{bc}	21.10^{bcd}	61.7^{a}	33.3^{abcd}	0.54^{bc}
4	12.87^{bc}	28.7^{bc}	3.21^{bc}	22.35^{abc}	59.0^{abc}	33.7^{abcd}	0.57^{bc}
5	14.73^{a}	34.0^{a}	3.79^{a}	20.85^{cd}	61.7^{a}	35.0^{abc}	0.57^{bc}
6	13.20^{b}	28.0^{bc}	3.28^{bc}	21.67^{abc}	60.3^{ab}	31.0^{cd}	0.51^{bc}
7	13.27^{b}	27.3^{bc}	3.21^{bc}	20.08^{d}	60.0^{ab}	34.7^{abc}	0.58^{b}
8	11.80^{c}	24.7^{c}	2.77^{d}	23.20^{a}	56.7^{cd}	37.7^{a}	0.67^{a}
9	11.87^{c}	25.0^{c}	3.05^{cd}	22.82^{ab}	60.7^{ab}	29.7^{d}	0.49^{c}
10	13.20^{b}	28.3^{bc}	3.17^{bc}	21.00^{bcd}	59.7^{ab}	32.7^{bcd}	0.55^{bc}
SEM	0.11	1.27	0.13	0.59	0.89	1.31	0.01
P-value	0.00	0.00	0.00	0.01	0.00	0.02	0.00

a, b, c: means on the same column bearing different superscripts differ significantly (P<0.05); SEM: Standard error of mean; Hb: Haemoglobin; PCV: Packed cell volume; RBC: red blood cell; WBC: white blood cell; H:L: Heterophil - lymphocyte ratio; Treatment 1 or Control (No dietary supplementation); T 2: 0.5 g *X. aethiopica* per kg basal diet; T 3: 1.0 g *X. aethiopica*; T 4: 1.5 g *X. aethiopica*; T 5: 0.5 g *T. tetraptera*; T 6: 1.0 g *T. tetraptera*; T 7: 1.5 g *T. tetraptera*/ T 8: 0.5 g *X. aethiopica*+0.5 g *T. tetraptera;* T 9: 1.0 g *X. aethiopica*+1.0 g *T. tetraptera;* T 10: 1.5 g *X. aethiopica*+1.5 g *T. tetraptera*

T4 which in turn did not differ significantly ($p > 0.05$). For H: L, hens in T1 and T8 recorded the highest values. They were followed by T7 (0.58) which did not differ significantly from values observed in T2, T3, T4, T5, T6, and T10, but was significantly higher than the value observed in T9 (0.49) which was the least value for this index.

DISCUSSION

Age at first egg (AFE) is an important reproductive trait in hens because it is an indicator of sexual maturity (Jambui *et al.*, 2017). In addition, AFE affects the overall performance of a hen over the laying period. Early AFE indicate early maturity, younger age at peak of lay and longer laying period compared to late AFE. The later AFE observed in the treated groups indicate dietary supplementation of *X. aethiopica and T. tetraptera* may have a retarding effect on development of reproductive system of the birds. Infectious bursal disease which the birds suffered may have contributed to the longer AFE in all groups compared to values reported in most studies (Lewis & Morris, 2007; Getiso *et al.*, 2016; Tan *et al.*, 2021). Infectious bursal disease negatively affects poultry health and production due to the resultant immuno-suppression (Orakpoghenor *et al.*, 2020). Tan *et al.* (2021) reported that the total number of eggs produced by late-maturing hens was significantly lower than those of early maturing hens. The observed lower weight of first egg in the

control group (T1) was due to the younger age at onset of lay as demonstrated by previous studies (Summers & Leeson, 1983; Ciacciariello & Gous, 2005; Getiso *et al*., 2016).

Haematological components are important in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals (Isaac *et al*., 2013; Salami *et al.*, 2021). Despite the significant differences in percent lymphocyte and H:L ratio between treatment groups at 10 WOA and in most of the differential counts and haematological indices at 50 and 73 WOA, the evaluated blood indices had values that are within the normal range (Olayemi, 2016; Sugiharto *et al.*, 2021). This shows that *X. aethiopica* and *T. tetraptera* fruit meal did not adversely affect the haematological values and hence health of the experimental birds. Enhanced micro-mineral absorption such as iron absorption may explain the observed higher values for haematological indices in the treated groups (Adegoke *et al*., 2018). Thus, *X. aethiopica and T. tetraptera* may have individually or jointly enhanced iron absorption and utilization hence leading to increased production of RBCs by the haematopoeitic cells. Obeagu *et al*. (2018) reported improved Hb count, PCV, TRBC, and TWBC in rats whose diets were supplemented with *T. tetraptera.* They concluded that extract of *T. tetraptera* can be used as a haematinic to treat anaemic patients instead of blood transfusion. Olayemi *et al*. (2016) showed that the use of *Morinda, Morinda plus Zysygium, and Morinda plus Xylopia* in broiler chickens led to improved PCV compared to birds on control diet. In the present study, leukocyte counts, and percentage lymphocyte were also improved in birds whose feed were supplemented with *X. aethiopica and T. tetraptera* fruit meal. This will result in enhancement of the immune system. Hens in T1 had the highest H:L ratio at 10, 50 and 73 WOA. Since H:L ratio is an indicator of level of stress in an animal, hens in the control group were under stress more than the supplemented hens. Invariably, hens in the control group will have lower resistance ability to infections than hens whose feed were supplemented with *X. aethiopica* and *T. tetraptera*, or a combination of the two. However, the mechanisms attributing the resistance ability to chickens with a low H:L ratio compared to those with a high H:L ratio remain unclear (Thiam *et al*., 2021).

CONCLUSION

The immunosuppression caused by infectious bursal disease brought about a delay in the onset of lay. The result of the ratio of heterophil to lymphocyte showed that layer chickens that were fed diets supplemented with *X. aethiopica* and *T. tetraptera* (T6 and T9) had a better effect on stress.

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