

HAEMATOLOGICAL INDICIES AND SERUM BIOCHEMISTRY OF BROILER BIRDS FED VARYING INCLUSION LEVEL OF MONK'S PEPPER (*VITEX AGNUS-CASTUS*) AND THYME (*THYMUS VULGARIS*)

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ABSTRACT

This study investigated the haematological and serum biochemical indices of broiler birds fed varying level of monk's pepper and thyme. A total of 90 day old Ross strain commercial broiler chicks were fed for 9 weeks. The birds were randomly allotted to nine treatment experimental diets with two replication of 5 birds each. The diet were formulated with the inclusion of monk's pepper and thyme at 0 inclusion, 1g of thyme/kg of feed, 1g of monk's pepper/kg of feed, 1g of thyme and 1g of monk's pepper, 3g of thyme/kg of feed, 3g of monk's pepper/kg of feed, 1g of thyme and 2g of monk's pepper/kg of feed, 1g of monk's pepper and 2g of thyme/kg of feed and 1.5g of thyme and 1.5g of monk's pepper/kg of feed for T1, T2, T3, T4, T5, T6, T7, T8, and, T9 respectively. The results indicated that dietary thyme and monks pepper significantly ($P<0.05$) affected all haematological parameters except haemoglobin and lymphocytes. PVC, RBC, TWBC in T7 and T8 were significantly ($P<0.05$) higher than those in other treatment. Thyme and monks pepper inclusion resulted in a significant ($P<0.05$) increase in total cholesterol, total protein, Albumin, Globulin, Uric acid, ALT and ALP. However, no significant difference ($P>0.05$) was observed in the AST across groups. From the result, it was concluded that inclusion of 1g thyme and 2g of monk's pepper/kg diet to broiler diet improved the blood parameters and general health of broiler chicken.

Key words: Broiler, Vitex, Thyme, Haematology, Serum biochemistry

INTRODUCTION

The increasing demand for animal meat products, as well as the need to improve livestock efficiency in converting feedstuff into meat, have caused a growth in intensive livestock husbandry systems, which rely heavily on antibiotics in livestock feedstuffs (Pan *et al.*, 2022). However, the major concern for livestock and human health is the increased risk of antibiotic resistance brought on by antibiotic overuse.

Therefore, the livestock and poultry industries are concentrating on reducing the use of antibiotics without compromising animal growth performance (Zhang *et al.*, 2021). Finding an alternative derived from plants with excellent antibacterial activity to replace the role of antibiotics in animal feeds seems to be a feasible approach to solve this challenge (Freitas *et al.*, 2020).

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 (Grigore *et al.*, 2010). Thymol, carvacrol, parasimol, linalool and cineol are the main components of thyme essential oil which both have been shown to possess potent antioxidant and antibacterial properties so this plant could be used instead of commercial antibiotics (Nahed *et al.*, 2014).

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. Species of *Vitex* are native throughout the tropics and subtropics, with a few species in temperate Eurasia. (Cathy, 2019). The *Vitex agnus-castus* fruit, known as monk's pepper, is about the size of a pepper corn. It contains among others, essential oils, diterpenes, flavonoids and iridoid glycosides which have demonstrated to have antioxidant, antimutagenic, antimicrobial, antifungal antinociceptive, opioidergic, antiepileptic, and anti-inflammatory properties (Chan *et al.*, 2018).

The objective of this study was to compare the effect of different levels of thyme and monks pepper on the haematological and serum biochemical indices of broiler birds

MATERIALS AND METHODS

Experimental Animals

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. A total of 90 day old Ross strain commercial broiler chicks were fed for 9 weeks. The birds were randomly allotted to nine treatment experimental diets with two replication of 5 birds each. The diet were

formulated with the inclusion of monk's pepper and thyme at 0 inclusion, 1g of thyme/kg of feed, 1g of monk's pepper/kg of feed, 1g of thyme and 1g of monk's pepper, 3g of thyme/kg of feed, 3g of monk's pepper/kg of feed, 1g of thyme and 2g of monk's pepper/kg of feed, 1g of monk's pepper and 2g of thyme/kg of feed and 1.5g of thyme and 1.5g of monk's pepper/kg of feed for T1, T2, T3, T4, T5, T6, T7, T8, and, T9 respectively. The Experiment was arranged in a Completely Randomized Design (CRD). 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 Assays

At the end of 8 weeks feeding trial, three birds were randomly selected from each treatment for blood analysis. 2ml of blood was collected from the wing veins of the birds. Red blood cell count (RBC), packed cell volume (PCV), haemoglobin, total white blood cell (WBC) and differential leukocytic counts were done according to the routine haematological procedures for avian (Feldman *et al.*, 2000). Serum samples were analyzed for total protein (TP), albumin (Alb), uric acid (UA) serum enzymatic activities of alanine amino transferase (ALT) alkaline phosphatase (ALP) and aspartate amino transferase (AST) using spectrophotometer and commercial test kits of Randox (United Kingdom) and following the manufacturer's instructions. Globulin concentration (Glob) in serum was computed by subtracting albumin concentration from total protein and consequently albumin to globulin ratio (A/G) was calculated.

RESULT AND DISCUSSION

Table 1: Effect of dietary inclusion of *Vitex agnus-castus* and thyme on Hematological indices of broilers

Parameters	PCV (%)	Hb (g/dl)	RBC ($10^6/\mu\text{L}$)	TWBC ($10^3/\mu\text{L}$)	Lymphocytes (%)	Heterophils (%)	Eosinophils (%)	Monocytes (%)
T1	28.60±1.96 ^{bc}	9.49±0.84	3.10±0.12 ^c	44.00±1.73 ^{bc}	54.50±3.18	32.50±4.33 ^{bc}	10.00±0.58 ^{ab}	3.00±0.58 ^c
T2	28.95±0.89 ^{abc}	9.23±0.18	3.22±0.10 ^{bc}	49.00±0.58 ^{bc}	43.50±0.29	32.00±1.16 ^{bc}	11.00±0.58 ^{ab}	13.50±1.44 ^a
T3	30.00±1.15 ^{abc}	9.87±0.96	3.46±0.15 ^{ab}	40.50±0.87 ^{bc}	39.50±0.87	44.00±2.31 ^a	11.00±0.58 ^{ab}	5.50±2.02 ^{bc}
T4	30.75±1.01 ^{ab}	9.99±0.62	3.55±0.13 ^{ab}	60.00±3.46 ^{ab}	47.00±0.00	39.00±1.73 ^{ab}	8.50±0.29 ^b	5.50±1.44 ^{bc}
T5	29.20±0.46 ^{abc}	10.47±0.28	3.30±0.04 ^{abc}	38.00±2.89 ^c	41.00±3.47	36.00±0.00 ^{ab}	13.00±1.15 ^a	10.00±2.31 ^{ab}
T6	28.00±1.15 ^{bc}	9.55±0.71	3.31±0.22 ^{ab}	46.50±4.33 ^{bc}	58.00±2.89	24.00±0.00 ^c	10.50±0.87 ^{ab}	7.50±2.02 ^{ab}
T7	31.00±0.00 ^{ab}	10.62±0.01	3.45±0.02 ^{ab}	75.00±15.59 ^a	48.00±12.12	29.50±6.64 ^{bc}	9.50±1.44 ^b	13.00±4.05 ^a
T8	32.25±0.43 ^a	10.14±0.15	3.70±0.11 ^a	70.00±6.35 ^a	53.00±0.00	34.00±1.15 ^b	8.00±1.73 ^b	5.00±0.58 ^{bc}
T9	27.00±1.15 ^c	8.94±0.62	3.18±0.11 ^{bc}	59.00±0.57 ^{ab}	54.00±1.15	31.00±2.31 ^{bc}	10.00±0.00 ^{ab}	5.00±1.16 ^{bc}
P-Value	0.04*	0.42	0.04*	0.00**	0.06	0.00**	0.04*	0.04*

PCV; packed cell volume, Hb; Haemoglobin, RBC; red blood cell, TWBC; total white blood cell. ^{abc}: means on the same column with different superscripts are significantly different (p<0.05).

The effects of inclusion of thyme and monks pepper on the haematological indices of broiler chicken is presented in table 1 above. The results depicts there was significant ($p<0.05$) differences were observed in means of PCV, RBC, TWBC, heterophils, eosinophil and monocytes across the various treated group. There was no significant ($p<0.05$) differences in means of haemoglobin and lymphocyte across the various treated group. Birds on T₈ (2g/kg of thyme and 1g/kg of monk's pepper), recorded the highest PCV, RBC. The significant increase in PCV values in could be explained by the presence of high content of iron in thyme and a variety of important flavonoids with antioxidant activity in thyme and monks pepper which responsible for improvement of complete blood count picture. The result of the present study was consistent with the results of Kaviarsan *et al.*, (2004), Nahed *et al.*, (2014) NR. check spelling *et al.*, (2015) and Adam *et al.*, (2020) who reported a significant increase in the PCV, RBC, TWBC, and Heterophils when *thymus vulgaris* was supplemented in the diets of broilers. However, no significant effect was observed on Hb values. This observation is in line with the results of Anvar *et al.*, 2012. The high WBC recorded in the treated groups is an indication of high degree of resistance to disease and enhanced adaption to local environmental conditions. This observation is in agreement with the research of Isaac *et al.*, (2013).

Table 2: Effect of dietary inclusion of *Vitex agnus-castus* and thyme on serum biochemical parameters indices of broilers.

Parameters	Total cholesterol (g/l)	Total Proteins (g/l)	Albumin (g/dl)	Globulin (g/dl)	Uric Acid (μ/mol/l)	AST (iu/L)	ALT (iu/L)	ALP(iu/L)
T1	119.81±5.09 ^{cd}	32.98±1.04 ^b	26.53±1.15 ^b	6.44±0.12 ^b	326.86±53.58 ^a	132.97±7.56	10.81±0.31 ^a	95.18±12.30 ^{ab}
T2	131.51±0.69 ^{bc}	34.61±0.64 ^b	26.30±0.75 ^b	8.31±0.11 ^b	279.58±78.83 ^{ab}	128.09±0.70	10.67±0.05 ^{ab}	89.30±9.76 ^{ab}
T3	117.09±0.20 ^{cd}	35.38±1.69 ^b	26.76±0.72 ^b	8.57±0.99 ^{ab}	108.76±5.45 ^c	135.94±8.46	10.33±0.16 ^{bcd}	85.08±7-80 ^{ab}
T4	120.29±1.52 ^{cd}	34.27±0.15 ^b	25.11±0.68 ^b	9.17±0.53 ^{ab}	280.17±40.26 ^{ab}	152.67±1.20	10.11±0.14 ^{cd}	85.36±6.49 ^{ab}
T5	106.09±8.47 ^d	36.33±0.65 ^b	25.72±0.24 ^b	10.47±0.32 ^{ab}	235.84±4.43 ^{abc}	136.29±13.69	10.19±0.08 ^{cd}	115.47±10.26 ^{ab}
T6	115.87±4.34 ^{cd}	33.23±0.02 ^b	26.35±0.10 ^b	6.93±0.12 ^b	235.84±33.10 ^{abc}	117.98±10.57	10.19±0.06 ^{cd}	102.73±2.73 ^{ab}
T7	123.86±0.84 ^{bcd}	42.86±4.32 ^a	28.78±0.20 ^a	14.08±4.53 ^a	161.95±42.99 ^{bc}	119.89±0.40	10.01±0.05 ^d	70.67±7.76 ^b
T8	141.41±3.79 ^{ab}	36.76±1.84 ^b	25.82±0.23 ^b	10.95±2.07 ^{ab}	147.18±20.82 ^{bc}	122.86±10.99	10.43±0.05 ^{abcd}	75.81±2.50 ^b
T9	150.12±14.05 ^a	37.53±1.49 ^b	26.84±0.53 ^b	10.69±0.96 ^{ab}	315.04±57.67 ^a	131.06±5.64	10.48±0.09 ^{abc}	126.53±9.11 ^a
P-Value	0.00**	0.02*	0.02*	0.01*	0.01*	0.71	0.00**	0.03*

^{a,b,c} – Means in the same row with different superscripts are significant at 5%(* p < 0.05

The effects of inclusion of thyme and monks pepper on the serum biochemical indices of broiler chicken is presented in table 2 above. The results showed a significant ($p < 0.05$) increase in mean total cholesterol serum proteins and globulin in the treatment groups compared to the control group. Serum protein consists of albumin and globulin which effectively reflects protein metabolism, feed condition and growth of the animal (NR. Check reference for spelling). The result of this study is in agreement with the findings of not referenced Uric acid is mainly generated by protein and nucleic acid degradation and is the main form of ammonia excretion in chicken (Zhu *et al.*, 2014). Serum uric acid directly reflects the level of protein catabolism in animals. From this study, uric acid level in the serum significantly decreased in the treatment groups thus showing that thyme and monks pepper improves protein synthesis, decrease protein decomposition speed and improve nitrogen use. Serum ALT and AST are very low under normal conditions. When liver damages or an increase in the permeability of liver cells are present, ALT and AST are released into the blood to increase their activity. Therefore, their serum levels are sensitive indicators of liver damage (Zhang, 2011). Alkaline phosphatase is known to catalyze the hydrolysis of some compounds, such as phosphomonoesters and nucleoside phosphates, and its activity is an important indicator of bone metabolism (Xu *et al.*, 2013). In this experiment the ALP activity showed a significant decrease in the treated groups compared with the control group. This result is consistent with the reports of Zhu *et al.* (2014).

Conclusion

From this study, it can be concluded that dietary inclusion of 1g thyme and 2g of monk's pepper/kg diet demonstrated the best influence on the haematological and serum biochemical parameters of broiler birds.

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