



Haematological Indices and Serum Metabolites of Broiler Chickens Fed *Zysygium aromaticum*, *Xylopia aethiopica* and *Morinda lucida* as Phytogetic Plants

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Authors' contributions

This work was carried out in collaboration between all authors. Author WAO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AOO reviewed the experimental design and all drafts of the manuscript. Authors OAA, FOO and AOM assisted in statistical analyses and field work. All authors read and approved the final manuscript.

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ABSTRACT

The objective of the study was to evaluate and compare the effect of phytogetic additives on haematological indices and serum metabolites of broiler chickens fed diet containing *Zysygium aromaticum*, *Xylopia aethiopica* and *Morinda lucida*. One hundred and eighty, 1- day- old hubbard broiler chicks obtained from a reputable hatchery were used. Birds were randomly divided into six dietary treatments in a completely randomized design (CRD). Each dietary group was replicated thrice with ten birds per replicate. Dietary treatment are as follow: 1. control (basal diet), 2. Antibiotics (basal diet + 1 g Amoxycol® wsp containing Amoxycillin 200 mg + Collistin sulphate 1000000 iu /kg diet), 3. *Morinda lucida* (basal diet + 1 g *Morinda lucida* / kg diet) 4. Basal diet + *Morinda lucida* and *Xylopia aethiopica* each at 0.5 g/diet, 5. Basal diet + *Morinda lucida* and

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Syzygium aromaticum each at 0.5 g/diet while 6 contained mixtures basal diet + *Morinda lucida*, *Xylopiya aethiopicum* and *Syzygium aromaticum* each at 0.33 g/kg diet. Birds were fed *ad libitum* with dietary treatment for the period of 56 days. The use of *Morinda*, *Morinda*+ *Zyzygium*, *Morinda* + *Xylopiya* and mixtures of all the phytogetic plants showed improved PCV concentration compared to control. Birds fed *Morinda*, *Morinda*+ *Xylopiya* had highest ($p<0.05$) neutrophil. Birds fed antibiotics and those fed *Morinda* + *Zyzygium* showed increased ($p<0.05$) lymphocyte counts. Highest ($p<0.05$) monocyte concentration was recorded in broiler fed *Morinda*+ *Zyzygium*. The serum biochemistry of the broilers were affected following dietary inclusion of the phytogetic plants. In conclusion, the use of *Morinda*, *Morinda*+ *Zyzygium*, *Morinda* + *Xylopiya* and mixtures of all the phytogetic plants showed improved PCV. The various phytogetic plants has significant ($p<0.05$) effect on serum biochemistry, total protein, globulin, albumin and serum cholesterol of the broiler chickens.

Keywords: Broilers; haematological indices; serum metabolites; phytogetic additives.

1. INTRODUCTION

The increasing cost of antibiotics and other drugs in addition to their residual effects has necessitated the need to research into natural herbal plants that could serve as cheap and good alternative to commercial (synthetic) antibiotics. The use of plants with phytogetic properties as additives in livestock nutrition is becoming popular due to its resultant effect on animals such as improved productivity, reproduction, and quality of animal products [1]. These plants are readily available and affordable to the farmers. Such materials would perform similar functions as the commercial feed additives without the adverse effects associated with indiscriminate use of antibiotics namely the development of antibiotic resistant microbial organisms [2,3] and residual contamination of the food chain with antibiotics [4,5]. Herbs or products containing plant extracts such as essential oils are among the alternative growth promoters that are already being used in practice [6,7]. This has led to the adoption of safety measures and a gradual withdrawal of antibiotic promoters to a more nutrition-based health strategy in future animal production development [8].

Blood examination are used as good indicators of the physiological status of animals [9]. The performance characteristics of animals are reflective of their blood picture [10]. The examination of blood constituents help to assess the physiological, nutritional and pathological status of animals [11,12]. Blood constituents were reported to change in relation to the physiological conditions of the animals [9,13]. Haematological components such as red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular

haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring feed toxicity as well as the health status of farm animals [14].

Zyzygium aromaticum (Cloves) constitutes one of the most popular spices used in human diet especially in Asians and African countries. The plant is an evergreen tree 10-20 m in height, belonging to the family Myrtaceae, indigenous to India, Indonesia, Zanzibar, Mauritius and Ceylon [15,16]. Clove is considered as a potential choice in this study as a result of its reported activities such as analgesic, anti-inflammatory, anesthetic, antimicrobial and antifungal properties [17]. It has also been shown to act as a digestive stimulant hence improving appetite [18]. *Zyzygium aromaticum* have been reported to have many therapeutic uses: they are used to control nausea and vomiting, cough, diarrhoea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, it also relieve pain and stimulate the nerves [19,20]. The phytogetic properties of *Zyzygium aromaticum* has been linked to the anti-nutritive contents such as tannins, saponins, phenolic compounds and flavonoids [21].

Xylopiya aethiopicum is a slim, tall, evergreen aromatic tree growing up to a height of 20 m of order Magnoliales and family Annonaceae. It has been used extensively as African cuisine and traditional medicine in the treatment of bronchitis, dysenteric or as a mouth wash for tooth ache. The dried fruits are used as a spice and herbal medicine. It has been reported that grains of selim has antimicrobial and anthelmintic activities and promotes growth in broiler chickens [22]. Improved growth following supplementation of *Xylopiya aethiopicum* dried fruits in diets of broilers have been reported [23].

Morinda lucida is a medium sized tree its leaves are use as the remedies against different type of fever, the bark infusion is used as an anti-malaria and anti-diabetic [24], anti-salmonella typhii [25]. Extracts of *Morinda lucida* had active antimicrobial activity, and a promising natural antibiotic when compared to the standard antibiotic drugs chloramphenicol and cipro-floxacin [26]. Extracts of *Morinda lucida* can also be used to treat Salmonella infections. Growth inhibition of *E. coli* both *in vitro* and *in vivo* studies using *M. lucida* have also been reported [27]. It has been reported that the plant contains saponins, tannins, antraquinones and alkaloids [28,29]. Phytochemical activity of *Morinda lucida* can help the body to neutralize both gram-positive and gram –negative bacteria [30].

This research was designed to evaluate the effects of *Syzygium aromaticum*, *Morinda lucida* and *Xylopi aethiopic a* on haematological indices and serum biochemistry of broiler chickens.

2. MATERIALS AND METHODS

2.1 Processing of Test Ingredient

Fresh *Morinda lucida* leaves were plucked from the Department of Agricultural Technology, Yaba College of Technology, Epe Campus, Lagos. These were air-dried and milled to yield *Morinda lucida* leaf meal (MLM). Dried *Xylopi aethiopic a* and *Zyzygium aromaticum* were purchased from market in Epe, Lagos. These were milled individually to yield *Xylopi aethiopic a* (XLM) and *Syzygium aromaticum* (SLM).

2.2 Animal, Diets and Experimental Design

One hundred and eighty, 1- day-old unsexed broilers (Hubbard strain) obtained from reputable hatchery were randomly divided into six dietary treatments in a complete randomized design (CRD). Each experimental diet was replicated thrice with ten birds per replicate. Treatment 1 was basal diet without antibiotics, Treatment 2 consist of basal diet supplemented with antibiotics (Amoxycol® wsp containing Amoxycillin 200 mg + Collistin sulphate 1000000 iu) at 1 gm/kg of feed, Treatment 3 consist of basal diet supplemented with *Morinda lucida* at 1 gm/kg of feed, Treatment 4 consist of basal diet supplemented with mixtures of 0.5 gm *Morinda lucida* and 0.5 gm *Xylopi aethiopic a*, Treatment 5 consist of basal diet supplemented with

mixtures of 0.5 gm *Morinda lucida* and 0.5 gm *Syzygium aromaticum* while Treatment 6 consist of basal diet supplemented with mixtures of 0.33 gm *Morinda lucida*, 0.33 gm *Xylopi aethiopic a* and 0.33 gm *Syzygium aromaticum*. The levels of dosage used in this study were based on previous research study [23]. The feeding trial lasted for 56 days. Birds were fed *ad libitum* with experimental diets for the starter and finisher Phases of the study. Feed were formulated according to nutritional requirements for starter (Table 1) and finisher phases (Table 2) [31] while experimental diets were analyzed according to the method of [32].

2.3 Measurement of Haematological Indices

No blood samples were collected at the starter phase of this study. Blood samples meant for haematological indices analysis were collected from *broilers* at 56 days from 6 birds per treatment through wing vein using sterilized syringe. 2.5 ml blood was collected from each bird into vials containing *ethytlene diamine tetra-acetic acid (EDTA)* as anticoagulant and used for the following hematological determinations: packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), total white blood cell count (WBC), and percentage distribution of white blood cells (differential leukocyte count) using standard techniques [33].

2.4 Measurement of Serum Metabolites

Blood samples were collected from 6 birds per treatment at the 56 days through wing vein using sterilized syringe. 2.5 ml blood was collected from each bird into vials without anticoagulants. The serum from the clotted blood samples were used to determine Total serum protein (TSP), Serum albumin (SA), Serum globulin (SG), Serum uric acid (SUA), Serum creatinine (SC), Serum glucose (SG), Serum enzyme activity (ALT and AST). The total serum protein was determined according to the method [34], while serum albumin and globulin were determined using bromocresol purple method [35]. Serum creatinine was determined using the principle of Jaffe reaction [36] while the serum uric acid was determined by the kit (Quinica Clinica Spam) [37]. Serum glucose was determined colorimetrically [38]. Serum cholesterol was determined by enzymatic end point method [39] while serum enzyme activity were determined using the colorimetric method.

Table 1. Percentage composition of the experimental diet (Broiler starter 0-4 weeks)

Ingredients	T1	T2	T3	T4	T5	T6
Maize	55.00	55.00	55.00	55.00	55.00	55.00
Soybean meal	30.00	30.00	30.00	30.00	30.00	30.00
Wheat offal	5.90	5.90	5.90	5.90	5.90	5.90
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Lime stone	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Premix	0.31	0.31	0.31	0.31	0.31	0.31
Amoxycol®	0.00	0.10	0.00	0.00	0.00	0.00
<i>Morinda lucida</i>	0.00	0.00	0.10	0.05	0.05	0.033
<i>Zysygium aromaticum</i>	0.00	0.00	0.00	0.05	0.05	0.033
<i>Xylopi aethiopic a</i>	0.00	0.00	0.00	0.00	0.00	0.033
Total	100	100	100	100	100	100
Determined analysis (%)						
Crude protein	22.43	22.43	22.43	22.42	22.43	22.42
Crude fibre	2.05	2.20	2.54	2.51	2.05	2.20
Ether extract	3.85	3.25	3.45	4.90	3.85	3.25
Calculated analyses (%)						
Calcium	1.78	1.77	1.76	1.76	1.78	1.77
Phosphorus	0.46	0.44	0.43	0.42	0.46	0.44
Lysine	1.49	1.38	1.26	1.15	1.49	1.38
Methionine	0.56	0.54	0.52	0.49	0.56	0.54
ME(Kcal/kg)	2832	2858	2882	2939	2832	2858

*Starter premix: - Vit. A 10,000,000 (iu), Vit D3 2,000,000 (iu), Vit. E 23,000 (mg), Vit K3 (mg), Vit B1 1,800 (mg), Vit. B2 5,500 (mg), Niacin 27,500 mg, Pantothenic acid 7,500 mg, Vit. B6 3,000 mg, Vit.B12 15 mg, Folic acid 750 mg, Biotin H2 60 mg, Chlorine chloride 300,000 mg, Cobalt 200 mg, Copper 3,000 mg, Iodine 1,000 mg, Iron 20,000 mg, Manganese 40,000 (mg), Selenium 200 mg, Zinc 30,000 mg, Anti-oxidant k1,250 mg

2.5 Statistical Analysis

All data generated were subjected to one-way Analysis of variance (ANOVA) in a completely randomized design, data was analysed using SAS statistical package [40] while significant differences among treatment means were separated using Duncan Multiple Range Test [41].

3. RESULTS AND DISCUSSION

3.1 Haematological Indices

Table 3 shows the haematological indices of the experimental birds under various treatments. The use of *Morinda*, *Morinda*+ *Zysygium*, *Morinda* + *Xylopi a* and mixtures of all the phytogetic plants showed improved PCV concentration compared to the birds on control diet. This implied that various phytogetic plants used improved the packed cell volume of the blood hence improved health status (REF). Results of hematological variables shows that the RBC, WBC, Hb, MCH, MCV, MCHC and basophil, eosinophil were not significant. However, neutrophils, lymphocytes

and monocytes counts were affected (p) by the dietary treatments. The birds fed *Morinda*, *Morinda*+ *Xylopi a* had highest ($P<0.05$) neutrophil as against others. Birds fed antibiotics and those fed *Morinda* + *Zysygium* showed increased ($P< 0.05$) lymphocyte counts. The increased lymphocyte ratio obtained could be due to the anti-nutritional content of *Morinda* which aggravated increased ($P<0.05$) production of lymphocyte cells. Highest ($P<0.05$) monocyte concentration was recorded in broiler fed *Morinda*+ *Zysygium*. All the haematological parameters had values within the normal range and mean of healthy broiler chickens [42]. The results of the present study are similar with those observed. Olugbemi et al. [43] reported that red blood cell count decreased due to the supplementation of *Moringa oleifera* leaf meal in cassava based diet in broilers. Similar findings have been observed [44] in chicken fed Neem leaf meal which did not revealed any detrimental effect on red blood cell count in broilers. It can therefore be inferred that the haematological indices were within safety limits for broilers in this experiment. These normal haematological values portray the nutritional status of the broiler chicken

and thus indicating adequate nourishment of the birds [45].

3.2 Serum Metabolites

Table 4 summarizes data obtained on the effect of experimental treatments on serum biochemical parameters. Serum components analyzed includes, total protein, albumin, creatinine, urea,

triacylglycerol, HDL, LDL, total cholesterol, alanine and aspartate. Broilers fed diet supplemented with Morinda + Zysygiu recorded the highest total serum protein and globulin. This implied that mixtures of morinda and szysygiu when supplemented in diets for broilers improved the utilization of dietary protein and indirectly health status. Blood serum proteins are a significant indicator of the health condition

Table 2. Percentage composition of experimental diet (Broiler Finisher 0-8 weeks)

Ingredients	T1	T2	T3	T4	T5	T6
Maize	56.00	56.00	56.00	56.00	56.00	56.00
SBM	24.00	24.00	24.00	24.00	24.00	24.00
Wheat offal	12.00	11.90	11.90	11.90	11.90	11.901
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Lime stone	3.00	3.00	3.00	3.00	3.00	3.00
Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30	0.30	0.30
Amoxycol®	0.00	0.10	0.00	0.00	0.00	0.00
<i>Morinda lucida</i>	0.00	0.00	0.10	0.05	0.00	0.033
<i>Zysygiu aromaticum</i>	0.00	0.00	0.00	0.05	0.05	0.033
<i>Xylophia aethiopica</i>	0.00	0.00	0.00	0.00	0.05	0.033
Total	100	100	100	100	100	100
Determined analyses (%)						
Crude protein	20.33	20.32	20.31	20.30	20.33	20.26
Crude fibre	4.08	4.18	4.24	4.32	4.08	4.18
Ether extract	3.91	4.25	4.62	4.97	3.91	4.25
Calculated analyses (%)						
Calcium	1.77	1.76	1.76	1.75	1.77	1.76
Phosphorus	0.44	0.43	0.42	0.41	0.44	0.43
Lysine	1.31	1.18	1.09	0.97	1.31	1.18
Methionine.	0.54	0.51	0.49	0.47	0.54	0.51
ME (Kcal/kg)	3100	3070	3070	3010	3100	3070

*finisher premix: - Vit. A 10,000,000 (iu), Vit D3 2,000,000 (iu), Vit. E 23,000(mg), Vit K3 (mg), Vit B1 1,800 (mg), Vit. B2 5,500 (mg), Niacin 27,500 mg, Pantothenic acid 7,500 mg, Vit. B6 3,000 mg, Vit.B12 15 mg, Folic acid 750 mg, Biotin H2 60 mg, Chlorine chloride 300,000 mg, Cobalt 200 mg, Copper 3,000 mg, Iodine 1,000 mg, Iron 20,000 mg, Manganese 40,000 (mg), Selenium 200 mg, Zinc 30,000 mg, Anti-oxidant k1,250 mg

Table 3. Hematological parameters of chicken broiler fed diets supplemented with *Zysygiu aromaticum*, *Xylophia aethiopica* and *Morinda lucida*

Parameters	T1	T2	T3	T4	T5	T6	SEM
PCV (%)	28.00 ^b	28.00 ^b	34.00 ^a	33.00 ^a	36.00 ^a	37.00 ^a	0.76
Hb(g/dl)	9.20	9.30	11.40	11.00	12.10	12.30	0.65
RBC($\times 10^{12}$ mil/mm ³)	1.46	1.44	1.76	1.70	1.87	1.92	0.30
MCH (pg)	63.01	64.58	64.77	64.71	64.71	64.06	1.04
MCHC (g/dl)	32.86	33.21	35.53	33.33	33.61	33.24	1.02
MCV(fl)	19.18	19.44	19.32	19.41	19.25	19.26	0.67
WBC(no/mm ³)	2900.00	3000.00	3300.00	2500.00	2700.00	2100.00	580.00
Neutrophils (%)	70.00 ^b	69.00 ^{bc}	76.00 ^a	64.33 ^c	80.00 ^a	71.00 ^b	0.87
Lymphocytes (%)	26.00 ^{bc}	28.00 ^{ab}	21.00 ^{cd}	33.00 ^a	16.00 ^d	24.00 ^{bc}	1.08
Eosinophils (%)	3.00	3.00	2.00	4.00	4.00	4.00	0.76
Monocyte (%)	1.00 ^b	0.00 ^c	1.00 ^b	2.00 ^a	0.00 ^c	1.00 ^b	0.15
Basophils (%)	0.00	0.00	0.00	0,00	0.00	0,00	0.00

^{abcd} Means on the same row having different superscript are significantly ($p=0.05$) different

Table 4. Biochemistry parameters of chicken broiler fed diets supplemented with *Zysygium aromaticum*, *Xylopia aethiopica* and *Morinda lucida*

Parameters	T1	T2	T3	T4	T5	T6	SEM
Total Protein (mg/dl)	37.00 ^b	31.00 ^c	38.00 ^b	39.00 ^b	52.00 ^a	38.00 ^b	0.99
Globulin (mg/dl)	17.00 ^b	14.00 ^b	15.00 ^b	16.00 ^b	28.00 ^a	16.00 ^b	1.02
Albumin (mg/dl)	20.00 ^b	17.00 ^c	23.00 ^a	23.00 ^a	24.00 ^a	22.00 ^{ab}	1.17
Uric acid (mg/dl)	7.80	6.70	8.60	9.30	12.50	8.80	0.82
Triacylglycerol	92.00	90.00	70.00	78.00	82.00	76.00	0.85
HDL (mg/dl)	47.00	42.00	48.00	40.00	38.00	43.33	1.19
LDL(mg/dl)	53.00	64.00	84.00	89.00	68.00	79.00	1.50
ALT (iu/L)	12.00	14.00	12.00	11.00	6.67	6.00	0.87
AST (iu/L)	15.00	16.00	12.00	18.00	14.00	8.00	1.13
Creatinine (mg/dl)	1.20	0.90	1.10	0.80	1.00	0.90	0.21
Cholesterol (mg/dl)	118.00 ^d	124.00 ^c	140.00 ^a	145.00 ^a	122.00 ^c	134.00 ^b	1.39

^{abcd} Means on the same row having different superscript are significantly ($p < .05$) different

and production features of animals because of their numerous roles in the body physiology. The significant difference noticed in other serum metabolites (except serum protein and cholesterol) following supplementation of phytobiotics in this study was also reported by other previous studies with spices and herb products [46,47]. Inclusion of morinda (T3) and supplementation of morinda and zysygium (T4) in the present study elevated the serum cholesterol. Although contrary reports by [48] indicated that dietary carvacrol reduces plasma triglycerides and phospholipids, increased serum cholesterol noticed could be attributed to the dosage used in the study.

4. CONCLUSION AND RECOMMENDATION

The main goal of the research was to find the effect of *Zysygium aromaticum*, *Xylopia aethiopica* and *Morinda lucida* on haematological indices and serum metabolite of broiler chickens. The use of Morinda, Morinda + Zysygium, Morinda + Xylopia and mixtures of all the phytogetic plants showed improved PCV. The various phytogetic plants has significant ($p < 0.05$) effect on serum biochemistry, such as total protein, globulin, albumin and serum cholesterol of the broiler chickens, despite the increase, the phytogetic plants has no detrimental effect on the health status of the broiler chickens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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