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***Original Research Article***

# Haematological indices and serum metabolites of broiler chickens fed *Zysygium aromaticum*, *Xylopia aethiopica* and *Morinda lucida* as phytogenic plants.


## Abstract

**The objective of the study was to evaluate and compare the effect of** effect of photogenic additives on haematological indices and serum metabolite of broiler chickens fed *zysygium aromatium, xylopia aethiopica and morinda lucida.* One hundred and eighty 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. Treatment 1, consisted of basal diets without antibiotics, Treatment 2, basal diet supplemented with antibiotics (Amoxycol® wsp containing Amoxycillin 200mg + Collistin sulphate 1000000 iu) Treatment 3, containing diet supplemented with *Morinda lucida* at 1%, Treatment 4, diet supplemented with combination of *Morinda lucida* and *Xylopia aethiopica* each at 0.5%, Treatment 5, diet supplemented with combination of *Morinda lucida* and *Syzygium aromaticum* each at 0.5% while Treatment 6 diet was supplemented with mixtures of *Morinda lucida*, *Xylopia aethiopica and Syzygium aromaticum* each at 0.33% inclusion level. Birds were fed *ad libitum* with dietary treatment for the starter and finisher phase of the study. The result shown that birds fed treatment 6 recorded highest increased pcv value of 37% at 8th es against birds on control diet with 28% value. The serum parameters shown increased in LDL and blood cholesterol Co cent ration, decreased in HDL and trial glycerol concentration compared to other treatment. Therefore, these phytogenic plant can be added as additives to feed, to enhanced health status and performance of broiler chickens.


## 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 Phytogenic feed additives (often called phytobiotics or botanicals) are plant-derived compounds, which are incorporated into diets of livestock in order to improve the feed properties, productivity, reproduction, and quality of animal products (Windisch *et* al*.,* 2008) as alternatives to growth promoters that are readily available and affordable to the farmers. Such materials would perform similar functions as the

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commercial feed additives without the adverse effects associated with indiscriminate use of antibiotics namely the development of antibiotic resistant microbial organisms (Wegener et al., 1998; Kyriakis *et al*., 1999) and residual contamination of the food chain with antibiotics (Chen *et al*.,2005; Roselli *et al*., 2005). Herbs or products containing plant extracts such as essential oils are among the alternative growth promoters that are already being used in practice (Lee *et al*., 2003; Bampidis *et al.,* 2005). 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 (Castanon, 2007).

The use of Clove (*Syzygium aromaticum* L.) is considered as one of the most versatile herbs due to its analgesic and anti-inflammatory, anesthetic, antimicrobial and antifungal effect (Dalkilic and Guller, 2009), antiseptic, appetite and digestion stimulation (Kamel, 2001). *Morinda lucida* has been reported as the most common herb used in traditional folk herbal medicine by Japanese, Korean and Chinese.

*Xylopia aethiopica* is a slim, tall, evergreen aromatic tree growing up to a height of 20m 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. Makeri *et al*., 2007 reported that grains of selim has antimicrobial and anthelminthic activities and promotes growth in broiler chickens. While *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 (Olajide *et al*., 1999), anti-salmonella typhii (Akinyemi *et al*., 2005). This research was hence designed to evaluate the effects of *Syzygium aromaticum, Morinda lucida* and *Xylopia aethiopica* on haematological indices and serum biochemistry of broiler chickens.

## Materials and Methods

### 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 *Xylopia aethiopica* and *Syzygium aromaticum* were purchased from a reputable farm in Epe, Lagos. These were milled individually to yield *Xylopia aethiopica* (XLM) and *Syzygium aromaticum* (SLM).

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**Animal, diets and experimental design**

One hundred and eighty, day-old unsexed broilers (Hubbard strain) obtained from reputable hatchery were randomly divided into six dietary treatments in a complete randomized design (CRD). Each dietary group was replicated thrice with ten birds per replicate. Treatment 1, consisted of basal diets without antibiotics, Treatment 2, basal diet supplemented with antibiotics (Amoxycol® wsp containing Amoxycillin 200mg + Collistin sulphate 1000000 iu) Treatment 3, containing diet supplemented with *Morinda lucida* at 1%, Treatment 4, diet supplemented with combination of *Morinda lucida* and *Xylopia aethiopica* each at 0.5%, Treatment 5, diet supplemented with combination of *Morinda lucida* and *Syzygium aromaticum* each at 0.5% while Treatment 6 diet was supplemented with mixtures of *Morinda lucida*, *Xylopia aethiopica and Syzygium aromaticum* each at 0.33% inclusion level. Birds were fed *ad libitum* with dietary treatment for the starter and finisher phase of the study. Feed were formulated according to their nutritional requirements for starter (Table 1) and finisher phases (Table 2) while samples were analyzed according to the method of AOAC (1990).

***Measurement of haematological indices***

*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. 2.5ml 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 ( Coles, 1986).

***Measurement of serum metabolites***

*Blood samples were collected from 6 birds per treatment at the 56 days through wing vein using sterilized syringe. 2.5ml 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 of Colowick and Kaplan (1955), while serum albumin and globulin were determined using bromocresol purple method of Varley et al. (1980). Serum*

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*creatinine was determined using the principle of Jaffe reaction as described by Bousnes and Tauslay (1945) while the serum uric acid was determined by the kit (Quinica Clinica Spam) (Wooton, 1964). Serum glucose was determined colorimetrically using the method described by Braham and Trinder (1972). Serum cholesterol was determined by enzymatic end point method as described by Roeschlau et al. (1974) while serum enzyme activity were determined using the colorimetric method.*

***Statistical Analysis***

All data generated were subjected to one-way Analysis of variance (ANOVA) in a completely randomized design using SPSS (version 17.0) Statistical package while significant differences among treatment means were separated using Duncan Multiple Range Test (Duncan, 1955).

## Results and Discussion

### Haematological indices:

Table3 shows the haematological indices of the experimental birds under various treatments. The data revealed that various haematological indices did not differ significantly except the PCV values as it increases as with the treatments. Birds fed diets that contains *Syzygium aromaticum, Morinda lucida* and *Xylopia aethiopica* (T6) had the highest PCV with a value of 37% as against the birds on control diet with value of 28% week 8. Results of hematological variables shown that the RBC, WBC, HB, MCH, MCV, MCHC and basophil, Eosinophil were not significant ($P$=.05).However, neutrophils, lymphocytes and monocytes counts were affected by the dietary treatments. The birds on T5 had the highest neutrophil value 80% as against birds on control diet with 70%. Haematological Parameters such as haemoglobin concentration (Hb), White blood cells (WBC) and white blood cell differentials such as Eosinophil and Basophil were not affected by the dietary treatments. All the haematological parameters had values within the normal range and mean of healthy broiler chickens as reported by Mitruka and Rawnsley, 1997. The results of the present study are similar with those observed by Olugbemi *et al*. (2010) who 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 by Zanu *et al*. (2012) 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

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status of the broiler chicken and thus indicating adequate nourishment of the birds as reported by (Church *et al*., 1984). This also implies that the immune system of the birds were active.

**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. There were no significant influence on total protein, albumin, triglyceride and LDL,cholesterol as observed. Broilers fed with 0.33% of *Syzygium aromaticum, Morinda lucida* and *Xylopia aethiopica* ( T6) resulted in a marked increase in LDL, blood cholesterol concentration and a decrease in HDL,Triacylglycerol concentrations compared to other treatments. The insignificant difference in total blood protein observed in this study was also reported by other studies with spices and herb products (Mehala and Moorthy, 2008; Toghyani *et al*., 2010).The results obtained agree with those reported by Ali (2007) who observed that adding thyme to hen's rations significantly decreased plasma HDL, total cholesterol, triglycerides and total lipids. The reduction in triglycerides and cholesterol noticed was attributed to the lowering effect of *Syzygium aromaticum* and *Xylopia aethiopica* on HMG-Co A reductase as the rate-limiting enzyme of cholesterol synthesis (Case et al.,1995; Lee et al., 2003b).The present study agreed with the findings of Lee et al. (2003b) which indicated that dietary carvacrol reduces plasma triglycerides and phospholipids and suggested that carvacrol may have more impact on lipogenesis than on cholesterol biosynthesis.This implies that the dietary treatments has no detrimental effect on the health status of the broiler chickens.

**Table 1: Percentage composition of the experimental diet (Broiler starter)**

| Ingredients | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|
| Maize | 55.00 | 55.00 | 55.00 | 55.00 | 55.00 | 55.00 |
| SBM | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 |
| Wheat offal | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| 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 |

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|---|---|---|---|---|---|---|
| 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 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| TOTAL | 100 | 100 | 100 | 100 | 100 | 100 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Determined analyses (%)* | | | | | | |
| *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 7,500mg, Vit. B6 3,000mg, Vit.B12 15mg, Folic acid 750mg, Biotin H2 60mg, Chlorine chloride 300,000mg, Cobalt 200mg, Copper 3,000mg, Iodine 1,000mg, Iron 20,000mg, Manganese 40,000(mg), Selenium 200mg, Zinc 30,000mg, Anti-oxidant k1,250mg.*

T1(control),T2 (Amoxicillin+ Collistin sulphate),T3 (1% *Morinda lucida*), T4 (0.5% *Morinda lucida* + 0.5% *Syzygium aromaticum*),T5 ((0.5% *Morinda lucida* + 0.5% *Xylopia aethiopica* ),
T6 (0.33% *Syzygium aromaticum* + 0.33% *Morinda lucida* + 0.33% *Xylopia aethiopica*)

**Table 2: Percentage composition of experimental diet (Broiler Finisher)**

| 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 | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 |
| 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 |
| TOTAL | 100 | 100 | 100 | 100 | 100 | 100 |

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|---|---|---|---|---|---|---|
| *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* |

**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 7,500mg, Vit. B6 3,000mg, Vit.B12 15mg, Folic acid 750mg, Biotin H2 60mg, Chlorine chloride 300,000mg, Cobalt 200mg, Copper 3,000mg, Iodine 1,000mg, Iron 20,000mg, Manganese 40,000(mg), Selenium 200mg, Zinc 30,000mg, Anti-oxidant k1,250mg.*

T1(control),T2 (Amoxicillin+ Collistin sulphate),T3 (1% *Morinda lucida*), T4 (0.5% *Morinda lucida* + 0.5% *Syzygium aromaticum*),T5 ((0.5% *Morinda lucida* + 0.5% *Xylopia aethiopica* ),
T6 (0.33% *Syzygium aromaticum* + 0.33% *Morinda lucida* + 0.33% *Xylopia aethiopica*)

**Table 3: Hematological parameters of chicken broiler fed diets supplemented with *Zysygium aromaticum*, *Xylopia 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*=.05)different.

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

| Parameters | T1 | T2 | T3 | | T5 | T6 | SEM |
|---|---|---|---|---|---|---|---|
| Total Protein(mg/dl) | 37.00 | 31.00 | 38.00 | 39.00 | 52.00 | 38.00 | 0.99 |
| Globulin(mg/dl) | 17.00 | 14.00 | 15.00 | 16.00 | 28.00 | 16.00 | 1.02 |
| Albumin(mg/dl) | 20.00 | 17.00 | 23.00 | 23.00 | 24.00 | 22.00 | 1.17 |
| Globulin(mg/dl) | 48.00 | 72.00 | 56.00 | 60.00 | 65.00 | 78.00 | 1.35 |
| Uric acid(mg/dl) | 7.80 | 6.70 | 8.60 | 9.30 | 12.50 | 8.80 | 0.82 |

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|---|---|---|---|---|---|---|---|
| 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 | 124.00 | 140.00 | 145.00 | 122.00 | 134.00 | 1.39 |

[abcd] Means on the same row having different superscript are significantly ($P < .05$)different

Conclusion and recommendation

The main goal of the research was to find the effect of photogenic additives on haematological indices and serum metabolite of broiler chickens fed *zysygium aromatium, xylopia aethiopica and morinda lucida.* It can be inferred that haematological indices were within safety limits for broiler chickens in this study.The serum metabolites values shown the improved nutritional status of the broiler chickens and thus indicating adequate nourishment. This implies that the dietary treatment has no detrimental effect on the health status of the broiler chickens.

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