

# Influence of phytogetic aqueous leaf extract of *Justicia carnea* and *Occimum gratissimum* on sexing accuracy, haematology and serum biochemistry of broiler chickens

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**ABSTRACT:** Sending chicks late to the rearing house and time spent on sexing at farm gates increased their mortality rate and negatively affected their general performance. This study investigates the effectiveness of feather and vents sexing methods for determining the sex of day-old Arbor Acres broiler chicks and the effect of feeding *Occimum gratissimum* and *Justicia carnea* on their blood parameters. Three hundred day-old arbor acres broiler chicks were sexed and randomly allotted to four dietary groups of seventy-five birds each, Treatment T<sub>1</sub>: water + enrofloxacin (Control), T<sub>2</sub>: 500g of *Occimum gratissimum* in 10 litres of water, T<sub>3</sub>: 500g of *Justicia carnea* leaves in 10 litres of water, T<sub>4</sub>: 500g each of *Justicia carnea* and *Occimum gratissimum* in 10 litres of water. The experiment lasted for six weeks. At the end of the experiment, blood samples were collected through the wing web into sample bottles with anticoagulants for haematology and sample bottles without anticoagulants for serum biochemical analysis, after blood collection, the selected birds were sacrificed and gonad observation was performed on the birds to validate their actual sex. The result reveals that feather and vent sexing is effective for Arbor Acres broiler chicks when handled by skilled personnel. However, feather sexing is recommended for its simplicity and speed in larger operations. Oral Administration of *Occimum gratissimum* and *Justicia carnea* enhance the red blood cells and does not negatively impair the serum metabolites.

**Keywords:** Arbor acre, broiler chicken, feather sexing, herbs, vent sexing, water.

## INTRODUCTION

The performance of broiler chickens can be influenced by a wide range of characteristics, such as breed, age, and sex; environmental factors, such as housing, stocking density, and nutrition; and the interactions between different parameters (England *et al.*, 2022). Even when studies have been published that clearly show a difference between male and female broiler growth performance in terms of body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), these differences are frequently disregarded as a source of variation, especially in mixed-sex broiler experiments (Otsuka *et al.*, 2016).

As a sector that provides wholesome food, such as meat and eggs, to people of all ages, castes, and religions, researchers are keen on conducting sustainable researches that optimizes the growth potentials of broiler

chicken but are confronted with the challenge that genetic changes have rendered sexing of day-old chickens in the predominant broiler breeds used in the commercial industry unfeasible, time consuming and less accurate.

A study by Disa *et al.* (2022) examined the impact of broiler chick hatching time and pull time on subsequent live performance, they concluded that sending chicks late to the rearing house and time spent on sexing at the farm gate increased their mortality and negatively affected their general performance. Time spent on sexing at the hatchery and further sorting at the farm has been identified as one of the major hatchery management operations that delay the early unboxing and settling of day-old chick at the broiler house (Veganzones Rodriguez *et al.*, 2024).

Feather sexing, vent (cloacal) sexing, and DNA sexing

**Table 1.** Methods of feather sexing adopted.

Method	Feather sexing	Male characteristics for prediction	female characteristics for prediction	References
1	Length difference between primary and secondary feathers on wing feathers	Primary and secondary feathers have almost the same length	Primary feather is longer than secondary feather	Dakpogan <i>et al.</i> , (2012)
2	Wing feathers' primary and convert feather length differences	Primary and covert feathers have almost the same length	Primary is longer than covert feather	Galli <i>et al.</i> , 2018

are the current techniques used in DOC sexing (Veganzones Rodriguez *et al.*, 2024). While feather sexing uses primary and secondary wing feather sexing procedures, vent sexing is a traditional approach primarily used to determine DOC sex (Iglesias *et al.*, 2019).

In separate experiments by Japan *et al.* (2022) and Akinlade *et al.* (2024), *Occimum gratissimum* and *Justicia carnea* were respectively reported to enhance the growth and carcass yield of broiler chickens, they attributed their result to the phytochemical constituents of the two herbs which have nutritional and health benefits. Therefore, the present study evaluated the influence of phytogenic aqueous leaf extract of *Justicia carnea* and *Occimum gratissimum* on sexing accuracy, haematology and serum biochemistry of broiler chickens.

MATERIALS AND METHODS

Experimental site

The research was carried out at the Poultry Unit of The Teaching and Research Farm of the Federal Polytechnic Ilaro, Ogun State.

Sex determination

Sex identification of four randomly selected chicks per replicate was conducted at day old using feather (Table 1) and vent sexing methods. Plastic tags with numbers were used to tag each chick according to the predicted sex and details recorded while vent sexing was conducted following the procedures of Otsuka *et al.* (2016) where the cloaca was opened with the hand; a smooth genital fold denotes a female, while a small genital bump suggests a male. At the end of six weeks, four birds per replicate were killed and gonad observation (testicles or ovaries) at the abdominal portion was used to validate the sex of the chicks (Dakpogan *et al.*, 2012).

Sources and preparation of *Justicia carnea* and *Occimum gratissimum* extraction

*Justicia carnea* and *Occimum gratissimum* were harvested from the Gbogidi Ilaro Forest, Ogun State, Nigeria. The plant samples were identified and authenticated as

*Justicia carnea* and *Occimum gratissimum* by a Botanist at the Biological Science Unit of Science Laboratory Technology, Federal Polytechnic, Ilaro. 500 g each of the leaves were removed from the stalk, rinsed in water to remove dirt and soaked in 10 litres of water in a different plastic tank for 72 hours (Akinlade *et al.*, 2024). The extracted solution was provided to the broiler bird as drinking water.

Experimental birds

Four dietary treatments of 75 birds each totalling three hundred arbor acre broiler chicks which were randomly selected were reared for a period of 6 weeks with adequate care which involved the regular feed and water supply, and adequate management practices observed. The treatment includes:

- T<sub>1</sub>: - water + enrofloxacin (Control)
- T<sub>2</sub>: *Occimum gratissimum* water
- T<sub>3</sub>: *Justicia carnea* water
- T<sub>4</sub>: *Justicia carnea* and *Occimum gratissimum* water

Statistical analysis

Data obtained was subjected to Analysis of Variance (ANOVA) and means were compared using Duncan's Multiple Range test with the aid of SPSS (1997) package. Descriptive analysis was also performed on the collected sexing data, and gonad observation was used as a validation to determine the proportion of accurate sexing success following sex confirmation (Kusumawati *et al.*, 2016).

$$\text{Level of accuracy} = \frac{\text{addition of corresponding sexing outcome with gonad observation} \times 100\%}{\text{The number of DOC samples}}$$

RESULTS

Accuracy of feather and vent sexing of six weeks broiler chicken

The outcome of sex prediction and gonad observation of six weeks old broiler chicken is presented in Table 2. The outcome revealed that feather sexing had more accuracy

**Table 2.** Outcome of sex prediction and gonad observation of six weeks old broiler.

Chick ID	Feather Sexing Result	Vent Sexing Result	Validated Sex (at 6 weeks)	Feather Sexing Accuracy	Vent Sexing Accuracy
1	Male	Female	Male	Correct	Incorrect
2	Female	Female	Female	Correct	Correct
3	Male	Female	Male	Correct	Incorrect
4	Female	Female	Female	Correct	Correct
5	Male	Female	Male	Correct	Incorrect
6	Female	Female	Male	Incorrect	Incorrect
7	Female	Female	Male	Incorrect	Incorrect
8	Female	Female	Female	Correct	Correct
9	Male	Female	Male	Correct	Incorrect
10	Male	Female	Male	Correct	Incorrect
11	Female	Female	Female	Correct	Correct
12	Male	Female	Male	Correct	Incorrect
13	Female	Male	Male	Incorrect	Correct
14	Male	Female	Male	Correct	Incorrect
15	Male	Male	Male	Correct	Correct
16	Male	Male	Male	Correct	Correct
17	Female	Male	Female	Correct	Incorrect
18	Male	Male	Male	Correct	Correct
19	Female	Male	Female	Correct	Incorrect
20	Male	Male	Male	Correct	Correct
21	Male	Male	Male	Correct	Correct
22	Male	Male	Male	Correct	Correct
23	Female	Male	Female	Incorrect	Correct
24	Female	Female	Female	Correct	Correct
25	Female	Female	Female	Correct	Correct
26	Male	Female	Male	Correct	Incorrect
27	Female	Male	Male	Incorrect	Correct
28	Male	Female	Male	Correct	Incorrect
29	Male	Male	Male	Correct	Correct
30	Male	Female	Male	Correct	Incorrect
31	Male	Male	Male	Correct	Correct
32	Female	Male	Female	Correct	Incorrect
33	Female	Male	Female	Correct	Incorrect
34	Female	Female	Female	Correct	Correct
35	Male	Male	Male	Correct	Correct
36	Female	Male	Female	Correct	Incorrect
37	Male	Male	Male	Correct	Correct
38	Male	Male	Male	Correct	Correct
39	Male	Male	Male	Correct	Correct
40	Female	Male	Female	Incorrect	Correct
41	Female	Female	Female	Correct	Correct
42	Female	Female	Female	Correct	Correct
43	Male	Female	Male	Correct	Incorrect
44	Female	Female	Female	Correct	Correct
45	Female	Male	Female	Incorrect	Correct
46	Male	Male	Male	Correct	Correct
47	Female	Male	Female	Incorrect	Correct
48	Female	Female	Female	Correct	Correct

**Table 3.** Accuracy of feather and vent sexing of six weeks old broiler chicken.

Parameters	Feather sexing accuracy	Vent sexing accuracy
Total number of accurate occurrences	40	30
Total number of maximum outcomes	48	48
Percentage of Accuracy	83.33	62.5

**Table 4.** Haematological parameter of broiler chicken administered *Justicia carnea* and *Occimum gratissimum* leaf extract.

Treatment	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM	P-value
PCV (%)	25	28.06	26.67	25.67	0.53	0.20
Hb (g/d)	8.37	9.43	9	8.67	0.19	0.22
RBCx10 <sup>12/L</sup>	2.30 <sup>b</sup>	3.20 <sup>a</sup>	3.07 <sup>ab</sup>	2.50 <sup>ab</sup>	0.14	0.03
WBCx10 <sup>9/L</sup>	13.3	14.63	13.43	13.8	0.36	0.63
NEUT (%)	32.33	31.67	32.67	33.33	0.9	0.95
LYM (%)	64.67	66.33	65.33	64.33	1.08	0.95
EOS (%)	0.2	0.33	0.67	1.33	0.29	0.17
BAS (%)	0.33	0	0.67	0	0.13	0.22
MONO (%)	0.67	1.67	0.67	1	0.3	0.67
MCVs (fl)	110.34	87.99	87.06	103.76	4.04	0.07
MCH (pg)	36.94	29.61	29.41	35.05	1.37	0.09
MCHC (g/d)	33.46	33.66	33.76	33.79	0.21	0.96

**Table 5.** Serum biochemical parameters of broiler chicken administered *Justicia carnea* and *Occimum gratissimum* leaf extract.

Parameters	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM	P-value
Creatinine (mg/d)	2.63	1.13	1.93	1.57	0.49	0.69
Cholesterol (mg/d)	351.20	282.33	219.63	299.49	10.50	0.50
AST (u/l)	197.07	201.37	197.17	200.77	10.83	0.83
ALT (u/l)	75.40	57.6	62.43	70.17	4.83	0.17
ALP (u/l)	34.10	35.63	35.47	34.28	2.78	0.78

than vent sexing. Table 3 shows the descriptive outcome of the accuracy of feather and vent sexing of six-week-old broiler chicken. Feather sexing has an 83.33% accuracy as opposed to vent sexing with only 62.5% accuracy.

#### Haematological parameters of broiler chicken administered *Justicia carnea* and *Occimum gratissimum* leaf extract

The results presented in Table 4 show that chickens in T<sub>2</sub> had the highest RBC count of 3.20, which is statistically different from other treatments indicating improved erythropoiesis.

#### Serum biochemical parameters of broiler chicken administered *Justicia carnea* and *Occimum gratissimum* leaf extract

Serum biochemical parameters of broiler chicken administered *Justicia carnea* and *Occimum gratissimum*

leaf extract are presented in Table 5. There was no significant difference in the serum biochemical metabolites measured in this study.

## DISCUSSION

Since broiler chickens are a heavy breed line early sex identification is crucial (Clark *et al.*, 2019) to guarantee fundamental animal management and nutrition. Reliable sexing methods are especially important in breeding operations for breeds because they can prevent issues with copulation or low hatching rates that arise from the negative impacts of high inbreeding in these minority populations (Gutiérrez-Reinoso *et al.*, 2020). In this sense, the early sex determination of broiler chicken may be advantageous for backyard farmers in Nigeria. The findings of this study reveal that feather sexing is a reliable and efficient method for sexing day-old Arbor Acres broiler chicks, with accuracy comparable to the traditional vent sexing method. Feather sexing's advantages include

faster processing times and reduced handling stress, making it a practical choice for large-scale hatchery and farm operations. The findings of Otsuka *et al.* (2016), who showed that feather sexing is a technique that could be utilized efficiently to discriminate day-old chicks in the poultry sector with an overall accuracy of 90.2%, corroborated this conclusion. Sex determination is based on feather growth patterns, commonly used in American poultry farms, in purebred chickens, studies have shown that feather growth control is linked to sex, with slow feathering being dominant over fast feathering (Galli *et al.*, 2018). Typically, female chicken's feathers quickly, while males feather more slowly (Galli *et al.*, 2018). By examining the primary and covert feathers on the wingtips, chicks can be sexed: if primary feathers are longer than coverts, they are identified as female; if primary and covert feathers are the same length or coverts are longer, they are male. This feather-sexing technique is simple and popular for certain large hybrid strains of chickens, though it may not be applicable to all breeds and is generally limited to specific strains of broilers (Iswati *et al.*, 2020). Ahiagbe *et al.* (2018) reported a lower outcome for vent sexing in guinea fowls where the accuracy was significantly lower, with 56% males and 81% females properly recognized out of 115 males and 100 females, respectively.

The haematological parameters analyzed revealed significant improvements in red blood cell (RBC) count, in the treatment groups compared to the control group. The highest values were observed in the group in T<sub>2</sub> and it's similar to groups that received extracts indicating enhanced erythropoiesis and better oxygen-carrying capacity of the blood in them. These improvements are attributed to the antioxidant and immunomodulatory properties of the bioactive compounds in *Ocimum gratissimum* and *Justicia carnea* (Onyeabo *et al.*, 2017; Oloruntola *et al.*, 2022).

The result of the serum biochemical analysis showed that there was no significant difference ( $p>0.05$ ) in broiler chickens fed with the phytogenic blend, but the values of creatinine, cholesterol and ALT decreased in the treatment groups. This corroborated the findings of Adegoke *et al.* (2018) and Malekizadeh *et al.* (2012). They found that adding powdered herbal leaves to the feed of laying hens can lower blood triglycerides, total cholesterol, and LDL cholesterol. They concluded that adding herbal plants to the diet can be used to manipulate the composition of poultry products and improve some good indexes of serum blood components (Malekizadeh *et al.*, 2012). By increasing the activity of liver cholesterol-7- $\alpha$ -hydrolase or inhibiting hydroxyl-methyl-glutarylcoenzyme-A (HMG-CoA) reductase, either by bile-acid conversion or faecal excretion of cholesterol, herbal supplements can lower total serum cholesterol (Malekizadeh *et al.*, 2012). These findings are consistent with previous research highlighting the hepatoprotective and metabolic benefits of plant-based feed additives (Miranda *et al.*, 2024).

However, no significant changes were observed in cholesterol (CHOL) levels across groups. Liver enzymes, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), remained relatively unchanged, suggesting no hepatotoxic effects. Alkaline phosphatase (ALP) levels also showed no significant changes. Notably, the combination treatment (T<sub>4</sub>) had the lowest CHOL level, although this was not statistically significant. Overall, the findings suggest that *Ocimum gratissimum* and *Justicia carnea* may have renoprotective effects, with no adverse effects on liver or kidney function.

## Conclusion

This study concluded that feather sexing is an effective and efficient method for sexing day-old Arbor Acres broiler chicks and oral administration of *Ocimum gratissimum* and *Justicia carnea* aqueous extract promote health and enhance the blood quality of broiler chicken.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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## REFERENCE

- Adegoke, A. V., Abimbola, M. A., Sanwo, K. A., Egbeyale, L. T., Abiona, J. A., Oso, A. O., & Iposu, S. O. (2018). Performance and blood biochemistry profile of broiler chickens fed dietary turmeric (*Curcuma longa*) powder and cayenne pepper (*Capsicum frutescens*) powders as antioxidants. *Veterinary and Animal Science*, 6, 95-102.
- Ahiagbe, K. M. J., Kayang, B. B., Naazie, A., Botchway, P. K., & Agbolosu, A. A. (2018). Comparison of vent sexing and polymerase chain reaction for reliable sex determination in guinea fowls. *Ghana Journal of Agricultural Science*, 52, 17-23.
- Akinlade, O. O., Olorunsogbon, B. F., Sangosina, M. I., & Okusanya, P. O. (2023). Effect of administration of aqueous extract of *Justicia carnea* (plume plant) on growth, carcass and blood profile of broiler chickens. *Nigerian Journal of Animal Production*, 50(5), 80-87.
- Clark, D., Nestor, K., & Velleman, S. (2019). Continual selection

- for increased 16-week body weight on turkey growth and meat quality: 50 generation update. *Journal of Applied Poultry Research*, 28(3), 658-668.
- Dakpogan, H. B., Salifou, S., Aboh, A., & Chrysostome, C. (2012). Effectiveness of a sexing technique on Free-range day-old chick. *Journal of Animal and Plant Sciences*, 16(2), 2336-2342.
- Dişa, R., Özlü, S., & Elilbol, O. (2022). Research Note: Interaction between hatching time and chick pull time affects broiler live performance. *Poultry Science*, 101(6), 101845.
- England, A., Gharib-Naseri, K., Kheravii, S. K., & Wu, S. B. (2023). Influence of sex and rearing method on performance and flock uniformity in broilers—implications for research settings. *Animal Nutrition*, 12, 276-283.
- Galli, R., Preusse, G., Schnabel, C., Bartels, T., Cramer, K., Krautwald-Junghanns, M. E., Koch, E. & Steiner, G., (2018). Sexing of chicken eggs by fluorescence and Raman spectroscopy through the shell membrane. *PLoS One*, 13(2), e0192554.
- Gutiérrez-Reinoso, M. A., Aponte, P. M., Cabezas, J., Rodríguez-Alvarez, L. & García-Herreros, M. (2020). Genomic evaluation of primiparous high-producing dairy cows: Inbreeding effects on genotypic and phenotypic production—reproductive traits. *Animals*, 10, 1704.
- Iglesias, P. C., Navas, G. F. J., Marín, N. C., Arando, A. A., González, A. A., León, J. J. M., & Camacho, V. M. E. (2019). Sexual dimorphism for coping styles complements traditional methods for sex determination in a multivariety endangered hen breed. *Animals*, 9(12), 1165.
- Iswati, Natsir, M. H., Ciptadi, G., & Susilawati, T. (2020). Sexing on a day-old chick of gold Arabic chicken (*Gallus turcicus*) using feather sexing method. *Journal of Animal and Plant Sciences*, 44(3), 7708-7716.
- Japan, E. H., & Ofongo, R. T. S. (2022). Breast meat yield response and organ weight of broiler chickens to *Ocimum gratissimum* (Lyn) administered orally and in feed. *Nigerian Journal of Animal Production*, 49(2), 75-85.
- Malekizadeh, M., Moeini, M. M., & Ghazi, Sh. (2012). The Effects of Different Levels of Ginger (*Zingiber officinale* Rosc) and Turmeric (*Curcuma longa* Linn) Rhizomes Powder on Some Blood Metabolites and Production Performance Characteristics of Laying Hens. *Journal of Agricultural Science and Technology*, 14, 127-134.
- Miranda, C. T. C. D. S., Soares, S. D., de Oliveira, W. Q., Lima, A. D. S., Neri Numa, I. A., & Pastore, G. M. (2024). Unconventional edible plants of the Amazon: bioactive compounds, health benefits, challenges, and future trends. *Foods*, 13(18), 2925.
- Oloruntola, O. D., Ayodele, S. O., Adeyeye, S. A., Fasuhami, O. S., Osowe, C. O., & Ganiyu, T. O. (2022). Proximate composition, phytochemical profile, antioxidant, antidiabetic and anti-inflammatory properties of *Justicia carnea* leaf powder. *Black Sea Journal of Agriculture*, 5(4), 415-423.
- Onyeabo, C., Achi, N. K., Ekeleme-Egedigwe, C. A., Ebere, C. U., & Okoro, C. K. (2017). Haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats. *Acta Scientiarum Polonorum Technologia Alimentaria*, 16(2), 217-230.
- Otsuka, M., Miyashita, O., Shibata, M., Sato, F., & Naito, M. (2016). A novel method for sexing day-old chicks using endoscope system. *Poultry science*, 95(11), 2685-2689.
- Veganzones Rodriguez, M., Phan, T., Fernandes, A. F., Breen, V., Arango, J., Kidd, M. T., & Le, N. (2024). Facial chick sexing: An automated chick sexing system from chick facial image. *arXiv e-prints*, arXiv-2410.