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Genetic Association of Broiler (Gallus Gallus Domesticus) Treated with a Disease Blocking Extract

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ABSTRACT

Broiler birds whose meat is considered healthy are assailed with diseases from hatch. However, vaccination protocols have been established to contain viral diseases. Unfortunately, bacterial diseases still menace poultry. In order to contain bacterial diseases, poultry farmers often abuse antibiotics and may not heed withdrawal periods before such birds are presented to consumers to purchase which sequels a community of poultry consumers unknowingly suffering from drug resistance. Guava leaf extract (GLE) is rich in phytochemicals. Therefore, this research was set up to assess the potential of guava leaf extract to block bacterial and viral diseases of poultry. Methods: A total of fifty day old chicks was procured. Forty were unvaccinated while 10 positive control) were vaccinated. They were acclimatized for one week. The 50 birds were divided into 5 groups of 10 birds per group. Group 1; 10 vaccinated birds. Group 2: unvaccinated untreated (negative control). Groups 3-5 were treated with 400mg/kg of GLE per os; daily, once in 7 days and once in 14 days respectively. Any death during the course of 13 weeks were microbially cultured. At 13th week, blood was collected from the birds for haematology and serology. Blood was also collected from 3 birds picked at random from groups 1, 2 and 4 for gene extraction. Result: There was gene modification of broiler birds treated with GLE which presented itself as gene deletions, insertions and single nucleotide polymorphism. The result of the microbial culture of dead birds incriminated klebsiella; an opportunistic bacteria as the main cause of death in groups 1 and 2. Differential white blood cell count showed heterophils significantly ($P \leq 0.05$) higher in guava leaf treated groups, but there was no established disease and no death. Serology showed GLE treatment led to significant ($P \leq 0.05$) increase in High Density Lipoprotein, significant ($P \leq 0.05$) decrease in Cholesterol, Aspartate aminotransferase, Alanine aminotransferase and creatinine. Conclusion: Guava leaf extract is healthy and successfully blocked bacterial and viral diseases of poultry.

Keywords: Drug resistance, Phytochemical, Gene modification, Gene mutation

INTRODUCTION

Chickens hold value from an evolutionary perspective as they provide information that bridges knowledge between mammals and other vertebrates [1]. To date, an array of specialized commercial populations and inbred chicken lines have subsequently been developed.

Thyroid stimulating hormone receptor (TSHR) is used as a prominent selection signature in all domestic chicken [2].

In the chicken, Z chromosome is in the male germ line two-thirds of the time while W chromosome is transmitted through the female germ line. Therefore, the chromosome pattern in avian species is ZW for female and ZZ for male [3].

The size of the chicken Z chromosome is approximately 83 Mb, accounting for 7.9% of the chicken genome. The Z chromosome contains 1345 genes, and some genes, including FGF10 (fibroblast growth factor 10), ELOVL7 (ELOVL fatty acid elongase 7) and ACO1 (aconitase 1, soluble), regulated fat deposition and development [4], [5]. It is therefore necessary to identify as many selection signatures as possible on the Z chromosome in chickens.

Guava is a medium sized fruit producing nature of tropical Africa. It has evergreen opposite, aromatic short petiole leaves. The stem has reddish brown, thin and smooth bark. The fruits are many seeded berries. Guava (*Psidium guajava L.)* is neotropical in distribution. However its evolutionary history and domestication processes are unknown [6]. *Psidium guajava* belongs to the order of *Myrtales*, family; *Myrtaceae,* genus; *Psidium* and species *Psidium guajava* [7]. Guava is a berry and has high quantities of macro and micro nutrients.

Guava leaf has many phytochemical in macro and micro quantities (Table 1):

Table 1: Phytochemicals found in Guava leaf

S/No	Phytochemicals	Qualitative	Quantitative (mg/100g)
1	Saponine	++	11.13 ± 0.28^{d}
2	Steroids	+	4.60 ± 0.19^{f}
3	Flavonoids	+++	28.81 ± 0.95^{b}
4	Phenols	+++	39.00 ± 0.31^{a}
5	Terpenoids	+	4.13 ± 0.04^{g}
6	Glycosides	+	1.96 ± 0.07^{h}
7	Alkaloids	++	13.02 ± 0.32^{c}
8	Tannins	+	5.82 ± 0.08^{e}

Source: Nwankudu and Agbo (Unpublished).

Flavonoids have the following effects in-vitro and in-vivo: anti-inflammatory [8], anti-oxidant [9], anti-diabetic [10], anti-bacterial [11] [12], antiviral [13], anti-cancer [14] and also effective against hyperlipidemia and atherosclerosis [15].

Phenols: Phenolic compounds from plant extracts had a strong correlation with antioxidant [16] and antimicrobial activities [17].

Numerous studies have highlighted the role of phenol and saponin derived from plants as antibacterial agents against both gram-positive and gram-negative bacteria. Based on the plethora of health benefits acclaimed to reside with the use of flavonoids and phenols as bactericidal and virucidal phytochemicals, this research was set up to evaluate the potential of Psidium guajava (Guava) leaf extract which possesses among others significant quantities of flavonoids and phenols to block bacterial and viral diseases of broiler birds.

MATERIALS AND METHODS

Fresh guava leaves were harvested early in the morning in Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. They were taken to the Department of Forestry for identification and confirmation as guava leaves in the same institution. The leaves were washed with clean running water and dried on laboratory bench for 5 days. The leaves were then pulverised. Hot water extraction was done; 220g of the pulverized leaf was put in 2.5 litres of boiling water at 100 ^{0}C for 5 minutes. The substance was sieved using Mounilex sieving cloth. The filtrate was allowed to cool. Five millilitres each of the filtrate was put in 3 crucibles and put in hot air oven at 220 ^{0}C to dry. The total weight over 15 millilitres was used to calculate the concentration of the extract per millilitre. The extract was stored at -4^o C in a freezer until needed for experimentation. (Fig. 1).

Figure 1: Dried guava leaves

Acute Toxicity Test of *Psidium guajava*

Acute toxicity test was conducted using Lorke's method [18]; Twenty-eight Albino rats of both sexes were grouped into seven (A-G) of four rats each. Group A (control group) was given 2ml/kg of distilled water while Groups B, C and D were given 10mg/kg, 100mg/kg, 1000mg/kg guava leaf extract (GLE) respectively. When no toxicity was observed, 1600mg/kg, 2900mg/kg and 5000mg/kg OF GLE was given to groups E, F and G respectively per os using gastric gavage. The animals were observed for 30minutes, 1hour, 3hours, 12hours, 24hours, 48hours, 72hours for signs of acute toxicity and probably death. The animals were allowed for extra 7days to observe signs of delayed toxicity.

The LD_{50} was calculated using:

$$LD_{50} = \sqrt{}\text{(least dose with mortality} \times \text{Highest dose without mortality)}.$$

In vivo Experiments with Guava Leaf Extract (GLE)

A total of fifty-day old chicks was procured. Forty were unvaccinated while 10 positive control) were vaccinated. They were acclimatized for one week. The 50 birds were divided into 5 groups

of 10 birds per group. Group 1; 10 vaccinated birds. Group 2: unvaccinated untreated (negative control). Groups 3-5 were treated with 400mg/kg of GLE per os; daily, once in 7 days and once in 14 days respectively. Any death during the course of 13 weeks were microbial cultured. At 13th week, blood was collected from the birds for haematology and serology. Blood was also collected from 3 birds picked at random from groups 1, 2 and 4 for gene extraction.

Ethical approval: The research was approved by College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Research Ethics Committee: MOUAU/CVM/REC/202407. Moreover, we ARRIVE guidelines for in vivo experimental animal research and reporting, which stresses that the methods used in research and reporting should be easy to replicate, was observed.

STATISTICAL ANALYSIS

The data collected were analysed using Statistical Package for Social Sciences (SPSS) version 20. Analysis of variance was done using Turkey HSD and values at ninety-five percent confidence interval ($P \leq 0.05$) were accepted as being significant.

RESULT

Result of Acute Toxicity Test of Broiler Birds Treated with Guava Leaf Extract (GLE)

The result observed in highest dose (5000 mg/kg) of guava leaf extract administered to albino rats showed toxicity and death post administration. The higher dose of 2900 were also toxic to the animals. Sign of toxicity observed were: Gasping for air, lethargy and death in 5000 mg/kg treated rats (Table 1). However, the dosage for this research was 400 mg/kg due the result obtained through experimenting with albino rats where graded doses of GLE (200, 400 and 800) mg/kg were used and 400 mg/kg gave the most significantly best result (Nwankudu and Agbo) Unpublished.

Table 2: Result of acute toxicity test in guava leaf extract treated rats

Groups	Dose mg/kg	Number of deaths	Percentage mortality
A	2ml/kg	0/4	0.00
B	10	0/4	0.00
C	100	0/4	0.00
D	1000	0/4	0.00
E	1600	0/4	0.00
F	2900	0/4	0.00
G	5000	4/4	100

Table 3 shows guava leaf extract is toxic at very high dose using Lorke's method.

Gene Extraction Result of Broiler Birds Treated with Guava Leaf Extract (GLE)

There was gene modification of broiler birds treated with GLE which presented itself as gene deletions, insertions and single nucleotide polymorphism (Fig. 2).

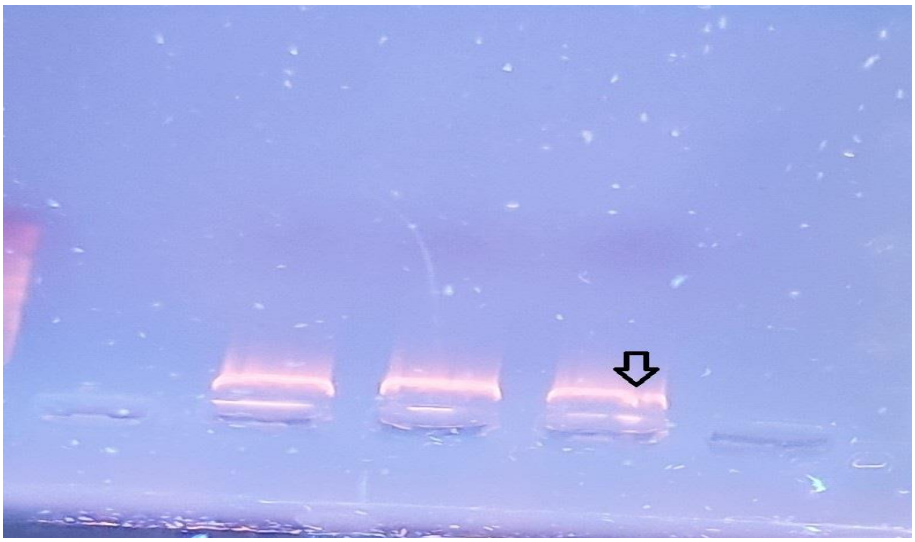

Figure 2: Shows gene deletion in guava leaf treated broiler birds once in 7 days.

The result of the microbial culture of dead birds incriminated klebsiella; an opportunistic bacteria as the main cause of death in group 1 (Vaccinated). The birds were vaccinated only at day old. Beyond 6 weeks, some birds from the group experienced death. Moreover, birds from group 2 (Unvaccinated and untreated birds) experienced death from day one. Resilient ones however persisted till 13 weeks but they were unthrifty. Differential white blood cell count showed heterophils significantly ($P \leq 0.05$) higher in guava leaf treated groups, but there was no established disease

Table 3: Microbial culture

Organs screened	Staphylococcus	Eucharichia coli	Baccilus species	Salmonella	Proteus	Streptococci	Klebsiella	Lactobaccillus
Buccal cavity	+ve	-	+ve	-	-	+ve	-	+ve
Respiratory	+ve	-	-	-	-	+ve	+ve	-
Intestine	+ve	+ve	-	+ve	+ve	-	+ve	+ve
Liver	+ve	-	-	-	-	-	-	-
cloaca	+ve	-	+ve	+ve	-	-	+ve	-

Table 3 shows organisms observed in microbial culture when buccal cavity, respiratory cavity, intestines, liver and cloaca were screened. However, Klebsiella; an opportunistic bacteria was found to be the cause of death in vaccinated and unvaccinated untreated birds that died. This is because all the other organisms encountered were normal micro flora.

Table 4: Haematology parameters of broiler birds treated with Guava leaf extract

Treatment group	Hb (g/dl)	PCV (%)	RBC ($\times 10^6 mm^3$)	TWBC ($\times 10^3 mm^3$)	MCV (fl)	MCH (Pg)	MCHC (g/dl)
1	12.86±0.35	33.66±2.02	3.81±0.22	22.58±0.90	89.89±0.80	33.87±1.12	38.37±1.28
2	11.33±0.37	27.33±1.76	3.05±0.18	19.01±1.09	90.35±0.84	37.87±1.17	41.64±1.40
3	13.00 ± 0.11^a	34.66 ± 0.33^a	3.97 ± 0.01^a	20.96 ± 0.32^b	87.24 ± 0.49^c	32.71 ± 0.14^b	37.49 ± 0.18^b
4	13.20 ± 0.16^a	35.66 ± 0.33^a	4.15 ± 0.02^a	21.96 ± 0.94^a	88.06 ± 0.42^b	31.80 ± 0.05^b	37.01 ± 0.17^b
5	13.46 ± 0.17^a	37.00 ± 1.00^a	4.24 ± 0.09^a	21.85 ± 0.72^a	89.21 ± 0.82^a	31.72 ± 0.26^b	36.42 ± 0.54^b

Superscript indicate significant ($P \leq 0.05$) difference in a column. Values are presented as mean ± S. E (Standard error of mean). Group 1: Vaccinated (Positive control); Group 2: Unvaccinated (Negative control); Group 3: Daily administration of *Psidium guajava*; Group 4: Weekly

administration of *Psidium guajava*; Group 5: Bi-weekly administration of *Psidium guajava.* Table 4 showed that broilers treated with GLE had significantly higher haemoglobin (Hb), Packed cell volume (PCV) and Red blood cell count (RBC) than both vaccinated and unvaccinated untreated. This shows that GLE could be an effective haematinic.

Table 5: Differential Leukocytes count of broiler birds treated with GLE

Treatment group	Lymphocytes (%)	Heterophils (%)	Monocytes (%)	Eosinophil (%)	Basophils (%)
1	66.00±2.08	25.66±1.85	5.66±0.33	2.33±0.33	0.33±0.33
2	53.66±1.45	32.66±0.66	5.33±0.88	8.33±0.88	0.00±0.00
3	63.00±1.73[a]	28.66±1.20[b]	5.33±0.33	2.33±0.66[b]	0.66±0.33
4	61.00±0.57[b]	31.66±1.20[a]	5.33±0.88	2.00±0.57[b]	0.00±0.00
5	61.00±1.00[b]	31.66±0.33[a]	4.66±0.33	2.33±0.33[b]	0.33±0.33

Values are presented as mean ± S. E (Standard error of mean). Superscript shows significant (P ≤ 0.05) differences in column. Treatments groups; 1 = Vaccinated, 2 = Unvaccinated, untreated, 3 = treated with Guava leaf (GLE) extract daily, 4 = treated with GLE once in 7 days and 5 = treated with GLE once in 2 weeks. Table 5 shows that group 3, 4 and 5; GLE treated birds had better immunity (increased number of lymphocytes) than group 2; unvaccinated, untreated. However, heterophils were significantly high in GLE treated birds than vaccinated birds which is an indication of bacterial infections, but they did not come down with disease which shows that the genes responsible for enhancing bacterial diseases might have been deleted.

Table 6: Lipid profile of broiler birds treated with Guava leaf extract (GLE)

Treatment group	T. cholesterol (mg/dl)	Triglyceride (mg/dl)	HDLC (mg/dl)	LDLC (mg/dl)	VLDLC (mg/dl)
1	119.30±4.90	75.10±1.96	40.51±0.50	63.76±4.95	15.02±0.39
2	116.72±4.78	75.42±2.50	39.59±0.71	62.18±4.84	14.95±0.59
3	103.08±1.47[a]	69.61±0.46[a]	43.55±1.16[a]	45.60±2.38[a]	13.92±0.09
4	113.88±2.59[b]	72.43±1.11[b]	42.42±0.90[b]	56.97±3.27[b]	14.47±0.20
5	115.17±2.86[c]	72.91±1.22[c]	41.88±0.34[c]	58.70±2.73[c]	14.58±0.24

Values are presented as mean ± S. E (Standard error of mean). Superscript shows significant (P ≤ 0.05) difference in a column. Group 1: Vaccinated (Positive control); Group 2: Unvaccinated, untreated (Negative control); Group 3: Daily administration of GLE, Group 4: Weekly administration of GLE and Group 5: Bi-weekly administration of GLE. Table 6 showed that GLE treatment significantly reduced, serum cholesterol, triglyceride and low-density lipoprotein-cholesterol (LDLC). However, serum High Density Lipoprotein-cholesterol was significantly increased. This shows that GLE, not only reduces cholesterol, but is also cardio-protective.

Table 7: Serum biochemistry parameters (Liver and Kidney functions)

Treatment group	T. protein (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dl)	Urea (md/dl)	Creatinine (mg/dl)
1	4.61±0.02	72.48±1.10	38.81±1.21	77.79±1.85	0.43±0.04	11.17±0.63	1.10±0.08
2	2.90±0.12	82.42±1.01	43.82±1.10	81.03±0.29	0.73±0.08	11.99±0.30	1.17±0.03
3	4.28±0.10[b]	71.22±0.97[a]	37.29±1.25[b]	78.12±2.26	0.44±0.05[b]	10.55±0.64	1.07±0.02[b]
4	4.10±0.11[c]	72.15±1.39[b]	37.35±0.91[b]	75.61±1.20	0.39±0.03[b]	10.99±0.81	1.01±0.03[a]

5	4.48±0.17[a]	72.43±0.59[b]	37.326±0.63[b]	78.16±1.62	0.48±0.02[b]	11.25±1.47	1.12±0.04[c]

Values are presented as mean ± S. E (Standard error of mean). Different superscript letters along treatment groups shows significant (P ≤ 0.05) differences in a column. Group 1: Vaccinated (Positive control); Group 2: Unvaccinated, untreated (Negative control); Group 3: Daily administration of GLE, Group 4: Weekly administration of GLE Group 5: Bi-weekly administration of GLE. Table 6 showed significant decrease is serum Aspartate amino transferase (AST). Elevated AST signal myocardial infarction, hepatic disease, muscular dystrophy and organ damage. However, Guava leaf extract treatment significantly reduced AST in broiler birds when compared to negative control which indicates that GLE does not cause liver damage. Moreover, ALT was lowest in GLE treated group which further showed that GLE did not cause any liver damage. Increased level of ALT in blood is an indication of liver damage. Moreover, Creatinine is significantly lower in GLE treated birds than in negative control which shows that Guava leaf extract protects the kidney.

DISCUSSION

- This research showed gene mutation which manifested as:
- Single nucleotide polymorphism
- Gene deletions and
- Gene insertions.
- It is possible that the gene responsible for accepting and enhancing bacterial and viral diseases were deleted.

Furthermore, guava leaf extract (GLE) treatment increases the immunity of broiler birds when compared to unvaccinated and untreated birds. Moreover, heterophils were significantly lower in GLE treated birds when compared to unvaccinated untreated birds, but, not as low as vaccinated birds, which leaves a strong hypothesis that chronic GLE treatment in broiler birds led to the deletion of genes responsible for establishing viral and bacterial diseases of broiler birds.

CONCLUSION

More research should be done to confirm gene modification due to chronic guava leaf therapy in vertebrates.

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Ethical Approval: The research was approved by College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Research Ethics Committee: MOUAU/CVM/REC/202407.

Consent of Publication: All the authors unanimously consented to this publication.

Authors Contributions: Oluchi Nnenna Nwankudu designed the research, funded it, supervised the research, analyzed part of the data collated and wrote the manuscript and is the

corresponding author while Daniel Chidi Ifenkwe saw to the day to day experimentation, took care of the birds and collated the data.

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Data Availability: Data are available upon request from the corresponding author.

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