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Growth Performance and Survival of Two Broiler Strains Treated with *Morinda lucida* Leaf Extract at Finisher Phase

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Abstract
*Antibiotic growth promoters (AGP) use in livestock production is indicted in residual antibiotics and antimicrobial resistance in animal and human populations necessitating search for safer alternatives. Morinda lucida (Brimstone tree), a Phytobiotic with antimicrobial activities, less toxicity and residue free product is a potential alternative growth promoter in broilers. Information is however scanty on its use in tropical broiler production as growth promoter. This study investigated growth performance and survival of broilers over 42-day period when drinking water is supplemented with Morinda lucida leaf extract (MLE). A total of 150 broiler day old chicks comprising of 75 each of Arbor acre and Ross were brooded for 3 weeks and randomly allocated to 5 treatment **groups** consisting of 3 replicates of 5 birds per replicate. A 2 x 5 factorial arrangement in completely randomized experimental design was used to test the effect of two strains of broiler and five treatment levels comprising of the negative and positive controls and three doses of MLE. The treatment groups include 0g/l MLE (negative control); 1g/l Oxytetracycline (positive control); 0.5g/l MLE; 1g/l MLE and 1.5g/l MLE. Data on survival and growth were analysed using Kaplan-Meier log rank test and two-way analysis of variance (ANOVA) respectively. The final body weight, daily weight gains and feed conversion ratio (FCR) of broilers were significantly ($p < 0.05$) increased by 30%, 34% and 25% respectively relative to the control when MLE was applied at 1.5g/l of water. MLE administered as 1.5g/l through drinking water is therefore recommended as alternative growth promoter to AGP in broiler production.*

Keywords: *Antibiotics; Growth Promoter; Nutrigenetic; Phytobiotics*

Introduction

The use of antibiotics as in-feed growth promoter in broiler production has been a practice of more than half century (Gibson and Fuller, 2000). Antibiotics growth promoter (AGP) is currently banned in the European Union because of problems associated with the misuse and overuse of antibiotics. The misuse and overuse of existing antimicrobials in humans, animals and plants are accelerating the development and spread of antimicrobial resistance which annually claims at least 700,000 lives globally (WHO, 2019). For instance, Adesina *et al.* (2018) reported the discovery of multi-drug resistant strains of salmonella in chicken eggs in Nigeria, Jammoul and Darra (2019) reported antibiotic residues in chicken meat while Abdelmoaty (2015) found antibiotic residues in both beef and poultry meat. To ensure prudent and safe use of antibiotics in both human and animal health, the Interagency Coordination Group on Antimicrobial Resistance (IACG) proposed an urgent multi-sectorial national One Health approach. This approach among others suggests the phase out of antimicrobials use as growth promoters as well as accelerating research into safe and effective alternatives in animal production (WHO, 2019). Many such alternatives have been studied and reviewed for the purpose of growth enhancement in animal production. These include probiotics (Apata, 2008; Ferdous *et al.*, 2019); prebiotics (Yang *et al.*, 2009) and phytobiotics (Rios and Recio 2005; Muthamilselvan *et al.*, 2016). Windisch and Kroismayr, (2006) defined phytobiotics as plant-derived products added to the feed in order to improve performance of agricultural livestock.

Morinda lucida (Brimstone tree) is one of the phytobiotics that have shown antimicrobial potential through *in vitro* studies because of the presence of phytochemicals. *In vitro* phytochemical screening carried out on *Morinda lucida* leaves indicated the presence of saponins, anthraquinones, cardenolides, alkaloids, sterols and tannin (Addy *et al.*, 2013) as well as β-sitosterol (Nweze, 2011). It was known for its common use in the treatment of malaria, diabetics, dysentery and jaundice in African folk medicine (Burkill, 1997). Because of its antimicrobial potentials, Ola-Fadunsin and Ademola, (2014)

suggested that *Morinda lucida* leaves could be used as feed additives in livestock production.

The evaluation of *Morinda lucida* for application in livestock as potential alternative to AGP must be done cautiously in the context of genotype – environment interaction. According to Toye *et al.* (2013), scientific evidence suggests that not all genotypes of farm animals respond equally to the same drug/ or nutritional regime. Farhud *et al* (2010) called this genotype – environment interaction Nutrigenetics which indicate heterogeneous response of gene variants to nutrients, dietary components and developing nutraceuticals.

Despite the potentials of *Morinda lucida* as growth promoter in farm animals, there is paucity of data on its role in growth enhancement in broiler chickens as well as possible genotype specific interactions indicative of nutrigenetical variations in the response of different strains of broilers to *Morinda lucida* especially under the tropical conditions. The study was therefore conducted to investigate the effect of *Morinda lucida* leaf extract (MLE) on the survival and growth performance of broiler chickens. It also evaluates the genotype – environment interaction between the strains of broilers and the dose of *Morinda lucida* leaf extract administered on survival and growth response of broiler chickens.

Materials and Methods

Experimental Site: The experiment was carried out at the poultry section of Teaching and Research Farm, Kwara State University, Malete, Nigeria. The University is located on geographical coordinates $08^0$42'0'' North and $04^0$28'0''East.

Ethical Statement: The study was conducted in compliance with institutional guidelines of Kwara State University on the care and use of animals for experimental research and based on ARRIVE guidelines (NC3RS) for reporting animal research (Kilkenny *et al.*, 2010).

Experimental Design: A 2 x 5 factorial arrangement in a completely randomized designed experiment was set up to investigate the effects of genotype (2 strains of broiler) and 5 doses of *Morinda lucida* leaf extract on survival and growth performance of broiler chicken.

Preparation of *Morinda lucida*: *Morinda lucida* leaves were sourced from Ibadan, Nigeria. The leaves were air dried for 7 days, packed, milled into powder and taken to Central Research Laboratory® Ilorin, Nigeria for methanol extraction and concentration of the leaf extract. The moisture content of the *Morinda lucida* leaf extract (MLE) was determined by weighing 10g of concentrated sample into aluminium dishes. This was replicated thrice and placed in the oven at 80 ^{0}C for 2 hours. It was removed, cooled and weighed using measuring scale balance (Furi, FEJ 5000). This was repeated until constant weight was achieved. 1.5g of the wet concentrated extract yielded 1g of the dried extract. The *Morinda lucida* leaf extract (MLE) was administered to the birds as concentrated paste on dry matter basis.

Experimental Birds: One hundred and fifty (150) broiler chicks, comprising of 75 each of Arbor acre and Ross, were purchased from Chemero® Hatchery, Ibadan, Nigeria. The birds were brooded in an experimental cage at 35^0C. Temperature was weekly reduced by 3^0C till room temperature of 26 ± 2^0C was attained. All routine vaccinations and medications were administered. Feed (Top Feeds®, Nigeria) and water were supplied *ad libitum*. At the end of 3-weeks brooding period, the birds were weighed (Avery Weight – Tronix) and randomly allocated to 5 treatment groups comprising of 15 birds per treatment in 3 replicates. The replicates, including 5 birds each, were randomly allocated to 8.6cm x 8cm experimental cages. The Five treatment groups include the negative and positive controls as well as the three doses of *Morinda lucida* leaf extract (MLE) administered through the drinking water of broilers at intervals in fortnight regime for 3 days (Grashorn, 2010). These include treatment one: negative control - 0g/litre MLE; treatment two: positive control - 1g/litre Oxytetracycline Hydrochloride (Kepro B.V, Holland); treatment three: 0.5g/litre MLE; treatment four: 1g/litre MLE and treatment five: 1.5g/litre MLE. Data were collected during 42-day study period.

Data Collection

Survival: Records of mortality/survival were taken daily for each strain of broilers and *Morinda lucida* treatment and control groups throughout the 42 days study period.

Growth Performance: The initial body weight (g) and final body weight (g) of the birds were taken (Avery Weight – Tronix). The feed intake (g) of the birds were determined by subtracting the subsequent left-over feed after 24 hours from the weight of feed supplied to the birds as daily ration. The data were used to evaluate the growth

performance of the birds by determining the weight gain per day (gain/bird/day (g)) and feed conversion ratio (FCR).

Data Analysis: Data collected on survival/ mortality were subjected to Log-rank test of Kaplan-Meier survival probability while data on initial body weight (IBW), final body weight (FBW), feed intake (FI), weight gain (g)/bird /day (WG) and feed conversion ratio (FCR) were subjected to two – way analysis of variance (ANOVA) using SPSS version 23 (IBM, SPSS 2015). Significant mean differences were separated using Duncan's Multiple Range Test module of the same statistical package.

Results

There was significant ($p < 0.05$) difference in survival rate between the two strains of broiler (Fig. 1). Ross had 100% survival rate throughout the study period, while Arbor acre had 95% survival rate (mortality rate of 1.3%, 2.7% and 1.4% was observed on days 23, 24 and 25 respectively). There was no significant effect of MLE on survival in broiler chickens (Fig.2). However, there was significant ($p < 0.05$) interaction between MLE and strain of broilers as improvement was observed in the rate of survival in Arbor acre when treated with MLE compared to the control while Arbor acre strain treated with doses of MLE ranging from 0.5g/l to 1.5g/l had 100% survival rate, 20% and 5% mortality rate was recorded in Arbor acre strain treated with 0g/l MLE and 1g/l Oxytetracycline, respectively (Fig. 3).

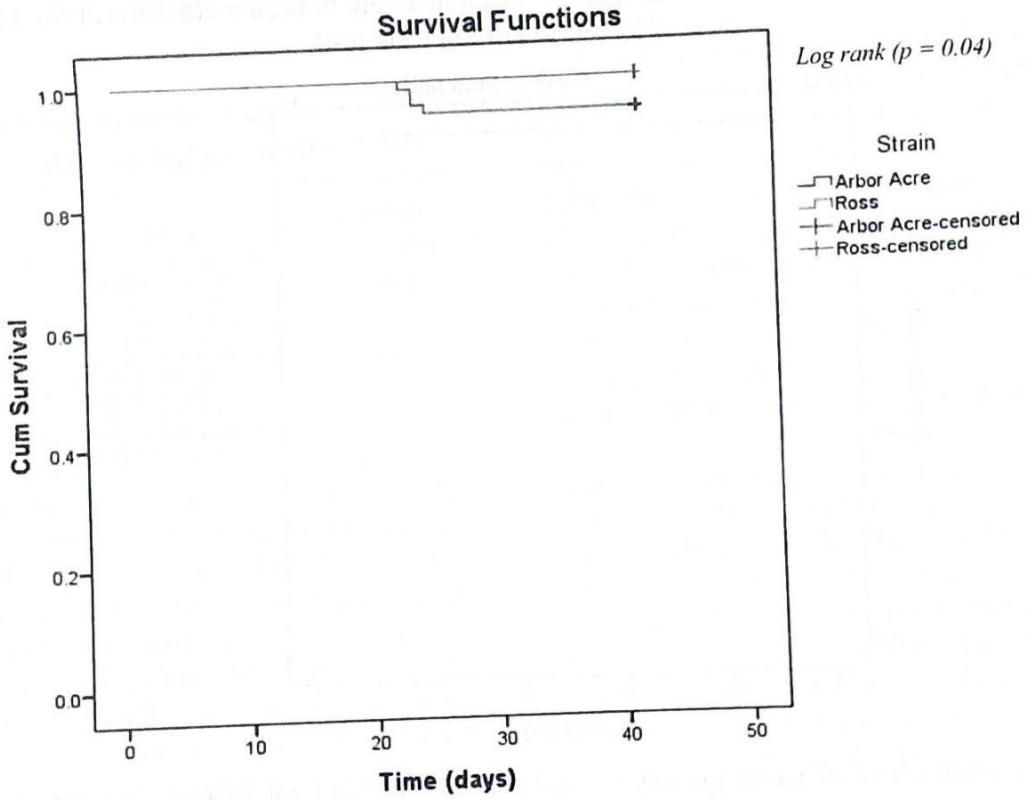


Figure 1: Effect of strain (genotype) on survival rate in broiler chickens at the finisher phase (Kaplan-Meier log rank test)

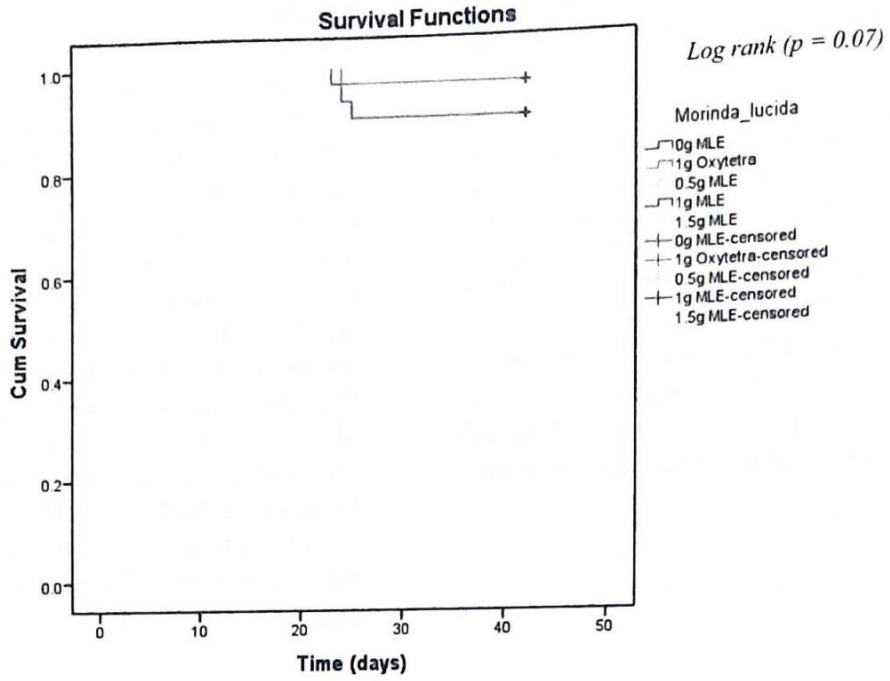


Figure 2: Effect of *Morinda lucida* Leaf Extract on survival rate in broiler chickens at the finisher phase (Kaplan-Meier log rank test)

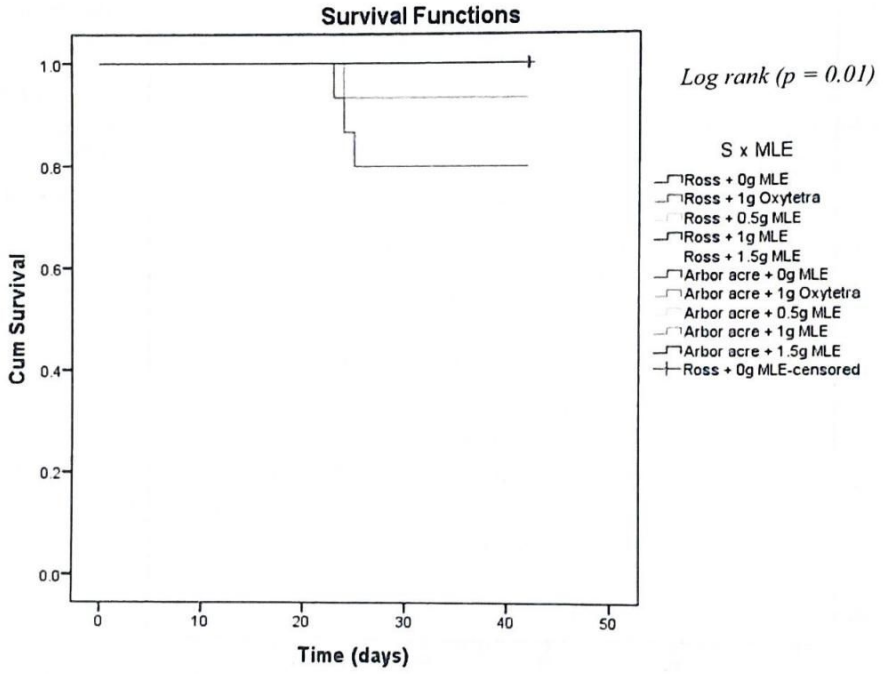


Figure 3: Interaction effect of strain (genotype) and *Morinda lucida* Leaf Extract on survival rate in broiler chickens at the finisher phase (Kaplan-Meier log rank test)

The present study revealed that there were no significant interaction effects of strain and MLE as well as genotype (strain) effects on the final body weight, feed intake, daily weight gains and feed conversion ratio in Arbor acre and Ross (Table 1). The administration of *Morinda lucida* leaf extract however significantly ($p < 0.05$) improved growth performance in broiler chickens (Table 1). There were data evidence of dose-dependent response of broilers to MLE. There was progressive improvement in growth performance as dose of MLE increased. While the feed intake was similar among all MLE treatment groups, data indicated that 0.5g; 1g and 1.5g MLE improved the final body weight by 10%, 17% and 30% respectively relative to the control. There was also an observed improvement of 17%, 20% and 34% in daily weight gains when 0.5g, 1g and 1.5g of MLE was administered and these same MLE doses significantly ($p < 0.05$) reduced the FCR by 7%, 15% and 25% respectively relative to the control.

Table 1: Effect of Morinda lucida leaf extract on growth performance of two strains of broiler chicken

Factors		IBW (g)	FBW (g)	FI (g)	WG (g)	FCR
Strain	Ross	350 ± 0	2984.33 ± 72.40	6483.05 ± 51.51	62.72 ± 1.72	2.49 ± 0.07
	Arbor Acre	370 ± 0	2993.00 ± 82.29	6493.20 ± 39.31	62.45 ± 1.96	2.51 ± 0.07
MLE	0g MLE	360 ± 4.47	2664.17^{d} ± 28.91	6402.85 ± 57.98	54.86^{d} ± 0.74	2.78^{a} ± 0.05
	1g Oxytetra	360 ± 4.47	2775.83^{d} ± 25.25	6423.63 ± 75.93	57.52^{d} ± 0.59	2.66^{ab} ± 0.02
	0.5g MLE	360 ± 4.47	2932.50^{c} ± 36.00	6656.43 ± 96.13	61.25^{c} ± 0.88	2.59^{b} ± 0.05
	1g MLE	360 ± 4.47	3120.83^{b} ± 59.31	6488.27 ± 45.58	65.73^{b} ± 1.41	2.36^{c} ± 0.06
	1.5g MLE	360 ± 4.47	3450.00^{a} ± 31.83	6469.45 ± 30.79	73.57^{a} ± 0.70	2.09^{d} ± 0.03
S x MLE		ns	ns	ns	ns	ns

Values are presented as mean ± standard error of mean (SEM). IBW = initial body weight; FBW = final body weight; FI = feed intake; WG = weight gain/bird/day;
FCR = feed conversion ratio. Means within the same column and factor with different superscript ^{abcd} are significantly different ($p < 0.05$).

Discussions

The interaction between strain of broilers and the dose of MLE on survival in the present study is indicative of nutrigenetic variations in the survival response of broilers to MLE. This Genotype-Environment interaction is suggestive of cautious approach in the recommendation of phytobiotics for general application in livestock production, especially in the context of *Morinda lucida* as alternative to AGP in broiler production (Toye *et al.*, 2013). The strain specific response of broilers in survivability in the present study confirms that there are underlining genetic factors responsible for variations in immune response of broilers to infections and their ability to adapt to stressful environmental and management conditions during production. The findings in this study conform with that of Awobajo *et al.* (2007); Shim *et al.* (2012) and Yerpes *et al.* (2020) in which there were significant ($p < 0.05$) differences in survival rate among broiler strains. However, the mortality rate of 5.4% recorded for Arbor acre and 0% mortality rate for Ross in the present 42-day study period were lower than 8.6% recorded for Arbor acre by Awobajo *et al.* (2007) and 1.85% recorded for Ross by Yerpes *et al.*, (2020) respectively. This may be due to the fact that the present study recorded mortality in broilers during the finisher phase while Awobajo *et al.*, (2007) and Yerpes *et al.*, (2020) recorded mortality in broilers during the starter phase. Although, mortality rate due to infections is expected to be lower during the finisher phase in broilers because of greater level of acquired immunity against diseases through passive exposure to pathogens in the production environment and through vaccinations, death as a result of metabolic disorder and oxidative stress could be higher. Phytochemicals contained in *Morinda lucida* (Addy *et al.*, 2013) with the phenolic compound components serving as potential antioxidants (Grashorn, 2010) which could help combat oxidative stress and consequently metabolic disorder related death in broilers which are genetically predisposed.

The administration of MLE to broilers at finisher phase in this study improved growth performance significantly ($p < 0.05$). This could be attributed to enhanced feed utilization since there was no significant difference in feed intake among the various treatment groups in the present study. There was evidence that the final body weight, daily weight gains and feed conversion ratio were MLE dose dependant. There was progressive improvement in these growth parameters as the dose of MLE was increased. The improved efficiency of feed utilization could be ascribed to improvement in gut morphology. Studies have shown that administration of phytobiotics to broilers increases the villus surface area which results in improved nutrient absorption (Khattak *et al.*, 2014). There is also empirical evidence from studies showing that improvement in growth performance of broilers treated with phytobiotics is due to enhanced secretion of digestive enzymes (Suriya *et al.*, 2012) and increased growth of

beneficial microbes as well as reduction of potential pathogens such as Coliforms and *C. perfringes* (Yang *et al.*, 2009) which result in improved gut functioning. Contrary to data cited by Grashorn (2010), where phytobiotics as plant extracts improved average daily weight gain by 2.6% and FCR by 0.14%, the present study indicates that the inclusion of *Morinda lucida* in drinking water of broilers resulted in considerable higher improvements of up to 34% and 25% respectively in these performance parameters. This could be suggestive of MLE as a potentially more efficient phytobiotic replacement for AGP.

Conclusion

This study demonstrated that the oral administration of *Morinda lucida* leaf extract via drinking water could improve growth performance in broiler chickens at finisher phase. The search for appropriate phytobiotics as potential alternative to the use of AGP in poultry production will translate to higher economic returns to farmers through improved growth performance in broilers without the associated adverse effect of antibiotic growth promoters. The administration of 1.5g MLE, in this study, significantly ($p < 0.05$) improved growth performance of broilers at the finisher phase. Consequently, 1.5g *Morinda lucida* Leaf Extract is recommended for use as phytobiotic growth promoter in commercial broiler production. It is also recommended for enhancing survivability in Arbor acre strains of broilers. Further studies to investigate potential adverse effect of *Morinda lucida* on the physiology of broilers as well as its effect on growth at broilers' starter phase are recommended.

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