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Effect of supplementing *Morinda lucida* (Brimstone) leaf meal in the diets on the Performance, Intestinal and Tissue Microbial count of broiler chickens

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Target audience: rural and organic poultry farmers

ABSTRACT

This study was conducted to evaluate the effects of dietary supplementation of Morinda lucida leaf meal on growth performance, tissue and gastro-intestinal tract microbial count of broiler chickens. A total of 198 one-day old Marshal Broiler chicks were randomly assigned into six treatments in a 3x2 factorial design of four replicates each. The six dietary treatments consist of the basal diet supplemented at 0, 0.1 and 0.2 g/kg Morinda lucida with or without routine medication. Body weight and feed intake were weighed per replicate on weekly basis for eight weeks while the microbiology assay of the tissue and intestine samples was determined by total viable bacterial and total coliform counts at the end of the fourth and eighth week. Addition of M. lucida to the diets significantly (P<0.05) improved weight gain and survivability at the starter phase with or without routine medication. Broiler chickens fed M. lucida in the diet with medication recorded higher feed intake than those without medication. M. lucida inhibited the growth of detected bacteria either at the starter or finisher phase with the exception of Staphylococcus saprophyticus and Escherichia coli. The total viable bacteria count was significantly (P<0.05) reduced in the intestine and tissue of chickens fed M. lucida supplemented diets than chickens fed control diet. Total coliform count in the intestine and tissue of the chickens did not show any significant (P>0.05) difference among all the treatment groups. The study reveals that Morinda lucida was able to reduce Psuedomonas aeruginosa Streptococcus spp, Micrococcus spp and Enterobacter spp. activities in the gastrointestinal tract resulting in improved performance.

Keywords: broiler chicken, *Morinda lucida*, microbiological assay

Description of Problem

Medicinal plants had been in use to improve the health of animals; this is as a result of the bioactive constituents of plants which include alkaloids, tannins, flavonoids and phenolic compounds

with a wide range of antimicrobial (1, 2), antioxidant (3), gut microflora manipulation (4), appetite and digestion stimulative properties (5) and immune enhancement (2) properties. Antibiotics have been added to poultry diets to stabilize the intestinal microbial flora in order to improve performance. However, because of the development of resistance by pathogenic bacteria, which have deleterious effect on public health, there is the quest for alternatives to antibiotics. The use of alternatives to replace antibiotics has gained increasing interest in animal nutrition given considerable attention to medicinal plants among the alternative growth enhancers. Compared with synthetic antibiotics or inorganic chemicals, herbal plants have proven to be natural, residue free and are thought to be ideal feed additives in food animal production (6).

The phytochemical components of *Morinda lucida also known as 'Oruwo' in south-western Nigeria* revealed the presence of saponins, tannins, anthraquinones and alkaloids. This result is similar to the report of (7) and (8). This suggests that they can be used for medicinal purpose since some extracts containing these active substances are being used as medicine (9, 10).

Food safety and public health are of particular interest in most countries around the world, this is as a result of the consumers' concerns about the relationship between diet and health. Studies have shown that antimicrobial drugs reduce part of the intestinal flora while potentially decreasing pathogen shedding (11, 12). As a result, producers believed that removing antibiotics could cause human pathogenic intestinal bacteria in food animals to increase. Becker (13) found that the main risks related to meat consumption perceived by consumers are chemical residues of growth hormones and antibiotics; high fat content and the related hazard of increased cholesterol; microbial infections and the resulting danger of food poisoning. Bacteria can be found in any kind of food but there is great tendency for it to flourish in protein rich foods such as meat and meat product which are sources of food borne disease in humans (14, 15, 16). This study was conducted to evaluate the effect of dietary supplementation of *Morinda lucida* on the growth performance, intestinal and tissue microbial counts of broiler chickens.

Materials and methods

Experimental location

The study was carried out at the poultry unit of the Teaching and Research farm, Federal University of Agriculture, Abeokuta Ogun State, Nigeria.

Collection and preparation of test ingredient

Leaves of *Morinda lucida* were collected from the nature around the Federal University of Agriculture Abeokuta, Ogun State, Nigeria. The leaves were air dried to 10 % moisture content and grinded to powdery form.

Experimental design and management

A total of 198, unsexed day old Marshal broiler chicks obtained from a reputable hatchery were randomly allocated to six dietary treatments of 33 birds per treatment. Each treatment was replicated thrice. Basal diets were formulated at the starter and finisher phases as shown in Table 1. The chickens were assigned to the six dietary treatments in a 3 x 2 factorial design of three *M. lucida* supplementation levels (0, 0.1 and 0.2 g/kg) with or without routine medications (antibiotics and anti coccidiostat) in two phases of starter (0-4 weeks) and finisher (4-8 weeks). Feed intake and live body weight were recorded weekly on replicate basis and feed conversion was calculated.

Table 1: Percentage composition of broiler chickens basal diets

Ingredients	**Starter (0-4 wks)**	**Finisher (4-8 wks)**
Maize	52.00	54.00
Soyabean meal	33.00	20.00
Fish meal (72% CP)	5.00	3.00
Wheat offal	5.00	17.00
Bone meal	3.00	3.00
Oyster shell	2.00	2.00
Broiler premix	0.25	0.25
Table salt	0.25	0.25
L-Lysine	0.20	0.20
DL-Methionine	0.30	0.30
Total	**100.00**	**100.00**
Determined analysis		
Crude protein (%)	22.90	20.02
Ether extract (%)	3.42	3.35
Crude fibre (%)	3.26	3.75
Metabolizable energy (MJ/kg)	11.83	11.72

Starter premix provided: Vitamin A -10,000,000iu, Vitamin D_3-2,000iu, Vitamin E -40,000mg, Vitamin K -2,000mg, Vitamin B_1-1,500mg, Vitamin B_2-4,000mg, Vi tamin B_6-40,000mg, Vitamin B_{12}-20mg, Niacin -40,00mg, Pantothenic -10,000mg, Folic -1,000mg, Biotin -100mg, Choline -300,000mg, Manganese -80,000mg, Zinc -60,000mg, Iron -40,000mg, Copper -80,000mg, Iodine-800mg, Selenium-200mg, Cobalt-300mg, Antioxidant-100,000mg.

Finisher premix provided: Vitamin A -12,000,000iu, Vitamin D_3-2,500,000iu, Vitamin E -30,000mg, Vitamin K -2,000mg, Vitamin B_1-2,250mg, Vitamin B_2-6,000mg, Vitamin B_6-4,500mg, Vitamin B_{12}-15mg, Niacin -40,00mg, Pantothenic -15,000mg, Folic -1,500mg, Biotin -50mg, Choline -300,000mg, Manganese -80,000mg, Zinc -50,000mg, Iron -20,000mg, Copper -5,000mg, Iodine-1,000mg, Selenium-200mg, Cobalt-500mg, Antioxidant-125,000mg.

Microbiological analysis

At the end of the 4th and 8th week of the experiment, the microbiological assay was assessed on the basis of Total Viable Bacterial Count (TVBC) and Total Coliform Count (TCC). Meat and gastrointestinal tract samples were analyzed for bacterial count according to method of (17) while bacteria organisms obtained from the gastrointestinal tract were identified according to (18).

Chemical analysis

The proximate composition of the experimental diets was determined using the methods of (19), while metabolizable energy was calculated.

Statistical analysis

All data were subjected to analysis of variance using the General Linear Model Procedure SAS software (20). Treatment means were tested using the Turkey's studentized range (HSD) test contained in the same package at $P<0.05$.

Table 2: Growth performance of broiler chickens fed *Morinda lucida* supplemented diets

Parameters	Non- medicated			Medicated						
	Morinda lucida inclusion level (g/kg)							P-values		
0 – 4 weeks	0	0.1	0.2	0	0.1	0.2	SEM	T	M	TxM
Average initial weight (g/bird)	36.35	36.36	36.33	36.32	36.36	36.37	0.01	0.5977	0.8491	0.5846
Average final weight (g/bird)	537.88^{b}	733.48^{a}	804.07^{a}	776.45^{a}	809.61^{a}	804.63^{a}	25.05	0.0013	0.0004	<.0001
Average weight gained (g/bird)	501.53^{b}	697.11^{a}	767.74^{a}	740.13^{a}	773.31^{a}	768.25^{a}	25.05	0.0003	0.0004	<.0001
Average feed intake (g/bird)	1679.00^{bc}	1858.00^{abc}	1624.67^{c}	2015.00^{ab}	2034.67^{a}	1747.00^{abc}	44.52	0.0030	0.0016	0.0056
Feed conversion ratio	3.40^{a}	2.70^{b}	2.12^{b}	2.72^{b}	2.63^{b}	2.27^{b}	0.11	0.0001	0.1778	0.0005
Mortality (%)	33.33^{a}	8.33^{b}	4.17^{b}	4.17^{b}	0.00^{b}	4.17^{b}	3.00	0.0029	0.0018	0.0003
4 – 8 weeks										
Average initial weight (g/bird)	537.88^{b}	733.68^{a}	804.07^{a}	777.45^{a}	809.61^{a}	804.63^{a}	25.05	0.0003	0.0004	<.0001
Average final weight (g/bird)	1438.90^{b}	1991.50^{a}	2130.70^{a}	2081.50^{a}	2196.50^{a}	2152.10^{a}	68.17	0.0002	0.0002	0.0002
Average weight gained (g/bird)	901.00^{b}	1258.10^{ab}	1326.60^{a}	1305.10^{a}	1386.80^{a}	1347.40^{a}	48.19	0.0098	0.0063	0.0114
Average feed intake (g/bird)	3461.10	3711.00	3462.00	3756.70	3802.50	3313.50	69.07	0.9560	0.3114	0.2246
Feed conversion ratio	3.91^{a}	2.96^{b}	2.64^{b}	2.91^{b}	2.76^{b}	2.47^{b}	0.15	0.0356	0.0794	0.0382
Mortality (%)	6.67	0.00	4.76	4.17	0.00	0.00	1.44	0.4156	0.4538	0.6847

[a- c] Means on the same row with different superscript are different ($P < 0.05$).

SEM = Standard error of mean

Results

The effects of the dietary inclusion of *M. lucida* on growth performance of broiler chickens are presented in Table 2. At the starter and finisher phases, final weight and weight gain were significantly ($P<0.01$) higher in medicated chickens and those fed *M. lucida* in their diets when compared to those fed the control diet without medication. Chickens in all the treatment groups given medication had significantly ($P<0.01$) higher feed intake than those chickens without medication. Feed conversion ratio at the starter and finisher phases improved in chickens in all the treatment groups with the exception of those fed control diet without medication. The mortality rate (%) indicated that there is no significant difference between *M. lucida* fed birds and medication birds but it differed significantly ($P<0.05$) in the control treatment.

The microbial bacteria count in the gastrointestinal tract of the broiler chickens fed varying levels of *M. lucida* supplemented diets as shown in Table 3 revealed that *M. lucida* inhibited the growth of *Psuedomonas aeruginosa Streptococcus spp*, *Micrococcus spp* and *Enterobacter spp*. with or without medication, while only chickens fed *M. lucida* with medication were able to inhibit the growth of *Enterococcus spp*. However, the growth of *Escherichia coli, Staphylococcus saprophyticus* and *Klebsiella spp*. were affected by neither *M.lucida* nor medication at the starter phase. At the finisher phase, *M. lucida* with or without medication was able to inhibit the growth of *Klebsiella spp* and *Enterococcus spp*. At the end of the experiment, either *M. lucida* or medication could inhibit the growth of *Escherichia coli* and *Staphylococcus saprophyticus.*

The total viable count showed that all samples were contaminated with microorganism (Table 4). Total viable bacteria count (TVBC) in the tissue at the starter phase significantly ($P<0.01$) reduced as the level of *M. lucida* increased in the diet. At the finisher phase regardless of *M. lucida* supplementation, the total bacteria count in the tissue and intestine were significantly ($P<0.05$) reduced with medication. However, no significant difference was found between the mean total coliform counts of samples obtained from all the treatment groups.

Discussion

The positive effects of *M. lucida* with or without medication on final weight, weight gain and mortality of broiler chickens could be attributed to the action of saponins present in the leaves. Herbal extraction of *M. lucida* leaves has been reported as a useful growth promoter of similar effects as antibiotics when used in broiler feeds (21). (22) reported that saponins are reputed to aid survival to stress; lower population of pathogenic bacteria, increase availability of nutrients which eliminate sub clinical infections and reduce production of growth depressing toxins or metabolites by intestinal microflora. (23) observed that antimicrobial activity and immune enhancement probably are the two major mechanisms by which phytobiotics exert positive effects on the growth

Table 3: Bacteria identification in the intestine of broiler chickens fed *Morinda lucida* supplemented diets

	Non- medicated			Medicated		
Parameters	*Morinda lucida* inclusion level (g/kg)					
0 – 4 weeks	0	0.1	0.2	0	0.1	0.2
Escherichia coli	+	+	+	+	+	+
Staphylococcus saprophyticus	+	+	+	+	+	+
Klebsiella spp	+	+	+	+	+	+
Pseudomonas aeruginosa	+	-	-	-	-	-
Streptococcus spp	+	-	-	-	-	-
Micrococcus spp	+	-	-	-	-	-
Enterobacter spp	+	-	-	-	-	-
Enterococcus spp	+	+	+	+	-	-
4 – 8 weeks						
Escherichia coli	+	+	+	+	+	+
Staphylococcus saprophyticus	+	+	+	+	+	+
Klebsiella spp	+	-	-	-	-	-
Pseudomonas aeruginosa	+	-	-	-	-	-
Streptococcus spp	+	-	-	-	-	-
Micrococcus spp	+	-	-	-	-	-
Enterobacter spp	+	-	-	-	-	-
Enterococcus spp	+	-	-	-	-	-

performance and health of animals.

Increased feed intake of chickens with medication could be attributed to the combination of the active ingredients in *M. lucida* and the medications.

The occurrence of *Escherichia coli* in the chickens' digestive system is not unexpected since the organism is one of the normal flora of the intestinal tract (24, 25). The presence of some metabolic compounds such as aromatic phenols may be responsible for the antimicrobial properties of *M. lucida* through inhibiting bacteria activities and proliferation in the chickens. (26) reported that Phenolic compounds are the main chemical group responsible for the antimicrobial activity of medicinal plants. (2009) (27) reported that the crude aqueous extract of *Morinda lucida* inhibited the *in vitro* growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* while it showed no inhibitory activity against *Salmonella typhi.*

Suppression of pathogenic bacteria including *Staphylococcus aureus, Salmonella paratyphi* and, *Klebsiella pneumoniaeas* by herbal plants was reported by (28). The significant reduction in the total viable bacteria count with M. lucida supplementation in the diet at the starter and finisher phase revealed the effectiveness of *M. lucida* in bacteria reduction in the gastrointestinal tract and the tissue.

Conclusion and applications

Morinda lucida supplementation in broiler diets had been found to

1. Improved the performance of the broiler chickens; however supplementation at 2g/kg was more effective in terms of weight gain and pathogen reduction.
2. This approach will be useful especially in organic poultry production and poultry production in the rural areas, where accessibility to conventional drugs is limited.

References

1. Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T and Arsenakis, M. 1996. Antimicrobial and cytotoxic activities of origanum essential oils. *Journal of Agriculture and Food Chemistry* 44, 1202-1205.
2. Guo, F. C., Kwakel, C. R. P., Soede, J., Williams, B. A., Verstegen, M. W., 2004.Effect of a Chinese herb medicine formulation, as an alternative for antibiotics, on performance of broilers. *British Poultry Science* ;45:793-797.
3. Botsoglou, N. A., Yannakopoulos, A.L., Fletouris, D.J., Tserveni-Gousi, A.S and P.D. Fortomaris 1997. Effect of dietary thyme on the oxidative stability of egg yolk. *Journal of Agriculture and Food Chemistry* 45, 3711-3716
4. Hashemi, S. R., Zulkifil, I. Hair-

Bejo, M., Karami, M., and Soleimani, A. F. 2009. The effect of Euphorbia hirta and acidifier supplementation on growth performance and antioxidant activity in broiler chickens. *Proceedings of the 21st Veterinary Association Malaysia (VAM) Congress, August 7-9, 2009, Port Dickson, Malaysia, pp: 215-217.*

5. Kamel, C. 2001. Tracing modes of action and the roles of plant extracts in non-ruminants. In: *Recent Advances in Animal Nutrition*. (Garnsworthy, P.C., Wiseman, J. Eds.) Nottingham University Press Nottingham, UK. pp. 135-150
6. Hashemi, S. R., Zulkifil, I., Hair-Bejo, M., Farida, A and Somchit, M. N. 2008. Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. *International Journal of Pharmacology* 4: 352-360
7. Nwinyi, O. C., Chinedu, N. S., Ajani, O. O. (2008). Evaluation of antibacterial activity of *Pisidium guajava* and *Gongronema latifolium Journal of Medical Plants Research* 2(8):189-192
8. Adejumobi, J. A., Ogundiya, M. O., Kolapo, A. L., Okunade, M. B. 2008. Phytochemical composition and *in vitro* antimicrobial activity of *Anogeissus leiocarpus* on some common oral pathogens, *Journal of Medical Plants Research* 2 (8): 193-196.
9. Sofowora A . 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria; pp289.
10. Balogun E. A and Akinloye D. I. 2012 Biochemical Effects of Methanolic Extract of *Morinda Morindoides a*nd *Morinda lucida* Leaves on Lipid Profile, Bilirubin and Some Marker Enzymes *Asian Journal of Medical Research* Vol-1 Issue-1; 12-16.
11. Mishra, S. K.1991. Effect of Livol powder on broiler chicks with aflatoxin contaminated feed. *Indian Journal of Medicine* 8:77-84.
12. Mujeeb, M. A. 1995. Poultry Guide. *British Microbiology Research Journal, 3(4): 623-634.*
13. Becker, T. (2000). Consumer perception of fresh meat quality: A framework for analysis. *British Food Journal*, 102, 158-176.
14. Bhandare, S. G., A. T. Sherikarv, A. M. Paturkar, V. S., Waskar, and R. J. Zende. 2007. A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control*. 18:854-868.
15. Podpecan, B., A. Pengov, and S.

Vadnjal. 2007. The source of contamination of ground meat for production of meat products with bacteria *Staphylococccus aureus*. *Slovak Veterinary Research* 44:24-30.

16. Easa, S. M. H. 2010. The Microbial Quality of Fast Food and Traditional Fast Food. *Nature and Science*. 8(10): 117-133.
17. Miles, A. A. and Misra, S. S. 1938.The estimation of the bactericidal power of the blood *Journal of Hygiene 38: 732*
18. Cowan, S. T and Steel, K. J. 1993. Enterobacteria In: G. I. Barrow, R. K.A. Felthan (Eds). Manual for the indentification of bacteria (3rd edition), Cambridge University Press, United *Kingdom, 213-218.*
19. A.O.A.C. 1995. Association of Official Analytical Chemists. Official Methods of Analysis. 17th edition Washington DC.
20. SAS Institute. (2000). SAS/STAT® User's Guide: Statistics. Version 6. 12 Edition. SAS Institute Inc., Cary. NC.
21. Peterolli, T. G., Albino, L. F. T., Rostango, H. S., Gomez, P. C., Tavernari, F. C and Balbino, E. M. 2012. Herbal extracts in diets for boilers. *Revista Brasileria de Zootechnica* 41(7):1683-1690
22. Shibata, S., Tanaka, O., Shoji, J and Saito H. 1985. Chemistry and pharmacology of Panax. *Economic and Medicinal Plant Research*; 1:218-284.
23. Cross, D. E., R. M. McDevitt, K. Hillman, Acamovic, T. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science* 48 (4): 496—506
24. Dozois, C. M., Daigle, F and Curtiss, R., 2003. Identification of pathogen-specific and conserved genes expressed in-vivo by avian pathogenic Escherichia coli strain. *Proceedings of the National Academy of Science, USA 100: 247-252*
25. Amit-Romach, E., Sklan, D and Uni, Z. 2004. Microflora ecology of the chicken intestine using 16s DNA primers. *Poultry Science 83: 1093-1098*
26. Karou, D. Simplice, Tchadjobo Tchacondo, Denise, P. Ilboudo and Jacques Simpore. 2011. Sub-Saharan Rubiaceae: A review of their traditional uses, phytochemistry and biological activities. *Paskistan Journal of Biological Sciences, 14: 149-169.*
27. Ogundare, A. O. and A. K. Onifade. 2009. The

antimicrobial activity of *Morinda lucida* leaf extract on *Escherichia coli Journal of Medicinal Plants Research* Vol. 3(4), pp. 319-323

28. Koul, O., Isman, M. B. And Ketkar, C. M. 1990. Properties and use of neem (Azadirachta indica) *Canadian Journal of Botany 68: 1-11*