GROWTH RESPONSE AND ANTIVIRAL POTENTIALS OF NEEM (*AZADIRACHTA INDICA* J.) AND SCENT (*OCIMUM SANCTUM* L.) LEAF MEALS AGAINST NEWCASTLE AND INFECTIOUS BURSAL DISEASES IN BROILER CHICKS

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ABSTRACT
Newcastle disease (ND) and infectious bursal disease (IBD) are the most feared viral disease of poultry. Viral reversion, vaccine failure, risk of autoimmune diseases and allergic disorder has led to the search for alternatives for vaccination. The effects of neem (Azadirachta indica) and scent (Ocimum santum) leaf meals fed singly or in combination on the growth performance and their antiviral potencies in broiler chicks were investigated. Two hundred 1-day old Ross 308 unsexed broiler chicks were randomly allotted to 5 treatments, consisting of 5 replicates with 8 birds each. Treatments 1 and 2 were basal diets with no leaf meals, treatments 3, 4 and 5 had the basal diets plus 3% neem leaf meal (NLM), 3% scent leaf meal (SLM) and the blend of 2% NLM + 2% SLM, respectively. Birds in treatments 2-5 were unvaccinated against Newcastle disease and Infectious bursal disease while birds in treatment 1 were vaccinated. Performance indices were measured on a weekly basis. At day-21, blood samples were randomly collected from 2 birds per replicate for haemagglutination inhibition and agar gel precipitation tests. Data were subjected to descriptive statistics and ANOVA at P<0.05. Birds fed diets supplemented with a combination of 2% neem + 2% scent leaves had the highest weight gain (819.49g/b) and lowest feed conversion ratio (1.83). In conclusion, birds fed diets supplemented with 3% neem and 3% scent leaf meals singly had haemagglutination inhibition titre values and conferred immunity above protective threshold against Newcastle disease and produced antibodies against Infectious bursal disease.
Keywords: Broilers, herbal plants, infectious diseases, vaccination.

INTRODUCTION

Poultry birds are prone to different types of diseases, most especially, the infectious poultry illnesses, which are serious concern to poultry farmers and the business at large, resulting in significant economic losses to the poultry industry (Nigest and Habin, 2020). Vaccination has been in use for decades and it is one of the most essential techniques for preventing infections and lowering poultry producers' financial losses (Bosha and Nongo, 2012). This involves injecting attenuated, dead, or recombinant organisms into the body to stimulate an immune response that detects the injected organism as a foreign antigen, which in turns, causes the antigen to be cleared and memory cells to grow (Ramirez *et al.,* 2013).

According to Daniel *et al.* (2009), Newcastle Disease and Infectious Bursal Disease are two of the Nigerian's most feared viral diseases of poultry, causing severe losses in their populations due to illness, reduced egg production, immunosuppression, and death after infection with pathogenic strains of their respective causative viruses. These two viruses never cease to wreak havoc on chicken flocks around the world and this led to the continual production of vaccines in a bid to combat them. However, there have been records of viral reversion and acute spread within the vaccinated flocks (Mortensen *et al.,* 2002). Some vaccines regarded as being stable also require strong adjuvants and several injections to induce the required level of immunity and are often effective in controlling only clinical signs rather than infections (Minke *et al.,* 2004), while some pose a greater risk of causing autoimmune diseases, allergic disorders, and vaccine injection site sarcomas (Day, 2006), hence the need for alternatives.

The growing need to bring about vaccines that would in no way have any ill consequences against lives of both the animals and final consumers, as well as enhance cost effectiveness in production, and encourage organic animal production, calls for the development of plant-based vaccines by maximizing the antiviral potentials of some herbal plants. Plants, such as *Azadirachta indica* (Neem),

Commiphora swynnertonii (Burtt), Aloe species, *Ocimum sanctum*, *Argemone mexicana*, *Dryopteridis crassirhizomatis* and *Fructus mume*, among others, have been found to be of immense medicinal importance in poultry production (Gill, 2022). However, information on the use of neem and scent leaf as viable alternatives to vaccines in poultry has not been fully documented. Therefore, this study evaluated the efficacy of *Azadirachta indica* (neem leaf) and *Ocimum sanctum* (scent leaf) as substitutes to vaccines, against Newcastle Disease Virus and Infectious Bursal Disease Virus in broiler chickens. Also, to determine the effects of neem and scent leaf meals on specific antibody titre levels in broiler chicks.

MATERIALS AND METHODS

Experimental site and preparation of test materials

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Oyo State, Nigeria. Fresh neem (*Azadirachta indica*) and scent (*Ocimum sanctum*) leaves were harvested from the surroundings of the University Teaching and Research Farm and were sorted, cleaned, and air-dried separately at room temperature until they became crispy but still retaining the greenish tint. The dry leaves were later milled with hammer mill and stored/labelled separately in different air-tight container until they were incorporated into broiler starter diets.

Experimental animals, diets, design, and management

A total of 200 one-day old Ross 308 unsexed broiler chicks were sourced from a reputable commercial hatchery. The birds were tagged, weighed, and randomly allotted to five dietary treatments, consisting of five replicates each with eight birds per replicate in a complete randomized design. The basal diet was a corn-soya bean meal diet formulated to meet the nutrient requirements (NRC, 1994) for broiler chickens {CP: 23.74%, 2914.00 ME (Kcal/kg) for starter diet}.

Experimental layout

Treatment 1: Positive control (Basal diet + 0% Leaf Meal + Vaccinated against NCD and IBD)
Treatment 2: Negative control (Basal diet + 0% Leaf Meal + Unvaccinated against NCD and IBD)
Treatment 3: Basal diet + 3% Neem Leaf Meal + Unvaccinated against NCD and IBD
Treatment 4: Basal diet + 3% Scent Leaf Meal + Unvaccinated against NCD and IBD
Treatment 5: Basal diet + 2% Neem Leaf Meal + 2% Scent Leaf Meal + Unvaccinated against NCD and IBD.

Vaccination procedure employed in the study

In this study, only the birds in the positive control were vaccinated. Gumboro vaccine (infectious bursal disease vaccine) was administered twice, on day 7 and day 18 of the experiment while Lasota vaccine (Newcastle disease vaccine) was administered on day 10 and day 20 of the experiment. The vaccines were administered orally through drinking water. The drinking water was withdrawn from the birds 6-8 hours prior to the vaccination, this was to ensure that the birds were thirsty and could finish the vaccine in their drinking water within 2 hours of administration.

Data collection

Growth parameters

Weekly body weight and feed consumption of the birds in each replicate were recorded. These values were used to estimate the feed conversion ratio and body weight gain.

Blood collection

Haemagglutination inhibition and agar Gel precipitation tests

About 2mL of blood samples were collected from each replicate into plain vials for the assessment of Newcastle Disease Virus and Infectious Bursal Disease Virus - specific antibody levels which were made by conventional haemaglutination- inhibition test (4 HA unit of Ag) and Agar gel precipitation test as described by Thayer and Beard (1998).

Statistical analysis

Data obtained were analyzed using ANOVA (SAS, 2012) at significant level of $P<0.05$. Treatment means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Growth performance of broiler chicks fed diets supplemented with neem and scent leaf meals with or without vaccination (d 0-21)

The results of the growth performance of broiler chicks are shown in Table 1. The birds fed blend of 2% neem + 2% scent leaf meal had the highest (P<0.05) final weight (859.73g/b) and weight gain (819.49g/b) while the least final weight (500.74g/b) and weight gain (460.56g/b) were recorded in birds that received neem leaf meal. Birds on the positive control consumed more feed (1897.56g/b) while lowest feed intake (1495.75g/b) and feed conversion ratio (1.83) were recorded in birds on the blend of 2% neem + 2% scent leaf meal. However, there was no significant difference in percentage livability across the treatment groups. The results obtained from this study revealed that the supplementation of neem and scent leaf meals in the diets of the experimental birds at the starter phase had positive effect on the performance of the birds than the control groups. This result is in agreement with the observation of Nweze (2020) who fed Marshal white broiler chicks with scent and neem leaf extracts and reported that the weight gain of the chicks on both extracts were higher than the control group and the mortality rate of the chicks on scent leaf extract was less than chicks without the scent leaf extract. The observed improved weight gains of the birds fed with the combination of 2% neem + 2% scent leaf meals from the current study could be attributed to the antimicrobial, digestion-stimulating and anti-bacterial properties of neem and scent leaves which help to improve the feed consumption and feed conversion efficiency of the birds (Prasannabalaji *et al*., 2012).

Table 1. Growth performance of broiler chicks fed diets supplemented with neem and scent leaf meals with or without vaccination (d 0-21)

Growth parameter	Treatment diet					SEM	P-Value
	PC+0%LM	NC+0%LM	3%NLM	3%SLM	2% NLM + 2%SLM		
Initial weight (g/b)	40.18	40.66	40.18	40.52	40.24	0.12	0.6323
Final weight (g/b)	734.24^{b}	742.56^{b}	500.74^{c}	771.84^{b}	859.73^{a}	26.23	<0.0001
Weight gain (g/b)	694.06^{b}	701.90^{b}	460.56^{c}	731.32^{b}	819.49^{a}	26.21	<0.0001
Feed intake (g/b)	1897.56^{a}	1727.80^{b}	1601.70^{c}	1767.38^{b}	1495.75^{d}	28.92	<0.0001
Feed conversion ratio	2.74^{b}	2.49^{b}	3.49^{a}	2.42^{b}	1.83^{c}	0.12	<0.0001
Liveability (%)	98.32	99.16	100	100	100	0.6	0.213

T1-Positive Control (0% Leaf Meal/Vaccinated); **T2**-Negative Control (0% Leaf Meal/unvaccinated); **T3**-3% Neem Leaf Meal/unvaccinated; **T4**-3% Scent Leaf Meal/unvacinated; **T5**-2% Neem Leaf Meal + 2% Scent Leaf Meal/unvaccinated

Haemagglutination Inhibition antibody titres against Newcastle disease virus

Table 2 represents the results of the haemagglutination inhibition antibody titres against Newcastle disease virus (NDV)**.** About 100% of the samples in the treatment fed 3% scent leaf meal indicated protective antibody levels against the NDV. Birds fed neem and scent leaf meal singly had higher HI titre values which was above the protective threshold. The result of the present study is similar to the findings of Sadekar *et al*. (1998) who reported that feeding of powdered neem leaves to broilers significantly enhanced antibody titers against NDV antigen. This result validates the immunomodulatory and immunopotentiation properties of both neem and scent leaves (Jeba *et al*., 2011).

Table 2. Haemagglutination Inhibition antibody titres against Newcastle disease virus (day 0-21)

Treatment	Number of Samples	Antibody titres obtained by HI test								
		(2^{1})	(2^{2})	(2^{3})	(2^{4})*	(2^{5})	(2^{6})	(2^{7})	(2^{8})	Mean ± SE
T1	5	-	-	2	-	-	3	-	-	41.60±13.72
T2	5	-	-	2	-	-	3	-	-	41.60±13.72
T3	5	-	-	2	-	3	-	-	-	22.40± 5.88
T4	5	-	-	-	-	-	5	-	-	64.00±0.00
T5	5	-	2	3	-	-	-	-	-	6.40±0.98

HI- Haemagglutination inhibition; * - Protective threshold, **T1**-Positive Control (0% Leaf Meal/Vaccinated); **T2**-Negative Control (0% Leaf Meal/unvaccinated); **T3**-3% Neem Leaf Meal/unvaccinated; **T4**-3% Scent Leaf Meal/unvaccinated; **T5**-2% Neem Leaf Meal + 2% Scent Leaf Meal/unvaccinated; **SE**-Standard Error.

Agar Gel Precipitation Test (AGPT) against Infectious Bursal Disease Virus at day 21

The results of the agar gel precipitation test (AGPT) are presented in Table 3. All the samples tested negative for the presence of infectious bursal disease (IBD) antibody at day 21. Pande (2000) also reported the significantly improved antibody titres against Newcastle disease in broilers treated with Zeetress (a polyherbal combination of *Withania somnifera* (aswaghandha), *Ocimum sanctum* (scent) and *Emblica officinalis* (amla).

Table 3. Agar Gel Precipitation Test (AGPT) against Infectious Bursal Disease Virus

Treatment	T1	T2	T3	T4	T5
Day 21	-	-	-	-	-
Number of Samples	5	5	5	5	5

Negative to AGPT (-), Positive to AGPT (+), **T1**-Positive Control (0% Leaf Meal/Vaccinated); **T2**-Negative Control (0% Leaf Meal/unvaccinated); **T3**-3% Neem Leaf Meal/unvaccinated; **T4**-3% Scent Leaf Meal/unvacinated; **T5**-2% Neem Leaf Meal + 2% Scent Leaf Meal/unvaccinated.

Conclusion

The results of this study showed that supplementation of neem (*Azadirachta indica*) and scent (*Ocimum sanctum*) leaf meals as plant-based vaccines improved the growth performance of broiler chicks. Neem and scent leaf meals also conferred immunity against Newcastle disease and infectious bursal disease when included singly.

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