ANTICOCCIDIAL POTENTIALS OF OREGANO (*ORIGANUM VULGARE* L.) AND NEEM (*AZADIRACHTA INDICA* J.) LEAF MEAL BASED-DIETS IN BROILER CHICKS

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ABSTRACT
Intensive rearing and high flock density of commercial poultry have increased the exposure to diseases such as coccidiosis. The protozoan parasite of the genus Eimeria multiplies in the intestinal tract and causes tissue damage. The use of herbal plants as an alternative natural product is a novel source of medications. Thus, this study was conducted to investigate the anticoccidial effects of oregano (Origanum vulgare) leaf meal and neem (Azadirachta indica) leaf meal on the incidence of coccidiosis in broiler chicks' production. Two hundred 1-day old unsexed Ross 308 broiler chicks were allotted to five dietary treatments, five replicates of eight birds each in a completely randomized design. T1 (basal0 diet, non-challenged), T2 (basal diet, challenged), T3 (basal diet + 0.5% oregano leaf meal, challenged), T4 (basal diet + 0.5% neem leaf meal, challenged), T5 (basal diet + 0.5% oregano leaf meal + 0.5% neem leaf meal, challenged). Performance indices were measured. On day 21, both the litter and excreta samples were collected for Eimeria counts and blood samples were collected for selected serum biochemical indices. Data were analyzed using descriptive statistics and ANOVA at P<0.05. The final weight, body weight gains, and feed conversion ratio of birds that received 0.5% Origanum vulgare or 0.5% Azadirachta indica diets singly were significantly (P<0.05) improved compared with those on the control diets. The highest albumin values were recorded for birds fed 0.5% Origanum vulgare *(1.91g/dL) and 0.5% Azadirachta indica (1.57g/dL) compared with the other treatment groups. The creatinine values recorded for birds fed the negative control group and 0.5%* Azadirachta indica *diets were similar to those of other treatment groups. The alanine aminotransferase of birds on leaf meal-supplemented diets was significantly (P<0.05) higher than the control diets. The egg per gram (litter) in birds fed positive control (580EPG) was substantially (P<0.05) higher than in other treatments. However, birds fed a blend of 0.5% Origanum vulgare + 0.5%* Azadirachta indica *leaf meal had the lowest egg per gram (160EPG). There was no oocyte per gram (excreta) observed in birds fed the leaf meals either singly or in combinations. In conclusion, supplementation of Origanum vulgare and Azadirachta indica at 0.5% in broiler chicks' diets lowered* Eimeria spp. *in the excreta and consequently enhanced the growth performance of the birds.*
Keywords: Phytogenics, broiler chicks, *Eimeria spp.*

INTRODUCTION

Coccidiosis has a significant economic impact on the poultry industry when extrapolated by geographical region, it indicates a global cost of approximately £10.4 billion at 2016 prices (£7.7–£13.0 billion), equivalent to £0.16/chicken produced (Blake *et al*., 2020). Avian coccidiosis is an enteric parasitic disease caused by multiple species of the protozoan parasite of the genus Eimeria **(**Silbergeld *et al.,* 2008**).** The protozoan parasite of the genus Eimeria multiplies in the intestinal tract and causes tissue damage, resulting in the disruption of digestive processes, poor nutrient absorption, dehydration, blood loss, loss of skin pigmentation, and increased susceptibility to other pathogenic diseases (McDougald *et al.,* 2008). However, the continual use of anticoccidial chemicals promotes drug resistance and results in tissue residues in chickens; therefore, a natural herbal plant with specific anticoccidial properties becomes necessary.

Phytogenic feed additives include a wide range of plants (e.g. thyme, oregano leaf, anise, neem leaf, ginger, turmeric, cinnamon) and plant-derived compounds such as essential oils and oleoresins (Windisch *et al.,* 2008.). The use of herbal plants as an alternative natural product is found as a significant source of novel medications because their derivatives possess several biological properties such as antimicrobial, antiviral, anti-stress, antifungal, and nutrigenomic effects on the development of the host's immunity (Bahadoran *et al.,* 2013; Jamil *et al*., 2022). Plant extracts were widely investigated and used for controlling avian coccidiosis and improving poultry performance (Silbergeld

et al., 2008). The objective of this study was to determine the effects of oregano (*Origanum vulgare*) and neem (*Azadirachta indica*) leaf meals on growth performance, some serum biochemical indices, and their anticoccidial efficacies, in broiler chicks.

MATERIALS AND METHODS

Experimental site

The research was carried out at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria.

Test materials

Fresh leaves of neem (*Azadirachta indica*) from trees surrounding the teaching and research farm of the University premises were harvested and air-dried until they became crispy while retaining the greenish coloration. The dried leaves were pulverized with a blender. Fresh oregano leaves (*Origanum vulgare*) were procured from a reputable farm. The leaves were sorted, cleaned, air-dried, and ground into a coarse powder. The coarse powder of the leaf meals was included at varying inclusion levels in the diet of broiler chicks.

Experimental animals, diets, design, and management

A total of 200 one-day-old Ross 308 unsexed broiler chicks were allotted to the five dietary treatments in a completely randomized design with each treatment having five replicates comprising eight birds per replicate. The basal diet was a corn-soybean meal diet formulated to meet the nutrient requirements (NRC, 1994) for broiler chickens {CP: 23.74%, 2914.00 ME (Kcal/kg) for the starter diet}.

Experimental layout

The dietary treatments formulated for broiler starter diets:

Treatment 1: Basal diet + 0% leaf meal (non-challenged) - Negative control
Treatment 2: Basal diet + 0% leaf meal (challenged) - Positive control
Treatment 3: Basal diet + 0.5% dried *Origanum vulgare* leaf meal
Treatment 4: Basal diet + 0.5% dried *Azadirachta indica* leaf meal
Treatment 5: Basal diet + 0.5% dried *Origanum vulgare* + 0.5% *Azadirachta indica* leaf meal

Birds in treatments 2 to 5 were challenged with *Eimeria spp.* on day 7 by adding an infected litter after analyzing the litter and ensuring that *Eimeria spp.* was present in the litter.

Data collection

Growth performance indices: Initial weights of the birds were recorded on day 1 of the experiment. Body weight and feed consumption of the birds were recorded weekly. The feed consumed and body weight was used to estimate the feed conversion ratio and weekly weight gain.

Blood collection: On day 21, about 1mL of blood samples per replicate were collected by puncturing the jugular vein, and blood was collected into a plain vial for serum biochemical assay.

Excreta oocyst count: Excreta samples were collected from birds in each replicate and stored until processed. *Eimeria* oocysts were isolated from the excreta using a saturated sodium chloride floatation solution following the procedures mentioned by Permin and Hansen (1998).

Statistical analysis

Data were analyzed using SAS (2012) software package. Repeated measures of ANOVA were used to compare differences among treatments. Means were separated using Duncan's Multiple Range test. Values were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Growth performance indices of broiler chicks fed diets supplemented *Origanum vulgare* and *Azadirachta indica* leaf meal

The results of the growth performance of broiler chicks fed *Origanum vulgare* and *Azadirachta indica*-supplemented diets for the starter diet are shown in Table 1. The inclusion of leaf meals (*Origanum vulgare* and *Azadirachta indica*) had a positive impact on the growth variables assessed. The final weight, body weight gains, and feed conversion ratio of birds that received 0.5% *Origanum vulgare* or 0.5% *Azadirachta indica* diets singly were significantly ($P<0.05$) improved compared with those on the control diets. This contradicts the findings of Bozkurt *et al.* (2016) who reported that the inclusion of oregano did not influence the feed conversion ratio and body weight gain of broilers compared to 100mg/kg of monensin sodium. However, Arshad *et al*. (2021) reported a higher final

weight gain in Japanese quail fed a diet supplemented with 0.5% neem powder. In this study, the improved feed conversion ratio in birds fed 0.5% *Origanum vulgare* or 0.5% *Azadirachta indica* could be attributed to the presence of carvacrol and thymol in the diet, which encourages nutrient digestion and gut passage rate in chickens (Lee *et al.,* 2004).

Table 1: Growth performance of broiler chicks fed diets supplemented with *Origanum vulgare* and *Azadirachta indica* leaf meals (d 0-21)

Parameters	Treatments					SEM	P-value
	NC	PC	0.5%OM	0.5%NM	0.5%OM+ 0.5%NM		
INWT (g/b)	39.38	39.98	40.00	40.24	40.08	0.17	0.5921
FWT (g/b)	709.68^{cd}	743.14^{bc}	761.58^{ab}	803.68^{a}	670.32^{d}	11.00	<0.0001
BWG (g/b)	670.30^{cd}	703.16^{bc}	721.58^{ab}	763.44^{a}	630.24^{d}	10.98	<0.0001
FI (g/b)	1462.59^{a}	1505.80^{a}	1152.73^{d}	1358.40^{b}	1259.63^{c}	28.96	<0.0001
FCR	2.19^{a}	2.15^{a}	1.60^{b}	1.78^{b}	2.00^{a}	0.05	<0.0001

^{a,b,c,d}: Means on the same row with different superscripts are significantly (P<0.05) different. INWT- Initial Weight, FWT- Final Weight, BWG- Body Weight Gain, FI- Feed Intake, FCR- Feed Conversion Ratio, NC- Negative control, PC-Positive control, OM-Origanum meal, NM-Neem meal, T1 (non-challenged) - Basal diet +0% Leaf meal, T2 (challenged) - Basal diet + 0% Leaf meal, T3 – Basal diet + 0.5% *Origanum vulgare,* T4 - Basal diet + 0.5% *Azadirachta indica,* T5 - Basal diet + 0.5% *Origanum vulgare* + 0.5% *Azadirachta indica*

Serum biochemical indices of broiler chicks fed diets supplemented with *Origanum vulgare* and *Azadirachta indica* leaf meals

The results of the serum biochemical indices of broiler chicks fed diets supplemented with *Origanum vulgare* and *Azadirachta indica* are presented in Table 2. There was no significant difference observed in the total protein and globulin of birds fed the experimental diets. The highest albumin values were recorded for birds fed 0.5% *Origanum vulgare* (1.91g/dL) and 0.5% *Azadirachta indica* (1.57g/dL) when compared with the other treatment groups. The leaf meals did not affect the total protein and globulin values of the birds; this is an indication that the protein in the diet was properly utilized by the birds across the treatments. However, all the values were within the acceptable range reported for broiler chicks and aligns with the findings of Obikaonu *et al.* (2011). The creatinine values recorded for birds fed the negative control group and 0.5% *Azadirachta indica* diets were similar to those of other treatment groups. The alanine aminotransferase of birds on leaf meal-supplemented diets was significantly (P<0.05) higher than the control diets. The highest aspartate aminotransferase (AST) value was recorded in the positive control group (131.84 i.u/L) while the least AST was recorded in birds fed *Origanum vulgar*e leaf meal (97.85 i.u/L) supplemented diet. Significantly lower (P<0.05) alkaline phosphatase (ALP) values were observed in birds fed 0.5% *Origanum vulgare* + 0.5% *Azadirachta indica* (20.17 i.u/L) but similar to the ALP of birds in the negative control group (21.09 i.u/L). The creatinine, Alanine aminotransferase, Aspartate aminotransferase, and Alanine phosphatase values were within the normal physiological range (Mitruka and Rawnsley, 1981). Values higher than normal may signify kidney malfunction and the extent of muscle wastage. The values within the normal range of the broiler chickens in this study suggest that the birds were not surviving at the expense of body reserves.

Table 2: Serum biochemical indices of broiler chicks fed diets supplemented with *Origanum vulgare* and *Azadirachta indica* leaf meals (d 0-21)

Parameters	Treatments					SEM	P value
	NC	PC	0.5%OM	0.5%NM	0.5%OM+ 0.5%NM		
Total protein (g/dL)	2.99	3.23	3.20	2.75	3.00	0.09	0.4295
Albumin (g/dL)	1.51^{c}	1.62^{bc}	1.91^{a}	1.75^{ab}	1.57^{bc}	0.04	0.0101
Globulin (g/dL)	1.48	1.60	1.29	1.00	1.43	0.09	0.2267
Creatinine (g/dL)	0.80^{ab}	0.63^{b}	0.66^{b}	0.72^{ab}	0.88^{a}	0.03	0.0459
ALT (iu/L)	25.37^{c}	16.37^{d}	30.78^{a}	29.80^{ab}	27.39^{bc}	1.12	<0.0001
AST (iu/L)	113.71^{c}	131.84^{a}	97.85^{d}	120.66^{bc}	122.85^{b}	2.61	<0.0001
ALP (i.u/L)	21.09^{cd}	24.53^{a}	23.48^{ab}	22.36^{bc}	20.17^{d}	0.39	<0.0001

[a,b,c,d]Means on the same row with different superscripts are significantly (P<0.05) different. T1 (non-challenged) - Basal diet +0% Leaf meal, T2 (challenged) - Basal diet + 0% Leaf meal, T3 – Basal diet + 0.5% *Origanum vulgare,* T4 - Basal diet + 0.5% *Azadirachta indica,* T5 - Basal diet + 0.5% *Origanum vulgare* + 0.5% *Azadirachta indica,* ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alanine phosphatase, NC- Negative control, PC-Positive control, OM-Origanum meal, NM-Neem meal.

Protozoan (*Eimeria spp.*) load of broiler chicks fed diets supplemented with *Origanum vulgare* and *Azadirachta indica* leaf meals

Table 3 represents the protozoan load of broiler chicks fed *Origanum vulgare* and *Azadirachta indica* leaf meal diets. The egg per gram (litter) in birds fed positive control (580EPG) was substantially higher (P<0.05) than in other treatments. However, birds fed a blend of 0.5% *Origanum vulgare* + 0.5% *Azadirachta indica* leaf meal had the lowest egg per gram (160EPG). There was no oocyte per gram (excreta) observed in birds fed the leaf meals either singly or in combinations. The lowered oocyst count in this study was similar to the findings of Alp *et al*. (2012) who reported that oocyst counts were significantly lower in birds receiving oregano oil-supplemented diets.

Table 3: Protozoan (*Eimeria spp.*) load of broiler chicks fed diets supplemented with *Origanum vulgare* and *Azadirachta indica* leaf meals (d 0-21)

Parameters	PC	NC	0.5%OM	0.5%NM	0.5%OM +0.5%NM	SEM	P-value
Litter (EPG)	580.00[a]	340.00[b]	180.00[c]	320.00[b]	160.00[c]	47.50	0.0232
Excreta (OPG)	60.00[b]	140.00[a]	0.00[c]	0.00[c]	0.00[c]	30.00	0.0528

[a,b,c,] Means on the same row with different superscripts are significantly (P<0.05) different. T1 (non-challenged) - Basal diet +0% Leaf meal, T2 (challenged) - Basal diet + 0% Leaf meal, T3 – Basal diet + 0.5% *Origanum vulgare,* T4 - Basal diet + 0.5% *Azadirachta indica,* T5 - Basal diet + 0.5% *Origanum vulgare* + 0.5% *Azadirachta indica,* NC- Negative control, PC-Positive control, OM-Origanum meal, NM-Neem meal, EPG-Eggs per gram, OPG-Oocyte per gram

CONCLUSION AND RECOMMENDATION

The findings of this study show that broiler chicks fed 0.5% *Origanum vulgare* and 0.5% *Azadirachta indica* singly improved the growth response and health status of broiler chicks. The leaf meals can be used as anticoccidial plants to mitigate the incidence of coccidiosis because a significant reduction in *Eimeria spp.* was recorded in the excreta of birds that received the leaf meal diets. Therefore, 0.5% *Origanum vulgare* or 0.5% *Azadirachta indica* is recommended.

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