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Full Length Research Paper

Dietary inclusion of dried *Artemisia annua* leaves for management of coccidiosis and growth enhancement in chickens

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Coccidiosis constitutes a major problem in poultry rearing. Recourse to the use of prophylactic chemotherapy, for example, is only a short-term solution. Ostensibly, the drugs used are effective but they are also expensive. In the present study, the influence of adding dried *Artemisia annua* L. leaves as a botanical coccidistat in coccidia-infested broilers was investigated. Concurrently, the feed consumption pattern and weight gain in broilers as well as egg production rate and size in layers, respectively, were also evaluated. In untreated broilers, trophozoites increased an average of four-fold over 84 days. Inhibition of parasite growth in birds fed with rations containing *A. annua* leaves was observed just like with a commercial anticoccidial therapy. In addition, there was a higher feed intake which resulted in higher weight gain in the broilers. Weekly egg production rate, size and intensity of egg yolk colour were equally improved in all the layers fed with a mixture of commercial mash and *A. annua* leaves. This is an implicit indication that the addition of *A. annua* leaves to poultry feed serves as a potentially rich source of medication and nourishment for the birds.

Key words: *Artemisia annua*, broiler chickens, coccidiosis, conventional feed, egg yolk, *Eimeria* spp.

INTRODUCTION

Avian coccidiosis is an intestinal disease which is a common health problem in poultry production systems around the world. It is considered to be one of the most economically devastating parasitic diseases that currently plague the industry as it is responsible for high mortality and morbidity rates as well as poor feed conversion of birds that survive outbreaks (Allen and Fetterer, 2002). Poultry operations all over the world are under threat of the disease and its prevalence results in the loss of millions of birds, accounting for more than 800 million US dollars in lost revenue annually all over the world (Williams, 1998). A central feature of avian coccidiosis is that it is caused by protozoan parasites, most notably *Eimeria* species, which have varied degrees of prevalence.

Up until recently, chemoprophylactic use of anticoccidials such as Amprolium® or chemotherapy has been the primary means of controlling the disease in most poultry farms all over the world. Predictably though expensive and cumbersome, drug regimens have played a significant role in the growth of the poultry industry (Allen and Fetterer, 2002). Unfortunately however, the emergence of drug-resistant strains of coccidia has made the currently available anticoccidials less effective and this has threatened the economic stability of the industry, especially in developing countries where the problem has become a major concern to resource-poor farmers. Consequently, many different types of substances have been investigated in the search for alternative methods for controlling coccidiosis.

Lately, various types of natural dietary additives based

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on botanical elements have been explored as sustainable alternatives of controls for coccidiosis and seen to be quite efficacious. Amongst the plant materials that have been tested and found to be effective for possible use as prophylactic feed additives are *Azadirachta indica*, *Sophora flavescens*, flaxseeds and, especially *Artemisia annua* (Tipu et al., 2002; Youn and Noh, 2001; Allen and Fetterer, 2002). Recently *Artemisia annua* has been found to play a very important role in the management of both animal and human healthcare needs (Brisibe, 2006; Turner and Ferreira, 2005; Allen and Fetterer, 2002) through one of the main active principles in the plant, artemisinin, which is a cadinane-type sesquiterpene lactone with an endoperoxide bridge that is presently the most potent and efficacious compound against the late-stage ring parasites and trophozoites of *Plasmodium falciparum*.

Artemisinin is a drug derived mainly from the leafy portions of *A. annua*. Together with its semi-synthetically prepared derivatives such as dihydroartemisinin, artesunate, artemether and arteether, these products are equally potent and efficacious against several other common infectious protozoan parasites including *Schistosoma mansoni, S. japonicum and S. haemotobium* (Xiao et al., 2004; Utzinger et al., 2001), which cause schistosomiasis that afflicts about 200 million people and causes 1.5 million disabilities annually (Kumar et al., 2004), *Cryptosporidium*, *Giardia intestinalis, Entemoeba histolytica*, and *Leishmania* species responsible for cryptosporidiosis, giardiasis, amoebiasis and leishmaniasis, respectively.

Preliminary studies have demonstrated that different parts of the plant, for example, leaves, inflorescence, stem and roots, contain not only comparable levels of primary metabolites and mineral elements with other forage plants but also a diverse array of secondary metabolites with antioxidant potential (Brisibe et al., 2008). Encouraged by these results, we were interested in trying to confirm whether dried leaves of *A. annua* would enhance the performance of chickens in terms of table meat, internal organs and egg production when included as feed supplements in poultry diets. The series of experiments reported here were, therefore, designed to evaluate the effects of the inclusion of *A. annua* as a botanical anticoccidial as well as a natural feed supplement to aid the productivity of broilers and layers by resource-poor farmers in developing countries. Essentially, we were interested in elucidating the potential of *A. annua* as a dietary source of protein and minerals in order to encourage the inclusion of the leaves as feed additives for the production of monogastric livestock. In the current study, our primary interest was in evaluating the effects of inclusion of the leaves of the plant in commercial broiler and layer rations on coccidial infection, especially with respect to oocyst count in faeces and lesion scores, chick mortality, feed conversion efficiency and weight gain in broilers as well as egg laying performance in layers, respectively.

MATERIALS AND METHODS

Plant material and tissue culture

Seeds of an hybrid line of *A. annua* (2/39 x IV) graciously provided by Dr. Pedro M. de Magalhães of CPQBA, University of Campinas, Campinas, Brazil with enhanced agronomic performance and high artemisinin content under humid lowland tropical conditions observed from preliminary trials were surface sterilized by soaking in 70% ethanol for 5 min. Thereafter, they were equally soaked in 0.1% mercuric chloride solution for 5 min. The seeds were thoroughly rinsed thrice with sterile distilled water and then germinated under aseptic conditions in 500 ml vessels containing 100 ml of half-strength MS (Murashige and Skoog, 1962) medium supplemented with 1% sucrose and gelled with 7% agar that had been autoclaved after the adjustment of the medium pH to 6.0. The seeds started to germinate within 3 – 4 days after transfer to this medium. After 4 weeks the seedlings were sectioned into small portions and transferred to MS basal medium supplemented with 3% sucrose, 0.05 mg/l naphthalene acetic acid and 2.0 mg/l benzylaminopurine to induce the formation of multiple shoots mainly from the leaves. These were later transferred after 8 weeks to another MS basal medium that was supplemented only with 1% sucrose and 0.01mg/l naphthalene acetic acid for induction of roots. Cultures were maintained at 28 ± 2ºC under a photoperiod of 16 hour (light)/8 h (dark) at 45 µmol photons m^{-2} s^{-1} and a relative humidity of 60% throughout this study. Fully developed plantlets were hardened and maintained for 4 weeks in plastic buckets in an air-conditioned glasshouse using fluorescent lamps (with a light intensity of 3,000 lux) at a temperature of 30 ± 2ºC and a relative humidity of 50% before transfer to a well irrigated field.

Plants were harvested shortly before the initiation of flowers in November 2005, after a 6-month vegetative growing period in the field. They were dried under shade for 3 – 5 days until moisture content dropped to about 8 - 10%, and then all the leaves were separated from the stems and weighed. The entire biomass of leaves was thoroughly pulverized to a coarse powder in an electric blender and stored in air-tight glass jars in a refrigerator until required for analysis or addition to the commercial poultry rations.

Proximate and chemical analyses

All assays of the proximate composition of the experimental materials were undertaken at the main soil analysis laboratory of Soil Science Department, Faculty of Agriculture, University of Calabar, Calabar, Nigeria. The ash and moisture contents were determined as described by AOAC (1990). Crude fat was extracted from about 4 g of each sample using the Soxhlet method with petroleum ether (40 - 60°C) for 8 h. The total nitrogen was determined using the microkjeldahl method and converted to crude protein content by multiplying with a factor of 6.25. The carbohydrate content was determined by the percentage difference of the various other proximate compositions summed together. Phytate was determined by the technique of Igbedion et al. (1994) while tannin was determined according to Makker and Goodchild (1996) and oxalate by the method of Day and Underwood (1986). All determinations were done in triplicates and results expressed as averages of percent values on dry weight basis.

Determination of mineral elements

The mineral composition of *A. annua* was determined at the Diagnostic Laboratory of Aluminium Smeltering Company of Nigeria (ALSCON), Ikot Abasi, Akwa Ibom State, Nigeria according to standard procedures. Essentially, two grams of dried pulverized leafy biomass were mixed with 20 ml of concentrated perchloric and nitric acids (5:1, v/v). The mixture was allowed to stand overnight and evaporated to dryness on a Kjeldahl apparatus. The digest was dissolved with distilled water and made up to 100 ml.

The digest solution was used for the determination of the following mineral elements: calcium, magnesium, zinc, iron, manganese, and copper in a Perkin Elmer atomic absorption spectrophotometer (AAS) using the appropriate wavelength and absorbents. Sodium and potassium were determined by flame emission techniques while phosphorus was determined as phosphate according to the vanadomolybdate colorimetric method of Pearson (1976).

Evaluation of artemisinin content

Quantitative analysis of artemisinin content was performed according to Zhao and Zeng (1985) with minor modifications. 1 gof pulverized dried plant material was extracted with 100ml of petroleum ether at 40 - 50°C for three hours in a Soxhlet apparatus (Fisher Scientific, Schwerte, Germany). The filtrate so obtained was again dried under vacuum and the residue dissolved in 1 ml methanol followed by derivatization by treating with 4 ml NaOH (0.2%) and incubating at 50°C for 30 min. After cooling to room temperature the solution was neutralized with 5 ml of 0.2 M glacial acetic acid in 20% methanol. Artemisinin was then analyzed with HPLC using methanol : 0.1 mM phosphate buffer at pH 7.9 (40:60) as mobile phase through a LunaC-18-ODS, 250 × 4.6 mm column, and using a photodiode array detector (Shimadzu, Japan) set at 260 nm. The average artemisinin content was observed to be 0.8% in the leaves.

Experimental animals and feed

Two independent experiments (I and II) were carried out concurrently in order to assess the potential of *A. annua* leaves in enhancing the growth performance of chickens, in association with fixed quantities of the commercial ration appropriate for the growth stage of the broiler or layer birds.

The two experiments consisted of 96 male broiler (leghorn strain) day-old chicks and 90 brown point of lay pullets, respectively, which were purchased from a commercial poultry farm in Calabar, Nigeria. All the day-old chicks which were vaccinated against Newcastle and Gumboro diseases using standard procedures and the point of lay pullets were allowed to acclimatize for a week on deep litter with wood shavings in two separate brooder pens at the poultry unit, Teaching and Research Farm, University of Calabar, Calabar, Nigeria, before the start of the experiments. Both groups of birds were maintained on normal broiler (Pfizer Nigeria PLC, Ikeja, Nigeria) and commercial layer diets, respectively, on tray feeders and provided with water *ad libitum* throughout the experimental period. Metabolisable energy of Artemisia leaves was estimated in accordance with the European Union system where the percentages of crude protein, crude fat, and digestible carbohydrates were multiplied by the factors 0.155, 0.343 and 0.167, respectively.

Experiment I

Before starting this experiment, all the broiler chicks were fed with a common starter diet and intentionally infected with coccidia at 1 week of age by administering a 1 ml suspension of sporulated *Eimeria tenella* oocysts obtained courtesy of the National Veterinary Research Institute, Vom, Plateau State, Nigeria, to each of the chicks. Thereafter the weight of each chick was noted and the effects of the coccidial infection were assessed at weekly intervals throughout the study period of twelve weeks. The investigation, which consisted of 96 chicks distributed randomly to one of four dietary treatment groups (A – D), was designed to study the effects of inclusion of two levels of dried *A. annua* leaves in the commercial mash on coccidial infection, especially with respect to the number of oocysts of *Eimeria* species in the faeces and lesion scores, chick mortality, feed conversion efficiency and weight gain in the broilers in comparison with the effects of Amprolium®, a commercial anticoccidial therapy that is commonly used for coccidiosis in the poultry industry as outlined below. Each treatment group had 24 birds. From the pulverized leaves of *A. annua*, four different feeds tagged A, B, C and D were compounded by varying the concentrations of the plant material in each group and having it mixed thoroughly with the commercial mash on a weight basis. Replications in the first group (that is, control feed group A) were fed with a regular broiler feed (hereafter referred to as Feed A), which contained 100% commercial feed with no *A. annua* leaves and no medication with Amprolium. Replications in the second group were fed with Feed B, which contained 100% commercial feed with no Artemisia leaves but had 10 g of Amprolium added to the drinking water. The chicks in the third group were fed with Feed C, which contained 10% dietary *A. annua* leaves and 90% commercial feed without Amprolium, while those in the fourth group were fed with Feed D that contained 20% dietary *A. annua* leaves and 80% commercial feed without Amprolium.

Oocyst count and lesion scores

The total number of oocysts was determined from duplicate counts of faecal droppings from the four groups of broilers fed with different rations at 6 weeks of age. Droppings were collected over a 24 h period, prepared for quantification of coccidial oocysts by mixing with water and having a smear examined under a light microscope according to Allen and Fetterer (2002). About the same period, 4 birds from each of the treatment groups were sacrificed and the number of lesions in the appropriate regions of their intestines assessed according to Johnson and Reid (1970).

Feed consumption

The daily feed consumption (in g) by a bird was obtained by measuring the amount of feed consumed by birds in each group and taking the average for a bird throughout the duration of the whole study.

Weight evaluation

As an index of the physical growth of the birds, the weight of the chicks in each of the groups was monitored at the beginning of the experiment and thereafter on a weekly basis every Saturday throughout the study. Growth parameters based on rate of weekly food consumption, mean weight gain and specific growth rate for each of the birds were evaluated according to Oyegoke et al. (2006).

Assessment of internal organs

In order to assess the influence of *A. annua* supplementation in the feed on the gross necropsy of internal organs of the birds, eight samples from each treatment unit were cut open at 12 weeks of age and the internal organs weighed and their lengths taken. The individual organs were also critically examined to study their shape, size and external characteristics. The data thus derived were summarized to obtain absolute and relative values for the different weights and lengths evaluated.

Experiment II

The second experiment consisted of point of lay pullets, which had

received routine vaccinations at the recommended ages. The 90 layers in this experiment were distributed randomly to one of three treatment groups (E – G); each with 30 birds. From the pulverized leaves of *A. annua*, three different feeds tagged E, F, and G were compounded by varying the concentrations of dried *A. annua* leaves and having it mixed with the commercial layers mash on a weight basis. Replications in the first group (that is, group E) were fed with a regular layer mash (hereafter referred to as Feed E or control), which contained pure or 100% commercial layers mash only. The layers in the second group (group F) were fed with Feed F, which contained 10% dietary *A. annua* leaves mixed with 90% commercial feed, while those in the third group were fed with Feed G that contained 20% dietary *A. annua* leaves and 80% commercial layers mash. All groups were fed *ad libitum* between 7 a.m. and 9 p.m. each day.

As with the broilers performance parameters based on feed consumption of birds, mean weekly egg production rate (as in the number of eggs laid per bird in a week), mean egg size and intensity of colour of the egg yolk were evaluated during the course of the experiment in order to determine the influence of addition of dried *A. annua* leaves in the commercial mash on egg laying performance in layers. Eggs from each experimental group were collected at 12 noon and 5.00 p.m. daily, respectively, and counted for each treatment group. Eggs collected were kept refrigerated at 4°C for 2 days and used for measurement of size by assessing the volume displaced when an egg is dipped into a 100 ml beaker with water. Thereafter, the eggs were broken and visually assessed on the intensity of yolk colour.

Statistical analysis

Triplicate results of proximate and biological data were examined by analysis of variance (ANOVA) using the general linear models procedure of SAS. Significant differences among group means were determined using the least significant difference (LSD).

RESULTS

Influence of broiler feed supplementation with *A. annua*

The gross compositions of the broiler starter and finisher diets are shown in Table 1 while the proximate compositions and mineral profiles of *A. annua* leaves and the four diets supplied to the broiler and layer chicks at the starter and finisher stages are shown in Tables 2 and 3, respectively. The results of the first experiment (that is, the mean values for oocyst count and lesion scores as well as feed consumption rate and weight gain after six weeks of treatment for broilers) are equally summarized in Tables 4 and 5, respectively. Not surprisingly, broilers in the control treatment group which neither received Amprolium through the drinking water nor dried Artemisia leaves through diet preparations gained less weight, consumed less feed and had the highest oocyst count in their faecal droppings. The number of oocysts in the fecal droppings and mean lesion scores of the broilers in groups B, C and D were significantly ($P < 0.05$) low and differences between them were very negligible. However, these were significant when compared with the numbers obtained from the birds in the control group with no medication or treatment with Artemisia. Interestingly, the symptoms of coccidiosis infection, which included enteritis, diarrhoea, and caecal lesions, were very visible in the intestines of the birds in the untreated (control) group.

The results presented in Table 5 show vividly that broilers treated with Amprolium as well as those fed with *A. annua*-supplemented feeds had a significantly ($P < 0.05$) higher live weight gain than those in the control group. The average body weight gain was significantly ($P< 0.05$) different for the various treatment groups, and was least in broilers in the control group when compared to the birds treated either with *A. annua* leaves or Amprolium. Compared to those in the control group with no treatment, birds maintained with Amprolium or on feed supplemented with the two levels (10 and 20%, respectively) of *A. annua* gained more weight during the first 8 weeks of the study. Surprisingly, an analysis of the collected data using a completely randomized design showed no significant ($P > 0.001$) difference in weight gain between the Amprolium and *A. annua*-treated birds, perhaps an indication that the effects of the products on the broilers were similar.

Rate of weekly food consumption among the broilers

Results of the feeding value of the four treatment diets are summarized in Tables 5 and 6. Significant differences in feed consumption pattern started being noticed between the four diets when the birds were at about 4 weeks of age. At this period differences in live weight began to be noticed between the different treatment groups. Regarding the feed conversion rate, both the Amprolium and *A. annua*-supplemented feeds were found to have significantly ($P < 0.05$) better feed gain ratio than the control group. Though the rate of feed consumption per week of the chicks fed with the diets containing dried leaves of *A. annua* (Feeds C and D) appeared slightly better than the values obtained for those treated with Amprolium, however, they did not differ significantly from one another but were significantly different when compared with those obtained for birds in the control treatment without medication or Artemisia supplementation.

Mean weight gain

Differences in body weight gain among the four treatment groups were significant ($P < 0.05$) with the birds on Amprolium and Artemisia treatments growing faster than the control. Though the differences in weekly mean body weight gain following the addition of 10% (Feed C) and 20% (Feed D), respectively, of Artemisia leaves in the commercial mash did not appear to be quite significant compared with the treatment containing Amprolium, however, they were highly significant ($P < 0.001$) when compared with the control treatment. On the average, broilers fed with the 10 and 20% Artemisia-

Table 1. Gross composition of broiler starter and finisher diets.

Ingredient (%)	Starter diet	Finisher diet*
Maize	48.00	50.00
Corn bran	6.85	8.00
Palm kernel cake	5.00	8.05
Soya bean meal	20.00	17.00
Groundnut cake	12.00	9.00
Fish meal	4.20	3.50
Bone meal	3.00	0.35
Vitamin and mineral premix*	0.30	0.30
Lysine	0.25	0.20
Methionine	0.15	0.10
Salt	0.25	0.25
Calculated analysis		
Crude protein (%)	23.07	21.00
Crude fibre (%)	3.42	4.07
Lysine	1.20	0.70
Methionine	0.71	0.50

*Source: Pfizer Feeds Limited.

Table 2. Proximate chemical and mineral composition of sweet wormwood (*Artemisia annua*) leaves in comparison with broiler starter and finisher diets (% DM basis).

Ingredient (%)	*A. annua* leaves	Starter diet*	Finisher diet*
Crude protein	19.66	23.07	21.00
Crude fibre	14.4	3.42	4.07
Crude fat	5.73	3.46	3.84
Ash content	10.26	10.87	10.97
Digestible carbohydrates	49.94	62.94	65.52
Phosphorus	0.46	0.40	0.34
Potassium	2.10	1.28	1.31
Calcium	1.60	0.96	0.80
Magnesium	1.00	0.26	0.21
Zinc	0.011	4.80	5.10
Iron	0.072	1.14	1.20
Manganese	0.0003	7.90	9.20
Sodium	0.03	0.26	0.28
Copper	0.002	-	-
Tannin (mg/100 g dry weight)	0.52	-	-
Phytin (mg/100 g dry weight)	120.00	-	-
Total oxalate (mg/100 g dry weight)	30.91	-	-
Metabolizable energy (ME in kcal/kg)	55.835	2,906	3,100

*Source: Pfizer Feeds Limited.

supplemented diets, respectively, consumed between 10 to 15% less feed in order to gain a kilogram body weight than the control group. Quite interestingly, this trend was observed in both growth phases of the birds in this study, whether at the starter or finisher stages. By comparison of the mean performance of the different treatment groups, a significantly higher live weight ($P < 0.05$) was equally observed in the birds treated with 20% Artemisia leaves at 12 weeks of age than all the other treatments.

Influence of *A. annua* supplementation on weights and sizes of internal organs of broilers

Visual examination of the birds showed that there were differences in weight between those fed with the conven-

Table 3. Proximate and mineral compositions of sweet wormwood (*A. annua*) leaves in comparison with the finisher diet of layer chickens (on % DM basis).

Ingredient (%)	*A. annua* leaves	Finisher diet*
Crude protein	19.66	20.00
Crude fibre	14.4	8.59
Crude fat	5.73	9.41
Ash content	10.26	15.56
Digestible carbohydrates	49.94	45.63
Phosphorus	0.46	0.70
Potassium	2.10	NA
Calcium	1.60	1.00
Magnesium	1.00	NA
Zinc	0.011	NA
Iron	0.072	NA
Manganese	0.0003	NA
Sodium	0.03	NA
Copper	0.002	-
Tannin (mg/100 g dry weight)	0.52	-
Phytin (mg/100 g dry weight)	120.00	-
Total oxalate (mg/100 g dry weight)	30.91	-

*Source: Ikot Omin Livestock Feeds Limited.
NA = Not available.

Table 4. Analysis of variance showing mean lesion scores in the caeca of broiler chickens after 6 weeks of treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Fcal
Total	15	0.256	-	-
Block	3	0.0133	0.0044	2.91^{NS}
Treatment	3	0.229	0.076	49.93**
Error	9	0.0137	0.0015	-

**Significant at 1% level of probability.

Table 5. Effect of addition of dried sweet wormwood (*Artemisia annua*) leaves to commercial starter feed on the growth performance of broiler chickens between 4 to 8 weeks of age.

Parameters	Feed A	Feed B	Feed C	Feed D
Weekly body weight gain (g)	110.95^{a}	156.21^{b}	158.79^{b}	165.18^{b}
Mean body weight gain (g)	107.52^{a}	164.87^{b}	162.50^{b}	177.55^{b}
Weekly feed consumption (g)	595.48^{a}	650.12^{b}	675.35^{b}	714.93^{b}

Means having the same superscripts within the same row do not differ significantly ($P > 0.05$) from one another.
Feed A – commercial broiler mash only, Feed B – broiler mash with Amprolium in the drinking water, Feed C – broiler mash supplemented with 10% of milled *A. annua* leaves, and Feed D – broiler mash supplemented with 20% of milled *A. annua* leaves.

tional feed alone and those fed with commercial feed supplemented with the two levels of *A. annua*. To investigate whether these differences were equally reflected in terms of sizes of internal organs of the birds, the mean absolute and relative weights of some of these organs and parts of the gastro-intestinal tract of the four groups of birds were equally evaluated. The gross necropsy did not reveal any effects on organ mass. In fact, mean absolute and relative weights of the heart, kidney, liver, lungs and gizzard of the *A. annua* treated and untreated

Table 6. Effect of addition of dried sweet wormwood (*A. annua*) leaves to commercial feed on the performance of layer chickens on weekly average egg laying capacity, egg size and intensity of colour of egg yolk after 18 weeks of treatment.

Parameters	Feed E	Feed F	Feed G
Weekly egg production rate	5.75 ± 1.52^a	6.01 ± 1.23^a	6.19 ± 0.87^b
Mean egg size (cm^3)	54.18 ± 2.32^a	54.59 ± 4.39^a	55.28 ± 3.31^a
Colour of egg yolk	Light	Intense	Intense

Means having the same superscripts within the same row do not differ significantly ($P > 0.05$) from one another.
Feed E – commercial layer finisher mash only (control treatment), Feed F – layer finisher mash supplemented with 10% of milled *A. annua* leaves, and Feed G – layer finisher mash supplemented with 20% of milled *A. annua* leaves.

birds were not significantly different ($P > 0.05$) from one another except for the liver that appeared slightly bluish in colour and length of the stomach, which indicated a higher value for the birds fed with *A. annua*-supplemented feed than for the other two treatments (data not shown).

Influence of supplementation of layer finisher diet with *A. annua* on egg production

The data on egg production rate, mean egg size as well as the intensity of colour of egg yolk are presented in Table 6. A consistent trend observed from these details is the fact that layers receiving diet preparations with Artemisia had a higher feed intake resulting from an increased appetite, which probably was responsible for the higher mean number of eggs, laid in a week and mean egg size observed than the birds maintained on the conventional diet. Statistical analysis equally showed that supplementation of the feeds with up to 20% of Artemisia leaves had a significant effect ($P < 0.05$) on egg size. Moreover, the intensity of the colour of the yolk of the eggs laid by the Artemisia-treated birds, though not statistically analysed as evaluation was empirical, was also higher than that of the control treatment.

DISCUSSION

Up until recently, *A. annua* was used as an herbal tea preparation in traditional Chinese medicine for the treatment of fevers associated with malaria that is caused by *Plasmodium*, a protozoan parasite, and was seen to be very effective largely as a result of the presence of anti-protozoan factors such as artemisinin and some of its sesquiterpenic precursors including artemisinic acid and dihydroartemisinic acid. Consequently, one of the major suppositions of the current study was that addition of pulverized dried *A. annua* leaves to poultry feed would exhibit anticoccidial activity since the causal organisms of coccidiosis, *Eimeria* species, are also protozoa. This initial objective in the utilization of dried leaves of *A. annua* as a natural anticoccidial agent was achieved in the current study even though the proper dosage range for effective control of coccidiosis remains to be determined. From the analysis undertaken in this study, however, it is obvious that the amount of artemisinin contained in 600 g of the commercial feed, which is about the average quantity of feed consumed by a broiler at about 6 weeks of age within a week, supplemented with 10 and 20%, respectively, of dried *A. annua* leaves with an initial artemisinin concentration of 0.8% was about 480 and 960 mg, respectively. Not surprisingly, these levels of artemisinin consumed through the leaves on a weekly basis, which were effective in reducing oocyst output in coccidian infections in the broilers, appear to be much lower than those required to clear Plasmodium infections in mice (138.8 mg/kg body weight per day for 3 days), monkeys (200 mg/kg body weight per day for 3 days) and humans (300 mg/kg body weight per day for 3 days) as reported earlier by Klayman (1985), perhaps on account of the longer treatment period in the current study.

Another interesting aspect of the results reported here appears to be the fact that there were no significant differences between the growth performances of the broiler chicks provided with the commercial anticoccidial drug through the drinking water and those maintained on the Artemisia-supplemented diets. Whether at the starter or finisher phases, diet preparations containing 10 and 20%, respectively, of dried *A. annua* leaves gave comparable, and sometimes even better, growth indices than the broiler chicks treated with Amprolium that were maintained on the conventional broiler mash.

Interestingly, the mean weight gains obtained in the broilers fed on the *A. annua* supplemented diets differed significantly from those fed with the commercial mash alone. This observation shows that the broiler chicks were able to utilize the Artemisia-supplemented diets quite more efficiently than the conventional feed, as the diet preparations containing Artemisia enhanced a better broiler performance in terms of higher live weight gains within a short period of twelve weeks. It could be inferred from this that the inclusion of dried *A. annua* leaves in broiler diets, as represented by Feeds C and D in the current study, were satisfactorily acceptable to the birds without any adverse effects. Moreover, the fact that there were significant differences between the various treat-

ment groups in terms of enhanced anticoccidial effects, feed consumption pattern and live weight gain showed that *A. annua* leaves can be used in compounding feed rations for broiler birds. This is not surprising since *A. annua* leaves are known to contain high levels of crude protein with values that are approximately about 20 to 24% (on % DM basis), essential amino acids, minerals, vitamins, antioxidants and flavonoids (Brisibe et al., 2008). Collectively, these are very crucial for growth and development of poultry as well as the enhancement of weight gain in animals. It is possible, therefore, that the growth of the broilers seen in the current study may have been enhanced following the addition of *A. annua* leaves in the starter and finisher diets probably because of its high protein content and presence of essential minerals such as sodium, potassium, zinc and manganese, amino acids and vitamins. The higher weight gain observed in broilers fed with *A. annua*-supplemented feeds might also be due to increased availability of carbohydrates (up to about 50% on DM basis) as well as crude fibre fraction. Usually, a typical commercial broiler feed contains 25% or more soluble (digestible) carbohydrate. An additional 3 to 6% carbohydrate may be generally present as crude fiber, which is considered to be very desirable in practical feed ingredients for poultry.

Now aside from its potential as a source of feed additive in poultry, *A. annua* equally has two other great advantages associated with enhanced feeding efficiency in animals. First and foremost, the plant has a high concentration of antioxidants just like has equally been reported in *Brassica chinensis*, *Allium cepa*, and *Artemisia vulgaris* (Bahorun et al., 2004), which are known to contain high levels of vitamins A, C, and E, and flavonoids such as quercetin. This is not surprising since Artemisia species are generally known as rich sources of antioxidants including flavonoids, coumarins (Zheng and Wang, 2001; El-Massry et al., 2002; Juteau et al., 2002; Liu et al., 2004; Canadanovic-Brunet et al., 2005; Kordali et al., 2005; Toda, 2005; Bilia et al., 2006) and estrogenic flavonoids (Lee et al., 1998; Nikolova et al., 2004).

Generally, antioxidants are very important as they help to block the action of free radicals which have been implicated in several stresses related to gastrointestinal mucosal injuries (Bagchi et al., 1999) and in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the entire aging process (Aruoma, 2003; Dasgupta and De, 2004; Coruh et al., 2007). Moreover, they are also known to be very good modulators of the immune system in humans (Bendich, 1993) and in animals (Chew, 1995). Aside from antioxidants, and compared to traditional forages, *Artemisia* species also have high concentrations of essential oils which are useful in the maintenance of a favourable microflora balance, suppression of rumen protozoa, increases nitrogen uptake and reduces methane production (Greathead, 2003). Taken together, it could be speculated from these and similar data that dietary inclusion of milled Artemisia leaves in poultry rations has the potential to increase feed efficiency and enhance weight gain in the animals. The additional advantages of increasing the feed conversion rate per kilogram body weight gain seen with diets supplemented with *A. annua* in the present study, therefore, are attributes that could be exploited in the formulation of poultry feeds.

Of equal interest and significance in the present study are the increased rate of egg production and higher egg sizes recorded with the Artemisia treatment. Obviously these data are clear manifestations of the fact that one or some of the several components amongst the beautiful portfolio of phytochemicals in Artemisia leaves could have an effect, which enhances the production of eggs in layers. Naturally, chickens laying eggs are known to have a high requirement for nutrients, especially calcium, magnesium and phosphorus (NRC, 1977). Ordinarily, the calcium (1% DM basis) and to a large extent, phosphorus (0.7% DM basis) contents of the commercial layers mash could be considered adequate to sustain good egg laying performance. However, the increased appetite observed in the birds in the present study, presumably resulting from the enhanced health status provided by antioxidants and essential oils in Artemisia (Greathead, 2003; Brisibe et al., manuscript submitted) coupled with the high percentage increases in the levels of calcium, magnesium and phosphorus in the diets that are supplemented with Artemisia when compared to that of the standard commercial layers mash, may have increased the availability of these essential nutrients to the birds, which may possibly be the reason for the observed increase in egg lay with increasing levels of Artemisia in the feed. Aside from this, the increased intensity of the colour in the egg yolk might equally be reflective of the presence of vitamin A or its precursors (β-carotenoids) and/or related compounds in the leaves, which may have a positive correlation on the nutritional quality of eggs.

The initial objective of this experiment was to find a natural product with anticoccidial properties that could be used as a feed additive with minimal processing (Allen et al., 1997). In fact, the study was conducted to compare the anticoccidial efficiency of *A. annua* with that of Amprolium®, a widely used synthetic anticoccidial drug. *A. annua* is a plant of great interest as it is currently the only source of the vital antimalarial drug artemisinin. This compound destroys parasitic organisms and cancer cells through the generation of highly reactive oxygen-based free radicals or electrophilic intermediates, by alkylating and oxidizing proteins and lipids of parasite membranes as well as inactivation of channel proteins (Ridley and Hudson, 1998). It has been demonstrated that the effect of artemisinin is equally mediated through disruption of membrane potential by interacting with the electron transport chain in the mitochondrial membrane, resulting in free radical damage and dysfunctional mitochondria (Li et al., 2005). However, more recently an alternative

mechanism of action based on inhibition of sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) of *Plasmodium* has been suggested (Eckstein-Ludwig et al., 2003), which has reconciled some intriguing observations on the actions of artemisinins against the parasite (Woodrow et al., 2005). It is likely that artemisinin and/or a few other constituents of Artemisia leaves, possibly polymethoxyflavones such as casticin, artemetin, chrysosplenetin and chrysosplenol-D, which are known to have a synergistic effect on artemisinin, might equally have exerted the anticoccidial properties observed in this study, acting in a manner similar to that reported in *Plasmodium*.

In conclusion, this study has clearly identified a number of advantages in supplementing daily rations of broilers and layers with up to 20% of dried pulverised *A. annua* leaves without any adverse effects. The opportunities afforded by the potent physiological effects of dietary inclusion of this plant material include an anticoccidial effect, a higher rate of feed consumption, the ease with which birds do gain weight, a better egg laying performance and an increased intensity in the colour of the yolk of the egg. It is most probable that the presence of a wide range of novel phytochemicals in the leaves of this innocuous, yet versatile plant with numerous pharmacological indications may be a pointer to the advantages inherent in its use. Its utility as a natural feed additive, therefore, would definitely complement current biotechnological efforts and help in advancing our understanding of the important role that medicinal plants (botanical elements) could play in the control of animal and human parasites through the identification of some of the exact components responsible for the parameters observed in this study.

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