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Efficacy of Diatomaceous Earth on *Ascaridia galli,* Blood Parameters: And on Ectoparasites In Chicken

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ABSTRACT

The efficacy of diatomaceous earth (DE) in the treatment of chicken against *Ascaridia galli* and ectoparasites; and its effect on blood parameters in chicken was investigated. Four hundred hens were divided into 5 treatment groups, A, B, C, D and E (n = 80 per group). Groups C, D and E were orally infected with 250 embryonated *A. galli* eggs while groups A and B were not. Meanwhile, groups A and C fed diets with 2% DE and group D was fed with piperazine; and groups B and E was neither fed with DE nor piperazine. Fecal samples and blood samples were analyzed at week 16 to 22 and; 16 and 36, respectively for fecal egg counts (FEC) and blood parameters (erythrocyte count, hemoglobin content and hematocrit value), respectively. In another experiment, to assess efficacy of DE in treating poultry ectoparasites (fleas, mites and lice) DE was topically applied. Results from biweekly fecal analyses showed significant differences in FEC ($P<0.05$); and treatment by group ($P<0.05$) implying that DE had a significant effect in treating *A. galli.* No significant effects on hematological values were noted ($P>0.05$). Findings from ectoparasite trials indicated that at certain time points DE eliminated all targeted parasites (efficacy =100%). This study concludes that DE has the potential to control *A. galli*; and ectoparasites in chicken. Further studies should target effect of DE on internal parasite dynamics for longer periods in chicken. Additionally, to quicken the action of DE against ectoparasites, organic approaches should be studied.

Keywords: Dermanyssus gallinae Echidnophaga spp, Haematological values, Internal parasites, Menopon gallinae and Organic.

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INTRODUCTION

This study sought to evaluate the efficacy of DE in the treatment of intestinal worms and ectoparasites, and its effect hematological parameters in deep-litter raised layer chickens in Uganda. Heavy intestinal parasite infestation can pose health implications in chicken, for instance, impaired weight gain and growth, decreased egg production, increased mortality and possibly anemia. As a result of poor husbandry standards and favourable climatic conditions, internal and external parasites flourish in the tropics (Imura et al., 2012). Gastrointestinal helminths and ectoparasites are a major cause of reduced productivity in poultry but since they rarely lead to death, they are usually neglected (Hunduma et al., 2010). The commonest gastrointestinal helminths in poultry are mainly in two categories, nematodes and cestodes. *Ascaridia galli* is the most prevalent nematode in poultry followed by *Heterakis gallinarum* and *Capillaria spp* (Asumang et al., 2019). The major poultry cestodes are *Raillietine spp*. (Ananda and Kavitha, 2016). In the traditional production system, the type of feed for

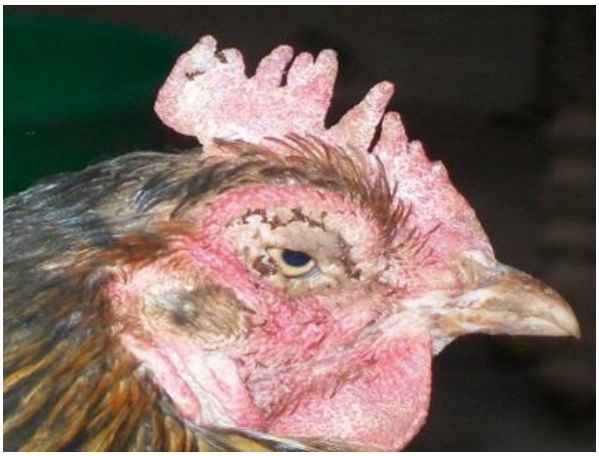

Figure 1. Typical stick-tight flea infestation on chicken.

Figure 2. Lice as seen under the feathers on chicken.

example grains, insects or fruits can be a major predisposing factor facilitating parasitic infections in poultry especially gastrointestinal parasites. This is mainly because these feeds may contain infective stages of parasites (Cervantes-Rivera et al., 2016). For proper diagnosis of the structure and functioning of an animal, blood parameters are very important (Elagib et al., 2011). On the other hand, to ascertain nutritional, pathological as well as environmental aspects, changes in blood parameters are paramount (Graczyket et al., 2003). Ectoparasites on the other hand and in addition to the above, they lead to reduced hatchability since the hens at times abandon the eggs after infestation (Zajac and Conboy, 2006). Stick-tight fleas *(Echidnophaga gallinacea)* which are a burrowing type of fleas cause detrimental effects to poultry production (Zoltan et al., 2007).

The females attach to the skin around the face and wattles to lay eggs (Figure 1). Ulceration and aggravation of the skin can occur and when the area around the eyes is affected, blindness can result (Zoltan et al., 2007). In severe cases, stick-tight fleas infestations can kill young birds. The chicken body louse (*Menacanthus stramineus*) and the shaft louse (*Menopon gallinae*) (Figure 2) are the two species of lice found on poultry. They lay eggs on the birds' feathers, typically near the base of the feather shaft. The eggs are cemented together and appear like white particles. When they hatch lice may live on a bird for several months, however, they sometimes go off the bird for only one week. The entire life cycle of the lice is on the host bird especially in the feathers (Kaufman, 2019). Poultry lice do not suck blood instead; they feed on dry skin scales, feathers, scabs and blood that appear on the surface of the skin (Jacob and Pescatore, 2006). Ectoparasites usually cause much irritation leading to poor health of the infested birds. Infested flocks usually have reduced feed intake, slowed body growth, decreased fertility, and reduced egg production (Zajac and Conboy, 2006). On the other hand, consumers are increasingly concerned with the safe and ethical production of food. The demand for organically produced animal products including organic poultry eggs has been steadily increasing (Berg, 2001; Bejaei and Cheng, 2010). This has led to the production of organic poultry in many countries (Oberholtzer et al., 2006). In organic farming, the routine use of prophylactic medications is not allowed (Wai, 2007). It is hence a requirement for a country like Uganda to emulate other countries by practicing organic farming as much as possible so as to access the international

market for its livestock products. This calls for use of natural products like DE which are organic in nature (Bunch et al., 2013). In recent years, the use of DE has been advocated as an alternative treatment of livestock diseases (Olusegun, 2019). DE is the fossilized remains of diatom shells (Fields, 2000). There are vast deposits of DE in Pakwach district in Northern Uganda (Isabirye et al., 2019), This resource, however, remains largely unexploited although DE has been reported to be essential in controlling aflatoxins in animal feeds (Kabak et al., 2006), insect pests in stored grains (Stathers et al., 2008) and ectoparasites in animals among other uses (McLean et al., 2005).

The action of DE on parasites is unclear but there are suggestions that the abrasive action of the powder pierces or scratches the outer protective layer of invertebrates, especially internal and external parasites, which later die as a result of dehydration and desiccation (Rigaux et al., 2001). A similar principle probably explains the fact that birds frequently take dust baths, presumably to rid themselves of parasites. Some scientists believe that DE is a de-ionizer or de-energizer of worms and parasites (Houyada et al., 2018). DE, therefore, has a unique physical rather than a chemical mode of action against parasites. This property is an important aspect of mammalian safety since DE has negligible toxicity to mammals (Subramanyam and Roesli, 2000).

MATERIALS AND METHODS

Study Area

Trials were conducted at Mukono Zonal Agricultural Research and Development Institute (MUZARDI) in Uganda between January 2016 and December 2017. The coordinates of the town of Mukono are 00 21 36N, 32 45 00E. Weather in Mukono is influenced by the Tropical Monsoon climate.

Source of Diatomaceous Earth (DE)

The DE was mined in Pakwach district (formerly Nebbi district) in Northern Uganda. After mining, the diatomaceous earth was ground into small manageable pieces by placing the DE lumps inside a polythene bag and banging them slightly using a wooden pole. These pieces were then crushed to powder form (with very tiny particles similar to those of talcum powder) to a particle size≤ 40µm using an electric powered milling machine. The powder was then packaged in 200 g paper sachets using a special pressurized packaging machine.

Study on Efficacy of DE on *Ascaridia galli* and on Blood Parameters

Management of Experimental Birds

Infective *A.galli* eggs were collected from fresh intestines of indigenous chicken during the processing of slaughtered chicken at a poultry abattoir found in Kalerwe, a suburb of Kampala Uganda. The study birds were purchased as day-old chicks from local hatcheries and raised on deep litter. Chicks were brooded on coffee husks and with charcoal-heated pots to supply heat for the first 5 weeks. In order to determine fecal egg counts, all the birds were dewormed using albendazole. This was required to ensure that the birds were free from worms before treatment with DE commenced. They were thereafter monitored for 2 weeks. By the end of this period, no worms were discovered. Antibiotics and vitamins were administered as and when required and vaccines against common diseases were given at specified time intervals. Vaccines include Newcastle disease, Infectious bronchitis, Infectious Bursal Disease, Fowlpox and Mareks disease. Deep litter was made up of dry coffee husks to maintain hygiene throughout the experimental period and control coccidiosis outbreak. The birds were fed *ad libitum* with chick mash (3000 Kcal/Kg/ME, 20% CP) from day one to eight weeks; growers mash (containing 2450 Kcal/Kg/ME, 14% CP) between 8 to19 weeks; and layers mash from 20 weeks onwards.

Infection Of Birds With *A.galli*

At 7 weeks of age, 400 birds were wing-banded and randomly allocated to 5 groups, A, B, C, D and E each composed of 80 birds in 2 replicate groups of 40 birds each. Groups C, D and E were each drenched with 250 *A. galli* embryonated eggs*;* while groups A and B were uninfected. Feeding trials with DE followed the following protocol: Group A – Non-infected birds on DE supplemented diet; Group B–Non-infected birds neither piperazine (a conventional dewormer) nor DE applied; Group C –Infected birds with worms and on DE supplemented diet; Group D – Infected birds with worms on piperazine; and Group E – Infected birds on neither DE nor piperazine. The composition of the piperazine used was 1000 mg per gram of powder. Its dosage and administration were 500 grams of powder per 250 kg of finished feed. The DE supplemented diet contained 2 kg DE per 100 kg of finished feed that is, 2%. The basal composition of the diets used in these experiments is presented in Table 1. Chick and duck mash was fed to the birds from Day 1 of age to week 8, while growers' mash was fed from week 9 to week 18; and layers' mash started at 19 weeks onwards until the birds were culled at 72 weeks of age.

Data Collection

Fecal Egg Counts

Table 1. Nutrient composition of diets (Before DE supplementation)

Parameter	Chick and Duck Mash	Growers Mash	Layers Mash
Moisture	7.96±0.79	8.56±0.49	8.23±0.71
Total ash	6.75±0.86	5.68±0.54	5.79±0.70
Crude protein	16.46±0.77	15.23±1.01	21.25±0.91
Crude fibre	4.25±0.54	5.26±0.88	5.56±0.73
Crude fat	2.32±0.45	2.30±0.46	4.60±0.51
Carbohydrate	62.13±2.91	61.43±2.36	59.55±3.0
Energy (MJ/kg)	13.89±0.23	14.19±0.16	13.81±0.23

Proximate analysis of diet determined according to AOAC (2000). All the diets were analyzed before addition of DE used in feeding trial.

Fifty hens (10 hens per group) were randomly selected and fecal samples repeatedly sampled and examined at biweekly intervals between 16 and 22 weeks of age. Approximately 4g of fecal matter was collected from the rectum of each selected hen into 50 ml centrifuge tubes. Fecal samples were transported to the Central Diagnostic Laboratory at the College of Veterinary Medicine, Animal Resources and Bio-security (COVAB), Makerere University. In the laboratory, fecal samples were weighed, preserved in 10% formalin using a 1:1 ratio of volume to fecal mass and refrigerated at 4°C till examination. Examination was always done every fortnight. Parasite eggs were quantified using a modified Wisconsin sugar flotation method (Cox and Todd, 1962; Cox and Lemiski, 1989). Briefly, the formalinized samples were diluted with distilled water to 35 ml, vortexed and centrifuged at 500 x g for 7 min. The mixture was homogenized with a glass rod. The egg counts were conducted in duplicate. The contents were loaded into both chambers of a McMaster slide (Paracount kit, Chalex Corporation, Issaquah, WA) as per the manufacturer's recommendations. The eggs were then counted under the light microscope using a magnification of 100x and the total eggs per gram (EPG) for each treatment was determined. Parasite eggs were then identified according to Soulsby (1982) Foreyt (2001) and Zajac and Conboy (2006).

Determination of Haematological Values

The basal composition of the diets used in these experiments is presented in Table 1. Chick and duck mash was fed to the birds from Day 1 of age to week 8, while growers' mash was feed from week 9 to week 18; and layers' mash started at 19 weeks onwards until the birds were culled at 72 weeks of age. The same feeding protocol as in experiments to determine the efficacy of DE on *A. galli* fecal egg counts described above was used. Data on the determination of the efficacy of DE on blood parameters in chicken was collected from the same groups of hens used in fecal egg counts experiments above. The blood parameters determined were: erythrocyte count – RBC (T/l), hemoglobin content – Hbg (g/l) and hematocrit value – PCV (l/l). In addition, mean corpuscular volume - MCV (fl), mean corpuscular hemoglobin– MCH (gp) and mean corpuscular hemoglobin concentration - MCHC (g/l) indices were calculated (Formulae 1, 2 and 3).

$$\text{MCV} = \frac{\text{hematocrit (\%) x 10}}{\text{RBC count (millions/cubic mm blood)}} \quad (1)$$

$$\text{MCHC} = \frac{\text{hemoglobin (g/100ml) x 100}}{\text{hematocrit (\%)}} \quad (2)$$

$$\text{MCH} = \frac{\text{hemoglobin (g/100ml) x 100}}{\text{erythrocyte count}} \quad (3)$$

Red Blood Cell Counts

In these experiments, another random selection of fifty hens (10 per group) was carried out to determine the red blood cell (RBC) counts. At weeks 16 and 36 of age About 2 ml of blood samples were taken from the wing vein of each selected hen and transferred immediately into a set of sterile glass tubes containing ethylene diamine tetra acetric acid (EDTA) anti-coagulant for hematological analysis. The samples were then prepared for automated electronic counting of total RBC using the coulter diluter dispenser 10 and counter (Coulter Z series Particle Counter and Size analyzer, Beckman Coulter, Inc. Fullerton, CA).

Determination of Packed Cell Volume

To determine the packed cell volume (PCV), about 2 ml aliquots of blood with EDTA from 10 randomly selected birds from each group were individually collected in micro-capillary tubes and then centrifuged for 10 min at 14000 rpm in an IEC MB micro Hematocrit centrifuge (Damon IEC Division). After centrifugation, samples were analyzed for PCV in a micro-capillary reader (Damon IEC Division). PCV analysis was done concurrently with RBC count analysis that is, at weeks 16 and 36 of age.

Statistical Analysis of Data from Feeding Experiments

Statistical analyses were performed using the Statistical Analysis System (SAS, V9.3). Analysis of variance (ANOVA) was carried out to determine significant differences among treatments. Dunnett's T-test was conducted for treatment comparisons in which groups B and E were used as non-infected and infected controls, respectively. Before analysis, outliers were removed from the original data using a special SAS macro program. Significance was determined at $P<0.05$.

Study on The Efficacy of Diatomaceous Earth on Ectoparasites In Chicken

Management of Experimental Birds on Ectoparasite Study

Local chickens naturally infested with fleas (*Echidnophaga spp)* on combs, eyelids and wattle; others with mites *Dermanyssus gallinae* and others with lice *(Menopon gallinae)* were screened. Hens 12 weeks of age were kept in separate cages to avoid cross-contamination by different ectoparasites and housed at a density of 2 sq ft per bird. The hens from each ectoparasite treatment were kept in adjacent poultry houses. The parasites were allowed to multiply on these chickens as much as possible before the commencement of treatment. Infested areas were marked and the external parasites on these areas counted. Pretreatment parasite density was determined by counting the number of parasites on individual birds. Pesticide treatments with efficacy against the 3 ectoparasites under trial were not permitted on the farm 3 months before the commencement; and throughout the study period.

Treatment of Birds on Ectoparasite Study

Hens, 12 weeks old were randomly chosen and divided into 3 groups A, B and C each representing control group (nothing to be applied); standard remedy (ultravin); and test group (to be treated using DE). Each group comprised of 5 birds which were replicated 3 times. 'Ultravin' a conventional commercial product for controlling ectoparasites in poultry commonly used in Uganda. Each bird on treatment was marked with three (3) rectangular demarcations designated a, b, and c, respectively, each totaling 20 cm^2 using a permanent marker pen for birds on lice and mites' trials. Meanwhile, for the case of hens on fleas control experiments demarcations were made on the comb, eyelids and wattle where fleas are commonly found on the bird's body and not on any other parts of their bodies. For mites and lice, the demarcations were made on the side of the hens under the left-wing (a); on the opposite side under the right-wing (b); and on the back between the wings (c). A magnifying glass was used to enhance accuracy in parasite counting. The chickens were each thoroughly dusted with 10 g of powdered DE per bird using a puff duster, and some DE was used to dust the cages. The hens dusted with DE were chosen at random. Another group of hens as dusted with 'Ultravin' a conventional commercial product for controlling ectoparasites in poultry commonly used in Uganda. Ultravin was applied by dusting the hens with a very fine powder (of about 2 grams per bird) under the feathers on the entire bird, and dusting in the cages. Ultravin is a combination of Permethrin 1% and Carbaryl 5% as its active ingredients. On the day scheduled for application of the respective pesticide, the ectoparasite counts were done before application. The dusting protocol followed the manufacturer's instructions. Some chickens were left out to act as controls. Birds infested with different parasites were housed separately. The day before the treatments started was named Day 1 while the day when the birds were treated with the materials under trial was called Day 0. The number of parasites for Day 0 was that number counted and recorded just before the first application was done. Treatment with different remedies (DE and ultravin in the different groups, respectively) was done within 14-day intervals thus, on Day 0; Day 14 and Day 28 beyond which no more treatments were carried out.

Data Collection on Ectoparasite Study

Observations on parasites dying or alive were made daily after treatments and ectoparasites counted and recorded accordingly. The counting continued once daily during the days that followed till 32 days post-treatment. The parasites were confirmed dead, especially for fleas, after observing them in the illuminated ring magnifier (Luxo, type LFM - 102β, 1* 22w). For comparison of the DE to that of ultravin, the results of DE (concentration?) at 10 g per bird were plotted against those obtained from treatment with ultravin (concentration?).

Determination and Assessment of Efficacy of DE and Ultravin

The determination of primary efficacy was based upon the counts of the different parasites per $20cm^2$ per hen from the units/group with DE treated chickens compared with the control units.

Percentage efficacy was calculated separately for each group. Post-treatment assessment time point was calculated using the Henderson-Tilton formula (Henderson and Tilton 1955) Formula (4)

$$\text{Efficacy}[\%] = \left(1 - \frac{\text{Tpost}}{\text{Cpost}} \text{x} \frac{\text{Cpre}}{\text{Tpre}}\right) \text{x } 100 \qquad (4)$$

Where Tpost = number of parasites per 20cm^2 per hen in a unit with DE treated hens for each post-treatment time point; Cpost is the mean number of parasites per 20cm^2 per hen for each post-treatment time point. Tpre is the mean number of parasites per 20cm^2 per hen in a unit with DE treated chickens on Day -1; Cpre is the mean number of parasites per hen in the control unit on Day -1. The mean number of parasites per hen denotes the arithmetic mean of all mobile stages that is, larvae, nymphs (both stages together) and adults. DE was considered effective at a certain time point where efficacy was at least 100% for fleas, lice and mites (*Dermanyssus gallinae)* in accordance with European Economic Commission Guidelines (EEC, 1994). The efficacy of the commercial product ('Ultravin') and that of DE was determined on the basis of percentage in the number of the ectoparasites per bird according to Khater and others (2013). Respective floors of cages and walls of housing units were also dusted with DE (20g per cage) or treated with ultravin by dusting the hens with a very fine powder (of about 2grams per bird) under the feathers; and dusting in the cages, as recommended by the manufacturer to treat the ectoparasites on the floors that is, by applying a very small amount of ultravin in those places.

Assessing the Residue Effect of Diatomaceous Earth

The long-term effects of DE, as well as that of ultravin, were assessed after Day 28 when the final application of DE or Ultravin was carried out on the respective groups. The day when the first ectoparasites on each chicken emerged (re-infestation) in each group was recorded and this was continued until all the chickens in the trial had each experienced re-infestation (Grist, 2004). Therefore, after Day 28 data collected only targeted the presence of any parasite on the marked areas and the day when the first parasite was seen on the birds under study. The number of parasites present of the different chickens during this period was not regarded as a contentious issue. Hence, the data were taken after Day 28 when the last application was made, mainly targeted presence or absence of parasite and Day when the first parasite was seen on a particular bird in a particular group.

Statistical Analysis of Ecto-Parasite Data

A general linear mixed model repeated measures procedure was used to analyze the mite, flea and lice numbers separately. The week was the fixed effect. The individual hen was used as the experimental unit that is, random effect. Week was the repeated measure in the model. All data were analyzed using the Statistical Analysis System (SAS, V9.3). The mean values, standard deviation and standard errors were calculated for each parasite and week.

Using a two-sided t-test, p-values were calculated to determine if the population of the different parasites was affected by either treatment (that is, with DE as well as with ultravin). The mean number of ectoparasites per group after treatment was compared with the mean number of ectoparasites the week before treatment using a t-test. To find out the extent of parasitic load and of re-infestation, the different days were also compared using a two-sided t-test. The significance level was set to $P<0.05$. Because of the different types of parasites, data from the different groups were analyzed separately.

RESULTS AND DISCUSSION

Efficacy of DE on *Ascaridia galli*

The results on the efficacy of DE on *A. galli* are presented in Figure 3. Error bars represent the mean±standard error (n=10). Biweekly fecal analyses showed significant differences in FEC $P<0.05$; and treatment by group $P<0.05$. The lower diagram represents birds infected with *A.galli* eggs. These are comprised of birds on DE supplemented diet (Group C); on piperazine supplementation (D); and on neither DE nor piperazine added to their diets (Group E). Meanwhile, the upper diagram represents results on birds that were not infected with *A. galli* eggs. Error bars represent the mean±standard error (n=10). Biweekly fecal analyses showed significant differences in FEC ($P<0.05$); and treatment by group ($P< 0.05$). The results of this study, therefore, indicate that dietary supplementation with DE had a significant effect on *A. galli* counts. These results contradict the findings of Bennett et al (2011), Tello-Velamazán and other workers (2015) who both concluded that the effect of DE on internal parasites in chicken was not significant ($P>0.05$). However, whereas the former used 2% DE in the experimental diet (the same concentration as in the current study), the latter used 1.35% DE which factor might have caused variation in the latter case. However, the DE application intervals in all three studies did not vary. Results of the present study, therefore, depict that inclusion of 2% DE in diets of layer chickens can help reduce their intestinal parasitic load.

Effect on Haematological Values

The hematological values in the five treatment groups are shown in Table 2. The present findings revealed that total erythrocyte counts, Hbg, PCV and MCV increased significantly ($P<0.05$) with the advancement in age being lower in week 16 and higher in week 36 of age in all treatment groups. The MCH and MCHC values decreased gradually with the advancement in age. There was no significant ($P>0.05$) difference in hematological

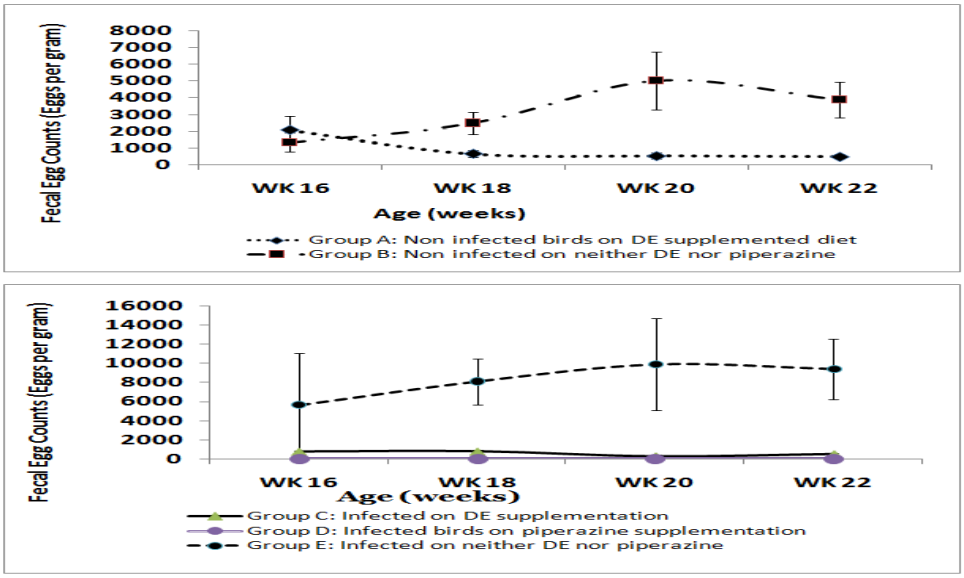

Figure 3. Fecal egg count (eggs per gram) of faeces from chicken infected and not infected with *Ascaridia galli* eggs.

Table 2. Means of haematological parameters in birds given treatments at different ages.

Parameter	Treatment	Age (weeks)	
		16	36
RBC (Erythrocyte number) (10^6/mm^3)	A	2.47±0.19	2.91±0.21
	B	2.5±0.17	2.94±0.19
	C	2.37±0.11	2.79±0.13
	D	2.36±0.13	2.78±0.17
	E	2.55±0.13	3±0.15
Hbg Concentration (g%)	A	9.48±0.43	10.66±0.68
	B	9.7±0.66	10.76±0.76
	C	9.36±0.47	10.25±0.54
	D	9.6±0.6	10.48±0.64
	E	10±0.49	10.93±0.58
PCV %	A	25.8±1.8	32.2±2.27
	B	25.63±2.01	32.09±2.54
	C	25.5±1.25	30.7±1.43
	D	24.5±1.74	30.83±2.11
	E	25.16±2.17	31.5±2.76
MCH (Microgram or Pictogram)	A	38.64±2.03	36.4±1.21
	B	38.78±0.92	36.54±0.49
	C	39.5±1.09	37±0.5
	D	40.62±1.84	37.5±.084
	E	39.25±1.33	36.54±1.31
MCHC (%)	A	36.94±1.5	33.1±0.74
	B	37.98±1.27	33.63±0.39
	C	38.23±0.99	32.8±0.76
	D	39.23±1.35	34±0.5
	E	40.28±5.4	32.83±0.6
MCV (%)	A	104.4±1.69	110.9±1.58
	B	102.18±0.90	108.91±0.93
	C	103.2±0.83	110.1±0.80
	D	103.33±0.84	110.83±0.70
	E	98.83±4.84	105.33±5.08

RBC, red blood cell count; Hbg, haemoglobin concentration; PCV, packed cell volume; MHC, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentrations; MCV, mean corpuscular volume.

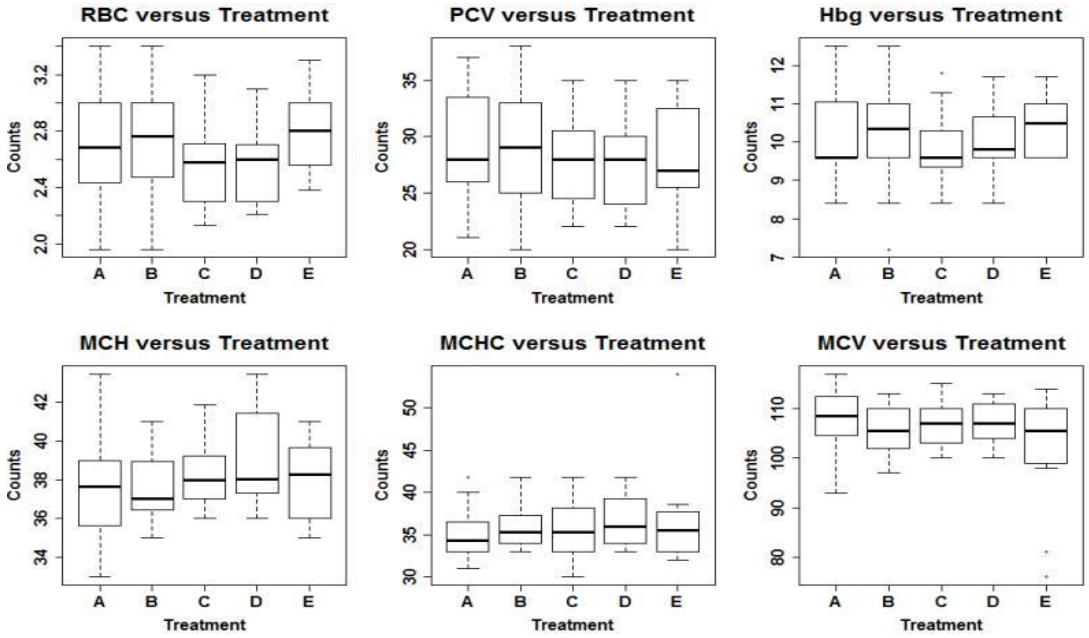

Figure 4. Box plots representing the age associated changes in blood parameters. Box plots depict counts of the respective haematological values namely RBC, PCV, Hbg, MCH, MCHC and MCV versus age in weeks.

values among treatment groups during a particular week. The 2% DE dietary inclusion did not significantly affect packed cell volume and hemoglobin count of the birds fed the experimental diets but their values fell within the normal range for chickens as defined in Wikivet (2012). This finding is in line with earlier findings by CCAC (1993). RBC count was non-significant (P>0.05) among the birds on the different treatments with and without DE supplementation. This contradicts findings by Adebiyi et al. (2010) who mentioned that inclusion of DE in diets of chicken had a significant increase in their RBC count. The total erythrocyte count, hemoglobin and packed cell volume increased with the advancement in the age in all treatment groups. The box plots in Figure 4 compare values of 6 hematological indices, namely RBC, PCV, Hbg, MCH, MCHC and MCV by age in weeks. The results depict that whereas MCV, MCH and MCHC values decreased with the advancement in age as evidenced in week 16 and week 36, respectively, there were no significant (P>0.05) differences in RBC, PCV, Hbg, MCH, MCHC and MCV among treatment groups. There were no significant differences (P>0.05) in results in a particular from birds on DE supplemented diets and their control groups; as well as in results from birds infected with *A. galli* and those that were not infected. The 2% DE inclusion did not significantly affect packed cell volume and hemoglobin count of the birds fed the experimental diets but their values fell within the normal range for chickens.

The results of this study are suggestive that inclusion or no inclusion of DE at 2% had no influence on blood parameters in layer chicken. The results also indicate that there were no cases of suspected anaemia in all treatment groups in this study including groups C, D and E which were infected with *A. galli*. These results, therefore, depict that the layer birds could still maintain the normal composition of their blood indices regardless of infection with *A. galli* or not regardless of dietary treatment. Literature reports on the characterization of the effect of dietary DE on blood indices in the chicken are scanty. In the present study however, no significant differences in blood parameters were found in birds infected with *A.galli* eggs and on their control groups after these two groups were fed on DE supplemented the diet (groups C and E, respectively). Similarly, no significant differences were observed in blood parameters in birds infected with *A. galli* eggs and on piperazine (a conventional drug for controlling *A. galli* in poultry in Uganda) that is, group D. On the other hand, no significant differences (P>0.05) in blood parameters were observed in birds non-infected groups on DE supplemented diet and non-infected birds not supplemented with DE in their diet (groups A and B, respectively). The inferential statistics on blood parameters are presented in Table 3.

Efficacy of DE on Ectoparasites In Chicken

The summary statistics of the 3 types of parasites are described in Table 4. The summary statistics show that the number of any of the 3 types of parasites exposed to treatment with DE and/or with ultravin at a particular point

Table 3. Inferential statistics on blood parameters.

Response Variable	Sum of Squares	Mean Sum of Squares	F-value	P-value
RBC	0.4767	0.1192	1.745	0.1483
PCV	31.10	7.780	0.8427	0.5022
Hbg	3.821	0.9553	1.122	0.3521
MCH	21.953	5.488	1.7046	0.1571
MCHC	28.59	7.150	1.3326	0.2651
MCV	108.2	27.05	1.570	0.4000

Table 4. Overall ectoparasites summary statistics (parasites per 20 cm^2).

Parasite Type	Mean Number	Minimum Number	Maximum Number	SEM
Fleas	3.42	0	14	0.12
Mites	12.49	0	30	16.31
Lice	4.39	0	22	0.16

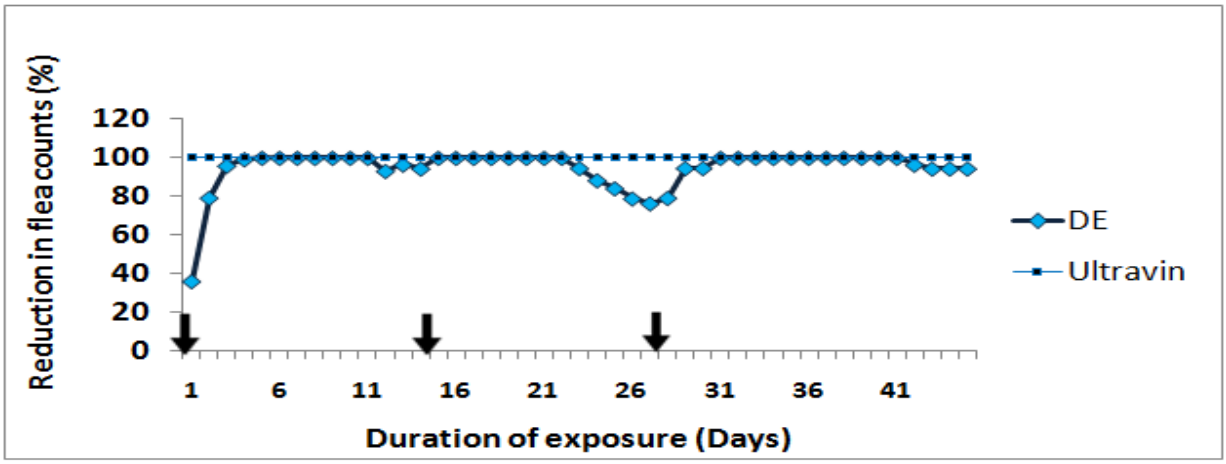

Figure 5. Reduction in flea numbers on hens treated with diatomaceous earth (DE).

in time reached a minimum of zero parasites per 20 cm^2. It is therefore evident from this finding that DE, each at a particular time point during the experiment, eliminated all the parasites of each type after application. Hence the efficacy of DE reached 100% at such time points. The statistics also depict that mites reached the highest proportions in terms of a number followed by lice then fleas.

Effect of DE on Fleas in Chicken

Between Day 4 and Day 11 after the first treatment, the reduction in flea counts was > 90%. On Day 14, the birds were again given another treatment with DE while another group was treated with Ultravin. The residual effect for DE was declining at this point until boosted on Day 14. This also explains the decline observed on day 21. The groups treated with Ultravin had a high advantage over those treated with DE since Ultravin has a higher residue effect as compared to DE (Figure 5).

Effect of DE on Mites in Chicken

Except on Day 3 where the mite counts were at 75% reduction, throughout the entire study reduction in mite counts in birds treated with DE was > 90%. The effect of DE on mite populations was observed at most 1 day after each DE application. This shows that DE has a gradual effect on mites. The efficacy of DE in controlling mites in chicken proved to be very high. This was evident from the 99% reduction in mite counts even 10 days after each DE application. The groups treated with Ultravin had a high advantage over those treated with DE since Ultravin has a higher residue effect as compared to DE (Figure 6).

Effect of DE on Lice in Chicken

The arrows indicate the particular days when treatments with DE or ultravin were carried out. The lice population reduced dramatically from Day 1 after treatment reaching

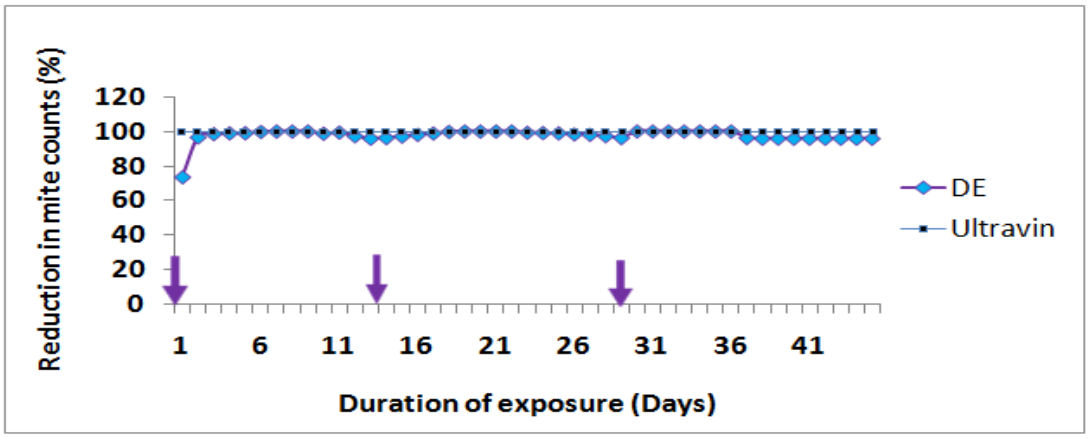

Figure 6. Reduction in mite counts on hens treated with DE.

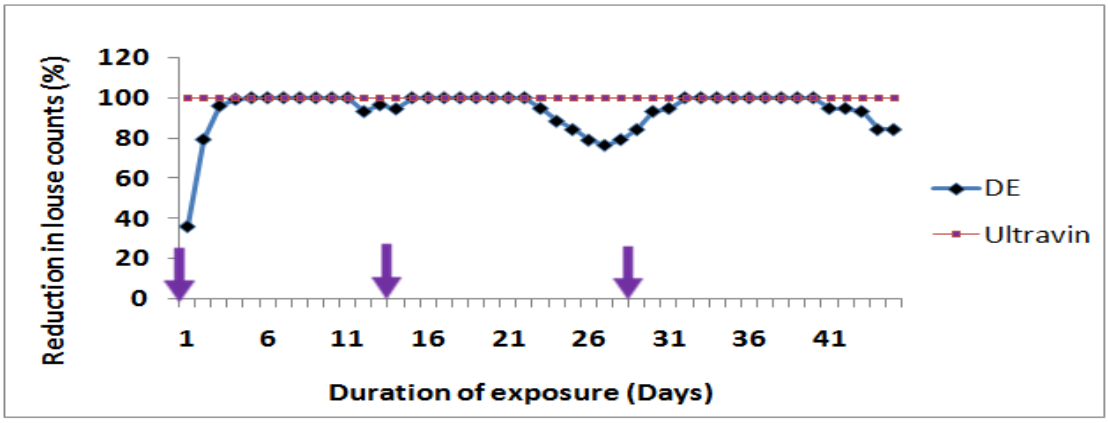

Figure 7. Reduction in louse counts on hens treated with DE.

99.3% and 100% reduction on Day 4 and Day 5, respectively. There was 100% reduction in lice counts between days 5 and 11; day 16 and 23; and between days 33 and 39. The effect of DE on lice populations was observed at most 1 day after each DE application. This shows that DE has a gradual effect on the parasites. The residual effect for DE was declining at this point until boosted on Day 14. This also explains the decline observed at day 12 to day 14. There was again a decline in the residual effect of DE again between days 22 to day 28, until this point when another application of DE was carried out on day 28. The groups treated with Ultravin had a high advantage over those treated with DE since Ultravin had a higher residue effect as compared to DE (Figure 7).

The Gradual Action of Diatomaceous Earth in Controlling Ectoparasites

In the present study, it was generally noted that the action of DE in controlling ectoparasites in chicken was gradual rather than instant. This might partly be associated with avoidance behaviour and/or repellent activity. These two phenomena were encountered in other studies on which were investigating the efficacy of desiccant dust on (Mohan and Fields, 2002; Faulde et al., 2006; Luz et al., 2012). It is believed that in these phenomena that members of the subclass acari, to which mites and many other arthropods belong, tend to avoid desiccant dust, hence taking longer to get exposure to the control agent. Diatomaceous earth is classified as desiccant dust since it is believed to cause death parasites mainly through desiccation and dehydration (Rigaux et al., 2001). The phenomena of avoidance behavior and repellent activity were also demonstrated in another study by Kilpinen and Steenberg (2016). In the present study, it took some hours before evidence of a reduction in numbers of parasites exposed to DE could be ascertained on the hens. Therefore, the elimination of targeted parasites using DE on hens tends to be more of

a gradual process. This demonstrates that the poultry red mite is reacting like other groups of arthropods such as beetles, cockroaches.

Residue Effect of DE on Chicken

The 100% threshold chosen to determine the duration of flea, mite and lice population control is in line with efficacy threshold levels accepted by veterinary regulatory agencies for the assessment of ectoparasite control remedies. At most two days after every treatment, there was a dramatic reduction in parasites population for all types of parasites under this study. There was close proximity of the untreated control groups of birds to the treated ones in all housing units. This might have led to a resurgence of the respective parasites, or this might have been as a result of no residual effect of DE at this particular point. This proximity was important in ascertaining any possible resurgence which aided in determining the residual effect of DE as compared to that of ultravin is a conventional poultry ectoparasiticide in Uganda. A faster parasite regrowth was observed in the treated groups a few days whenever there appeared to be total elimination of parasites. This might have been due to the movement of personnel and equipment between the 2 units, even if all efforts were made to ensure protocol adherence, and this would have greatly increased the risk of parasite cross-contamination to the treated groups. DE proved more effective in controlling mites in chicken as compared to lice and fleas. The action of DE in controlling ectoparasites in chicken was gradual rather than instant. Findings of the present study, therefore, depict that the dusting of hens with DE had a significant effect in reducing the population of ectoparasite under investigation. The findings also give enough evidence that whereas DE did not have a residue effect as high as that of ultravin, the efficacy, as well as the residual effect of DE, could still be improved at reduced application intervals, say a weekly application for better results.

CONCLUSION

Biweekly fecal analyses showed significant differences in FEC ($P<0.05$); and treatment by group ($P<0.05$) in birds infected with *A. galli* and treated with DE as compared to those infected and not supplemented with DE in their diet. This, therefore, gives evidence that dietary supplementation with DE had a significant effect on *A. galli* counts. The results of the present study, therefore, depict that inclusion of 2% DE in diets of layer chickens can help reduce their intestinal parasitic load.The 2% DE dietary inclusion did not significantly affect packed cell volume and hemoglobin count of the birds fed the experimental diets but their values fell within the normal range for chickens. The total erythrocyte count, hemoglobin, packed cell volume and MCV increased with the advancement in the age in all treatment groups. The MCH and MCHC values decreased gradually with the advancement in age. The results of the present study therefore depict that dietary inclusion of 2% diatomaceous earth does not have a significant effect on blood parameters in chicken. These results also depict that the layer birds could still maintain a normal composition of their blood indices regardless of infection with *A. galli* or not regardless of dietary treatment. At a particular time point during the experiments, DE eliminated all the parasites of each type (mites, lice or fleas) after application. Hence the efficacy of DE reached 100% at such time points. The groups treated with Ultravin had a high advantage over those treated with DE implying that the conventional drug Ultravin had a higher residue effect as compared to DE. Diatomaceous earth proved more effective in controlling mites in chicken as compared to lice and fleas. The action of DE in controlling ectoparasites in chicken was gradual rather than instant. This might have occurred as a result of avoidance behavior and/or repellent activity exhibited by arthropods exposed to DE. From the findings, this study concludes that DE has the potential to be used in an integrated approach to reduce or eliminate lice, mites and fleas in chicken. DE can also be used as an effective remedy in controlling intestinal parasites such as *A.galli* in chicken. Further studies comprising the use of DE at varying concentrations for longer periods should be conducted to evaluate the effect of dietary DE on internal parasite dynamics and on blood parameters in chicken. Additionally, organic approaches should be used to mitigate the effects associated with avoidance behaviour and repellent activity exhibited by arthropods when exposed to DE. This will greatly quicken its mode of action against ectoparasites.

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