Study the efficacy of neem leaves powder on *Ascaridia galli* in chickens with meat sensory evaluation

Shaker A. Seddiek[1], Magdy T. Gerges[2], Amany F. Zayed[3] and Ahmed A. Mobarak[4]

[1]Animal Health Research Institute (Benha Branch-Avian Diseases Department)
[2] Animal Health Research Institute (Benha Branch-Food Control Department)
[3]Animal Health Research Institute (Alexandria Branch-Food Control Department)
[4] Benha University, Faculty of Science (Botany Department)

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Abstract

Two hundreds and ten (210), one-day-old Baladi chicks (males and females) were divided into six equal groups and allocated in their cages (35 chicks per each). Group 1 (G1) was considered as a negative control (none infested and none treated). Group 2 (G2) were infested with 250 embryonated eggs of *A. galli* per bird at 1 day old using stomach tube and not treated (positive control). Group 3 (G3) fed ration supplemented with neem dry leaves powder (5 kg/ton from 1-84 consecutive days) and not inoculated with *A. galli* eggs. Group 4 (G4) fed ration supplemented with neem dry leaves powder (5 kg/ton from 56-84 and not inoculated with *A. galli* eggs. Group 5 (G5) fed ration supplemented with neem dry leaves powder (5 kg/ton from 1-84 days) and inoculated with 250 embryonated eggs of *A. galli* per bird at 1 day old. Group 6 (G6) fed ration supplemented with neem dry leaves powder (5 kg/ton from 56-84 days) and inoculated with 250 embryonated eggs of *A. galli* per bird at 1 day old. Chicks of all groups were fed balanced ration and weighed every two week till the age of 12 weeks, and the cumulative body weight-gain was calculated. The fecal egg count per gram feces (FEC) and adult worm burden of *A. galli* were counted on the 56, 63, 70, 77 and 84 days of age. Serum samples were prepared from chicken's wing veins blood of at the termination of experiment (84 day-old) for determination of ALT, AST and creatinine.

The results revealed that the Fecal egg count per gram feces (FEC) and worm burden in the small intestine were completely absent in the chickens of G5 when compared with G6 at 70 day of age. The body weight and cumulative body weight-gain were improved in G5 versus G6 at the end of experiment. These results clearly indicated that dry leaf powder of neem has an anthelmintic effect resulting in an improvement of the growth performance of chicks (body weight and body weight-gain). This was elucidated by the complete expulsion of *A. galli* worms from the intestine at 70 days of age and augmented gain in the body weight. ALT, AST and creatinine of G3, G4 and G5 were significantly lowered than that of G6 but there is no a significant change when compared with G1 (negative control). There were significant differences in the tenderness and overall acceptability of breast muscle. For tenderness, meat sample from G3, G4, G5 and G6 were moderately tender than those with G1 and G2 which were slightly tender. Similarly, for overall acceptability parameter, broiler breast muscle sample from G3, G4, G5 and G6 were higher than samples that of G1 and G2 which fed neem free diet. The aroma, juiciness, and overall flavor were not significantly affected by addition of neem to diet.

It could be concluded that the dry leaf powder of neem *(Azadirachta indica)* is considered as an anthelmintic alternative therapy and recommended in the control of ascaridiosis in poultry industry,

since it is effective, cheap and safe (not toxic) with significant differences in the tenderness and overall acceptability of breast muscle.

Key words: *Neem leaves, Ascardia galli, chicken, meat sensory, analysis*

Introduction

Ascaridiosis is still a cause of economic losses in modern poultry farming **(Permin and Raving, 2001)**. *Ascaridia galli (A. galli)* is the most prevalent helminth species in different poultry production systems **(Permin *et al.*, 1999; Abdelqader *et al.*, 2008 and Kaufmann *et al.*, 2011-b)**, it can cause extensive economic losses in weight, meat production, egg production and mortality of birds **(Kamal, 1989)** and may cause anorexia, weight loss, haemorrhages in the intestinal mucosa, obstruction of the intestinal lumen, altered hormone level and eventually death in a wide range of avian species **(Ikeme, 1971)**. Heavily infected chickens may show signs of droopiness, hemorrhages, and diarrhea **(Permin *et al.*, 1998 and Ruff, 1999)**. In severe infection there can be actual intestinal blockage, and death may occur **(Ramadan and Znada, 1991)**. Economic losses are usually associated with *A. galli* infections because of treatment cost, decreased feed efficiency and impaired performance **(Martín-Pacho *et al.*, 2005)**. The chief economic importance lies in the ability of *A. galli* to act as a vector for transmission of other infectious organisms like *Salmonella enterica* and *Escherichia coli* **(Chadfield *et al.*, 2001)**. The drug resistance by helminthes against chemotherapeutical products and associated risks of chemical residues in poultry products has drawn attention to alternative approaches. Different approaches were employed to sustainable control of *A. galli*, for example, utilization of genetic resistance **(Gauly *et al.*, 2002 and Abdelqader *et al.*, 2007 and Kaufmann *et al.*, 2011-a)**, biological control **(Braga *et al.*, 2011)** and use of plants with antiparasitic properties **(Siamba *et al.*, 2007)**.

The neem (*Azadirachta indica*) belonging to the *Meliaceae* family and a fast growing evergreen tree has a potential to provide medicinal and nutritive value to broilers **(Schmutterer, 1990)** and has been found to exert some beneficial effects in chicks **(Chand, 1987)**. Various parts of the tree have been reported to contain chemicals like azadiractin, nimbin, nimbindin and quercetin **(Makeri *et al.*, 2007)** which have antimicrobial, antihelminth, antioxidant, antifungal, insecticidal, antiprotozoa and spermicidal properties **(Elangovan *et al.*, 2000)**. Neem leave powder treatment caused cent percent (100%) evacuation of the worms on 56th day post treatment **(Patra et *al.*, 2010)**. Also, neem leaves extract has anthelmintic activity and could be used in poultry ascariasis **(Begum *et al.*, 2010)**, beneficial effect on body weight gain and food conversion efficiency as well as significantly increase in liver and gizzard weights **(Chakravarty and Prasad, 1991 and Zanu *et al.*, 2011)**.

Neem leaf meal (NLM) can be a good herbal medicine for broiler production aside its nutritional importance. Carcass characteristics were not significantly influenced by the NLM although mild bitter taste was sensed in high inclusion level (2.5%) when the meat was not salted **(Bonsu *et al.*, 2012)**.

Hence, the present study has been proposed to evaluate the anthelmintic efficacy of neem against *Ascaridia galli* infestation in chickens, assessing the effect of neem on sensory acceptipility.

Material and Methods

Birds

Two hundreds and ten (210), one-day-old Baladi chicks (native breed) chicks were purchased from a commercial hatchery. The commercial starter ration was obtained from the Islamic centre company (not less than 21% crude protein, 2.7% crude fat, 2950 Kcal/kg ration metabolizing energy and not more than 2.7% crude fibers) and the water were offered

ad libitum along the experimental period. All chicks were vaccinated against Newcastle disease virus (NDV), Infectious bronchitis (IB), Infectius bursal disease (IBD), Infectious laryngetracheaitis (ILT), avian influenza and pox diseases as followed by the routine vaccination program. The birds were kept under the routine management practice and optimum hygienic environment until slaughter.

Preparation of *A. galli* embryonated eggs

Adult *A. galli* female worms were collected from naturally infected chickens according to standard parasitological techniques **(Permin *et al.*, 1997 and Abdelqader *et al.* .(2008 ,**The uteri of graved female worms were dissected and *A. galli* eggs were recovered. The eggs were embryonated by incubation in 0.1% (w/v) potassium dichromate solution at room temperature (26-30C°) for 18-21 days to permit the eggs to embryonate. Larvae viability was assessed by observing the larvae spontaneous movement inside the egg after increasing the surrounding temperature. Infective *A. galli* L3 eggs were stored at 4°C until use. On the day of infection another viability test was performed. Only larvae that were well developed, motile and not hatched were counted as viable and infective. Number of eggs/ml suspension was determined using a hemocytometer slide. Birds were inoculated intra crop (using stomach tube) with 250 embryonated eggs of *A. galli* at 1 day old according to **Permin *et al.* (1997)**.

Preparation of dry leaf powder of neem

The dry leaf powder of neem *(Azadirachta indica)* was prepared according to **Parta *et al.*, (2010)**. Leaves of neem plant were collected, washed thoroughly with clean water for several times followed by double distilled water. Leaves were chopped into small pieces and then dried under shade at a well-ventilated place until they became crispy while retaining its greenish coloration. Then the leaves were ground to powder form and were stored at -4°C until use. The dry leaf neem powder was offered to the birds after its mixing with the ration in a dose of 5 k gm /ton **(Ogbuewu *et al.*, 2010 and Nayka *et al.*, 2013)** from 1 day till the termination of the experiment (84 days old) as prophylaxis or from 56 to 84 days of age as treatment.

Experimental design

Chicks' total of 210 one-day-old Baladi (native breed) were divided into six equal groups (each of 35 birds) and allocated in their separate units in metal wire-floored clean batteries after arranging them using the ranking method **(Gardiner and Wehr, 1950**) as follow:

1- Group 1 (G1) was considered as a negative control (not infested and not treated).
2- Group 2 (G2) were infested with 250 embryonated eggs of *A. galli* per bird at 1 day old using stomach tube and not treated (positive control).
3- Group 3 (G3) fed ration supplemented with neem dry leaves powder (5 kg/ton from 1-84 consecutive days) and not inoculated with *A. galli* eggs.
4- Group 4 (G4) fed ration supplemented with neem dry leaves powder (5 kg/ton from 56-84 and not inoculated with *A. galli* eggs.
5- Group 5 (G5) fed ration supplemented with neem dry leaves powder (5 kg/ton from 1-84 consecutive days) and inoculated with 250 embryonated eggs of *A. galli* per bird at 1 day old.
6- Group 6(G6) fed ration supplemented with neem dry leaves powder (5 kg/ton from 56-84 consecutive days) and inoculated with 250 embryonated eggs of *A. galli* per bird at 1 day old.

Experimental design

Group	Design	No. of chicks	Inoculation with 250 embryonated *A. galli* eggs at 1-day-old	Treatment	
				Treatment with neem dry leaves powder (5 kg / ton) in ration from 1 to 84 days of age	Treatment with neem dry leaves powder (5 kg / ton) in ration from 56 to 84 days
Group (1)	Negative control	35	Non	Non	Non
Group (2)	Positive Control	35	+	Non	Non
Group (3)	Experimental	35	Non	+	Non
Group (4)	Experimental	35	Non	Non	+
Group (5)	Experimental	35	+	+	Non
Group (6)	Experimental	35	+	Non	+

Experimental infection	
Time of infection	At 1-day-old
Infective dose per chick	250 embryonated *A. galli* eggs
Route of infection	Directly inoculated intra-crop by the stomach tube

Sampling		
Parameters	No. of samples per group	Time of sampling
Fecal egg count (FEC)	All birds	At age of 56, 63, 70, 77 and 84 days
Worm burden	5	At age of 56, 63, 70, 77 and 84 days
Growth performance	All birds	At one-day old and then every two weeks interval till 12 weeks
Biochemical	5	At termination of experiment (84 day-old)
Carcass characteristics and sensory analysis	10	At termination of experiment (84 day-old)

Two to eight weeks post-infestation (PI), the clinical sings and post-mortum manifestations were described from diseased, dead or sacrificed birds according to **Alcorn (2001)** and the detected pathological lesions were recorded. The fecal egg count per gram feces (FEC) from each group were counted on the 56^{th}, 63^{rd}, 70^{th}, 77^{th} and 84^{th} day of age to evaluate the degree of infestation using modified McMaster technique **(MAFF, 1986)** according to the following equation:-

$$\text{Calculation of FEC} = \frac{\text{Total no. of eggs counted}}{\text{No. of counting chambers}} \text{ X } 200$$

Decrease in % of FEC describes the percent of reduced FEC between positive control and treated groups. It was calculated on days 56, 63, 70, 77 and 84 of age according to **Yazwinski *et al.* (2003)** from the following formula:

$$\text{FEC reduction \%} = \frac{\text{FEC positive control - FEC treated}}{\text{FEC positive control}} \text{ X } 100$$

Evaluation of efficacy of treatment

Efficacy of treatment was determined based on worm count. Adult worms of *A. galli* were counted in 5 birds at 56th, 63rd, 70th, 77th and 84th day of age according to **Permin and Hansen (1998)**. After decapitation, the entire gastrointestinal tract including the oesophagus was collected from each bird. Ggastrointestinal tract was opened in a longitudinal section and the contents carefully washed through a sieve for collection of mature parasite. Efficacy of the drug on the basis of post mortem worm count was evaluated as the method of **Soulsby (1982)**:

$$\text{AE (\%)} = \frac{\text{A - B}}{\text{A}} \text{ X } 100$$

Where:
AE = Anthelmintic efficacy
A = Number of parasites in infected untreated (control) birds
B = Number of parasites in treated birds

The chicks were weighed every two weeks and the cumulative body weight-gain was calculated. Blood samples were collected from wing veins of chickens at the termination of experiment (84 day-old). The serum was prepared and kept in deep freezer at -20 ºC until analysed. The biochemical parameters of the blood were determined in the serum by using commercial kits for Alanine transaminase (ALT), Aspartate transaminase (AST) and creatinine.

Carcass Studies

At the termination of experiment (84 days old), ten birds from each group were randomly selected and deprived of food but not water for 14 hour perior to slaughter and eviscerated for organ weight determination and detection the effect of neem on carcass characteristics and sensory analysis of chicken breast meat. Weight of organs and various parts were taken and expressed as percentage of the final live weight of the birds **(Odunsi *et al.*, 2009)**. Chicken breast meat samples were steamed separately with 60 ml of water to a temperature of 100°C for 10 minutes to produce cooked meat samples. Equal quantities of salt (that is 2.0 g of granulated iodated salt) were added to chicken breast meat samples to produce salted cooked breast meat. The temperature-time treatment was monitored using a Digital Cooking Thermometer with Probe and Timer with 0°C to 200°C range. Cooked chicken breast meat samples were sliced into cubes of uniform sizes and served warm to 8 trained sensory panelists. The trained sensory panelist evaluated meat color intensity, aroma, juiciness, texture, flavor, abnormal flavor and overall acceptability of meat. The sensory qualities of the meat were evaluated with 7 points category scale (1-7) according to **(Sendecor and Cochran, 1987)** as follows:

Item / Point	Yellowish Meat Color Intensity	Juiciness	Tenderness	Flavor Intensity	Abnormal Flavor Intensity	Overall Acceptability
1	Extremely light	Extremely dry	Extremely tender	Extremely weak	Extremely weak	Extremely unacceptable
2	very light	very dry	very tender	very weak	very weak	very unacceptable
3	slightly light	slightly dry	slightly tender	slightly weak	slightly weak	slightly unacceptable
4	moderate intense	moderate juicy	moderate tough	moderate intense	moderate intense	moderate acceptable
5	slightly intense	slightly juicy	slightly tough	slightly intense	slightly intense	slightly acceptable
6	very intense	very juicy	very tough	very intense	very intense	very acceptable
7	extremely intense	extremely juicy	extremely tough	extremely intense	extremely intense	extremely acceptable

Results and Discussion

A. galli is an intestinal worm and the chickens under three months of age are mostly susceptible to this worm. Control of gastro-intestinal nematodes especially of *A. galli* is mainly based on regular anthelmintic treatment **(Walter *et al.,* 1987)**. The administration of piperazine and herbal anthelmintic as neem and pineapple leaves in the birds caused removal of *A. galli* and as the treatment continued, the disturbances in the small intestine were lowered or removed which ultimately restored the capacity of small intestinal activities **(Parta *et al.,* 2010)**. In this context, investigations on indigenous medical plants (neem) might contribute to develop effective and low cost herbal anthelmintics.

Clinical sings and findings were noted in chicks experimentally infested with 250 *A. galli* embryonated eggs. Observed sings were depression, dullness, and loss of appetite, ruffled feathers, emaciation and unthriftness after two weeks PI. The post-mortem examination of dead and sacrificed chicks showed emaciation and anaemic carcasses. Moreover, the intestines were inflamed indicating enteritis, thickening and granulomatous changes in the intestinal mucosa. Adult worms were detected in the small intestine at the 55th day PI. Similar results are reported by **Soulsby (1982)**.

Table (1) shows the mean number of fecal egg count per gram feces (FEC) in all groups at 56, 63, 70, 77 and 84 days of age. The mean of FEC in chickens fed ration supplemented with neem dry leaves powder from 1-84 consecutive days and inoculated with *A. galli* eggs (G5) was significantly decreased from 63 to 70 days PI and become zero at 77 days PI when compared with the positive control (G2) and G6. Moreover, the mean of FEC in chickens inoculated with *A. galli* eggs and fed ration supplemented with neem dry leaves powder from 56-84 consecutive days (G6) was significantly decreased at the same periods PI when compared with the positive control (G2) but did not become zero even at 84 days PI ($P \leq 0.05$). The reduction percent of FEC in G5 was significantly increased than that of G6 at 63 days of age (89.68% vs 36.84%), at 70 days of age (97.50% vs 67.48%), at 77 days of age (100% vs 82.23%) and at termination of the experiment (84 days of age) (100% vs 90.69%). In the present study, the mean of FEC in chickens fed ration supplemented with neem dry leaves powder from 1-84 consecutive days and inoculated with *A. galli* eggs (G5) was significantly

decreased from 63 to 70 days PI and become zero at 77 days PI when compared with the positive control (G2) and G6. This is attributed to inhibitory effects of neem on nematode growth, parasite enzymes or plasma membrane pumps, and it might also interfere with metabolic pathways. Similar results are reported by **Patra *et al.* (2010)**. Moreover, the mean of FEC in chickens inoculated with *A. galli* eggs and fed ration supplemented with neem dry leaves powder from 56-84 days (G6) and was significantly decreased at the same periods PI when compared with the positive control (G2) but did not become zero even at 84 days PI. The FEC reduction percent of G5 was significantly increased than that of G6 at 63, 70, 77 and 84 day PI (89.68%, 97.50%, 100% and 100% versus 36.84%, 67.48%, 82.23% and 90.69%, respectively). So, the prophylactic use of neem dry leaves powder (from 1 to 84 days of age) was better than the treatment use (from 56 to 84 days of age). The present results are in agreement with similar observations of **Mostofa *et al.*, (1995); Hossain *et al.*, (1996) and Patra *et al.* (2010)**. Also, oral administration of neem leaves extract (20 mg/ml) was effective against *A. galli* infection in broiler chickens after 21 days PT without showing any toxic effect compared to piperazine and levamisole **(Begum *et al.*, 2010)**.

Table (2) shows the mean number of collected *A. galli* worms (worm burden) in all groups at 56, 63, 70, 77 and 84 days PI. The mean number of worm burden in chickens of (G5) was significantly decreased from 63 to 84 days PI and the worms completely expelled at 77 days PI when compared to G2 and G6. Meanwhile, *A. galli* worms in chickens intestines of G6 did not expel even at 84 days PI. Anthelmintic efficacy (AE) percent of G5 was significantly increased when compared with G6 at 63 days of age (85.12 % vs 34.82 %), at 70 days of age (96.15% vs 53.93%), at 77 days of age (100% vs 73.19%) and at termination of the experiment (84 days of age) (100% vs 86.65%). Post mortem parasites count gave clear indicator on neem dry leaves powder anthelmintic activity. The neem dry leaves powder has a little significant activity against *A. galli* at short period treatment (56-84 day old). However, the long period of treatment (1-84 day-old) was more effective. The mean number of worm burden in chickens of G5 was significantly decreased from 63 to 70 days PI and the worms completely expelled (absent) at 77 days PI when compared with G2 and G6. Meanwhile, the *A. galli* worms in chickens' intestines of G6 did not expel even at 84 days PI. At 84 days of age, the anthelmintic efficacy (AE) percent of G5 was excellent when compared with that of G6 (100 % vs 86.65 %, respectively). The present results agreed with findings of **Begum *et al.* (2010) and Patra *et al.* (2010)**. Neem and pineapple leaves powder treatment caused evacuation of the worms on the 28th and 56th day PT, respectively **(Patra *et al.*, 2010)**. In the present study, AE of neem leaves powder in baladi chickens (G6) was 34.82%, 53.93%, 73.19% and 86.65% at 63, 70, 77 and 84 days PI. However, **Patra *et al.* (2010)** found the AE of neem leaves powder in broilers was 39.53%, 64.44%, 70%, 84.44, 100% at 7, 14, 21, 28 and 56 days PT. Neem leave powder treatment in G5 caused cent percent evacuation of the worms at 77 days PI. However, **(Patra et *al.*, 2010)** applied neem leave powder treatment in broilers caused cent percent evacuation of the worms on 56th day PT. Round worms in fecal samples (little numbers) and in gastrointestinal tract (profuse numbers) of broiler chickens fed the control diet whereas those birds fed the diet that contained the neem leave meal (NLM) had no such worms **(Bonsu *et al.*, 2012)**. Some of the birds infected with coccidiosis and worms were isolated and given the diet that contained NLM at 2.5% and these birds recovered although minute traces of the coccidian were detected from laboratory analysis. NLM could also serve as herbal medicine in poultry production as a way of reducing the cost of medication **(Bonsu *et al.*, 2012)**.

Table (3) shows the mean body weight and cumulative body weight-gain in the chickens. Body weight in G5 was significantly improved when compared to G6 at the time from 14 day-old till termination of the experiment (2-12

weeks-old). However, body weight and body gain of G6 was significantly improved when compared with G2. At 84 days-old, body weight and body gains of G5 was significantly improved than that of G6 (body weight: 763.6±2.06 vs 610.5±3.23 and body gain: 734.9±1.98 vs 586.3±1.50, respectively). Meanwhile, body weight of G3 was markedly increased (improved) when compared to G4 (966.9±2.07 vs 878.5±2.14). Similarly, cumulative body gain of G3 was 938.7±2.09 vs 850.9±2.23 in G4. The improvement percent of G1 (100%) body weight-gain was better than G5 (90.92%) and G6 (72.55%). However, at 84 days of age, G3 and G4 were improved in their body gains (116.13% & 105.27%, respectively) at $P \leq 0.05$. Body weight in G5 was significantly improved when compared with that of G6 at the time from 14 day-old till termination of the experiment (2-12 weeks-old). Similar results are reported by **Castanon (2007)** and **Zanu *et al.* (2011)** in broilers. Meanwhile, body weight of G3 was markedly increased (improved) when compared with that of G4 (966.9±2.07 vs 878.5±2.14). Similarly, the cumulative body weight-gain of G3 was 938.7±2.09 vs 850.9±2.23 in G4. Those results are similar to the results obtained by many authors on broilers who used the phytogenic additives in the chickens' diets leading to stimulation of the digestibility that can in turn improve feed efficiency **(Lee *et al.*, 2003 and Onyimonyi *et al.*, 2009)**. Also, **Nayaka *et al.* (2013)** found that the chickens fed ration supplemented with neem (8gm/kg feed) for 42 days showed an improvement in growth performance and hematological parameters. The mortality recorded did not exhibit significant difference between control and neem leaves diets and the overall health of dietary neem leaves-treated birds appeared to be excellent, which was shown by the relative weights of major organs remaining stable. The neem (*Azadirachta indica*) has beneficial effect on body weight gain and food conversion efficiency **(Elangovan *et al.*, 2000)**. Administration of neem dry leaves powder from 1-84 days of age would ameliorate the negative impact of *A. galli* on chickens rather than 56-84 days PT. So, this anthelmintic property represents a promising alternative solution to chemical treatments. Rabbits fed diets containing 10, 20 and 30% raw neem seed cake resulted in similar performance till 20% and reversed at 30% level **(Fuzinimi *et al.*, 1989)**. Similarly, the neem seed meal at 10 and 20% inclusion level resulted in superior and similar performance than control, respectively but 30% level showed poor performance **(Salawu *et al.*, 1994)**. However, raw neem seed meal (20%) for 12 week period adversely affected the performance in rabbits **(Vasanthkumar *et al.*, 1995)** and in layers **(Gowda *et al.*, 1998 and Verma *et al.*, 1998)**.

Alanine transaminase (ALT), Aspartate transaminase (AST) and creatinine levels of G5 had no significance than that of G6 (27.68±0.29 vs 29.96±0.28, 62.13±0.35 vs 63.38±0.27and 0.072±0.002 vs 0.083±0.003, respectively) but there is no a significant change when compared with G1 (negative control) (Table 4). There is no significant increase in ALT, AST and creatinine levels in chickens of G3 to G6 compared to G1. This indicates the absence of the toxic effect of the neem leaves powder on the liver and kidney cells of the birds. This may be due to application of small dose of dry leaves neem powder (0.5%) although it is administered for a long period (84 days). Similar results are reported by **Ali *et al.* (2011)** who reported oral administration of neem leaves extract (20 mg/ml) was effective against *A. galli* infection in broiler chickens after 21 days PT without showing any toxic effect. This may be attributed to the small dose in this study (5gm/kg feed) (0.5 %) similarly to the results reported by **Nayaka *et al.* (2013)** who found that the chickens fed ration supplemented with neem (8gm/kg feed) (0.8%) for 42 days showed an improvement in growth performance and hematological parameters without toxic effect. These results ensure the *A. indica* leaf powder has not toxic effects while it has a hepatoprotective activity. The present results are supported by **Aruwayo and Maigandi (2013)** who proved the neem seed cake in the ruminants was safe and not toxic. Neem kernel cake inclusion up to 20% level

did not interfere with the liver and renal functions of the buffalo calves **(Gangopadhyay *et al.*, 1981)**, lambs **(Aruwayo *et al.*, 2010)**, male kids for 180 days **(Baxi, 1976)** and sheep **(Vajjan *et al.*, 1982 and Merck Veterinary Manual of Biochemical Reference Ranges, 2008)**. However, the toxicity and degenerative changes in liver and kidney were reported in birds fed 20 % water extracts of neem fruits **(Singh *et al.*, 1985)** or neem kernel meal **(Gowda *et al.*, 2000)**.

The control diet stimulated production of heavier carcass and lighter gizzard and kidneys compared to those of chickens fed neem diets, but the differences did not attain significance. However neem diets significantly increased the weight of liver. Organ weights were not significantly influenced by the neem in G3, G4, G5 and G6 indicating that inclusion at the present level (5 kg/ton) may not cause any detrimental effect on the organs (Table 5). These results are agree with the results obtained by **Adeyemo and Akanmu (2013)**, they reported that the values for liver, heart, gizzard and lungs did not differ significantly by the neem, this could be linked to the absence of anti-nutritional factors in the diets, because higher physiological activities by these organs is triggered by the presence of anti-nutritional factors and their concomitant effect. The percentage weights of liver and gizzard obtained with inclusion of neem was in agreement with work by **Esonu *et al.* (2006)** on layers who recorded the highest liver and gizzard weight at 5% dietary levels of neem leave meal. Also, These results are similar to the results obtained by **Awaad and Zouelfeker (2001)**, they reported that the mortality recorded did not exhibit significant difference between control and neem treated birds, the overall health of dietary neem treated birds appeared to be excellent, which was shown by the relative weights of major organs remaining stable.

Fat deposition had a positive correlation with energy concentration of the diet. Fat isolated from the lower abdomen was significantly higher in the control birds (G1) when compared with that of birds fed the neem for 84 days (G3 & G5) but did not differ significantly from G4 and G6 which fed neem for 28 days (Table 5). Similar results were reported by **Bonsu *et al.* (2012)**, where they found that fat isolated from chicken fed NLM in the diets was significantly higher in the control birds when compared with that of birds fed the NLM 2%. Also, **Uko and Kamalu (2008)** reported that raw and autoclaved neem diets reduced abdominal fat deposition contrary to the effect of control.

Visual appraisal of cooked chicken breast meat showed that breast meat from chicken fed neem-free diets (G1 and G2) had a pale colour for both the skin and breast meat while meat from chicken fed neem-based diets had yellowish skin and breast meat. The variation in skin and meat colour could be attributed to the carotenes in the neem-based diets. There was however no appreciable difference in colour intensity as the duration of neem usage in the diet was increased. Also, there were no significant differences in the aroma, texture and juiciness of cooked meat samples (Table 6). A few sniffs at cooked chicken breast meat samples did not show any differences in aroma of the treatments. They all had the characteristic "chicken smell". Cooked chicken breast meat did not show any differences for tenderness or toughness when they were bitten through. Likewise, cooked meat did not show any differences in exudation of juices or differences in dryness when they were lightly chewed. Fed ration supplemented with neem dry leaves powder 5 kg/ton from 1-84 (G3) consecutive days did not alter the aroma, texture and juiciness of chicken meat (Table 6). Similar results were reported by **Bonsu *et al.* (2012)** who studied in 8 week trial the effect of diets containing neem leaf meal on meat sensory characters of broilers and reported that the inclusion of neem at rates of 1.0%, 2.0% and 2.5% did not alter the aroma, texture and juiciness of chicken meat.

Sensory evaluation showed no differences among all cooked chicken breast meat samples once they were salted (Table 7). These results are similar to the results obtained by **Vasanthkumar *et al.* (1995 and 1999)** they

reported that neem bitters present in NSKC did not impart any bitter taste or odour and comparable scores and no-untoward taste were reported in meat of rabbits fed processed NSKC **(Sushil kumar *et al.*, 1989 and Khan, 1994)**. However, unsalted cooked meat from chickens fed neem-based diets for 84 days (G3 and G5) were found to be mildly bitter. The high duration of inclusion of neem might have resulted in the deposition of azadiratin in muscle fibers resulting in mild bitter taste. This indicated the cumulative effect of residual neem bitters. Similar results were reported by **Bonsu *et al.* (2012)**.

Generally, all the chicken breast meat samples from the fed neem birds were acceptable for consumption. The mildly bitter taste of chicken breast meat from chickens fed neem-based diet for long duration was masked and unnoticed if meat is adequately salted during cooking. Similar results were reported by **Bonsu *et al.* (2012)**. Also, **Vasanthkumar *et al.* (1999)** reported that NSKC could be used as a wholesome vegetable protein supplement up to 10% in the diet of rabbits without any adverse effect on commercial carcass traits. Many authors made attempts to debitterize the neem cake for improving its palatability in feeding of animals **(Gowda and sastry, 2000)** in buffalo calves, **(Katiyar *et al.*, 1993)** in adult cattle and buffaloes, and **(Nagalakshmi, 1993)** in broiler chicks. So, further investigation including chemical treatment would be attempted to avoid slight bitter taste of neem.

Conclusion

In conclusion, treating *A. galli* infected birds with neem dry leaves powder could partially substitute chemotherapeutical treatment. Use of neem dry leaves powder in feed from 1-84 days (prophylactic) might be not toxic to birds and better than the treatment use (56-84 days) in addition to good meat sensory analysis but the neem leaves would require chemical treatment before use to avoid slight bitterness in the taste and flavor. However, further investigations are needed to determine active principles, mode of action and chemical treatment to avoid its bitterness.

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Table 1. Fecal egg count per gram feces (FEC) in chicks infested with *A. galli* invasive eggs and fed ration supplemented with neem dry leaves powder. (Mean ± SE, n=12) (no. x 1000)

Group Age (day)	G1	G2	G3	G4	G5	G6	LSD
65	0.00^{b} ±0.00	91.75^{a} ±1.46	0.00^{b} ±0.00	0.00^{b} ±0.00	30.40^{b} ±1.42	90.50^{a} ±1.39	30.40^{*}
63	0.00^{d} ±0.00	100.92^{a} ±1.22	0.00^{d} ±0.00	0.00^{d} ±0.00	10.42^{c} ±0.89	64.75^{b} ±1.74	10.42^{*}
Reduction% at 63 days PI	----	0 %	----	----	89.68 %	36.84 %	------
70	0.00^{c} ±0.00	113.25^{a} ±1.90	0.00^{b} ±0.00	0.00^{b} ±0.00	2.83^{c} ±0.39	36.83^{b} ±1.53	33.92^{*}
Reduction% at 70 days PI	----	0 %	----	----	97.50 %	67.48 %	------
77	0.00^{c} ±0.00	116.33^{a} ±1.88	0.00^{c} ±0.00	0.00^{c} ±0.00	0.00^{c} ±0.00	21.83^{b} ±1.04	21.83^{*}
Reduction% at 77 days PI	----	0 %	----	----	100 %	82.23 %	------
84	0.00^{c} ±0.00	119.00^{a} ±1.13	0.00^{c} ±0.00	0.00^{c} ±0.00	0.00^{c} ±0.00	11.08^{b} ±0.84	11.08^{*}
Reduction% at 84 days PI	----	0 %	----	----	100 %	90.69 %	------

-G1 = Chicks not infested with *A. galli* and not treated (-Ve Control)
-G2 = Chicks infested with *A. galli* and not treated (+Ve Control)
-G3 = fed ration supplemented with neem dry leaves powder (5 kg/ton) from 1-84 days and not inoculated with *A. galli* eggs
-G4 = fed ration supplemented with neem dry leaves powder from 56-84 and not inoculated with *A. galli* eggs.
-G5 = fed ration supplemented with neem dry leaves powder from 1-84 days and inoculated with *A. galli* eggs.
-G6 = fed ration supplemented with neem dry leaves powder from 56-84 consecutive days and inoculated with *A. galli* eggs.
-Means with different alphabetical superscripts in the same row are significantly different.

Table 2. Worm burden in chickens infested with *A. galli* invasive eggs and fed ration supplemented with neem dry leaves powder. (Mean ± SE, n=10)

Group Age (day)	G1	G2	G3	G4	G5	G6	LSD
56	0.00^{c} ±0.00	52.90^{a} ±1.59	0.00^{c} ±0.00	0.00^{c} ±0.00	15.00^{b} ±1.03	52.80^{a} ±1.50	15.00^{*}
63	0.00^{d} ±0.00	53.10^{a} ±1.90	0.00^{d} ±0.00	0.00^{d} ±0.00	7.90^{c} ±0.67	34.61^{b} ±1.24	7.90^{*}
AE % at 63 days PI	----	0 %	----	----	85.12 %	34.82 %	------
70	0.00^{c} ±0.00	51.88^{a} ±1.80	0.00^{c} ±0.00	0.00^{c} ±0.00	2.00^{c} ±0.42	23.90^{b} ±1.27	21.90^{*}
AE % at 70 days PI	----	0 %	----	----	96.15 %	53.93 %	------
77	0.00^{c} ±0.00	52.60^{a} ±1.32	0.00^{c} ±0.00	0.00^{c} ±0.00	0.00^{c} ±0.00	14.10^{b} ±0.97	14.10^{*}
AE % at 77 days PI	----	0 %	----	----	100 %	73.19 %	------
84	0.00^{c} ±0.00	48.67^{a} ±1.26	0.00^{c} ±0.00	0.00^{c} ±0.00	0.00^{c} ±0.00	6.50^{b} ±0.48	6.50^{*}
AE % at 84 days PI	----	0 %	----	----	100 %	86.65 %	------

-G1 = Chicks not infested with *A. galli* and not treated (-Ve Control)
-G2 = Chicks infested with *A. galli* and not treated (+Ve Control)
-G3 = fed ration supplemented with neem dry leaves powder (5 kg/ton) from 1-84 days and not inoculated with *A. galli* eggs
-G4 = fed ration supplemented with neem dry leaves powder from 56-84 and not inoculated with *A. galli* eggs.
-G5 = fed ration supplemented with neem dry leaves powder from 1-84 days and inoculated with *A. galli* eggs.
-G6 = fed ration supplemented with neem dry leaves powder from 56-84 consecutive days and inoculated with *A. galli* eggs.
-Means with different alphabetical superscripts in the same row are significantly different. AE = Anthelmintic efficacy.

Table 3. Mean body weight and cumulative body weight-gain in chickens infested with *A. galli* invasive eggs and fed ration supplemented with neem dry leaves powder. (Mean ± SE, n=35)

Group / Age (Days)	Parameters	G1	G2	G3	G4	G5	G6	LSD
1 day old	Body weight	27.89^{a} ±0.49	28.81^{a} ±0.45	27.60^{a} ±0.48	28.66^{a} ±0.43	27.51^{a} ±0.49	27.71^{a} ±0.37	NS
14 Day old	Body weight	91.23^{a} ±0.79	75.17^{b} ±1.11	90.17^{a} ±1.03	90.54^{a} ±0.84	88.86^{a} ±0.96	72.97^{b} ±1.17	13.6^{*}
28 day old	Body weight	251.2^{b} ±4.19	164.1^{d} ±1.03	270.9^{a} ±1.09	252.9^{b} ±0.92	226.7^{c} ±1.60	167.5^{d} ±1.22	18.7^{*}
42 day old	Body weight	344.9^{b} ±1.49	244.8^{d} ±1.46	371.6^{a} ±1.38	345.9^{b} ±1.34	310.6^{c} ±1.31	241.7^{d} ±3.86	25.7^{*}
56 day old	Body weight	533.5^{b} ±3.39	368.1^{d} ±1.55	560.7^{a} ±2.78	533.6^{b} ±1.27	460.5^{c} ±1.89	364.6^{d} ±1.47	27.1^{*}
70 day old	Body weight	759.1^{b} ±1.77	406.5^{e} ±1.02	791.9^{a} ±1.57	763.6^{b} ±2.05	600.7^{c} ±1.74	490.9^{d} ±1.76	28.3^{*}
84 day old	Body weight	836.5^{c} ±1.58	505.3^{f} ±1.30	966.9^{a} ±2.07	878.5^{b} ±2.14	763.6^{d} ±2.06	610.5^{e} ±3.23	42.0^{*}
	Cumulative body weight -gain	808.3^{c} ±1.81	478.0^{f} ±1.49	938.7^{a} ±2.09	850.9^{b} ±2.23	734.9^{d} ±1.98	586.3^{e} ±1.50	42.6^{*}
Improvement rate (%) of body weight-gain		100 %	59.14%	116.13%	105.27%	90.92%	72.55%	-----

-G1 = Chicks not infested with *A. galli* and not treated (-Ve Control).
-G2 = Chicks infested with *A. galli* and not treated (+Ve Control).
-G3 = Chicks fed ration supplemented with neem dry leaves powder (5 kg/ton) from 1-84 days and not inoculated with *A. galli* eggs.
-G4 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 and not inoculated with *A. galli* eggs.
-G5 = Chicks fed ration supplemented with neem dry leaves powder from 1-84 days and inoculated with *A. galli* eggs- .
-G6 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 consecutive days and inoculated with *A. galli* eggs .
Means with different alphabetical superscripts in the same row are significantly different.

Table 4. Some biochemical parameters at 84 days in chickens infested with *A. galli* invasive eggs and fed ration supple mented with neem dry leaves powder. (Mean ± SE, n=10)

Group / Parameter	G1	G2	G3	G4	G5	G6	LSD
ALT (U/L)	28.17^{bc} ± 0.27	32.67^{a} ± 0.42	27.29^{c} ±0.39	28.34^{b} ±0.23	27.68^{bc} ±0.29	29.96^{b} ±0.28	0.92^{*}
AST (U/L)	61.52^{c} ± 0.36	71.43^{a} ± 0.31	61.14^{c} ±0.37	62.13^{c} ±0.47	62.13^{c} ±0.35	63.38^{b} ±0.27	1.25^{*}
Creatinine (mg/dl)	0.071^{c} ± 0.009	0.092^{a} ± 0.009	0.070^{c} ±0.002	0.082^{b} ±0.003	0.072^{c} ±0.002	0.083^{b} ±0.003	0.010^{*}

-G1 = Chicks not infested with *A. galli* and not treated (-Ve Control).
-G2 = Chicks infested with *A. galli* and not treated (+Ve Control).
-G3 = Chicks fed ration supplemented with neem dry leaves powder (5 kg/ton) from 1-84 days and not inoculated with *A. galli* eggs
-.G4 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 and not inoculated with *A. galli* eggs.
-G5 = Chicks fed ration supplemented with neem dry leaves powder from 1-84 days and inoculated with *A. galli* eggs.
-G6 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 consecutive days and inoculated with *A. galli* eggs.
-Means with different alphabetical superscripts in the same row are significantly different.

Table (5). Effect of neem on carcass characteristics of chickens (weight of some organs)

Group / Weight organ (%)	Treatments					
	G1	G2	G3	G4	G5	G6
Liver weight %	1.98	1.94	2.48	2.31	2.34	2.35
Heart weight %	0.48	0.46	0.48	0.44	0.42	0. 44
Kidney weight %	0.65	0.66	0.82	0.74	0.78	0.76
Gizzard weight %	1.56	1.36	1.87	1.53	1.76	1.64
Abdominal Fat %	3.13	2.93	2.19	2.50	2.43	2.65

Table (6). Sensory attributes of unsalted cooked chicken meat (n=10)

Descriptor	Treatments						LSD
	G1	G2	G3	G4	G5	G6	
Appearance	6.00 ^{ab} ± 0.21	5.70 ^{b} ± 0.22	6.40 ^{a} ± 0.16	5.70 ^{b} ± 0.21	6.00 ^{ab} ± 0.22	5.80 ^{ab} ± 0.25	0.70*
Aroma	4.90 ^{a} ± 0.23	5.00 ^{a} ± 0.26	5.30 ^{a} ± 0.21	4.90 ^{a} ± 0.23	4.90 ^{a} ± 0.23	4.60 ^{a} ± 0.31	NS
Juiciness	5.10 ^{bc} ± 0.23	5.30 ^{a} ± 0.21	5.10 ^{bc} ± 0.23	5.00 ^{bc} ± 0.21	5.00 ^{bc} ± 0.21	4.50 ^{c} ± 0.27	0.80*
Tenderness	4.60 ^{bc} ± 0.22	4.40 ^{c} ± 0.22	5.40 ^{a} ± 0.22	4.90 ^{abc} ± 0.18	5.10 ^{ab} ± 0.23	5.10 ^{ab} ± 0.18	0.70*
Flavor	5.10 ^{a} ± 0.23	5.20 ^{a} ± 0.36	5.00 ^{a} ± 0.21	5.00 ^{a} ± 0.21	5.30 ^{a} ± 0.21	4.90 ^{a} ± 0.18	NS
Abnormal flavor	1.60 ^{a} ± 0.16	1.70 ^{a} ± 0.15	1.40 ^{a} ± 0.16	1.80 ^{a} ± 0.13	1.70 ^{a} ± 0.15	1.70 ^{a} ± 0.15	NS
Overall acceptability	5.90 ^{a} ± 0.28	5.70 ^{a} ± 0.26	6.00 ^{a} ± 0.21	5.90 ^{a} ± 0.23	6.10 ^{a} ± 0.18	6.20 ^{a} ± 0.25	NS

-G1 = Chicks not infested with *A. galli* and not treated (-Ve Control).
-G2 = Chicks infested with *A. galli* and not treated (+Ve Control).
-G3 = Chicks fed ration supplemented with neem dry leaves powder (5 kg/ton) from 1-84 days and not inoculated with *A. galli* eggs
-G4 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 and not inoculated with *A. galli* eggs.
-G5 = Chicks fed ration supplemented with neem dry leaves powder from 1-84 days and inoculated with *A. galli* eggs.
-G6 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 consecutive days and inoculated with *A. galli* egg.
-Means with different alphabetical superscripts in the same row are significantly different.

Table (7). Sensory attributes of salted cooked chicken meat (n=10)

Descriptor	Treatments						LSD
	G1	G2	G3	G4	G5	G6	
Appearance	6.18 ^{c} ± 0.02	6.10 ^{d} ± 0.02	6.34 ^{a} ± 0.03	6.23 ^{bc} ± 0.02	6.27 ^{b} ± 0.02	6.15 ^{cd} ± 0.01	0.07*
Aroma	5.35 ^{c} ± 0.04	5.06 ^{c} ± 0.04	5.61 ^{a} ± 0.04	5.43 ^{b} ± 0.03	5.63 ^{a} ± 0.02	5.54 ^{a} ± 0.03	0.11*
Juiciness	5.14 ^{b} ± 0.03	4.89 ^{d} ± 0.02	5.34 ^{a} ± 0.03	5.01 ^{c} ± 0.02	5.11 ^{b} ± 0.02	5.03 ^{c} ± 0.04	0.09*
Tenderness	4.98 ^{ab} ± 0.16	4.58 ^{b} ± 0.26	5.63 ^{a} ± 0.30	5.45 ^{a} ± 0.32	5.42 ^{a} ± 0.26	5.40 ^{a} ± 0.27	0.82*
Flavor	5.64 ^{a} ± 0.33	4.50 ^{b} ± 0.27	5.54 ^{a} ± 0.22	5.62 ^{a} ± 0.26	5.73 ^{a} ± 0.21	5.32 ^{a} ± 0.30	0.82*
Abnormal flavor	1.32 ^{b} ± 0.03	1.19 ^{c} ± 0.02	1.30 ^{b} ± 0.02	1.53 ^{a} ± 0.03	1.34 ^{b} ± 0.05	1.57 ^{a} ± 0.05	0.11*
Overall acceptability	6.22 ^{c} ± 0.03	6.11 ^{d} ± 0.03	6.60 ^{a} ± 0.05	6.39 ^{b} ± 0.04	6.54 ^{a} ± 0.03	6.35 ^{b} ± 0.04	0.12*

-G1 = Chicks not infested with *A. galli* and not treated (-Ve Control).
-G2 = Chicks infested with *A. galli* and not treated (+Ve Control).
-G3 = Chicks fed ration supplemented with neem dry leaves powder (5 kg/ton) from 1-84 days and not inoculated with *A. galli* eggs.
-G4 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 and not inoculated with *A. galli* eggs.
-G5 = Chicks fed ration supplemented with neem dry leaves powder from 1-84 days and inoculated with *A. galli* eggs.
-G6 = Chicks fed ration supplemented with neem dry leaves powder from 56-84 consecutive days and inoculated with *A. galli* egg.
-Means with different alphabetical superscripts in the same row are significantly different.

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