Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2019) 43: 206-211
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doi:10.3906/vet-1807-39

Evaluation of the in vivo efficacy of pumpkin (*Cucurbita pepo*) seeds against gastrointestinal helminths of chickens

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Received: 09.07.2018 • **Accepted/Published Online:** 04.03.2019 • **Final Version:** 04.04.2019

Abstract: The present study was conducted to evaluate the in vivo efficacy of pumpkin seeds as an alternative natural anthelmintic for chickens. Ninety Philippine Jolo native chickens of mixed sexes, aged 4–5 months and weighing 1–2 kg, were randomly distributed into three treatment groups with 30 chickens per group. Control group A was fed basic mash feed, group B received feed mixed with ground pumpkin seeds (2 g/bird per day), and group C received mebendazole-medicated feed (30 mg/kg body weight). Fifteen randomly selected chickens from each group were euthanized and necropsied before treatment, and the remaining fifteen in each group were euthanized and necropsied at 3 days after the end of the treatment. Gastrointestinal worm and fecal egg counts were determined. Three genera of helminths were identified from necropsy: *Ascaridia* spp., *Heterakis* spp., and *Raillietina* spp. Results indicate that compared to mebendazole, pumpkin seed was moderately effective in reducing worm counts of *Ascaridia* spp. and *Raillietina* spp., marginally active in reducing worm counts of *Heterakis* spp., and moderately effective in reducing egg output of the worms. The results suggest that pumpkin seed has the potential to be used as an alternative anthelmintic for chickens.

Key words: Anthelmintic, chicken, helminths, pumpkin seed

1. Introduction

Gastrointestinal helminths are a major problem in animal health, and control is heavily dependent on the use of synthetic anthelmintic drugs. In chickens, the major pathogenic intestinal parasites are *Ascaridia galli*, *Heterakis gallinarum*, and *Capillaria* spp., infections of which result in listlessness, emaciation, and diarrhea (1). Unfortunately, due to indiscriminate and excessive use of chemotherapeutics, anthelmintic resistance in helminth populations has now become a global problem (2), and the amount of evidence verifying incidences of anthelmintic resistance continues to rise (3). Resistance to all the major families of broad-spectrum (benzimidazole, levamisole, avermectin, milbemycin) and some narrow-spectrum anthelmintics, such as closantel, has been reported (4).

Over the last decade, a rising number of problems with modern veterinary drugs, such as development of modern veterinary drug resistance, meat and environmental residues, and the growing demand for alternative sources of anthelmintics, have been addressed by increased efforts at evaluating medicinal plants such as garlic, ginger, and papaya for their anthelmintic potential as they may be utilized for sustainable control of helminth infections with low environmental impact and low toxicity to both animals and man (2,5,6).

Among the plants studied as possible anthelmintics are representatives of the family Cucurbitaceae, which, in traditional medicine, are administered as antiparasitic agents. Pumpkin (*Cucurbita moschata*, or *C. maxima*, or *C. pepo*) stands out as it is used worldwide as a natural vermifuge, and its seeds are known to have anthelmintic properties when used in humans and animals (6,7). In the ethnoveterinary medicine literature, pumpkin seeds have been reported to be extensively used in traditional medicine for their midlevel anthelmintic activity in both humans and livestock, owing to the secondary metabolite cucurbitin (8). However, reports on the efficacy of pumpkin seeds against helminths affecting poultry are still limited despite their ethnopharmacological applications in the treatment of parasitical diseases in humans and livestock (2,7).

Although studies using herbal dewormers for poultry have been conducted and published, there are no reports in the literature concerning the sole use of pumpkin seeds on chickens. The aim of this study is to evaluate the in vivo efficacy of pumpkin seeds as a dewormer in naturally infected chickens.

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2. Materials and methods

All procedures performed on the birds in this study had been reviewed and approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of the Philippines Los Baños, in accordance to applicable institutional, local, and national guidelines.

2.1. Native chickens

Ninety Philippine Jolo native chickens of mixed sexes, aged 4–5 months old with around 1–2 kg body weight (BW), and none of which were previously treated with anthelmintics, were acquired from the Bureau of Animal Industry-National Swine and Poultry Research and Development Center (BAI-NSPRDC) in Tiaong, Quezon. The chickens were transported to Victoria (Laguna Province), housed by groups in traditional bamboo chicken houses, and allowed about 60 days to acclimatize to the environment and to acquire natural infection prior to treatment, during which time they were fed corn mash and provided tap water ad libitum. Mebendazole (Parafre®, Peakhealth Inc., Quezon City, Philippines) was acquired from the supplier closest to the study site.

2.2. Preparation of pumpkin seed

Three whole *Cucurbita pepo* pumpkins (one for each day of treatment) weighing 1.5–2.0 kg were acquired from Puentespina Farms, Davao City. Raw pumpkin seeds were coarsely ground (2–4 mm) every treatment day and fed (2 g seeds/bird per day) first before offering commercial corn mash (UNIFEEDS® Chicken Layer Mash; crude protein 17% min., metabolizable energy NLT). The dosage of 2 g pumpkin seeds/bird per day follows the results of Feitosa et al. (6), where the dosage of 1g/kg BW was most effective in controlling ostrich nematodes.

2.3. Experimental design

The experimental design followed the guidelines of Groot et al. (9). The 90 birds were conveniently randomized into one of three treatment groups, each with 30 birds:

A. Control (no-treatment) group. Group A birds remained on mash throughout the study.

B. Pumpkin seed-treated group (2 g/bird per day). Group B birds received ground pumpkin seeds and mash from Day 0 to Day 2.

C. Mebendazole-treated group (30 mg/kg BW). On Day 0, the feed for Group C was replaced with mebendazole-medicated feed until Day 2.

2.4. Determination of gastrointestinal helminths and fecal egg counts

The parent flock was subjected to fecalysis, and worm counts and fecal egg counts (FECs) of the birds obtained before treatment and 3 days after treatment were also determined. Speciation was done by stereomicroscopic examination and based on morphologies described by Permin and Hansen (10). The procedures for necropsy, parasite collection, and parasite counting were taken from the guidelines by the World Association for the Advancement of Veterinary Parasitology with a few adaptations: (a) cervical dislocation was used for euthanasia, (b) residue was preserved with 70% alcohol, and (c) the same number of samples were collected before treatment and 3 days after treatment. The fecal analyses were done using the McMaster method (11).

2.5. Data analysis

The variance between both worm count and egg count data were calculated by one-way ANOVA with $\alpha = 0.05$, and significant differences of the treatment groups' means were analyzed by Student's t-test using IBM SPSS Statistics Version 24.0 (IBM Corp., Armonk, NY, USA).

Percentage effectiveness of the treatments against each species (based on both worm counts and FECs) were calculated using equations derived from Henderson and Tilton (12):

$$\%\,Efficacy = \frac{N2-N1}{N2} \times 100$$

where N1 = geometric mean number of worms/eggs in treated birds and N2 = geometric mean number of worms/eggs in controls (13); and

$$\%\,Efficacy = \frac{a-b}{b} \times 100$$

where a = arithmetic mean number of worms/eggs in controls and b = arithmetic mean number of worms/eggs in treated birds (13).

Fecal egg count reduction (FECR) was calculated using the Henderson-Tilton equation (12):

$$FECR\,(\%) = 100\left(1 - \frac{T2}{T1} \times \frac{C1}{C2}\right)$$

where T, C, 1, and 2 refer to treated, control, pretreatment, and posttreatment mean egg counts, respectively.

Percentage effectiveness and percent FECR were regarded according to Kassai's classification of claims for anthelmintic efficacy (14).

3. Results

The parent flock (n = 20) was subjected to fecal analysis via the McMaster method to verify history of infection. Out of 20 chickens, 16 were positive for *Ascaridia* sp. and/or *Heterakis* sp. ova, and EPG counts ranged from 0 to 2600 with a mean of 830 ± 867.2.

Worms from all necropsied chickens were collected and quantified. Three genera of gastrointestinal helminths were identified: *Ascaridia* spp., *Heterakis* spp., and *Raillietina* spp. (Figures 1A–1C). Pre- and posttreatment arithmetic (AM) and geometric mean (GM) worm counts of all three helminths are shown in Tables 1–3.

Percentage effectiveness of both pumpkin seed and mebendazole treatments were calculated by the % Efficacy equations adopted from Kassai (14) and Yazwinski et al. (13). In reducing worm load, mebendazole was 100% effective against all three species by both equations. From arithmetic means, pumpkin seed demonstrated 80.90% effectivity against *A. galli*, 75.78% effectivity against *H. gallinarum*, and 88.10% effectivity against *Raillietina* spp. From geometric means, pumpkin seed demonstrated 83.21% effectivity against *A. galli*, 79.94% effectivity against *H. gallinarum*, and 73.43% effectivity against *Raillietina* spp. Finally, for corrected percentage effectiveness, pumpkin seed demonstrated 63.53% effectivity against *A. galli*, 88.46% effectivity against *H. gallinarum*, and 88.10% effectivity against *Raillietina* spp.

Comparing pre- and posttreatment mean worm count values (Table 4), pumpkin seed treatment showed a significant decrease in worm load (P = 0.008). Comparing pre- and posttreatment EPG mean values (Table 4), pumpkin seed treatment did not demonstrate significant decrease in egg load (P = 0.305); nevertheless, individual pre- and posttreatment values for pumpkin seed EPG revealed that birds infected were reduced by 50%.

Since *Ascaridia* spp. and *Heterakis* spp. ova resemble one another morphologically (15), they were tallied together for FECs. Pre- and posttreatment arithmetic and geometric mean EPG values per treatment group are presented in Table 5. There was 0.00% reduction observed in the control group, while the arithmetic means of pumpkin seed and mebendazole treatment yielded 80.02% and 100% reduction in egg output, respectively. Likewise, the geometric means of pumpkin seed and mebendazole treatment yielded 98.66% and 100% reduction, respectively.

4. Discussion

Parasites recovered from necropsy of Philippine Jolo native chickens revealed three species of helminths, namely *Ascaridia galli*, *Heterakis gallinarum*, and *Raillietina* spp. From the study of Rabbi et al. (16) on helminth infections in different types of poultry, the three aforementioned species are the most prevalent causes of helminthiases of backyard poultry, which they stated are more susceptible to infection than layers and broilers. Correspondingly, Pattison et al. (17) stated that *A. galli* and *H. gallinarum* are the two most common helminths infecting backyard chickens in tropical countries.

Worm counts and FECs are usually asymmetric in distribution, and efficacy calculations in anthelmintic research require estimates of the central tendency of the nematode populations. Hence, both arithmetic and geometric means were calculated to assess the efficacies of the employed treatments.

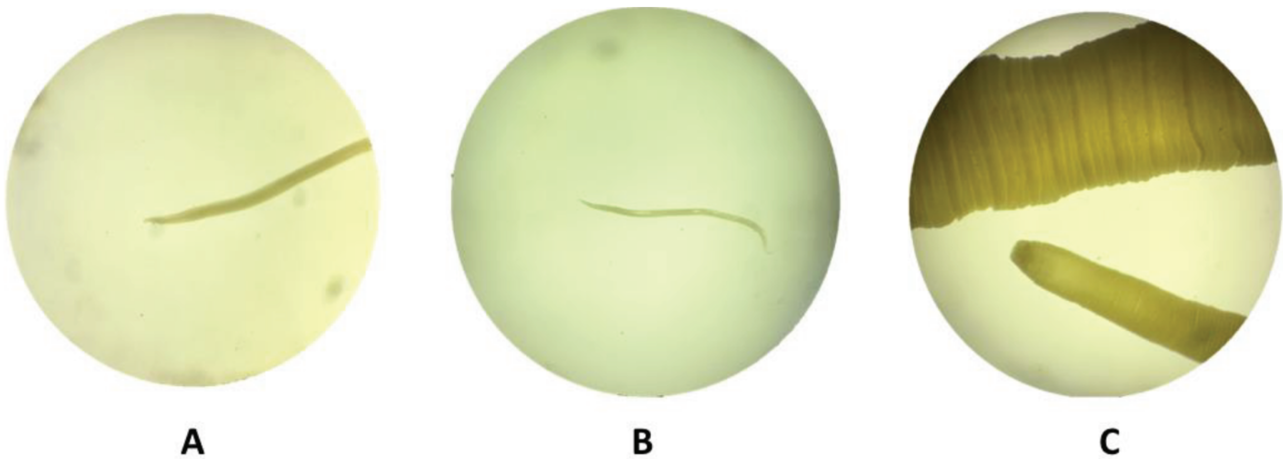

Figure 1. Stereomicroscopic images of (A) *Ascaridia galli*, (B) *Heterakis gallinarum*, and (C) *Raillietina* spp. collected from necropsy, fixed magnification.

Table 1. Pre- and posttreatment arithmetic and geometric mean (±SD) worm counts of *Ascaridia galli* in Philippine Jolo native chickens given pumpkin seeds and mebendazole.

		Control	Pumpkin seeds	Mebendazole
Worm count (arithmetic mean)	Pretreatment	5.6 ± 9.20	2.9 ± 3.26	4.4 ± 5.31
	Posttreatment	5.9 ± 7.40	1.1 ± 2.53	0.0 ± 0.00
Worm count (geometric mean)	Pretreatment	2.0 ± 9.20	1.6 ± 3.26	2.0 ± 5.31
	Posttreatment	5.9 ± 7.40	0.5 ± 2.53	0.0 ± 0.00

Table 2. Pre- and posttreatment arithmetic and geometric mean (±SD) worm counts of *Heterakis gallinarum* in Philippine Jolo native chickens given pumpkin seeds and mebendazole.

		Control	Pumpkin seeds	Mebendazole
Worm count (arithmetic mean)	Pretreatment	10.8 ± 11.13	22.7 ± 18.31	7.1 ± 8.06
	Posttreatment	29.7 ± 27.60	7.2 ± 15.03	0.0 ± 0.00
Worm count (geometric mean)	Pretreatment	2.6 ± 11.13	12.2 ± 18.31	2.6 ± 8.06
	Posttreatment	29.7 ± 27.60	2.2 ± 15.03	0.0 ± 0.00

Table 3. Pre- and posttreatment arithmetic and geometric mean (±SD) worm counts of *Raillietina* spp. in Philippine Jolo native chickens given pumpkin seeds and mebendazole.

		Control	Pumpkin seeds	Mebendazole
Worm count (arithmetic mean)	Pretreatment	0.5 ± 0.81	0.5 ± 18.31	0.8 ± 1.05
	Posttreatment	2.8 ± 1.53	0.3 ± 0.60	0.0 ± 0.00
Worm count (geometric mean)	Pretreatment	0.5 ± 0.81	0.3 ± 0.72	0.6 ± 1.05
	Posttreatment	2.8 ± 1.53	0.2 ± 0.60	0.0 ± 0.00

Table 4. Significant differences of pre- and posttreatment worm counts and egg counts in Philippine Jolo native chickens given pumpkin seeds and mebendazole.

Pairwise comparison	P-value	
	Worm count	Egg count
Pre-Control vs. Post-Control	0.030093085	0.008781226
Pre-Pumpkin Seed vs. Post-Pumpkin Seed	0.00807091	0.304713162
Pre-Mebendazole vs. Post-Mebendazole	0.005478963	0.000033729
Post-Control vs. Post-Pumpkin Seed	0.017879251	0.000249741
Post-Control vs. Post-Mebendazole	0.000531333	0.000106
Post-Pumpkin Seed vs. Post-Mebendazole	0.066643946	0.02660758

Table 5. Pre- and posttreatment arithmetic and geometric mean (±SD) fecal egg counts in Philippine Jolo native chickens given pumpkin seeds and mebendazole.

		Control	Pumpkin seeds	Mebendazole
Egg count (arithmetic mean)	Pretreatment	5.6 ± 9.20	2.9 ± 3.26	4.4 ± 5.31
	Posttreatment	5.9 ± 7.40	1.1 ± 2.53	0.0 ± 0.00
Egg count (geometric mean)	Pretreatment	2.0 ± 9.20	1.6 ± 3.26	2.0 ± 5.31
	Posttreatment	5.9 ± 7.40	0.5 ± 2.53	0.0 ± 0.00

Mebendazole, as a positive control, generated 100% reduction in all worm counts. Results showed that pumpkin seed treatment was moderately effective against *A. galli*, marginally active against *H. gallinarum*, and marginally active to moderately effective against *Raillietina* spp.

Pumpkin seed treatment showed a significant decrease in worm load (P = 0.008). Additionally, though pumpkin seed compared to mebendazole is not significant (P = 0.067), the difference is fairly close to the significance cutoff. This suggests that pumpkin seed treatment decreases worm load, likely due to the presence of cucurbitin, the active constituent responsible for the anthelmintic effects of the seeds. Besides damaging the tegument through proteolysis, cucurbitin also paralyzes worms by interfering with energy generation, uncoupling the oxidative phosphorylation process and causing a worm-expelling effect by detaching the parasites from the intestinal wall of the host (18). This agrees with the results reported by Sharma et al. (19), Lans et al. (20), Marie-Magdeleine et al. (21), and Mythili and Kavitha (22).

In asymmetric distributions, the geometric mean approximates the median, and in the case of FECs, this mean is a better estimate of the count of the "average" bird in the flock. However, in FECR tests, the appropriate estimates to use are the arithmetic means of pre- and posttreatment egg counts as they are derived from the sum of individual worm egg counts and are directly proportional to the total egg output of the group (23).

While the difference of mebendazole compared to pumpkin seed treatment is significant (P = 0.027), it does not disprove the anthelmintic effect of pumpkin seeds on helminth ova. This is reinforced by the works of Mahmoud et al. (24) and Al-Bayati et al. (25), where their results showed pumpkin seed remedies not only damaged but also reduced parasite eggs. Reduction in EPG counts could also be attributed to the death and expulsion of worms.

The experimental design employed a dosage of 2 g pumpkin seeds/bird per day. At the aforesaid dosage, efficacy of pumpkin seeds in reducing worm load and egg output ranged from insufficiently active (less than 80%) to highly effective (greater than 98%). In 2017, Ozaraga and Ozaraga (26) studied the efficacy of ipil-ipil (*Leucaena leucocephala*), betel nut (*Areca catechu*), and papaya (*Carica papaya*) seeds in reducing EPG values in Darag native chickens. Highest %FECR was observed in birds given a dosage of 6 g/kg BW. From their results, they concluded that the efficacy of different ethnobotanicals is dependent on dosage. This agrees with the dosage of 6 g pumpkin seeds/bird suggested by Groot et al. (9).

The above results suggest that pumpkin seed treatment was moderately effective in reducing worm counts of *A. galli* and *Raillietina* spp. and was marginally active in reducing worm counts of *Heterakis gallinarum*. In reducing egg output of the flock as a whole, it was moderately effective, with percentage effectiveness of 80.02%, and in reducing the egg output of the average bird in the flock, it was highly effective, with percentage effectiveness of 98.66%. Overall, the anthelmintic effect of pumpkin seed is in agreement with its effect on other helminths. Thus, it may find ethnomedicinal use in the prevention and control of helminthic infections in poultry.

Based on the results of the study, the following are hereby recommended: (a) studies to determine the optimal dose of pumpkin seeds; (b) toxicity studies to investigate the safety profile of pumpkin seeds; (c) preformulation and formulation studies to develop stable, effective, and acceptable preparations for pumpkin seeds; (d) palatability/consumption studies on different preparations; (e) production performance studies on pumpkin seed-treated birds; (f) efficacy studies on pumpkin seed treatment for other poultry species; (g) a cost-benefit analysis of using pumpkin seed treatment over synthetic drugs; and, lastly, (h) studies to determine the anthelmintic efficacy of *Cucurbita maxima* seeds, as it is the predominant cultivar of pumpkin in the Philippines.

Acknowledgments
The authors would like to extend their gratitude to Antonio Beltran, Bernadette Idanan, and Regie Mansano for their assistance in both the husbandry and the processing of the experimental birds.

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