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Comparative efficacy of ivermectin and *Nigella sativa* against helminths in Aseel chickens (*Gallus gallus domesticus*)

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Research Paper

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Abstract

In this study, we evaluated the *in vivo* comparative efficacy of ivermectin and *Nigella sativa* extract against helminths in Aseel chickens, and the effects of helminths on blood parameters before and after treatment in Aseel chickens. Forty naturally infected adult Aseel chickens were randomly divided into four groups ($n = 10$ each): group A (ivermectin at 300 µg/kg); group B (*N. sativa* extract at 200 mg/kg); group C (ivermectin at 300 µg/kg + *N. sativa* extract at 200 mg/kg); group D was kept as a positive control to monitor time-related changes. On day 28 post treatment, the mean percentages of faecal egg-count reduction (FECR %) in groups A, B and C were recorded as 93.58, 88.09 and 100.00%, respectively. Further data analysis showed significantly higher efficacy in group C (100 ± 0.00%) than in groups A and B ($P < 0.001$). Highly significant ($P < 0.001$) improvements in mean percentage values of packed cell volume (PCV %) were recorded in groups A and C on days 14 and 28 post treatment. Meanwhile, the improvements in mean values of haemoglobin (Hb) concentration in groups A, B and C were highly significant ($P < 0.001$) when compared to that of group D on day 28 post treatment. The synergistic combination of ivermectin and *N. sativa* extract possessed greater efficacy than either ivermectin or *N. sativa* extract used alone. Furthermore, both PCV % and Hb concentration values gradually increased in the treated groups compared to the control group, in which PCV % and Hb concentration gradually decreased throughout the trial.

Introduction

The Aseel (*Gallus gallus domesticus*) is the native, tallest and largest chicken breed of Pakistan, and is found especially in Punjab and Sindh provinces (Babar *et al*., 2012). Amid the indigenous chicken breeds of the Indo-Pak subcontinent, the Aseel is the most popular and significant breed, and is a major source of income for rural people. The Aseel is an excellent meat producer and is the ancestor of the White Cornish and Plymouth Rock breeds, both of which are parents of the present-day commercial broiler (Platt, 1925; Dohner, 2001; Jatoi *et al*., 2015). However, in spite of its enormous production potential and significance, the Aseel breed has been overlooked by researchers.

Helminthiasis has been regarded as an important factor related to the poor production of rural chickens. Parasites are a problem wherever poultry are raised, whether in large commercial systems or in rural backyard farming, and can result in huge economic losses (Ruff, 1999). In backyard poultry farming, indigenous chickens are reared in a free-range scavenging system, which poses a relatively higher risk of parasitic infections, in particular gastrointestinal helminth infections, occurring (Aini, 1990).

Ivermectin (a semi-synthetic anthelmintic) belongs to the family of drugs known as avermectins (Fisher *et al*., 1989). These compounds are reported to have at least 25 times more potency compared to the preceding generation of anthelmintics, and exhibit a broad spectrum of activity against nematodes and arthropod parasites of domestic animals (Shoop *et al*., 1995). To date, very few studies have been conducted to elucidate the efficacy of ivermectin against helminths in various avian species (Okaeme, 1988; Shahadat *et al*., 2008; Ibarra-Velarde *et al*., 2011; Khayatnouri *et al*., 2011; Mirhadi *et al*., 2011; Islam *et al*., 2012; Zia-ur-Rehman *et al*., 2014), and evidence of resistance of gastrointestinal helminths against ivermectin in poultry has not been reported. However, due to the mounting development of anthelmintic resistance, limited availability and high cost of commercial anthelmintics, there is increasing interest in

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the ethno-veterinary approach to screening the anthelmintic properties of traditionally used medicinal plants (Kaplan, 2004; Mali and Mehta, 2008), giving impetus to the search for new compounds from plant origin as alternative methods of helminth control (Veerakumari, 2015). *Nigella sativa* (Linn.) is an indigenous herbaceous plant that is commonly known as the fennel flower plant and belongs to the buttercup or *Ranunculaceae* family (Ahmad *et al.*, 2013). *Nigella sativa* seeds are reported to contain many chemical compounds and active ingredients, including nigellone, thymoquinone and essential oil (Akhtar, 1988). The anthelmintic efficacy of *N. sativa* has been reported in some earlier studies (Akhtar and Javed, 1991; Kailani *et al.*, 1995; Al-Shaibani *et al.*, 2008).

Considering the need for efficient and cost-effective treatment of helminth infections that are causing significant production losses in backyard poultry (Ruff, 1999; Katoch *et al.*, 2012), and the increasing anthelmintic resistance worldwide (Waller *et al.*, 1996; Jabbar *et al.*, 2006), it would be of great value to scrutinize the synergistic efficacy of modern and safer anthelmintic drugs and important medicinal plants possessing broad-spectrum anthelmintic properties. The current study was therefore conducted to (1) evaluate the comparative efficacy of ivermectin and *N. sativa* in adult Aseel chickens, and (2) assess the effects of helminths on percentage of packed cell volume (PCV %) and haemoglobin (Hb) concentration (g/dl) in naturally infected adult Aseel chickens before and after treatment with ivermectin and *N. sativa* extract.

Materials and methods

Study animals

In order to obtain a pool of naturally infected birds, a total of 228 adult Aseel chickens (irrespective of sex) were randomly subjected to coprological examination. The chickens were obtained from local farmers in our vicinity. Backyard poultry are usually reared in free-range or semi-intensive rearing systems, and poultry farmers keep small flocks of 5 to 15–20 birds.

From these 228 Aseel chickens, forty adults were randomly selected and divided into four groups, A, B, C and D ($n = 10$ each). Birds in all four groups were naturally infected with various helminth species: *Ascaridia galli*, *Heterakis gallinarum* and *Syngamus trachea* (pure or mixed infection).

For further experimental procedures, the selected birds were maintained at the poultry demonstration farm of the Shaheed Benazir Bhutto University of Veterinary and Animal Sciences (SBBUVAS), Sakrand, Pakistan under semi-intensive rearing conditions, i.e. birds were provided with sheds and a large fenced area for ranging. At night they were usually shut in the sheds. Moreover, all birds were offered *ad libitum* water and feed of broken wheat, rice and barley grains. Birds in all four groups were separated from each other by chicken mesh wire partitions. The night before faecal sample collection, birds of each group were individually confined to a specified floor area covered with brown chick paper in order to minimize the chances of mixing of faeces.

Collection of faecal samples

Fresh faecal samples were collected early in the morning (pre-feeding) and transferred to wide-mouth screw-capped plastic jars containing 10% formalin solution for preservation. Then the samples were brought to the Department of Veterinary Parasitology, SBBUVAS, for coprological examination.

Coprological examination

Faecal samples were processed for qualitative and quantitative examinations. Qualitative examination of faecal samples was performed by direct smear and flotation methods, and the McMaster technique was used for quantitative examination (Urquhart *et al.*, 1996). Samples found to be negative using the direct smear method were subjected to the flotation method for confirmation. The McMaster technique was performed to quantify eggs per gram (EPG) in positive samples (Whitlock, 1948; Urquhart *et al.*, 1996). Helminth species were identified using the standard key as described by Thienpont *et al.* (1979) and Soulsby (1982).

Preparation of Nigella sativa extract

Authenticated dried seeds of *N. sativa* were purchased from a local market in Sakrand, Pakistan. The extract was prepared as described by Shalaby *et al.* (2012) and Jamila and Al-Malki (2013). The condensed *N. sativa* extract was preserved in a tightly corked, labelled bottle and stored in a refrigerator at 4°C for further use in experiments (Beshay, 2018).

Experimental design

On the basis of faecal egg count (FEC), 40 naturally infected adult Aseel chickens were used for the evaluation of anthelmintic activity of ivermectin (Ivomec®, 1% sterile solution, by Merial) and *N. sativa* extract alone and in combination. The dose of *N. sativa* extract was adopted from a previous study published by the researchers from our group (Al-Shaibani *et al.*, 2008), and the dose of ivermectin was adopted from Sharma and Bhat (1990). However, bearing in mind the scavenging behaviour of backyard poultry and the high chance of continuous re-infection, we used a booster dose in all the treatment groups. At the time of treatment, a number of parasitic larvae may be passing through the histotrophic stage and are therefore not well exposed to the anthelmintics. The histotrophic phase of many nematode species in birds ranges from 3 to 54 days before final maturation (Herd and McNaught, 1975).

Forty naturally infected adult Aseel chickens were randomly divided into groups A, B, C and D ($n = 10$ each). Group A was treated with ivermectin at 300 µg/kg by subcutaneous route on day 1, followed by a booster dose on day 15. Group B was treated with *N. sativa* extract at 200 mg/kg by oral route on days 1 and 2, followed by a booster dose on days 15 and 16. Group C was treated with ivermectin at 300 µg/kg by subcutaneous route on day 1, followed by a booster dose on day 15, along with *N. sativa* extract at 200 mg/kg by oral route on days 1 and 2, followed by a booster dose on days 15 and 16. Group D was kept as a positive control to monitor time-related changes.

Throughout the experiment, fresh faecal and blood samples were collected from each bird on days 0 (pre-treatment), 7, 14 and 28 (post-treatment). Furthermore, EPG values were recorded by the McMaster technique, as described previously. Haematological indices, namely PCV % and Hb concentration (g/dl), were determined by the Microhematocrit method and Sahli's hemoglobinometer method, respectively (Coles, 1986). For haematological studies, *c.* 0.5 ml of venous blood was collected from wing vein in a vial containing EDTA.

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Estimation of anthelmintic efficacy

Estimation of anthelmintic efficacy of ivermectin and *N. sativa* extract was carried out according to the field controlled faecal egg count reduction test (FECRT) (Coles and Roush, 1992; Taylor *et al.*, 2002).

Data analysis

Data were analysed using GraphPad Instat software (version 3.05). Variations among weekly intervals and groups under study were analysed through one-way analysis of variance (ANOVA) and Tukey's test. A P value < 0.05 was considered to be statistically significant. Data are presented as mean ± standard deviation (SD).

Results

The mean values of EPG (pre- and post-treatment) and faecal egg count-reduction percentage (FECR %) in groups A, B, C and D are shown in table 1. Briefly, the mean values of EPG were significantly reduced in groups A, B and C on days 7, 14 and 28 post treatment. Reduction in EPG was highly significant ($P < 0.001$) in groups A and C throughout the post-treatment period. However, the difference in mean values of EPG in group B was very significant ($P < 0.01$) on day 7 and highly significant ($P < 0.001$) on days 14 and 28 post treatment. Meanwhile, no reductions were observed in mean values of EPG in group D; instead a gradual increase ($P > 0.05$) was recorded on days 7, 14 and 28.

Moreover, a rapid increase in FECR % was recorded in group C compared to groups A and B on days 7, 14 and 28 post treatment. On day 28 post treatment, the mean percentages of faecal egg count reduction (FECR %) in groups A, B and C were recorded as 93.58, 88.09 and 100.00%, respectively. Further data analysis showed significantly higher efficacy ($P < 0.001$) in group C (100 ± 0.00%) than in groups A and B (table 1).

The mean values of PCV % in groups A, B, C and D are shown in table 2. Briefly, the mean values of PCV % in group A were significantly ($P < 0.05$) improved on day 14 post treatment, however, highly significant ($P < 0.001$) improvements in PCV % were recorded on day 28 post treatment. The mean values of PCV % in group B were improved very significantly ($P < 0.01$) on day 28 post treatment. Meanwhile, the improvements in PCV % in group C were very significant ($P < 0.01$) and highly significant ($P < 0.001$) on days 14 and 28 post treatment, respectively. On day 28 post treatment, further data analysis showed that the variations in mean values of PCV % in groups A, B and C were non-significant ($P > 0.05$). However, the improvements in mean values of PCV % in groups B and C were highly significant ($P < 0.001$) compared to group D. Furthermore, the mean values of PCV % in group A were very significantly improved ($P < 0.01$) compared to that of group D on day 28 post treatment.

The mean values of Hb concentration in groups A, B, C and D are presented in table 3. Highly significant ($P < 0.001$) improvements were recorded in mean values of Hb concentration in groups A and C on days 14 and 28 post treatment. Meanwhile, the improvements in mean values of Hb concentration in group B were very significant ($P < 0.01$) on day 28 post treatment only. On day 28 post treatment, further data analysis indicated that the variations in mean values of Hb concentration in groups A and B were very significant ($P < 0.01$). Furthermore, the improvements in mean values of Hb concentration in groups A, B and C were highly significant ($P < 0.001$) compared to that of group D on day 28 post treatment. However, the improvements in mean values of Hb concentration were very significantly different ($P < 0.01$) in groups A and C on day 28 post treatment.

Discussion

The findings of the present study provide evidence that ivermectin and *N. sativa* possess significant efficacy against helminths in backyard poultry. As for the efficacy of ivermectin, the results of our study are consistent with those of Zia-ur-Rehman *et al.* (2014) and Khayatnouri *et al.* (2011), who reported 94.11% and 99% efficacy of ivermectin against *Ascaridia galli* (in commercial layer birds) and *Heterakis* species (in native chicken), respectively. Similarly, Shahadat and colleagues also reported a comparable (90–100%) efficacy of ivermectin against *Ascaridia galli* in indigenous chickens (Shahadat *et al.*, 2008).

We also observed a significant anthelmintic efficacy of *N. sativa* extract against the helminth species infecting the Aseel chickens. However, despite the apparent lack of relevant studies available in the literature, the results of our study are consistent with findings of Kailani *et al.* (1995) and Maqbool *et al.* (2004), who evaluated the anthelmintic activity of *N. sativa* against *Fasciola hepatica* in buffaloes and reported 88.2% and 80.8% efficacy, respectively. Furthermore, the results of our study are also supported by a previous study reporting the significant anthelmintic activity of essential oils of *N. sativa* against earthworms, tapeworms and nodular worms (Agarwal *et al.*, 1979). Similarly, in a recent study, 100% efficacy of *N. sativa* oil has been observed against *Hymenolepis nana* in mice (Al-Megrin, 2016). The anthelmintic efficacy of *N. sativa* has also been evaluated in other mammals, with varying degrees of effectiveness. Al-Shaibani *et al.* (2008) reported 69.5% efficacy of ethanolic extracts of *N. sativa* against helminths in sheep. Similarly, Ayaz *et al.* (2007) reported 66.6% efficacy of *N. sativa* against *Aspiculuris tetrapetra* in mice. Discrepancy in the results may be due to the differences in dose quantity, nature of preparation used, study animal and parasite species and genetic variation of individual plants.

Among the bioactive constituents present in *N. sativa* seeds and oil, thymoquinone has been regarded as an important phytochemical anthelmintic arsenal. Thymoquinone has been reported to cause surface tegumental damage in a number of helminth species, including the tropical liver fluke, *Fasciola gigantica* (Shalaby *et al.*, 2012). However, at present, the mode of action of thymoquinone is largely unknown. Recently, Ullah and colleagues reported that the oxidative damage of parasite proteins as revealed by their carbonylation, which serves as a biomarker of protein damage, was significantly increased in *F. gigantica* worms following treatment with thymoquinone in a concentration-dependent mode. The recognition of carbonylated proteins may furnish the biomarkers for oxidative impairment induced by thymoquinone in helminths (Ullah *et al.*, 2017). In addition to this, the anthelmintic activity of *N. sativa* may also be attributed to its other bioactive compounds, through improvement in nutritional status and boosting effects on host immunity. It has been reported that after ingestion of condensed tannins by adult worms, the intestinal mucosa is disturbed at varying levels and leads to the destruction of parasites (Athanasiadou *et al.*, 2001). Furthermore, *N. sativa* seeds are reported to inhibit egg-laying in adult female worms and exhibit active biocidal effects against miracidia, cercariae and adult worm stages of *Schistosoma mansoni* (Mohamed *et al.*, 2005).

In order to evaluate the synergistic effects, *N. sativa* has been used in combination with certain chemical anthelmintic agents in

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Table 1. Mean values of eggs per gram (EPG) and percentage faecal egg count reduction (FECR %, in parentheses) of helminth species in Aseel chickens before and after different treatments: group A, ivermectin at 300 μg/kg (2 doses) by subcutaneous route; group B, *Nigella sativa* oil extract at 200 mg/kg (4 doses) by oral route; group C, ivermectin combined with *Nigella sativa* oil extract at 300 μg/kg (2 doses) by subcutaneous route and 200 mg/kg dose rate (4 doses) by oral route, respectively; group D, control without treatment.

	Pre treatment	Post treatment		
Group	0 day	7th day	14th day	28th day
A	1675 ± 429.63^{a}	640 ± 172.88^{a} (61.1)	375 ± 153.21^{a} (77.2)	105 ± 76.19^{a} (93.6)$^{\wedge\star}$
B	1235 ± 444.75^{ab}	745 ± 302.26^{b} (40.1)	410 ± 202.48^{a} (67.4)	160 ± 117.38^{a} (88.1)$^{\wedge\dagger}$
C	1300 ± 300.00^{a}	465 ± 194.44^{a} (65.3)	115 ± 62.58^{a} (91.3)	0 ± 0.00^{a} (100)*†
D	950 ± 217.31^{bc}	1030 ± 222.61^{c} (−8.7)	1120 ± 193.22^{c} (−19.4)	1265 ± 182.65^{b} (−36.1)

[a]Values with same letters in same rows have highly significant difference ($P < 0.001$).
[b]Values with same letters in same rows have very significant difference ($P < 0.01$).
[c]Values with same letters in same row have non-significant difference ($P > 0.05$).
[†]Values with same superscripts in same column have highly significant difference ($P < 0.001$).
[*]Values with same superscripts in same column have significant difference ($P < 0.05$).
[^]Values with same superscripts in same column have non-significant difference ($P > 0.05$).

Table 2. Mean values of PCV (%) of Aseel chickens before and after different treatments: group A, ivermectin at 300 μg/kg (2 doses) by subcutaneous route; group B, *Nigella sativa* oil extract at 200 mg/kg (4 doses) by oral route; group C, ivermectin combined with *Nigella sativa* oil extract at 300 μg/kg (2 doses) by subcutaneous route and 200 mg/kg dose rate (4 doses) by oral route, respectively; group D, control without treatment.

	Pre treatment	Post treatment		
Group	0 day	7th day	14th day	28th day
A	24.16 ± 1.02^{abc}	24.94 ± 1.03^{c}	25.87 ± 0.97^{a}	$28.45 \pm 1.58^{bd\star}$
B	26.51 ± 2.27^{ac}	27.26 ± 2.29^{c}	28.85 ± 1.86^{c}	$30.06 \pm 1.98^{a\star\Delta}$
C	25.49 ± 1.39^{abc}	26.49 ± 1.33^{c}	27.79 ± 1.34^{a}	$29.28 \pm 1.48^{b\star\surd}$
D	26.51 ± 1.91^{c}	26.50 ± 2.12^{c}	25.87 ± 1.96^{c}	$25.20 \pm 1.87^{cd\Delta\surd}$

[a]Values with same letters in same rows have very significant difference ($P < 0.01$).
[b]Values with same letters in same rows have highly significant difference ($P < 0.001$).
[c]Values with same letters in same rows have non-significant difference ($P > 0.05$).
[*]Values with same superscript in same column have non-significant difference ($P > 0.05$).
[d]Values with same letters in same column have very significant difference ($P < 0.01$).
[Δ]Values with same superscript in same column have highly significant difference ($P < 0.001$).
[√]Values with same superscript in same column have highly significant difference ($P < 0.001$).

Table 3. Mean values of Hb concentration (g/dl) of Aseel chickens before and after different treatments: group A, ivermectin at 300 μg/kg (2 doses) by subcutaneous route; group B, *Nigella sativa* oil extract at 200 mg/kg (4 doses) by oral route; group C, ivermectin combined with *Nigella sativa* oil extract at 300 μg/kg (2 doses) by subcutaneous route and 200 mg/kg dose rate (4 doses) by oral route, respectively; group D, control without treatment.

	Pre treatment	Post treatment		
Group	0 day	7th day	14th day	28th day
A	7.05 ± 0.63^{ab}	7.68 ± 0.55^{a}	8.45 ± 0.57^{b}	$9.65 \pm 0.61^{b\star\dagger\wedge}$
B	8.82 ± 1.54^{ac}	9.52 ± 1.61^{a}	10.16 ± 1.68^{a}	$11.17 \pm 1.32^{c\surd}$
C	7.73 ± 0.60^{ab}	8.33 ± 0.56^{a}	8.97 ± 0.48^{b}	$11.18 \pm 0.78^{b\star\wedge\Delta}$
D	8.42 ± 0.90^{a}	8.26 ± 0.88^{a}	8.07 ± 0.93^{a}	$7.57 \pm 1.01^{a\dagger\surd\Delta}$

[a]Values with same letters in same rows have non-significant difference ($P > 0.05$).
[b]Values with same letters in same rows have highly significant difference ($P < 0.001$).
[c]Values with same letters in same rows have very significant difference ($P < 0.01$).
[*]Values with same superscript in same column have very significant difference ($P < 0.01$).
[†]Values with same superscript in same column have highly significant difference ($P < 0.001$).
[^]Values with same superscript in same column have very significant difference ($P < 0.01$).
[√]Values with same superscript in same column have highly significant difference ($P < 0.001$).
[Δ]Values with same superscript in same column have highly significant difference ($P < 0.001$).

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the past. *Nigella sativa* oil used in combination with praziquantel in mice produced better results (in terms of reduction in number of eggs produced by *Schistosoma mansoni*) compared to praziquantel used alone (Mahmoud *et al*., 2002). Furthermore, in the present study we observed that the synergistic combination of ivermectin and *N. sativa* extract possessed greater efficacy than either ivermectin or *N. sativa* extract used alone. Before this, no *in vivo* study investigated the synergistic combination of ivermectin and *N. sativa* in chickens. However, the results of our study are in conformity with previous studies reporting the synergistic effect of ivermectin and *N. sativa* against helminths *in vitro*. In 2012, Shalaby *et al*. tested the combination of ivermectin and *N. sativa* and reported that the combination of these two agents is far more efficacious than either ivermectin or *N. sativa* when used alone (Shalaby *et al*., 2012). Jamila and Al-Malik (2013) used the same combination and reported a significant efficacy against *Moniezia expansa*. However, despite the enticing evidence from these two *in vitro* studies and the findings of our present *in vivo* study, further experimentation is required to fully elucidate the exact underlying mechanisms responsible for such synergistic anthelmintic effects of ivermectin or *N. sativa* extract.

Parasitic infections constantly affect the overall health and productivity of animals, and haematological studies may provide significant entropy regarding such damage (Hafeez, 1996). The total blood Hb concentration is an indicator of the capacity of birds to meet their oxygen demands. In avian species, regenerative anaemias are usually induced by parasite-stimulated haemorrhages and haemolysis (Boyd, 1951). As such, Hb concentrations are hypothesized to serve as a strong indicator of physiological conditions in avian species (Bańbura *et al*., 2007). Assessment of PCV % is a good indicator of developing anaemia and associated problems induced by helminths. The haematological observations of our study are consistent with the findings of Fatihu *et al*. (1991), Rehman (1993), Khan *et al*. (2006), Mazur *et al*. (2007), Deka and Borah (2008), and Adang *et al*. (2012), who have all reported a decline in Hb concentrations in infected birds compared to treated birds. Similar findings have also been reported by Krivutenko (1980), Matta and Ahluwalia (1982), and Kumar *et al*. (2003), who reported lowered PCV % and Hb concentrations in infected birds. This may be attributed to the larval migration and accompanying disruption of mucus membrane of small intestines in penetrative infective stages and the resultant rupture of small blood vessels in the tissue phase of the life cycle of helminth parasites, which involves some blood loss. The decline in Hb concentrations may also be attributed to metabolic disturbance caused by the helminths, rather than direct blood loss (Kumar *et al*., 2003). Consistent with our findings, improvements in PCV % and Hb concentration have been observed in infected chickens following ivermectin treatment (Sufian *et al*., 2006; Shahadat *et al*., 2008). Similarly, Hassan and colleagues observed significant improvements in PCV % and Hb concentration in Bengal goats following ivermectin treatment (Hassan *et al*., 2012). These gradual improvements in haematological indices may be ascribed to a gradual decrease in number of parasites in the treated groups compared to the control group. The post-treatment upswing in mean PCV % is most probably associated with the rising Hb concentration, as these are closely related.

To sum up, our study is the first to demonstrate a synergistic anthelmintic effect of ivermectin and *N. sativa* in backyard poultry, particularly the Aseel chicken, and may serve as a foundation for future research focused on potential anthelmintic remedies in poultry. In general, the results of our study also support the hypothesis of using drug combinations against helminth infections in domestic birds, particularly backyard poultry. Moreover, in order to examine the broader anthelmintic potential of ivermectin and *N. sativa*, further controlled experimental studies involving other species of avian helminths (one parasite species being studied at a time) would be of great importance. However, for a comprehensive and multidirectional understanding of the anthelmintic potential of *N. sativa* and its active ingredients, detailed studies are warranted. Being cheaper and easily available in rural areas, further studies on the anthelmintic potential of *N. sativa* will greatly contribute to the design of practical and cost-effective solutions to reduce the ignored helminth infections in backyard poultry.

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Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the animal ethics committee governed by Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Pakistan.

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