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Effects of Some Alternative Plant Extracts Used as Natural Coccidiostat for Pigeons

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ABSTRACT

The aim of the study was to determine the effect of the coccidiostats used in pigeon lofts. A total of 60 disease-free young pigeons of 4 months of age were taken from different areas of Lahore Pakistan and were divided into 4 groups A, B, C, and D having 15 birds in each group. The trial was conducted to find out the comparative chemotherapeutic efficacy of extract of leaves of neem in group A, crude extract of leaves of guava in group B, anticoccidial drug in group C, whereas group D remained as a control group. The pigeons of group A had better weight gain and feed conversion ratio as compared to other groups but the difference between the groups was non-significant (P>0.05). Moreover, the crude extract of neem (*Azadirachta indica*) leaves boosted the immunity level of diseased pigeons that resulted in reduction of oocyst count while the crude extract of guava (*Psidium guajava)* leaves prevented the diseased pigeons from getting secondary bacterial infection. Therefore, the crude extract of neem leaves can be recommended for the treatment of coccidiosis and, also, to enhance the prophylactic levels in pigeons.

Doğal Koksidiostat Olarak Kullanılan Bazı Alternatif Bitki Ekstraktlarının Güvercinlere Etkileri

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ÖZET

Bu araştırmanın amacı, güvercinlerde kullanılan koksidiostatların etkisini belirlemektir. Çalışmada, Pakistan'ın Lahor kentinin farklı bölgelerinden temin edilen 4 aylık 60 adet sağlıklı genç güvercin kullanılmıştır. Güvercinler 15'er adetlik 4 gruba ayrılmıştır (A, B, C, D). Çalışmada, A grubunda neem (*Azadirachta indica*) yaprakları ekstraktının, B grubunda guava (*Psidium guajava*)yaprakları ekstraktının, C grubunda antikoksidiyal ilacın etkileri koksidiyoz hastalığına karşı karşılaştırılmalı olarak incelenirken, D grubundaki güvercinler ise kontrol grubudur. Araştırmada, enfekte olmayan gruptaki güvercinlerin, tedavi edilen gruplara göre daha iyi (P<0.05)

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canlı ağırlık artışına sahip olduğu tespit edilmiştir. A grubundaki güvercinlerin diğer gruplara göre daha iyi canlı ağırlık artışına ve yemden yararlanma oranına sahip oldukları, ancak görülen farkın anlamlı olmadığı tespit edilmiştir (P>0.05). İlave olarak, guava yaprakları ekstraktı verilen gruptaki hastalıklı güvercinlerde sekonder bakteri enfeksiyonları önlenirken, neem yaprakları ekstraktı verilen gruptaki güvercinlerde, bağışıklık düzeyinde önemli ölçüde artış görülmüştür. Bu gruptaki güvercinlerde diğer gruplarla karşılaştırıldığında ookist sayısı daha düşük bulunmuştur. Bu nedenle güvercinlerde neem yaprakları ekstraktı, koksidiyoz tedavisi için ve profilaktik seviyenin arttırılması için önerilebilir.

Introduction

Coccidia are common protozoa pathogens in pigeons. The literature around the world describes nine species of the genus *Eimeria* and one of the genus *Isospora*, but only three species are of significance: *Eimeria columbae*, *E. columbarum* and *E. labbeana*, which are characterized by fluctuating degrees of virulence found in domestic pigeons (*Columba livia domestica*) and rock pigeons (*Columba livia livia*). Throughout the world, the coccidiosis is 5.1%-71.9% seen in pigeons while the mortality varies from 5%-70% in young pigeons the disease is acute and most of the deaths happen in the 3-4 month of life of pigeons (Krautwald- Junghanns et al., 2009). Both the pigeons and doves have been reared for food (eggs and meat are eaten by people). Research involving columbids had led to increased knowledge about the inheritance of morphological and behavioral characteristics, endocrinology, learning, evolution, orientation and navigation.

Pigeon racing is, also, common in most of the countries of the world particularly the Asian countries e.g. Pakistan, India, etc for income purpose (Baptista et al., 1992; Lack, 2003). In racing pigeons faster tiring and watery diarrhea occur. Sick pigeons severely suffer feathers are dead and brittle, weight loss and expelling feces streaked with blood are characteristic signs. Coccidiosis mainly affects thoroughbred and wild birds aged from 4 weeks to 4 months. One infected pigeon may expel from a few to hundreds of millions of oocysts per day in feces. Oocysts may also be found in water, litter and feed (Szeleszczuk, 1995). In European countries, the prophylactic use of anticoccidial chemicals as feed additives has been strictly limited since 2006 and a full ban has been proposed to be effective in 2021. To cope with this global situation, composed of one or more strains of wild-type or attenuated *Eimeria* species, is successfully developed as another approach to prevent coccidiosis though their cross-species protection and efficacy may need to be improved.

The plant of neem (*Azadirachta indica*), belongs to the family of Meliaceae and is abundantly found in Asian countries including Pakistan, India, etc. The neem contains variety of bioactive compounds including nimbin,

nimbidin, nimbolinin, nimbolide, nimbidol, sodium nimbinate, gedunin, salannin and limonoids and these have significance in altering various genetic pathways to manage the disease. However, azadirachtin is the most important active ingredient of neem plant. The Quercetin and ß-sitosterol are also extracted from leaves of neem which have antifungal and antibacterial activities (Govindachari, 1998), antibacterial (Singh et al., 1997), antifungal (Kher et al., 1997), anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, and anticarcinogenic activities (Bandyopadhyay et al., 2004; Sultana et al., 2007; Ebong et al., 2008; Paul et al., 2011)

The leaves of neem contain ingredients such as nimbin, nimbol, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, (Ali, 1993; Hossain et al., 2011; Kokate et al., 2010). Neem leaves and its constituents have immunomodulatory, anti-inflammatory, antimalarial, antifungal, antibacterial, antiviral, antioxidant properties (Subapriya and Nagini, 2005). The crude extract of neem leaves contains nimbim, nimbinene, 6-desacetylnimbinene, nimbandiol, nimbolide and quercetin (Mitra et al., 2000). In another trial it was observed that leaves, fruits and bark of neem tree had more antioxidant potential (Sithisarn et al., 2005). Similar research trial was performed to observe the *in vitro* antioxidant activity of crude extract of neem leaves and the results showed that crude extract of neem leaves could be used as natural antioxidant (Hossain et al., 2013).

In another study trial, the immunomodulatory effects were observed by administering the decoction of neem leaves in broiler poultry birds and results indicated improved antibody titre, growth performance (Durrani et al., 2008). Similarly it was reported in a trial that feeding powder of finely ground dry neem leaves at the rate of 2 g/kg of feed improved the humoral and cell mediated immune responses in broilers by enhancing the antibody titre new castle disease. An aqueous extract (10%) of neem leaves is reported to possess antiviral activities and significantly enhances antibodies production against diseases in birds (Sadekar et al., 1998). Historically, neem has been used to rid the body of all forms of parasites. Neem quickly kills external and internal parasites. Neem extracts have hormone which interferes with the life cycle of parasites, inhibit their ability to feed and prevent the eggs from hatching (Youn and Noh, 2001). Guava contains chemical ingredients, i.e. tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. The guava leaves are a rich source of flavonoids and phenols and it can be used to cure diarrhea. Another essential phytochemical ingredient found in guava leaves “quercetin” is thought to be contributive in anti-diarrheal effects of guava; it causes the intestinal smooth muscle to be relaxed and hampers the bowel contractions (Oh et al., 2005). The

present study was executed to evaluate the anticoccidial effect of infusion prepared from the leaves of neem and guava compared to Baycox (toltrazuril) and, also, to see the impact of this treatment on immunity levels of diseased pigeons.

Material and Methods

The current study was performed at the Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore-Pakistan after the approval of the Local Ethics Committee of Faculty of Veterinary Medicine under approval No: UVAS-546 on 10-04-2013.

Experimental birds

A total of 60 apparently disease-free young domestic pigeons, having "bar" wing pattern, of 4 months old were taken from different places of Lahore and before the start of trial, their "disease-free" status was checked from their feces with the help of McMaster egg counting technique for fecal examination to detect the presence of *Eimerian oocysts*. For the selection of pigeons for this trial, , only the coccidiosis disease free pigeons were selected which did not have considerable amount of *Emieria* oocysts in their feces. The selected pigeons were randomly divided into four groups viz; A, B, C, and D each group was composed of 15 birds. The pigeons were kept in disinfected cages in laboratory Department of Clinical Medicine and Surgery, University of Veterinary and Animal Science, Lahore. The birds were provided with water and coccidiostat free feed ad libitum throughout the research period. Their bedding of white paper was changed daily for two times, i.e. at morning and at evening time. All of the groups were housed in separate chamber of the cage equipped with a lamp, feeder and drinkers. The cages were kept at room temperature and before start of the trial and the birds were vaccinated against Newcastle disease and infectious bronchitis.

Preparation of aqueous extract of leaves

Fresh leaves of neem and guava 100 grams each were taken, 50 g of the leaves were taken from the botanical garden of Govt. College University Lahore whereas remaining 50 g of the leaves were taken from the local vegetable market of Lahore. The leaves were taken from two different localities just to have a diversified effect. The leaves were chopped into small pieces with the help of metallic grinder and kept in two separate non-metallic jars. The leaves were not exposed to direct sunlight just to prevent the deterioration of active ingredient so the leaves were put in oven at 37^0C for 24 hours. After that, 1 L of boiled water was put in both of the jars and kept for overnight at room temperature following the procedure prescribed by Santiago-Flores (1977). The aqueous extract of leaves thus collected was stored in separate labeled bottles, mixed in drinking water and given to the pigeons when required.

Preparation of inoculums and experimental infection

Eimerian spp was isolated from the caeca of coccidiosis infected pigeon which was brought to the University Diagnostic Laboratory, UVAS and the oocysts were identified based on size, shape and location of lesions. These were homogenized in a blender or by stirring with a rod and filtered using a 106 µm mesh sieve. *Eimeria* oocysts were harvested using the saturated NaCl floatation method (for 10 min). The harvested oocysts were re-suspended in distilled water and washed by centrifugation three times to remove the flotation solution (for 5 min). The sediment containing the oocysts was transferred into beakers, suspended in 2% (w/v) $K_2Cr_2O_7$ solution and allowed to sporulate at room temperature for seven days with regular stirring. After sporulation, oocysts from each sample were cleaned with sodium hypochlorite (4% active chlorine) and washed with distilled water three times as described before Eckert et al. (1995). The inoculum of 1 ml contained 30.000 viable sporulated oocysts of *Eimerian spp* in saline water directly injected into the crop of pigeons of group A, B and C via oral gavage.

A clean polyethylene sheet, placed daily under each cage, was used for the collection of excreta for oocyst analysis. Total fecal samples for each 24 hours from each subgroup, were placed in separate airtight plastic bags, homogenized thoroughly with a domestic mixer and kept refrigerated until assessed for total oocyst counts. The homogenized samples were ten-fold diluted with tap water to be further diluted with saturated NaCl solution at a ratio of 1:10. The oocyst counts were determined using modified McMaster chambers and presented as the number of oocysts per bird (Hodgson, 1970).

Protocol of Medication

The clinical sign and symptoms of the diseased pigeons were observed at 7^{th} day post inoculation of *Eimerian oocysts.* The below mentioned treatment protocols started at 8^{th} day post inoculation. Each group was given specific treatment protocol whereas the dose rate was calculated on the basis of body weight of pigeons. In a similar kind of trial, (Van Reeth and Vercruysse, 1993) used the standard anticoccidial drug on the experimentally infected racing pigeons.

- The pigeons of group A were given the herbal aqueous extract of neem leaves (2 ml extract mixed in 1 L drinking water).
- The pigeons of group B were given the herbal aqueous extract of guava leaves (2 ml extract mixed in 1 L drinking water).
- The pigeons of groups C were treated with the anticoccidial drug Baycox (20 mg per kg body weight).
- Control pigeons (groups D) had no treatment.

The experiment lasted for 7 days when there were almost no clinical signs present in pigeons. The 7-d period was usual period of chemotherapeutic trial for avian coccidiosis using the standard anticoccidial drug.

Evaluation parameters

The efficacy of different treatments applied on different pigeon groups was evaluated on the basis of body weight gain, feed consumption, feed conversion ratio (FCR) and bloody diarrhea. This drug trial was performed to check the effects of decoction of leaves of neem and guava on the mitigation of clinical signs of coccidiosis in pigeons and therefore the main evaluation parameter was to check the reduction of number of oocysts per gram of the feces of pigeons and also to check the healing effects of the plant based medication from the recovery of diseased pigeons to normal and healthy pigeons. Feed consumption, FCR, and weight gain were not the focus of study, the main focus of the trial was the reduction of oocysts and to check the healing effects or immunity enhancement of diseased pigeons through the decoction.

- The body weight gain and FCR was determined just before the start of the trial and, also, at the end of trial.
- Bloody diarrhea was investigated at the 3rd day of challenge and the extent of bloody diarrhea score was assigned from 0 (-) to 3 (+++). Zero was normal status while 1, 2, and 3 corresponded to 33%, 33-66% and 66-99% blood in total feces.
- The droppings of all birds from all the cages were collected daily and examined by using a modified McMaster counting technique. After the collection of feces from all the cages separately, one gram of feces from each of the group were subjected to McMaster counting technique and rest of feces of each group were discarded. The oocysts count per grams of feces was done after the pigeons were experimentally infected with the *Eimeria* oocysts and this continued during the period of medication till the end of the trial.

Statistical analysis

The results obtained were analyzed statistically by using analysis of variance and Duncan's multiple range test (Marin and Robert, 2007). The model assumptions of normality and homogeneity of variance were examined by Shapiro-Wilk and Levene tests, respectively. The statistical analysis was performed with MedCalc (1996). ANOVA was used for group comparison followed by Tukey-Kramer for post-hoc.

Results

The status of bloody diarrhea is shown in Table 1; it is evident that the severity of bloody diarrhea was maximum on the 1st day of treatment and as the treatment progressed the severity of haemorrhagic diarrhea was gradually reduced. The severity and frequency of haemorrhagic diarrhea was seen more in group C as compared to rest of all other groups while the group A had less severity. As for as the group B was concerned it had less severity of bloody diarrhea than group C and more than group A.

Table 2 shows the mean of feed intake of groups A, B, C, and D was 13.27 g, 12.91 g, 12.02 g, and 13.15 g respectively. Feed intake of group A was significantly higher ($P<0.05$) than that of the rest of all groups (Table-2). Higher

feed conversion ratio FCR was found in group A and lower FCR was of group C.

Table 1. Bloody diarrhea in pigeons post-treatment

Groups Treatment		Blood in feces (days of treatment)			
		Day 1	Day 2	Day 3	Day 4
A	Infected +aqueous extract of neem leaves	+	+	-	-
B	Infected +aqueous extract of guava leaves	++	++	+	-
C	Infected + Baycox	+++	++	+	
D	Control	-	-	-	-

Table 2. Performance parameters of the experimental groups

Groups	Treatment protocol	Mean feed intake (g/day)	Mean body weight gain (g)	FCR
A	Infected + extract of neem leaves	$13.27^{a} \pm 0.029$	27.60 ± 0.021^{a}	2.08 ± 0.010^{a}
B	Infected + extract of guava leaves	$12.91^{b} \pm 0.012$	22.76 ± 0.015^{b}	1.76 ± 0.021^{b}
C	Infected + Baycox	$12.02^{b} \pm 0.145$	21.63 ± 0.021^{b}	1.8 ± 0.014^{b}
D	Control	$13.15^{a} \pm 0.001$	27.18 ± 0.014^{a}	2.06 ± 0.081^{a}
P values		0.036	0.051	0.021

Table 3. Post-infection oocyst count

Groups	Oocysts count after the infection						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A	250	650	2000	3850	6000	8350	9850
B	300	700	1850	4050	6500	8600	10250
C	200	550	2100	3950	5950	7850	10050

Table 4. Oocyst count during treatment

Groups	Treatment	Oocysts count at respective days of treatment						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A	Extract of neem leaves	9850	7000	5850	4550	3000	1200	800
B	Extract of guava leaves	10250	8950	6100	5000	3350	1850	1050
C	Baycox	10050	8500	6150	4050	2000	1350	950

Droppings of all infected pigeons were collected from on days 1, 2, 3, 4, 5, 6 and 7 of post-challenge for estimation of egg per gram (EPG) of feces by applying McMaster egg counting technique (Soulsby, 1982). Table 3 shows daily gradual increase in number of egg per gram of feces of pigeons. The Table 4 shows the effects of treatments in the form of reduction in number of egg per grams of feces of different groups. Lower oocyst count was seen in the group treated with standard anticoccidial and herbal aqueous extract. The pigeons treated with the aqueous extract of neem leaves were comparatively healthier since it boosted the immunity level and birds became healthy and survived under coccidiosis contaminated condition.

Discussion

The results obtained in our trial regarding the feed intake and FCR were in alignment with the results obtained in a trial conducted by Chakarverty and Prasad (1991) where better FCR of the birds who were fed commercial ration and were also given decoction of neem leaves. The increased FCR could be due to the good effects of plant based medication on the intestinal microbes which improved better feed consumption.

In the present study the results obtained regarding the weight gain were in accordance with the results obtained by Chakarverty and Prasad (1991) who used decoction of neem leaves and similar results were obtained in a trial conducted by Tipu et al. (2002) in which he used salinomycin and neem fruit as feed additive and made comparison with anticoccidial drug and found better result weight gain.

The neem plant has the ability to dismantle the external as well as internal parasites, it contains hormones which interfere with the life cycle of parasite, inhibits the ability of parasite to feed and ultimately it prevents the egg from being hatched (Youn and Noha, 2001). Luong et al. (2012) reported that the slurry of neem leaves is an organic product acts as larvicidal and used in Africa. On the other hand the guava leaves have been used to cure diarrhea particularly in village areas. The important active ingredient quercetin is supposed to have antidiarrheal effects and causes the relaxation of intestinal smooth muscle and also causes the relaxation of bowel contraction.

The clinical signs of young pigeons infected with coccidiosis include anorexia, dehydration, weight loss, acachexia, fetid, watery and bloody diarrhea (McDougald, 2003). The mortality ratio can be 5%-70% in young pigeons of 1-4 months old, and majority of deaths happen in 3-4 months (Mennemeier,1985). The pathogenicity of causative agent of coccidiosis is negligible in adult pigeons due to more immunity status of adult pigeons as the they grow however this infection could mitigate the flying or racing performance of pigeons.

One of the important clinical signs of pigeons infected with coccidiosis include bloody diarrhea which was also seen in the undergoing trial. The bloody diarrhea was seen in all

groups but its severity varied. The pigeons of group A had bloody diarrhea but to lesser extent whereas it was heavily reported in group C. The treatments suppressed the clinical signs particularly the bloody diarrhea. The pigeons of group A, when treated with decoction of neem leaves, recovered from bloody diarrhea effectively which could possibly be due to the synergistic effects of active ingredients of neem leaves. The active ingredients of neem leave have the antidiarrheal ability and also due to antioxidant ability, the active ingredients empowered the intestinal microbiota which ultimately boosted the immunity of birds and due to this the birds effectively recovered from bloody diarrhea. Similar type of findings were observed in a trial conducted by (Mennemeier, 1985) where he reported mild catarrhal enteritis and reddening of intestinal mucosa on 5^{th} or 6^{th} day of inoculation of oocysts of E labbeana, in pigeons. The results obtained in our study trial indicated rapid reduction of *Eimeria oocysts* when the decoction was given to the diseased pigeons and that is possibly due to the ovicidal effects of extract of neem leaves. It is supposed that the ovicidal activity might be due to the interference and dominant affect of azadirachtin with the embryonic development of egg of protozoan. The same is reported by as reported by Dimetry et al. (1993) who stated that neem based products had ovicidal effects against mites.

The results found in this study were aligned with the results obtained by Pilarczyk et al. (2006) where the anticoccidial drug Baycox was mixed in drinking water and it proved to be effective against coccidiosis in pigeons. They found 25000 oocysts in 1 g of feces, after seven days 200 oocysts, and after 14 days he found 50 oocysts in 1 g of feces. Similarly, Van Reeth and Vercruysse (1993) and Vercruysse (1990) reported 97% reduction in oocysts to count when administered anticoccidial drug toltrazuril (Baycox) @ 20 mg/kg body weight. Szeleszczuk (1995) and Michalczyk et al. (2011) were also in agreement with this study which confirmed high efficacy of the medicament in parasitological prevention. The leaves contain querectin and β sitosterol having antifungal and antibacterial properties to prevent the secondary bacterial infection (Govindachari et al., 1998). Moreover, these leaves contains higher amount of antioxidant (Hossain et al., 2013). All parts of neem plant (leaf, bark, fruit, seed, etc.) can be used to cure diseases but the leaves are used because these are easily and readily available, since this study was conducted on pigeons and in particularly in the province of Punjab Pakistan, the neem trees are mostly found in village areas. Moreover, in Punjab, the pigeons are mostly used for racing purpose as game birds so it is easy for the people involved in this kind of sports to use decoction of neem leaves as quickly as possible whenever there are signs of coccidiosis in pigeons so therefore neem leaves were used in this trial.

Conclusion

During the trial it was noted that the anticoccidial drug Baycox only inhibited the clinical signs and did not boost the immunity and the immunity level was boosted by the aqueous extract of neem leaves. This study trial in the form of prophylaxis and therapy for treating coccidiosis comprise anticoccidial chemicals and herbal products. The plants are biologically available rich source of phytochemicals which can be used to treat coccidiosis in pigeons as well as in poultry birds. In this scenario, some further research trails need to be conducted to get to know more dimensions of prophylactic levels at different concentration of these herbal preparations in treating avian coccidiosis.

Moreover, this way of treating coccidiosis of pigeon can also be beneficial for the people living in village areas of the province of Punjab Pakistan because the neem tress are mostly found in village areas whereas the to raise fancy pigeons and also for racing purpose is the hobby of most of the villagers and it is easy, readily available and economical for the pigeon fanciers to use the way of treatment whenever there are signs of coccidiosis in their pigeons

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