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Ameliorative Effect of Poly-Herbal Mixture on *Caecal coccidiosis* in Broiler Chicks

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Abstract

Caecal coccidiosis is disease of poultry which is caused by the *Eimeria tenella* (family of protozoan belongs to genus *Eimeria*). This is most prevalent ailment and results in decrease production and high death rates in broiler chicks**.** Extensive use of drugs as preventive therapy has created antimicrobial resistance. Poultry experts are thinking about the use of herbal plants and their combinations for the control of *Coccidiosis* in commercial poultry as alternatives. Holarrhena Antidysenterica and Azadirachta indica were reported to have anthelmintic, antifungal, antibacterial and antiviral activity. A total of 270 broiler chicks were equally divided into nine group from A to I. they were experimentally infected with a challenge of Eimeria tenella from group A to H. Group A to G were treated with poly herbal preparations and a routinely used drug. Group H was positive control but group I was negative control. Birds were observed for live body weight, hematological parameters and structural changes in cecum. Birds of group G and H showed highest ($P<0.05$) body weights, RBC, Hb and PCV as compared to positive control group. It was concluded that poly herbal mixture showed more anticoccidial effects to control this problem as compared to routine therapeutic drugs and hence they can be used as alternatives.

Keywords: Eimeria, broilers, hematology, histopathology

Introduction

Coccidiosis is a parasitic affliction caused by apicomplexan protozoa of the genous *Eimeria.* It is a fowl ailment caused by microscopic protozoan which causes global economic losses of about $ 3 billion per year in commercial poultry (Dalloul and Lillehoj, 2006). The protozoan resides and multiplies within intestinal tract and cause tissue damages. It causes subclinical to clinical infection in birds with lower production and shedding infectious oocysts in the surrounding environment (Gerhold, 2015).

Poultry sector is the second biggest industry and is a feasible source of animal protein in Pakistan. The poultry industry was suffered by different destructive problems in term of mortality and morbidity. Coccidiosis is inversely affecting the economy and it has been reported round the year. A study was conducted to know the seasonal prevalence of the disease and it was found that the increased prevalence of infection during the rainy season enhanced the oocyst sporulation and subsequent spread (Dalloul and Lillehoj, 2006). High mortality and low weight gain in birds due to coccidiosis are major reasons for economical losses of farmers (Kothavade *et al*. 1996).

Coccidial infection can be diagnosed by observing the oocysts in the droppings of the live birds and by taking swabs from the dead birds. The location of lesions in the intestinal tract helps in estimating the specie of Eimeria. Age of birds and mortality can also help in estimating the Eimeria species. Eimeria species can also be identified on the basis of clinical signs and lesions (Habibi *et al*. 2016). Coccidiosis is a general infection in broiler industry and has been considered as a management problem around the world. Broilers are mostly affected by Coccidiosis and it is considered as the most detrimental disease because it caused an annual loss of US$ 127 million (Takara *et al*. 2002; El-Shall *et al*. 2022). Coccidia when enter into the body of birds, they start their replication and cause lesions in the intestinal tract (Ros *et al*. 2009). In the domestic fowl, *E. tenella* produced severe losses (Chapman, 2014) due to enteritis and hemorrhagic diarrhea. It has been reported that *Eimeria tenella* infected the cells of intestinal wall of caecum along with immunosuppression in broiler chicks (Vermeulen *et al.* 2001).

Coccidiosis might be controlled by excellent management practices, bio-security and bedding material (Nilsson *et al*. 2012). However, it is not possible to completely abolish and stop the disease without effective medication. A number of anticoccidial medicines are available which can either stop the coccidia by breaking their life cycle or directly kill them directly (Mallick *et al*. 2007). Several chemicals and ionophores were used in feed (Nogueira *et al*. 2009) which may cause hazardous effects in birds (Abbas *et al*. 2013) and drug residues for consumers. Eimeria tenella is an oval shaped protozoan and affect the epithelial lining cells of small intestine (Vermeulen *et al*. 2001) but it causes severe losses when attached on epithelial cells of cecum which may include decreased feed and water consumption, bloody diarrhea, weight loss, pigmentation loss, decreased feed conversion, high mortality and ruffled feathers in broiler chicks (Molina *et al.* 2000; Bachaya *et al.* 2012;Sharma *et al*. 2001).

People are using anticoccidial drugs in feeds since the start of poultry industry in Pakistan. But on the other side, a continuous use of synthetic drugs even in small quantity has negatively affected the defense mechanisms and produced resistance in birds against coccidiosis (Chapman 2009). The widespread emergence of antimicrobial resistance has developed in recent years about the safety of anticoccidial drugs and hazards on health of human, animals, birds and the environment (Zidar and Zizek, 2012). On 1st January 2008, The European Commission submitted a report (Arczewska-wlosek and Swaitkiewicz 2013) on the exodus of coccidiostats and histomonostats for its use as feed additives. According to the EC, there is presently no comparably effective substitution to coccidiostats. While the search for their natural replacement is direly needed.

In the backdrop of above, researchers have suggested the use of herbs for treatment of cecal coccidiosis (Arczewska-wlosek and Swaitkiewicz 2013). Some of the workers are involved in investigation of plants and plant derived products for the control of coccidiosis to save the poultry industry from heavy losses (Abdelnour *et al*. 2020). For this, Abbas *et al*. (2012) scientifically proved the anticoccidial effects of diverse herbs as a probable substitute to manage the avian coccidiosis. After this, many herbal products were experienced by the poultry farmers and found good results but they were unable to give scientific explanations about these products. But it is documented that these herbal products possess the anticoccidial, antimicrobial, antioxidant and anti-stress properties. They have been showing beneficial effects on gut micro flora with nutrigenomic effects and immune enhancement properties in birds (Hashemi and Davoodi, 2010).

In these circumstances, utilization of herbs is thought to be a safe, effective and economical way of controlling Coccidiosis (Abbas *et al.* 2012). *Azadirachta indica* has been used to cure

jaundice and liver diseases in many countries of the world. It has been traditionally used to treat ulcers, gastrointestinal disorders and acne vulgaris. Many researchers reported that it possessed the strong adaptogenic action and anti-oxidant properties (Craig, 1999). *Holarrhena antidysenterica* had many biological properties including immune stimulation, anti-inflammatory, vaccine adjuvant, anti-oxidant (Takara *et al.* 2002), anti-thrombosis (Molina *et al.* 2000), modulation of acetylcholine release (Barocci, 1999) and anti-stress effects (Bachaya *et al.* 2012) in animals and human. So, scientists are thinking that these plants may be a safe and effective substitution to control coccidiosis.

Keeping in view the above findings, the present study was planned to investigate the effects of Poly-herbal mixtures in controlling the Caecal coccidiosis in broiler chicks. This study has revealed the effects of herbal product on haematological parameters and cellular changes in intestinal mucosa at different doses over a period of 42 days in broiler chicks.

Materials and Methods

The current study was planned to investigate the effect of Poly-herbal mixture on *Caecal Coccidiosis* and its impact on haematology and histopathology of intestine in broiler chicks. Two hundred and seventy (n=270) healthy, day old broiler chicks in good health were purchased from local market and were kept at experimental poultry farm, College of Veterinary and Animal Sciences (CVAS), Jhang for 42 days. Feed and water were given ad-libitum. Birds were vaccinated against ND and IBD at day one and the booster dose of IBD at Day 8 and ND at day 10 were given to all birds.

The chicks were divided randomly into 9 equal groups from A to I with 30 chicks in each group at day 12. All groups were given same management, environment, feed and water at experimental farm. The groups of birds from A to F were given herbal mixtures but the group G was given an anticoccidial drug (Table 1). The group H had received only infection but no herbal treatment and had served as a positive control while the group I had given neither infection nor herbal treatment and had served as a negative control (Ros *et al*.2009).

The field isolate of *Eimeria tenella* was extracted from the broiler birds at sale points in the local market. The suspected cecum samples were examined in Parasitology Laboratory of CVAS, Jhang for *Eimeria tenella*. The positive samples were processed for extraction and sporulation of *Eimeria tenella* and the oocysts present in positive samples were separated through salt floatation technique as described by Bachaya *et al.* (2012) and preserved in 2.5% potassium dichromate solution. To make the challenge dose, counting of sporulated oocysts were executed by using Mac Master Chamber (Ros *et al.* 2009). For the induction of infection, 75000 sporulated oocysts per ml of distilled water were adjusted. The challenge dose of 1ml was administered to the broiler birds by using crop tube on day 14 as a single dose.

Leaves of *Azadirachta indica* (NL) and bark of *Holarrehna antidysenterica* (KB) were collected from the local market, dried and then grinded to form powder separately (Tipu *et al*. 2002). The powder was given to treatment groups in feed for 5 days from day 19 to day 23 daily in feed as given in Table 1.

Birds were examined for clinical signs continuously specially after administration of infection. All birds were weighed at the start and end of experiment. The clinical observations were recorded in the form of general body condition in terms of average body weight, morbidity and mortality throughout the period after infection.

Fifteen birds from each group were randomly selected and slaughtered at day 35 and remaining 15 birds were slaughtered at day 42. The blood samples were collected in vacutainer tubes coated with EDTA from each bird for hematological studies before slaughtering. Hematological parameters like red blood cell count (RBC), white blood cell count (TLC), hemoglobin (Hb) and Packed cell volume (PCV) were assessed by using hematology analyzer (Abbas *et al*. 2012).

Gross changes were noted in ceca in each slaughtered bird. The cecal length, hemorrhages on mucosal and serosal surfaces were observed carefully. The tissues (ceca) were processed for routine H and E staining (Bancroft and Gamble, 2008) for examination of microscopic changes like inflammation, congestion and necrosis of epithelium. After mounting, the tissue sections were examined under light microscope at different magnifying lenses i.e. 4x, 10x, 20x and 40x. Lesion scoring was done as following criteria.

No lesions found -

Mild intensity of lesions +

Moderate lesions ++

Severe lesions +++

The data were analyzed by ANOVA (Repeated measure ANOVA) and multiple comparisons were tested by Tukey's Test of statistical SPSS computer.

Results

The present study was planned to find out the anticoccidial effects of phytochemicals in broiler birds. The birds were experimentally infected with cecal coccidiosis to compare the curative effect of phytochemicals with a routine anticoccidial drug. The broiler chicks have the ability to gain weight in a short period of about 42 days. Birds suffering from coccidiosis showed impaired growth and low weight gain. Amprolium is used to treat this disease in broiler chicks on commercial level. Our results showed that the birds of group F and G receiving treatment of polyherbal mixture and amprolium respectively gained highest ($P<0.05$) live body weight (Table 2). The birds of group E showed less live body weight at day 35 but the weight was improved gradually over a period of one week and they gained higher weight as compared to groups A to D.

All the infected groups showed morbidity and mortality as given in Table 2. The morbidity in treatment groups was significantly ($P<0.05$) higher than negative control group but comparable with positive control group in our experiment. Our results showed that there was lowest mortality of birds in group F which was treated with higher doses of polyherbal mixture.

The results of blood parameters at day 35 and 42 are given in Table 3 and 4 respectively. The blood parameters are affected with coccidial infection as there is blood loss in this disease. An early ameliorative effect of anticoccidial agents can prevent the blood loss with normal blood parameters. Our results showed that red blood cell count, hemoglobin concentration and packed cell volume in birds of groups F and G were comparable with negative control group. The white blood cells perform a defensive role against infections and their number is increased in infections. In our study, the values of white blood cells in group F and G were significantly ($P<0.05$) lower than positive control but comparable with negative control group.

There are many Eimeria species which are affecting the intestine on specific locations. Eimeria tenella is specifically damaging the cecum and producing lesions in this part of intestine. The lesions are produced in wall of intestine which can be observed with naked eye and confirmed by microscopic examination. In our study, the stained tissue sections of group G showed the lowest percentage at day 35 with 46.6% of positive cases. Group G was treated with highest dose of polyherbal mixture and this group showed highest recovery from the disease and only 6.6% positive cases were observed at day 42 of this trial.

Discussion

Coccidiosis is caused by Protozoan parasites of the genus *Eimeria* (Coccidia: *Eimeriidae*) which live and multiplies in the intestinal tract. These parasites caused an enteric disease of chickens which caused economical losses in poultry worldwide (El-Shall et al. 2022). Economic importance of this disease is because of production losses and high morbidity resulting from an acute, bloody enteritis and high mortality rates. Eimeria species show specific predilection sites in intestinal tract and hence intestinal lesions of the infection are different depending on the species (Blake *et al*. 2020). About 1800 *Eimeria* species affect the intestinal mucosa of different mammals and birds but seven species of *Eimeria* including *E. tenella, E. necatrix*, *E. acervulina,*

E. maxima, E. brunetti, E. mitis, and *E. praecox* are the causative agents of coccidiosis in chickens.

This topic is specifically important due to the manifestation of a widespread anticoccidial resistance of *Eimeria* species and the problems linked with drug residues (Hashemi and Davoodi, 2010). There is a long list of chemotherapeutic agents which are being used in poultry industry all over the world. Many hazardous effects of drugs have also been reported on health and performance of broilers. This is the reason that people are thinking about alternatives to avoid antimicrobial resistance and health hazards of chemotherapeutic agents.

Performance of broiler chicks is denoted by live body weight (LBW) gain in a specific period of 42 days (Remmal *et al*. 2011). In our experiment, coccidiosis affected the live body weight of broilers chicks and different herbal products were used to control the disease separately and in combinations. Herbal products were compared with a drug (Amprolium) in our research work. The herbal mixture was equally effective against coccidiosis and the live body weight was similar to amprolium treated group. Our these findings were strongly supported by Zaman *et al*. (2012) who found *A. indica* as a cheap alternative agent against Eimeria species. They also observed similar effects of amprolium of live body weight of broiler chicks. But their work was differernt to our research as they workded on Eimeria tenella only. In another stuyd, Biu *et al*. (2006) observed the same effects of A indica on live body weight of broiler chicks but the number of birds per group was only four and histopathological changes were not correlated with coccidiosis in their study.

In our study, there was significant reduction in red blood cell count, hemoglobin concentration and packed cell volume in experimentally infected group of birds. This is similar to the findings of Ellakany *et al*. (2011) who observed the lower values of RBC, Hb and PCV in broiler chicks infected with E. tenella. The birds treated with herbal mixture showed higher values of red blood cell count, hemoglobin concentration and packed cell volume in our stuyd which was in line with the findings of Oyagbemi and Adejinmi (2012) who observed an increase in RBC, Hemoglobin concenration and packed cell volume of birds treated with A. indica. The reports of National Research Council showed that *A.indica* caused an increase in RBC count, WBC

count and antibodies production when given orally to birds (Council, 2002) which also support our findings.

The structural changes in cecum of positive control were more pronounced as compared to treatment group with polyherbal mixture and amprolium in our study. The similar histological changes were observed by Tsiouris *et al*. (2021) where they experimentally infected birds with Eimeria species and used a poly herbal mixture containing H. antidysentrica as a major constituent of the herbal preparation. In another study, Dkhil *et al*. (2013) reported the ameliorative effects of *A. indica* in Eimeria infection in rats which also support our findings of less histologic changes in intestine of birds in *Eimeria tenella* infection in broilers.

In conclusion, the constant use of chemotherapeutic substacne as feed additives against coccidiosis are creating drug resistance in broilers. They are producing a threat to consumers in terms of drug residues in meat. In this scario, the the herbal products are the best alternatives which are cost-effective and readily available in many areas of developing countries like pakistan. The herbal products are equally effective against coccidiosis as that of drugs but they are quite safe for birds as well as consumers. We summarized that herbal plants used in our research were effective and showed preventive, therapeutic and growth promoting effects against coccidiosis.

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Table 1 Groups of broiler chicks receiving infection and treatments of herbal mixture.

Groups	Infection	Treatment	Dose	No. of birds
A	*E.tenella* oocysts	NL powder	2g/kg of feed	30
B	*E.tenella* oocysts	NL powder	3g/kg of feed	30
C	*E.tenella* oocysts	KB powder	3g/kg of feed	30
D	*E.tenella* oocysts	KB powder	4g/kg of feed	30
E	*E.tenella* oocysts	NL + Mixture KB	2g/kg of feed + 3 g/kg of feed	30
F	*E.tenella* oocysts	NL + Mixture KB	3g/kg of feed + 4g/kg of feed	30
G	*E.tenella* oocysts	Amprolium	1ml/liter of water	30
H	*E.tenella* oocysts	------	-------	30
I	----------	----------	-------	30

Table 2. Clinical signs of coccidiosis observed in broiler chicks from day 14 to 42 of the trial.

Groups	Average body weight at Day 35 (g)	Average body Weight at day 42 (g)	Morbidity (%)	Mortality (%)
A	**1462**	**2014**	**76.6**	**36.6**
B	**1665**	**2215**	**80**	**33.3**
C	**1575**	**2115**	**86.6**	**40**
D	**1645**	**2232**	**83.3**	**30**
E	**1427**	**2565**	**83.3**	**30**
F	**1968**	**2605**	**90**	**13.3**
G	**1967**	**2570**	**86.6**	**20**
H	**1320**	**1690**	**90**	**46.6**
I	**1987**	**2680**	**6.67**	**6.67**

Table 3 Mean values of blood parameters at day 35 in experimental birds.

Groups	Hb (g/dl)	RBC	WBC	PCV (%)
A	8.9 ± 0.4[a]	1.81±0.8[a]	7.94±3.55[a]	26.5±1.0[a]
B	8.8 ± 0.6[a]	2.59 ±0.4[b]	7.68±6.61[a]	27.0±0.9[a]
C	8.9 ± 0.4[a]	1.80 ± 0.5[a]	7.94±3.55[a]	26.8±0.9[a]
D	9.0 ± 0.4[a]	2.61 ± 0.4[b]	7.94±3.55[a]	27.9±1.0[a]
E	9.1 ± 0.4[a]	1.78±0.4[a]	7.94±3.55[a]	28.3±0.7[b]
F	9.4 ± 0.4[b]	3.50 ±.33[c]	5.83±3.69[a]	31.0±2.0[c]
G	9.6 ± 0.3[c]	3.47 ± .12[c]	5.68±5.13[b]	34.7±0.7[d]
H	7.4 ± 0.1[d]	0.67 ± .05[d]	8.71±5.15[c]	21.7±1.0[c]
I	10.3 ± 0.4[e]	4.22 ± .12[c]	5.83±3.69[b]	34.6±0.7[d]

Mean ± SD, the values with different superscripts has significant difference at P<0.05

Table 4 Mean values of blood parameters at day 42 in experimental birds

Groups	Hb (g/dl)	RBC	WBC	PCV (%)
A	9.3±0.5[a]	1.83±.09[a]	7.89±4.31[a]	28.5±.6[a]
B	9.5±0.7[ab]	2.59±.04[b]	7.63±7.19[a]	28.3±1.0[a]
C	9.5±0.1[a]	1.81±.07[c]	7.63±7.19[a]	28.1±0.7[a]
D	9.4±0.7[a]	2.59±.05[b]	7.56±9.04[a]	28.5±0.7[a]
E	10.0±0.4[bd]	1.77±.04[a]	7.63±7.19[a]	30.2±0.9[b]
F	9.9±0.4[a]	3.48±.40[c]	5.62±5.62[b]	34.2±0.9[c]
G	9.9±0.4[ab]	3.42±.14[c]	5.66±5.49[b]	34.3±0.8[c]
H	7.1±0.5[c]	0.66±.06[d]	8.86±5.02[c]	21.8±0.7[d]
I	10.5±0.2[d]	4.27±.14[c]	5.84±3.21[b]	34.4±0.7[c]

Mean ± SD, the values with different superscripts has significant difference at P<0.05

Table 5 Histopathological findings at Day 35 in broiler birds of different groups.

Groups	Total no of Birds	Nil (-)	Mild (+)	Moderate (++)	Severe (+++)	Positive %
A	15	5	5	2	3	66.6
B	15	6	5	2	2	60.0
C	15	4	6	3	2	73.3
D	15	4	7	3	1	73.3
E	15	5	7	1	2	66.6
F	15	8	5	1	1	46.6
G	15	7	4	3	1	53.3
H	15	1	3	4	7	93.3
I	15	14	1	0	0	6.6

Table 6: Histopathological findings at Day 42 in broiler birds of different groups

Groups	Total no of Birds	Nil (-)	Mild (+)	Moderate (++)	Severe (+++)	Positive %
A	15	6	4	3	2	60.0
B	15	7	5	2	1	53.3
C	15	6	5	2	2	60.0
D	15	9	3	2	1	40.0
E	15	8	4	2	1	46.6
F	15	8	4	2	1	6.6
G	15	9	4	1	1	40.0
H	15	2	4	3	6	86.6
I	15	13	1	1	0	13.3