Evaluation of anticoccidial activity of aqueous extract of *Fomes fomentarius*

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Abstract: The aims and objectives of the present study were to evaluate the in vivo anticoccidial effects of aqueous extract of wild mushroom *Fomes fomentarius* in comparison to the reference drug amprolium against coccidiosis in broilers on the basis of oocysts per gram of faeces, weight gain and feed conversion ratio. This study showed that treatment with *F. fomentarius* resulted in a marked reduction in the number of coccidian oocysts shed in the faeces, leading to improved weight gain and better feed conversion ratio. The results confirmed the virulence of coccidian oocysts and the effectiveness of both amprolium and *F. fomentarius* extract against coccidian oocysts.
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Key Words: Coccidiosis; Poultry; *Fomes fomentarius*

Introduction

Coccidiosis is the most important protozoan disease affecting the poultry industry worldwide. It has been documented that coccidiosis is the most consistently reported health problem in poultry (Biggs, 1982; Williams, 1999). In our country, it is considered a serious problem causing huge economic loss to poultry industry, especially in the production of broiler chicken. Over the past 100 years, much research has persisted on coccidiosis because of its significance in the animal industry. In all parts of the world where confinement rearing is practiced, coccidiosis represents a major disease problem demanding the attention of poultry producers, feed manufactures, and poultry disease experts (Reid, 1978). Coccidiosis is believed to be a commonest depreciator or even a potential killer of our poultry.

Material and methods

Fomes fomentarius (Polyporaceae)

Fomes fomentarius (commonly known as the tinder-fungus, hoof fungus, tinder conk and tinder polypore or ice man fungus) is a species of fungal plant pathogen found in Europe, Asia, Africa and North America. The species produces very large polypore fruit bodies which are shaped like a horse's hoof and vary in colour from a silvery grey to almost black, though they are normally brown. It grows on the side of various species of tree, which it infects through broken bark, causing rot. The species typically continues to live on trees long after they have died, changing from a parasite to a decomposer. *Fomes fomentarius* has a fruit body of between 5 and 45 centimetres across, 3 and 25 cm wide and 2 and 25 cm (0.8 and 9.8 in) thick (Phillips and Roger 1981).

Mushrooms collected were firstly washed with distilled water. After washing they were processed for shade drying in a well ventilated room. To facilitate complete drying, the fruit bodies of mushroom were cut into small pieces and then dried in shade conditions. The dried mushrooms were milled to a fine powder using an electric blender. The mushroom powder was again dried for about 3 h in an oven at 40°C and then stored in plastic polythene bags and kept at room temperature until required for extraction.

Preparation of aqueous extract

The crude aqueous extracts of the selected mushroom was prepared according to the techniques described by Iqbal *et al*. (2004). The powdered mushroom parts (100 g) were extracted with distilled water (500 ml) at 90-100^{o}C in a Soxhlet extractor for 8 h. The aqueous extract was filtered, and stored at 4 °C until used.

Broilers and experimental design

Day-old broiler chicks were purchased from local market and screened for coccidial infection. The broiler chicks were reared under standard management practices in the animal house of the Department of zoology, University of Kashmir, for five weeks. The birds were maintained in a coccidian free atmosphere. The method of housing the broilers was an intensive deep-litter system. Before birds were placed, the houses were cleaned, washed, disinfected and provided with saw dust. The ambient temperature in experimental house was maintained at 29°C during the first week and after than gradually decreased by 3°C in the third week, and finally fixed at 22°C thereafter. All birds were reared in cages, kept in strictly isolated room. To meet the nutrient requirements of the broiler chicken during the entire experimental period, a complete basal diet was formulated for each of the 2 stages of growth; starter and grower. The diets were formulated to meet the nutrients requirements of

broilers as recommended by the National Research Council (NRC, 1994). The chicks were provided with standard coccidiostat free feed. The feed and water was provided *ad libitum* during the study period. Lighting of the environment was provided for 24 hrs. At 22nd day age, the birds were used for experimental purpose. All the birds were tagged to maintain their identity.

On day 22 the body weight of all chicks were taken and grouped into four experimental groups A, B, C and D each having 10 chicks by random allocation. Underweight and weak chicks were excluded from the experiment. The birds in groups A, B and C were inoculated with mixed coccidial oocysts of *Eimeria* species at the rate 3850-4000 sporulated oocysts per bird (Williams, 2001) using insulin syringe introduced directly into the crop of each bird at 22nd day of age. By day 6 post-inoculation (PI), they were treated with mushroom extracts and recommended medicine according to the following schedule:

Group-A: Infected and treated with extract of mushroom (1) in water for 5 consecutive days.

Group-B: Infected and treated with recommended medicine for 5 consecutive days.

Group-C: Infected and un-medicated group.

Group-D: Uninfected and un-medicated group.

Group D served as uninfected and un-medicated control, groups A to C were infected with sporulated oocysts of *Eimeria* on the 22nd day of age. Group C was infected and left untreated. Group B was infected, and treated with the allopathic drug amprolium. The Group A was infected and treated with aqueous extract of *Fomes fomentarius.* Drinking water was provided *ad-libitum* throughout the entire period of study.

An inventory of birds for procuring infection

An inventory of poultry birds in nature was made for getting the coccidian infection in nature. Coccidiosis suspected guts were collected from different poultry Farms. All the intestines and caeca were opened and their contents (faeces) were collected in a beaker. The oocysts thus procured will be kept in a medium for experimental infection.

Parasite inoculation

Feacal samples from all experimental groups were collected and examined for any contamination by coccidia parasites prior to the experimental infection. All groups were found negative for coccidial oocysts. On 22nd day of age each group was inoculated by coccidial oocysts of *Eimeria* species obtained from the guts of infected chicks directly into crop or by giving oral infection. The sporulated oocysts were given at the dose rate of 3850-4000 oocysts per bird (Williams, 2001). One ml of oocyst suspension in distilled water was orally inoculated directly in to the crop using a flexible plastic tube fitted to 5ml syringe.

Determination of weight gain and feed conversion ratio

Performance of broilers was evaluated by recording body weight (BW), daily body weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) during the entire experimental period. Mortality was recorded as it occurred. Weight gain of the broilers was monitored using a weighing balance (made in China by Hana) every morning prior to feeding. The feed: gain ratio per group was determined, where feed: gain per bird=total feed consumption by the birds in a cage divided by weight gain of surviving birds + weight gain of dead birds in the cage. The group with the highest value indicates evidence of depression of feed intake due to infection with *Eimeria*. The broiler mash contained maize, groundnut cake, wheat chaff, rice bran, fishmeal, bone-meal, limestone and premix, giving about 22 % crude protein and 2800 Kcal/kg metabolisable energy. The feeders and drinkers were washed daily using boiling water to reduce the risk of contamination.

Collection of faecal samples and laboratory examination

The birds started shedding oocysts 128 hours post infection. The fecal droppings in each cage were collected on a polyethylene sheet placed on the fecal tray of the cages. The faecal samples were continuously observed after a time interval of 24 hrs, 48 hrs and 72 hrs and severity of infection is confirmed. Diagnosis of *Eimerian* oocysts in faeces is an easy to get an impression of the infection level, direct smear method and both qualitative and quantitative techniques can be done to faecal sample. McMaster's oocyst counting technique was used for counting the coccidian oocysts (Soulsby, 1982). Faeces from each group were thoroughly mixed in plastic bottles using a spatula. One gram of the faecal sample was placed in a sterile bottle and homogenized by mixing with 1 m of flotation sodium chloride (NaCl) salt solution to make a suspension that was then mixed with 9m of the salt solution, sieved in gauze wire mesh or muslin, the solid matter discarded and the filtrate collected in clean sterile plastic tubes filled to the brim and a cover slip was placed on top taking care to exclude air bubbles. The bottles were allowed to stand upright for 15 min to enable coccidia oocysts to float to the cover slip before examination under a light microscope at ×10 and ×40 magnifications. A portion of the positive sample only was used to fill the McMaster counting chamber and allowed to stand for about 15 min to enable oocysts to float and settle at the top of the chamber to facilitate identification and counting of the oocysts under the microscope using a differential counter. Absolute numbers of coccidia oocysts counted per ml of the solution were recorded.

Oocysts counting

To obtain accurate information with regard to severity of an infection, egg counting methods were carried out to determine number of eggs per gram (EPG) of faeces. For this purpose McMaster counting chamber was used. This method is generally used in litter oocyst counting procedures since the percentage of Sporulation and oocyst dimensions are not required in this measurement.

McMaster chamber method

The McMaster chamber method is documented by Hodgson (1970), Long and Rowell (1958), and Long *et al.* (1976).

Equipment: Centrifuge, cheesecloth (muslin), beaker, a jar with a lid, or Parafilm, McMaster counting chamber, hand tally counter, 10 or 15ml graduated test tubes, saturated sodium chloride.

Procedure:

1. 10 g of litter are soaked in 100 ml of distilled water for 24 hours at 4◦C in a 200 ml beaker that is tightly covered (either with a lid or Parafilm).

2. The beaker is shaken vigorously and the litter is filtered through a single thickness of muslin cloth.

3. A 15 ml centrifuge tube is filled with filtrate to 1 cm from the top and centrifuged for five minutes at a speed that concentrates the solids.

4. The supernatant is discarded. The pellet was resuspended in 100ml of saturated salt solution (NaCl).

5. Two chambers of McMaster counting slide were filled with the suspension with the help of plastic transfer pipette and allowed 3-5 minutes for floatation of oocysts before examination. The oocysts float to the top of the solution, and the total number is counted.

Calculation:

Number of oocysts per gram of litter = n / 0.15 × volume × 0.1

Where n = number of oocysts counted, 0.15 = volume of the McMaster counting chamber, volume = 100 ml of water that the litter is soaked in, and 0.1 = correction for 10 g of litter originally taken.

Therefore, each oocyst counted is equivalent to 67 oocysts per gram of sample. When calculations of oocysts per bird are done, the number of oocysts per gram is divided by the number of birds in the pen to give the number of oocysts per gram per bird.

Statistical Analysis

The whole data was fed into Microsoft Excel 2010, a computer program (SPSS 11.5 for windows) and Primer software was used for data analysis. The data was represented as mean of replicates followed by standard deviation i.e. Mean ± standard deviation (SD).

Results and Discussion

Oocyst per gram (OPG) counts

The OPG counts of different groups of chickens are represented in Table 1. The highest oocyst count per gram of faeces (OPG) was recorded in group C as it was untreated group. Prior to treatment at 26th day the oocyst output of birds was 5010.71 ± 28.029 oocysts/g faeces (group A), 4879.40 ± 25.87 oocysts/g faeces (group B) and 5187.21 ± 23.825 oocysts/g faeces (group C). The faeces of uninfected group D were free of coccidial oocysts. After treatment the oocysts detected in the *F. fomentarius* treated group (A) on 27th day had reduced significantly in number (2800.58 ± 16.920 oocysts/g faeces) compared to un-treated group (C) which showed increase in oocysts released (5730.42 ± 26.150 oocysts/g faeces). By 28th day, the oocysts released in A and B group had reduced to 986.0 ± 11.230 and 15.36 ± 1.129 and by day 29 the birds in these groups were almost free of infection (group A 210.34 ± 6.099) (group B 3.26 ± 0.053), while group C continued discharge high number of oocysts.

Body weight gain records and Feed conversion ratio

The impact of oral administration of sporulated coccidial oocysts on body weight gain of different groups of chickens followed by administration of *F. fomentarius* extract are represented in Table 2. The mean initial weight of chicks for all groups was almost similar which was recorded on day 1-22nd day. Among the treated groups the significant improvement in body weight was recorded in group B. Chickens of group A gained the next highest body weight on the same day. The results further showed that infection with coccidial oocysts results in the decrease of feed intake of birds in all the infected groups, but this was followed by a compensatory increase in feed intake in group A and B after treatment. Feed conversion ratio was higher in group C as compared to all the other groups. The mean weight gain of the birds in group C at day 35 was also significantly lower (1201.34 ± 12.981 grams) than other treated groups.

The experimental infection of the broiler chickens with coccidial oocysts showed clinical signs of weakness, reduced appetite, diarrhoea, and presence of oocysts in faeces. The experimental trials in all the infected birds showed a significant reduction in faecal oocyst output in birds that were treated with either aqueous extract of *F. fomentarius* or amprolium. However the lowest OPG was recorded in amprolium treated group indicating the highest prophylactic efficacy among all groups. The reason for better efficacy of amprolium could be that it is already in the pure state and we can expect a bit low efficacy in the crude extracts of *F. fomentarius.* In this study a gradual but significant oocyst output in both infected-

untreated and infected-treated groups was recorded. The results in terms of use of aqueous extract of *F. fomentarius* to suppress oocysts of coccidia in broilers was in full agreement with Conway, *et al.*, (1993) who studied the effects of different levels of oocysts inocula of *Eimeria acervulina, E. tenella* and *E. maxima* on plasma constituents, packed cell volume, lesion scores and performance in chickens and Elmusharaf *et al.*, (2006) who investigated the effect of a Manna-oligosaccharide (MOS) preparation on *Eimeria tenella* infection in broiler chickens. Moreover the results of this study are also in agreement with Wills, *et al.*, (2010), they strongly suggest that a diet supplemented with 5% FMG as an alternative control method in reducing *Eimeria* oocyst numbers during grow out.

Table 1. Oocyst output of broilers infected with coccidian oocysts (*Eimeria*) and treated with aqueous extract of *Fomes fomentarius*

Oocyst output per gram of faeces	Age in Days	Different treatment groups			
		Group A	Group B	Group C	Group D
After infection	26th day	5010.71 ± 28.029	4879.40 ± 25.827	5187.21 ± 23.825	0
During treatment	27th day	2800.58 ± 16.920	2274.91 ± 19.345	5730.42 ± 26.150	0
	28th day	986.01 ± 11.230	15.36 ± 1.129	6088.03 ± 23.384	0
	29th day	210.34 ± 6.099	3.26 ± 0.053	6552.27 ± 28.196	0
Significance		**	***	NS	NS

(* less significant; ** more significant; *** highly significant; NS not significant)

Group A = Infected and treated with aqueous extract of *Fomes fomentarius* at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 1. Graphical representation of Table 1

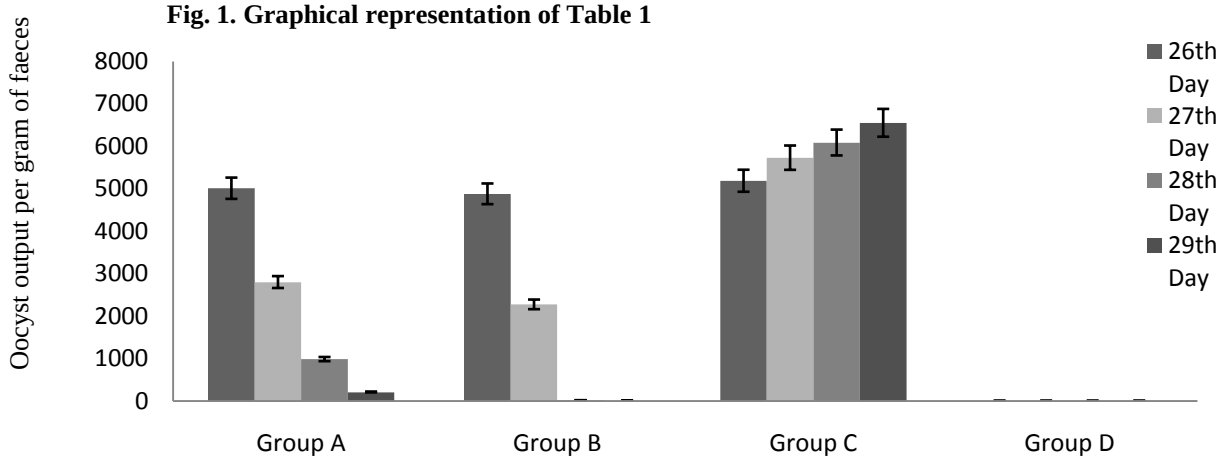
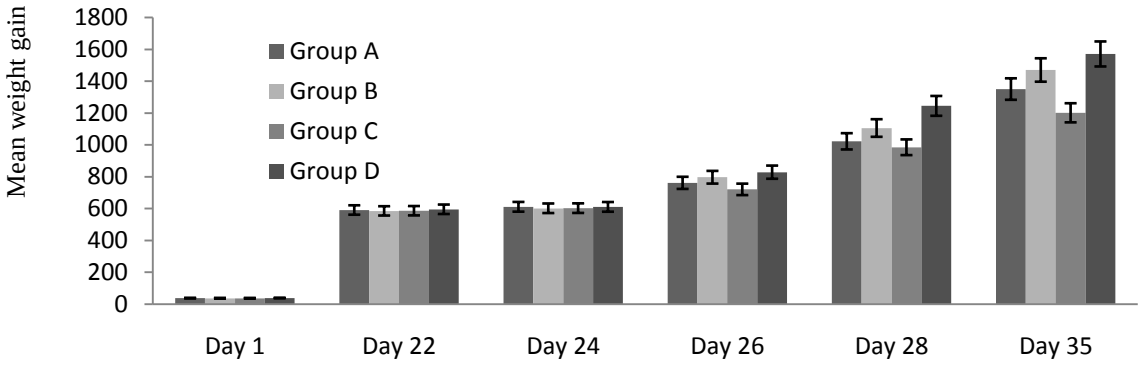


Table 2.Group mean weight gain (in grams) of broilers infected with coccidia *(Eimeria*) and then treated with aqueous extract of *Fomes fomentarius* and amprolium

Parameters	Age in days	Group mean weight gain (in grams)			
		Group A	Group B	Group C	Group D
Initial weight	1	37.55 ± 0.541	36.98 ± 0.643	36.8 ± 0.585	38.04 ± 0.621
At pre-infection	22	590.84 ± 2.85	585.32 ± 2.69	586.28 ± 3.15	595.32 ± 2.52
At infection time	24	610.92 ± 3.762	601.66 ± 3.20	602.49 ± 2.983	610.45 ± 3.045
Before treatment	26	761.33 ± 4.322	796.57 ± 4.172	720.32 ± 4.165	828.35 ± 3.873
Three days after the treatments	28	1022.11 ± 8.643	1105.73 ± 7.653	984.9 ± 8.960	1244.76 ± 7.924
Seven days after the treatments	35	1350.41 ± 12.402	1470.25 ± 13.122	1201.34 ± 12.981	1570.86 ± 11.137

Group A = Infected and treated with aqueous extract of *Fomes fomentarius* at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 2. Graphical representation of Table 2



The highest feed conversion ratio observed in the infected, untreated birds (2.483) was observed provides an evidence of depression of feed intake due to infection with coccidian oocysts. The highest feed conversion ratio reported in infected broilers resulted in significant reduction in the body weight. The study revealed that groups of birds not infected with coccidial oocysts consume more feed, while infected groups showed lower feed intake was due to coccidial stress. Hayat *et al.*, (1991) supported the results of the present study and reported that coccidial infection decreased feed intake. Conway *et al.*, (1993) also reported that a significant reduction in body weight occurred in broilers infected with a dose of 10000 sporulated oocysts of *E. tenella*. The less effect of infection on growth performance may be related to the mildness of the infection. Under conditions of more severe infection with *Eimeria*, weight gain is generally reduced (Johnson and Reid, 1970; Conway *et al.*, 1993; McDougald, 2003; Chapman *et al.*, 2004).

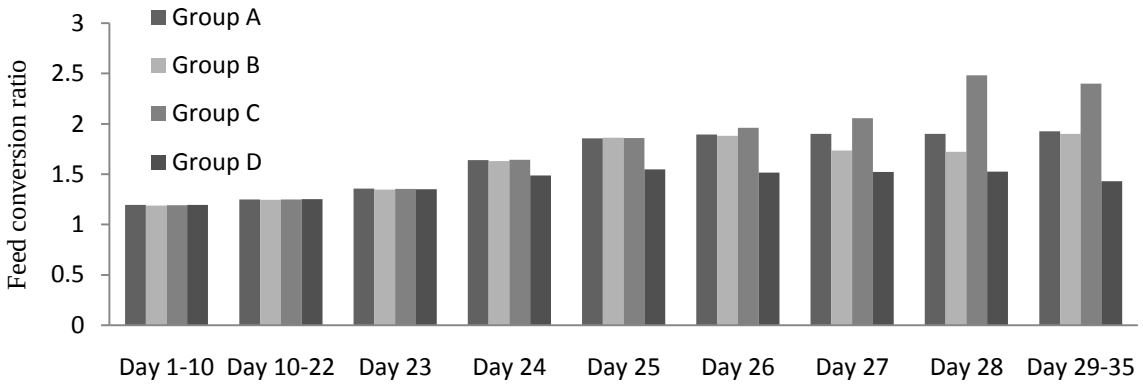
The results of the present work showed in first experiment the birds of group A, infected and treated with aqueous extract of *F. fomentarius* extract had significantly higher mean weight gain (1350.41 ± 12.402 g) and lower feed conversion ratio (FCR) (1.927), whereas birds of group C, infected but not treated gained lowest weight (1323.9 g) and highest FCR (2.399). The poorest FCR was observed in birds which were infected but non-medicated. These results are supported by Voeten et al., (1988) who found that coccidiosis adversely affected growth and feed conversion.

Table 3: Feed Conversion ratio of different treatment groups

Parameters	**Age in Days**	**Feed Conversion Ratio = Feed consumed / Weight gained**			
		Group A	**Group B**	**Group C**	**Group D**
Not infected	1-10	1.195	1.189	1.191	1.194
	11-22	1.250	1.246	1.248	1.252
Infected	23	1.358	1.347	1.354	1.352
	24	1.639	1.631	1.642	1.486
	25	1.855	1.862	1.858	1.548
During treatment	26	1.894	1.881	1.961	1.517
	27	1.902	1.736	2.057	1.523
	28	1.901	1.721	2.483	1.524
After treatment	29-35	1.927	1.901	2.399	1.431

Group A = Infected and treated with aqueous extract of *Fomes fomentarius* at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 3. Graphical representation of Table 3



Bioactive compounds or polysaccharides are known to play vital roles in enhancing health; they block colonization of the intestine by pathogens, thereby improving their elimination from the body (Elmusharaf *et al.*, 2006; Guo *et al.*, 2004., Hughes, *et al.*, 1958). Some biologically active compounds or organic acids, resins, and glycosides which include steroid and triterpenoid saponins are known to have therapeutic uses against microbes and parasites (Anon, 2006; Die *et al.*, Guo *et al.*, 2004; Hobbs, 1995). The mushrooms used in this study were reported to possess these active compounds. Other studies have shown that some mushrooms have polysaccharides that play a role in stimulating the activities of many interdependent cell types such as T and B-lymphocytes, macrophages, and natural killer (NK) cells, inducing production and secretion of cytokines and complement (Guo, *et al.*, 2004). Other mushrooms (e.g. *Fraxinella*, *Boletus* and *Lactarius* spp.) have also been reported to prevent intestinal coccidiosis in poultry (Guo *et al.*, 2004*;* Harkonen, 1998; Pang, *et al.*, 2000). Other authors reported that some mushrooms contain chemical substances that enhance the immune response and control certain parasitic and viral diseases (Anon, 2006; Guo *et al.*, 2004*;* Oei, 2003; Wachtel *et al.*, 2004; Wasser, 2002; Zakhary *et al.*, 1983). However, the active principles and the mechanisms of action of these mushrooms have not been fully elucidated, and should be the subject of future studies. This study showed that treatment with *F. fomentarius* resulted in a marked reduction in the number of coccidian oocysts shed in the faeces, leading to improved weight gain. The results confirmed the virulence of coccidian oocysts and the effectiveness of both amprolium and *F. fomentarius* extract against coccidian oocysts. Hence, the utilization of *F. fomentarius* has potential as an alternative to other methods in coccidiosis intervention in elimination of clinical *Eimerial* infection in broiler chickens.

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