

Anticoccidial effects of acetic acid on performance and pathogenic parameters in broiler chickens challenged with *Eimeria tenella*¹

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ABSTRACT.- Abbas R.Z., Munawar S.H., Manzoor Z., Iqbal Z., Khan M.N., Saleemi M.K., Zia M.A. & Yousaf A. 2011. **Anticoccidial effects of acetic acid on performance and pathogenic parameters in broiler chickens challenged with *Eimeria tenella*.** *Pesquisa Veterinária Brasileira* 31(2):99-103. Department of Parasitology, University of Agriculture, Faisalabad 38040, Pakistan. E-mail: raouaf@hotmail.com

The objective of the present study was to evaluate the anticoccidial effect of the different concentrations of the acetic acid in the broiler chickens in comparison with the amprolium anticoccidial. A total of 198 chicks were placed 11 per pen with three pens per treatment. The different concentrations (1%, 2% and 3%) of acetic acid and amprolium (at the dose rate of 125ppm) were given to the experimental groups in drinking water from 10–19th days of age. One group was kept as infected non medicated control and one as non infected non medicated control. All the groups were inoculated orally with 75,000 sporulated oocysts at the 12th day of age except non infected non medicated control. Anticoccidial effect was evaluated on the basis of performance (weight gain, feed conversion ratio) and pathogenic (oocyst score, lesion score and mortality %age) parameters. Among acetic acid medicated groups, the maximum anticoccidial effect was seen in the group medicated with 3% acetic acid followed by 2% and 1% acetic acid medicated groups. Amprolium and 3% acetic acid were almost equivalent in suppressing the negative performance and pathogenic effects associated with coccidiosis (*Eimeria tenella*) challenge. In summary, acetic acid has the potential to be used as alternative to chemotherapeutic drugs for *Eimeria tenella* control.

Concentration-dependent anticoccidial effect of acetic acid suggests that further studies should be carried out to determine the possible maximum safe levels of acetic acid with least toxic effects to be used as anticoccidial.

INDEX TERMS: Coccidiosis, *Eimeria tenella*, acetic acid, anticoccidial.

INTRODUCTION

Poultry sector is one of the most vibrant segments of agriculture sector in Pakistan. It generates direct or indirect employment for about 1.5 million people. Poultry meat

contributes about 19% of the total meat production in the country (Ahmad et al. 2010, Ghafoor et al. 2010) and is one of the best available sources for the production of high biological value animal protein. Commercial poultry farming in Pakistan is expanding day by day. However, this sector is still confronted with many problems like coccidiosis which are hindering its progress (Saima et al. 2010).

Avian coccidiosis is caused by obligate intestinal protozoan parasites belonging to genus *Eimeria*. *Eimeria tenella* primarily invades and resides in the lining of the cecum of exposed chickens (Allen 1999, Laurent et al. 2001). Infective sporozoites enter the cecal mucosa by penetrating villus epithelial cells, resulting in extensive destruction of the cecal epithelium, hemorrhagic feces, reduction in body weight gain, and decrease in feed efficiency and eventually mortality which

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lead to serious economic consequences with an estimated worldwide annual loss of more than \$3 billion (Williams 1999, Dalloul & Lillehoj 2006). Thus far, chemoprophylaxis and anticoccidial feed additives have controlled the disease but have been complicated by the emergence of drug resistance (Dutta et al. 1990, Kawazoe & Fabio 1994) and their toxic effects on the animal health (Nogueira et al. 2009).

To prevent the emergence of drug resistance, new drugs have been developed and administered on a rotational basis with existing drugs. However, this has resulted in the increased cost of poultry products. Furthermore, drug or antibiotic residue in the poultry products is potentially annoyance to consumer. Therefore, alternative strategies are being sought for more effective and safer control of coccidiosis in chickens (Dalloul et al. 2006, Williams 2006, Abbas et al. 2010). Acids such as fumaric, propionic, sorbic, acetic, and tartaric acid have also been reported for positive influence on feed conversion ratio and growth performance (Vogt et al. 1981, 1982).

In Pakistan, acetic acid is being used extensively as anticoccidial agent in the local poultry industry. According to poultry producers, acetic acid has been found effective against avian coccidiosis, but there is no scientific report available regarding its anticoccidial activity. Therefore, this study has been planned to evaluate the possible anticoccidial effect of acetic acid, if any, employing standard parasitological procedure.

MATERIALS AND METHODS

Experimental birds, feed and management. One hundred and ninety eight (1-day-old) broiler chicks (Hubbard Al-Noor Chicks, Pvt) were purchased from local hatchery. Chicks were reared under standard management practices. All the chicks were kept on broiler starter ration up to 2 weeks of age and then fed a broiler finisher. The feed and water were provided *ad libitum*. The temperature was maintained at 85-90°F during the first week of age and was reduced by 5°F on weekly basis. Lighting was provided for 24 hours through out the experimental period. All the birds were vaccinated for New Castle disease on 5th day, for Infectious Bursal disease on 14th day, and for Hydro pericardium syndrome on 18th day of age.

Parasite. Coccidial oocysts were obtained from the caeca of infected chickens and were propagated in broiler chickens by giving oral infection. After obtaining sufficient amount of oocysts, they were sporulated by placing in 2.5% $K_2Cr_2O_7$ in the presence of suitable humidity and temperature. Sporulated oocysts were cleaned and counted by the McMaster technique (MAFF 1986). The required concentration of the sporulated oocysts (75,000/ml) was maintained with phosphate buffered saline.

Study design. The chicks (n=198) were placed 11 per pen with three pens per treatment. Treatments were randomized within blocks. Treatments were as follows:

1% acetic acid medicated group; 2% acetic acid medicated group; 3% acetic acid medicated group - in Pakistan, the poultry farmers use 2% acetic acid in drinking water to prevent the coccidiosis, therefore, in the present study; we used one concentration up (3%) and one down (1%) which is the normal approach for the scientific validation of the ethno veterinary practices. Amprolium medicated group - amprolium was used at

the recommended dose rate of 125ppm; infected non medicated control; non infected non medicated control. All the groups were inoculated orally with 75,000 sporulated oocysts at the 12th day of age except non infected non medicated control. Amprolium (at the dose rate of 125 ppm) and different concentrations of acetic acid were given in water from 10 to 19th days of age.

Evaluation of anticoccidial effects. Five chicks from each group were weighed on day of inoculation (12th day of age) and then reweighed on 7th day post inoculation (19th day of age).

Feed conversion was calculated as the grams of feed consumed to produce one gram of live weight. Statistical analysis of FCR was not possible because of group feeding of birds.

Five chicks from each group were sacrificed for post mortem examination at 7th day of post inoculation (19th day of age). Caecal lesions were scored by the lesion scoring technique described by Johnson and Reid (1970).

An oocyst index (0 to 5) was determined by microscopical examination of scrapings from the caeca of chicks sacrificed for lesion scoring at 7th day of post inoculation (Hilbrich 1978).

Caecal contents of three chicks from each group were removed as soon as possible for the pH measurements on days 3, 5 and 7 post inoculation following procedures outlined by Ruff et al. (1974).

Statistical analysis. Data obtained on various parameters were analyzed by analysis of variance, and the mean values were compared by Tukey test. The differences among group means were considered significant at $P < 0.05$.

RESULTS

The results of weight gain (Table 1) showed that the body weight gains in all the medicated groups were significantly ($P < 0.05$) higher than infected non medicated control. Among acetic acid medicated groups, the maximum weight gain was shown by the group medicated with 3% acetic acid followed by the groups medicated with 2% and 1% acetic acid. There was no significant ($P < 0.05$) difference between the weight gains of groups medicated with 3% acetic acid and amprolium.

Table 1. Comparative values of the mean (and SEM) weight gain, feed conversion ratio, lesion score, oocyst index, and mortality percentage

Treatments	Weight gain	Feed* conversion ratio (g/g)	Lesion score	Oocyst score	Mortality % age
Acetic acid (1%)	277 ^b	1.27	2.6 ^b	2.7 ^b	9.2
Acetic acid (2%)	289 ^{ab}	1.21	2.3 ^b	2.3 ^b	7.3
Acetic acid (3%)	300 ^a	1.15	2 ^b	1.6 ^{bc}	5.8
Amprolium (125ppm)	285 ^{ab}	1.34	2.3 ^b	1.3 ^c	5.2
Infected non medicated	257 ^c	1.43	3.6 ^a	4.6 ^a	23.4
Non infected non medicated	287 ^{ab}	1.32	-	-	-
S.E.M.	14.5	-	0.22	0.38	-

^{a-c} Means shearing similar superscripts within a column do not differ ($P < 0.05$).

* Statistical analysis was not possible because of group feeding of chicks.

The results of FCR (Table 1) revealed that the FCR values of acetic acid medicated groups were numerically lower compared with infected non medicated group, although a statistical comparison could not be made due to group feeding. Acetic acid medicated groups showed better FCR compared with amprolium medicated group. Among acetic acid medicated groups, the lowest FCR was observed in the group medicated with 3% acetic acid followed by the groups medicated with 2% acetic acid and 1% acetic acid.

The results of the lesion scores are indicated in Table 1. The uninfected control group showed zero score. All the groups medicated with acetic acid and amprolium showed significantly ($P < 0.05$) lower lesion scores than infected non medicated group. Among all medicated groups, the lowest lesion scores were shown by the group medicated with 3% acetic acid.

The results of the oocyst scores (Table 1) revealed a pattern relatively similar to that of lesion scores among different groups. The oocyst scores were lower ($P < 0.05$) in medicated groups compared with infected non medicated group. Among medicated groups the maximum reduction in oocyst scores were seen in the amprolium medicated group followed by 3% acetic acid, 2% acetic acid and 1% acetic acid medicated groups respectively. The results revealed that all the acetic acid medicated groups have anticoccidial activity in terms of oocyst score.

The percent mortality (Table 1) was higher in the infected non-medicated control group compared with medicated groups. Among medicated groups, the mortality was numerically lower in amprolium medicated group followed by groups medicated with 3%, 2% and 1% acetic acid respectively.

The results on the pH of cecal contents in the different experimental groups are shown in (Table 2). On all days (3, 5 and 7 post inoculation), a significant ($P < 0.05$) difference was observed among intestinal pH of the acetic acid medicated groups, amprolium medicated group and non infected non medicated control, and infected non medicated control. The maximum pH was observed in the infected non medicated control followed by amprolium medicated group and acetic acid medicated groups. There was no significant ($P < 0.05$) difference of pH between amprolium medicated group and non infected non medicated control.

Table 2. Effect of acetic acid treatment on mean (n = 3) pH of caecal contents (on 3, 5 and 7th days post inoculation) in broiler chickens

Treatments	3 rd day	5 th day	7 th day
Acetic acid (1%)	6.24 ^c	6.15 ^c	6.30 ^c
Acetic acid (2%)	6.18 ^c	5.92 ^c	6.38 ^c
Acetic acid (3%)	5.81 ^c	6.25 ^c	5.69 ^c
Amprolium (125ppm)	6.53 ^a	6.75 ^a	6.82 ^a
Infected non medicated	6.96 ^b	7.23 ^b	7.42 ^b
Non infected non medicated	6.52 ^a	6.64 ^a	6.92 ^a
S.E.M.	0.001	0.004	0.003

^{a-c} Means shearing similar superscripts within a column do not differ ($P < 0.05$).

DISCUSSION AND CONCLUSIONS

To prevent and control coccidiosis, the poultry industry has relied heavily upon prophylactic chemotherapy resulting in the development of resistant strains of *Eimeria* to all introduced anticoccidial drugs (Chapman 1997). Therefore, recent research has focused on the alternative strategies for the control of avian coccidiosis such as adding acids in the diet (Runho et al. 1997, Thompson & Hinton 1997, Vale et al. 2004). In the present study, acetic acid administered in drinking water has shown the anticoccidial effects against *Eimeria tenella* in terms of improved weight gains, better FCR, lower oocyst and lesion scores. The coccidiosis can lead to enormous economic losses in the poultry industry due to the intestinal lesions caused by the *Eimeria* species and subsequent malabsorption of nutrients. The performance improvements observed while using acetic acid (particularly 3%) could overcome these losses.

Acetic acid (CH_3COOH) also known as ethanoic acid, is a weak organic acid, which gives vinegar and is a partially dissociated acid in an aqueous solution. Very limited research exists on the effects of acetic acid on poultry health particularly during coccidiosis. However, few reports (Chaveerach et al. 2004, Van Immerseel et al. 2004) are available regarding the antimicrobial effects of organic acids other than acetic acid. These organic acids showed promise in altering bacterial activities and cecal environment in chicken.

In the present study, drinking of acetic acid resulted in protective effects against *E. tenella* probably by declining pH of ceca and obliteration of oocysts in chickens. Indeed, the improved body weight gains and FCR shown in this work could be due to growth promoting effects of acetic acid that enhanced the feed intake and digestibility.

A number of similar reports of positive effects of organic acids on weight gains (Vogt et al. 1981, Henry et al. 1987, Manickam et al. 1994, Runho et al. 1997, Yeo & Kim 1997, Gunes et al. 2001) support the findings of the current study. On the other hand, there are also some reports (Watkins & Kratzer 1984, Izat et al. 1990, Lee et al. 1993) which differ from the results of present study. According to these researchers, the supplementation of an acid in feed or drinking water did not show any effect on weight gain.

The acetic acid medicated chickens also showed significantly reduced oocyst and lesion score. According to several researchers, organic acids have antimicrobial (Hinton & Linton 1988, Thompson & Hinton 1997, Entani et al. 1998, Chaveerach et al. 2004, Van Immerseel et al. 2004) and antibacterial (Chaveerach et al. 2004, Van Immerseel et al. 2004) activities. However, the anticoccidial activity of the acetic acid has been reported for the first time.

The pH in the ceca was significantly higher in infected non medicated group than non infected non medicated and all medicated groups. Ruff et al. (1974) suggested that this increase reflects changes in the cecal flora since: 1) the "normal" ceca contain a predominance of acetic-acid-producing bacteria (Moore 1969); and 2) the pH in the ceca is significantly greater in germ-free birds than in conventional birds.

A little bit is known regarding the mode of action of acids against bacteria. In this aspect many researchers have different views. According to some researchers, the antibacterial activity of acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pKa-value of the respective acid, and the pH of the surrounding milieu. Acids are lipid soluble in the undissociated form, and they easily enter the microbial cell by both passive and carrier-mediated transport mechanisms. Once in the cell, the acids release the proton H⁺ in the more alkaline environment, resulting in a decrease of intracellular pH. This influences microbial metabolism, inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to export the excess of protons H⁺, ultimately resulting death by starvation. In the same matter, the protons H⁺ can denature bacterial acid sensitive proteins and DNA. Generally lactic acid bacteria are able to grow at relatively low pH, which means that they are more resistant to acids than other bacterial species, such as *Escherichia coli* and *Salmonella*. Lactic acid bacteria, like other gram-positive bacteria, have a high intracellular potassium concentration, which counteracts acid anions (Russell & Diez-Gonzalez 1998).

In general, potential bacterial targets of biocidal compounds include the cell wall, cytoplasmic membrane, and specific metabolic functions in the cytoplasm associated with replication, protein synthesis, and function (Denyer & Stewart 1998, Davidson 2001). Although the antibacterial mechanisms for acids are not fully understood, they are capable of exhibiting bacteriostatic and bacteriocidal properties depending on the physiological status of the organism and the physicochemical characteristics of the external environment. Given the weak acid nature of most of these compounds, pH is considered primary determinant of effectiveness because it affects the concentration of undissociated acid formed (Davidson 2001). It has been traditionally assumed that undissociated forms of acids can easily penetrate the lipid membrane of the bacterial cell and once internalized into the neutral pH of the cell cytoplasm dissociate into anions and protons (Eklund 1983, 1985, Cherrington et al. 1990, 1991, Davidson 2001). Generation of both of these species potentially presents problems for bacteria that must maintain a near neutral pH cytoplasm to sustain functional macromolecules. Export of excess protons requires consumption of cellular adenosine triphosphate (ATP) and may result in depletion of cellular energy (Davidson 2001).

Data from the present study indicate that acetic acid has anticoccidial activity against *Eimeria tenella* in broiler chickens; therefore, it has the potential to be used as an alternative coccidiosis control agent. But the exact mode of action against *Eimeria* species is not clear. So, further research should be carried out to find out the exact mode of action of acetic acid against coccidia.

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