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Original Article

Comparative anticoccidial activity of hydrochloric acid (HCl) and formic acid against *Eimeria tenella* in broiler chickens

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ABSTRACT

The objective of the present study was to evaluate the anticoccidial effects of formic acid in contrast to HCl in broiler chickens challenged with *Eimeria tenella* infection in comparison with the amprolium anticoccidial. For this purpose, a total of 225 chicks were placed 15 per pen with three pens per treatment. HCl (2000ppm), formic acid (5000ppm) and amproilum (at the dose rate of 125ppm) were given to the experimental groups in drinking water from 10 to 19th days of age. One group was kept as infected non medicated control and one as non infected non medicated control. At the 12th day of age, all the groups were inoculated orally with 75,000 sporulated oocysts except non infected non medicated control. Anticoccidial activity was evaluated on the basis of performance (weight gain, feed conversion ratio) and pathogenic (oocyst score, lesion score and mortality %age) parameters. Among medicated groups, the maximum anticoccidial effect was seen in the group medicated with 125ppm amprolium followed by 2000ppm HCl medicated group and 5000ppm formic acid medicated group. However, formic acid (5000ppm) showed maximum positive influence on weight gains and FCR. In summary, the lower doses of HCl and formic acid have the potential to be used as alternative to chemotherapeutic drugs for *Eimeria tenella* control. It is therefore suggested that further studies should be carried out to determine the possible minimum safe levels of HCl and formic acid with least toxic effects to be used as anticoccidial.

1. Introduction

Poultry industry is one of the most expanding sectors in Pakistan that coupled around 1.5 million people and contributes about 19% of the total meat production in the country [1, 2]. In Pakistan, poultry farming is expanding steadily and provides high biological value animal proteins to humans [3]. However, various diseases such as coccidiosis are a great challenge for this sector [4].

Avian coccidiosis is a fatal disease caused by intracellular protozoan parasites of genus Eimeria. In Pakistan, Eimeria tenella is the most common species that attacks primarily to the cecum and resides there for prolification [5, 6]. Infective sporozoits penetrate the cecal mucosa through villus epithelial cells and cause massive damage to cecal epithelium which results in severe hemorrhagic feces. All these destructive changes lead to rapid weight loss, poor feed efficiency and eventually death of the birds. The estimated annual cost deriving directly or indirectly from diseases caused by Eimeria species in the raising of fowls is about US $3 billion [7, 8]. Coccidiosis is generally controlled by the use of anticoccidials in poultry industry. These compounds have diverse mode of actions but polyether ionophores like sulphanamides, pyrimidine derivatives, triazinetrions and benzenacetonitryls are used widely that modify ion transport system and interrupt osmotic balance. Although these compounds are effective, resistance to them appears to be growing [9, 10, 11, 12].

New drugs have been developed and administered on a rotational basis with existing drugs to overcome the emergence of drug resistance, but this approach lead to high cost of poultry products. In addition, drug residue in the poultry products is potentially annoyance to consumer. Thus, alternative strategies are essential for better control of avian coccidiosis [8, 13]. Many acids such as fumaric, propionic, acetic, sorbic and tartaric acid have been reported for positive influence on growth performance and feed conversion ratio [14, 15].

In Pakistan, hydrochloric acid [HCl] and formic acid are being used extensively at dose rate of 2000ppm and 5000ppm

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respectively as anticoccidial agent in the local poultry industry. Recently, we have reported the anticoccidial effect of HCl but activity of formic acid against coccidiosis is still not reported scientifically. Therefore, the present study has been planned to evaluate the possible anticoccidial effect of formic acid if any, and to compare the various anticoccidial parameters with HCl employing standard parasitological procedure.

Materials and Methods

Chemicals

HCl [Sigma-Aldrich, Germany], formic acid [Apex Chemicals (S) Pte Ltd, Singapore] were purchased from local market of Pakistan.

Experimental birds, feed and management

Two hundred and twenty five [1-day-old] broiler chicks [Hubbard Al-Noor Chicks, Pvt] were purchased from local hatchery. Chicks were reared under standard managemental practices. All the chicks were offered broiler starter ration up to 2 weeks of age and then fed a broiler finisher. The feed and water were provided ad libitum. Temperature was maintained at 85-90oF during the first week of age and then reduced by 5oF on weekly basis. Lighting was provided for 24 hours through out the experimental period. All the birds were vaccinated for New Castle disease, Infectious Bursal disease and Hydro pericardium syndrome on 5th, 14th and 18th days of age respectively.

Parasite

Ceca of infected chickens were selected for coccidial oocysts and infection was propagated in broiler chickens orally. After obtaining ample amount of oocysts, they were sporulated by placing in 2.5% K2Cr2O7 in the presence of suitable humidity and temperature. Sporulated oocysts were cleaned and counted by the McMaster technique [16]. The required concentration of the sporulated oocysts [75,000/ml] was maintained with phosphate buffered saline.

Study design

The chicks [n=225] were placed 15 per pen with three pens per treatment. Treatments were randomized within blocks. Treatments were as follows:

2000ppm HCl medicated group, 5000ppm formic acid medicated group, 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 125ppm], HCl [at dose rate of 2000ppm] and formic acid [at dose rate of 5000ppm] were given in water from 10 to19th 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]. Cecal lesions were scored by the lesion scoring technique [17].

An oocyst index [0 to 5] was determined by microscopical examination of scrapings from the ceca of chicks sacrificed for lesion scoring at 7th day of post inoculation [18].

Cecal 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 [19].

Statistical analysis

Data obtained on various parameters were analyzed by analysis of variance [ANOVA], and the mean values were compared by Tukey test.. The differences among group means were considered significant at $P<0.05$. Graph Pad prism v 5.00 was used for statistical test.

Results

The results of weight gain [Fig 1] revealed that the body weight gains in all the medicated groups were significantly [$P<0.05$] higher than infected non medicated control. Among the medicated groups, the maximum weight gain was shown by the group medicated with formic acid followed by the groups medicated with amprolium and HCl respectively.

The results of FCR [Table 1] showed that the FCR values of all the medicated group were numerically lower compared with infected non medicated group, although a statistical comparison could not be made due to group feeding. Formic acid medicated group displayed numerically lower FCR compared with amprolium medicated group and HCl medicated group.

The results of the lesion scores are also indicated in Table 1. The uninfected control group showed zero score. All the medicated groups exhibited significantly [$P<0.05$] lower lesion scores than infected non medicated group. Among medicated groups, the lowest lesion scores were seen by the group medicated with amprolium followed by group medicated with HCl and group medicated with formic 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 HCl medicated group and formic acid medicated group though both of these groups are statistically significant [$P>0.05$] with amprolium medicated group.

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 formic acid and HCl 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], cecal pH of all the groups were significantly [P<0.05] lower than infected non medicated control group. Among the groups the maximum reduction in pH was observed by HCl medicated group followed by formic acid medicated group, non infected non medicated group and amprolium medicated group. The cecal pH of formic acid medicated group, amprolium medicated group and non infected non medicated group were statistically non significant [P<0.05] with each other, though significantly [P<0.05] higher than HCl medicated group except formic acid medicated group.

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

Treatments	Feed conversion ratio (g/g)*	Lesion Score	Oocyst score	Mortality %age
HCl (2000ppm)	1.59	2.8[b]	2.8b[c]	13.2
Formic acid (5000 ppm)	1.43	3.4[b]	3.2b[c]	11.9
Amprolium (125 ppm)	1.51	2.3[c]	1.9[c]	6.3
Infected non medicated	1.91	3.9[a]	4.7[a]	24.5
Non infected non medicated	1.63	-	-	-
S.E.M	-	0.27	0.16	-

[a-c] Means shearing similar superscripts within a column do not differ (P<0.05).

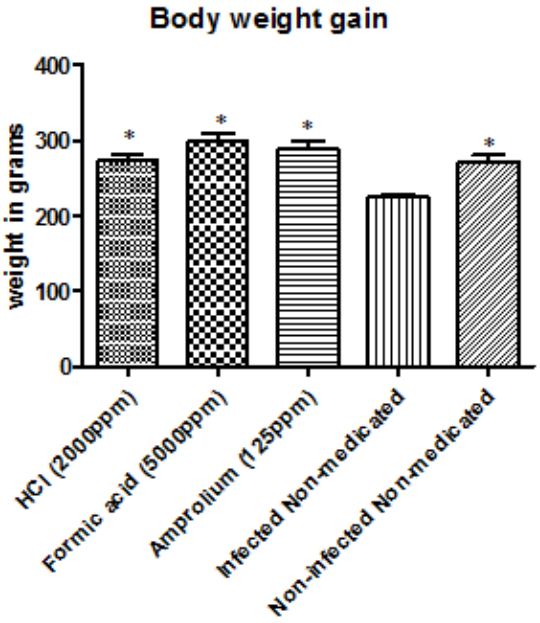
[*] Statistical analysis was not possible because of group feeding of chicks.

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

Treatments	3rd day	5th day	7th day
HCl (2000ppm)	6.16[c]	5.83[c]	5.96[c]
Formic acid (5000ppm)	6.35[bc]	5.96[bc]	6.13[bc]
Amprolium (125ppm)	6.58[b]	6.75[b]	6.82[b]
Infected non medicated	6.99[a]	7.25[a]	7.44[a]
Non infected non medicated	6.53[b]	6.67[b]	6.96[b]
S.E.M.	0.051	0.063	0.056

[a-c] Means shearing similar superscripts within a column do not differ (P<0.05).

Fig 1. Body weight again of the different groups on 7th day post inoculation. The bars represent the mean standard deviation. The asterisks indicate the significant difference compared to infected non-medicated (*p<0.5).



Discussion

In the present study, lower doses of formic acid and HCl administered in drinking water have shown the anticoccidial activity in broiler chickens challenged with Eimeria tenella by means of improved weight gains, better FCR, lower oocyst and lesion scores. Owing to substantial damage of intestinal epithelium, subsequent malabsorption of nutrients and high mortality rate, avian coccidiosis can cause serious losses in the poultry industry. The performance improvements observed while using formic acid and HCl could overcome these losses.

Hydrochloric acid is a monoprotic acid [it can ionize only once to yield one single H+ ion] and found naturally in the gastric juice. It is a strong acid and in the presence of water it is completely dissociated in to hydronium [H3O+] ion and chloride [Cl-]ion.

Formic acid [HCOOH] also known as methanoic acid, is a weak organic acid and is the simplest form of carboxylic acid. It is an important intermediate in chemical synthesis and occurs naturally, most notably in the venom of bee and ant stings. It is a versatile player in the chemical industry and its action is unique, because it acts both as an aldehyde and as a carboxylic acid. It reacts readily with many oxidizing and reducing compounds. A major use of formic acid is as a preservative and antibacterial agent in livestock feed. In the poultry industry, it is sometimes added to feed to kill E. coli bacteria [20, 21]. The first character of carboxylic acid is acidity due to dissociation into H+ cations and RCOO- anions in aqueous solution. The two oxygen atoms are electronegatively charged and the hydrogen of a carboxyl group can be easily removed. The

presence of electronegative groups next to the carboxylic group increases the acidity.

Very limited research exists on the effects of acids on poultry health principally during coccidiosis. However, only some reports [22, 23] are available regarding the antimicrobial effects of acids. These acids exhibited promise in altering bacterial activities and cecal environment in chicken.

The results on the improved body weight gains and FCR presented in this work are in line with a number of previous reports [14, 24, 25, 26, 27] indicating that acids at low concentrations result in the better weight gain and FCR by improving the solubility of feed ingredients, digestion and absorption of nutrients. However, higher concentrations were associated with loss of body weight gains and poor feed intake. In comparison with HCl, formic acid showed improved body weight gain and better FCR reflecting that formic acid has more beneficial effect on weight gain than HCl. There are some reports of positive influence of formic acid on growth and FCR in chickens, which are in parallel to the results of present study [21].

In the stomach, HCl secretion is desired for the digestion of protein by the activation of pepsinogen to pepsin. HCl also renders the stomach sterile against orally-ingested pathogens and prevents bacterial or fungal overgrowth of the small intestine. It encourages the flow of bile and pancreatic enzymes, and facilitates the absorption of a variety of nutrients. It is also known that when the hydrochloric acid production falls short the required amount necessary to maintain the acidity of the white cells and the acid-base balance becomes insufficient and hydrogen chloride eventually vanishes from the circulation. When hydrogen chloride disappears from the circulation some other acid must take its place immediately in order to maintain the pH of the circulating fluids. The acid wastes assume the role of hydrogen chloride in the blood chemistry. This is followed by an imbalance of the blood chemistry.

The HCl and formic acid medicated chickens also showed significantly reduced oocyst and lesion score. According to several researchers, acids have antimicrobial [22, 23, 28, 29, 30] and antibacterial [22, 23] activities. HCl displayed reduced oocyst and lesion score than formic acid, though non significant difference exists there, indicating that HCl has better anticoccidial activity than formic acid. However, this anticoccidial activity of the formic acid has been reported for the first time.

In the present study, drinking of HCl and formic acid provided protection against pathogenic effects of E. tenella in the intestine probably by declining pH of ceca, eradication of oocysts and healing of intestinal mucosa in chickens. White cells are integral part in the healing of wounds, on the chemical side the good effects come from a glandular and cellular stimulation by the HCl, so making these factors in the production of the acid return to their normal production. On the other hand, while using acid solution against coccidiosis one must find out the correct dosage for even a slight excessive dose may cause serious intestinal inflammation along with severe diarrhea and dysentery. In the HCl and formic acid medicated groups mortality was also very high and these results are in concurrent with the results as described [31] that increasing level of acids cause high mortality. Formic acid showed relatively less mortality than HCl suggesting that formic acid is somewhat safer than HCl.

The pH in the ceca was significantly higher in infected non medicated group than non infected non medicated and all medicated groups. Some researchers [19] suggested that this increase in pH reflects changes in the cecal flora since: 1) the "normal" ceca contain a predominance of acid-producing bacteria [32]; and 2) the pH in the ceca is significantly greater in germ-free birds than in conventional birds. Being strong acid, HCl medicated group showed non significantly lowered pH than the group medicated with formic acid.

Regarding the mode of action of acids against bacteria, many researchers have different views. Some researchers have the view that decrease in pH and the ability of the acids to dissociate might play vital role in antibacterial action of acids. Degree of dissociation determines the pKa-value of the respective acid along with the pH of the surrounding milieu. In the undissociated form, acids are lipophilic and easily cross the microbial cell membrane through both passive and carrier-mediated transport mechanisms. Once in the cell, the acids liberate 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. All these changes force the bacterial cell to use extra energy for the expulsion of excessive protons H+, ultimately resulting death by starvation. In the same matter, the protons H+ can denature bacterial acid sensitive proteins and DNA [33]. Likewise, Hydrochloric acid and formic acid also ionize to release H+ ions and these protons H+ might have some role in the destruction of the pathogen.

Conclusion

Data from the present study propose that lower doses of HCl and formic acid have anticoccidial activities against Eimeria tenella in broiler chickens; hence, they can be used as an alternative coccidiosis control agents. Formic acid is less effective than HCl in the control of coccidiosis though posses greater positive effect on growth and FCR than HCl. But the exact mode of action against Eimeria species is not clear. So, further research should be conceded to find out the exact mode of action of these acids against coccidia.

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