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Impact of Acidifier on Florfenicol Pharmacokinetics and their Tissue Residues in *Escherichia coli* O78-Infected Chickens

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ABSTRACT

Florfenicol (FF) is a broad-spectrum antibiotic and has been associated with high therapeutic efficacy and low toxicity; therefore, it is widely used in poultry farms. Acidifier has been reported to limit multiplication of enteropathogenic *Escherichia coli*. The study was conducted to investigate the effect of acidifier on pharmacokinetics of FF and also examine their tissue residues in *E. coli* O78-infected broiler-chickens. A total of 136 healthy broiler-chickens were used for the study. The broiler-chickens were grouped for pharmacokinetic (A-F), FF tissue residual (G-L) and colony forming unit (CFU; M-N) studies. They were infected with *E. coli* O78 and FF was administered orally for 3 consecutive days at 30 and 60 mg/kg, with / without acidifier. Faecal CFU of *E. coli* O78 was determined. At intervals of 1st, 3rd, 5th, 7th and 9th day post FF treatment, chickens were slaughtered and tissue specimens collected for analysis. High performance liquid chromatography (HPLC) was used to measure plasma of FF levels. The results showed that FF serum level was significantly lower in infected broiler-chickens compared with the healthy control group at the different time intervals. The outcome of the CFU showed a significant decrease in infected broiler-chickens with acidifier only (2.77±0.015 CFU/g) in the 4th day after infection in relation to those treated with FF, FF supplemented with acidifier (2.62±0.033 and 2.58±0.036 CFU/g, respectively) while in non-treated infected group was (4.00±0.008 CFU/g). Our findings recommend feed supplementation of acidifier (30 mg/kg BW) with FF for the treatment of *E. coli* O78-infected broiler-chickens.

***Keywords*:** Acidifier, *E. coli* O78, Florfenicol, HPLC, Pharmacokinetics, Tissue residue.

Introduction

Florfenicol (FF) is a synthetic, broad-spectrum antibiotics, derived from thiamphenicol, and it is considered for veterinary use. Its broad-spectrum, high therapeutic efficacy, and low toxicity, makes it unique among the most commonly used medications for poultry farms and animals produced for human consumption.[1] There are several reports on its efficacy against enteropathogenic *E.* coli.[1,2] FF can be processed into amine (FFA), alcohol, and oxamic acid. Among different species, the ratio between them differs and (FFA) is considered the major metabolite is in most animal species[3] and has a high bioavailability (F>80%).[4] Pharmacodynamics in domestic animals have been studied against several infectious diseases relating to widespread tissue distribution and rapid elimination.[5] From a medicinal plasma, the amount of FF must be compared with the least inhibitory concentration of possible pathogens. FF pharmacokinetics have been investigated in many species, including cattle, sheep, pigs, rabbits, dogs, broiler-chickens, turkeys, ducks, pigeons, and quail.[4] However, the kinetic data for broiler-chickens are limited. FF used in poultry treatment must not be tested alone in terms of the ideal therapeutic impact but also in terms of the FFA tissue residue.There are strong laws put in place to regulate the usage of antimicrobial agents in animals for food so as to reduce health danger connected to FF remains during consumption.[6] Therefore, for human food safety, the "Maximum Residue Limit" (MRL) for these drugs set by the European Union (EU) for the amounts of FF and FFA in muscle (100 part per billion [ppb]), skin and fat (200 ppb), kidney (750 ppb) and liver (2,500 ppb) for poultry intended for personal consumption.[5] Higher or multiple doses of FF might result in longer period for detection of FF residue, and so the withdrawal period would be extended. FF and its metabolites in broiler-chickens plasma, kidneys, livers, and muscles homogenate have been reported to be transformed to FFA salts by acid catalyzed hydrolysis according to EMEA.[5]

Acidifier is used to increase the amount of endogenous acid in the stomach, thereby decreasing pH value in gastrointestinal tract. This could limit multiplication of enteropathogenic *E. coli*, especially the acid-intolerant species, thus providing a good medium for multiplication of some useful gastro microbiota, such as yeasts or lactic acid bacteria.[8] Therefore, the present research was aimed at investigating the effect of some acidifiers on pharmacokinetics of FF and also determining tissue residue for fit in broiler-chickens infected with enteropathogenic *E. coli* O78.

Materials and Methods

Sources of chemical reagents

All reagents used for extraction and other analyses were of analytical grade. Water and acetonitrile used in the preparation of mobile phase of HPLC procedure were obtained from Thermo Fisher Scientific.

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Also, 97.6% FFA standard was purchased from Sigma Chemical Co. (St. Louis, MO).

Ethical approval
This study was approved by the Ethical Committee of Animal Health Research Institute, Giza, with serial number 165712, and performed in accordance with all international regulations and guidelines.

HPLC system
The high-performance liquid chromatography (HPLC) system used for the study was Agilent Series 1200 quaternary incline drive, Series 1200 auto technician, Series 1200 UV detector. HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France).

Source of drugs
FLORIBIOTIC® (10 %) oral solution was supplied by Atco Pharma for Pharmaceutical Industries, Egypt. Acidifier (GALLIMIX®) encapsulated feed additive was obtained from MG2Mix Company, France. The micro encapsulation consisted of active ingredients of organic acids (Fumaric acid, Sorbic acid, DL Malic acid, and Citric acid) and essential oils (aroma substances) in a vegetable hydrogenated triglyceride matrix (Palm oil).

Experimental grouping of broiler chickens
A total of 136 healthy, day-old broiler-chickens were used in this study. They were fed on medicine-allowed ration and water *ad libitum*. One week before infection, the broiler-chickens were separated into six different chicken cages. Thirty-three of the broiler-chicken were used for pharmacokinetic studies (6 groups of A - F, comprising of 5 chickens/group and 3 for the control group). Ninety-three broiler chickens were used for studying FF residual distribution in different tissues (6 groups of G – L, comprising of 15 chickens/ group and 3 for the control group). Ten broiler chickens were used for CFU experiment in the presence of acidifier only (Groups M-N). The experimental set up is presented in Table 1.

Infection of experimental broiler-chickens with E. coli O78
Enteropathogenic *E. coli* O78 was obtained from Bacteriology Department, AHRI, Dokki, Giza, Egypt. It was inoculated on Beef Infusion Broth and incubated at 37^{o}C for two days. Then, the bacteria were sub cultured on MacC agar and incubated at 37 ^{o}C for a day. At the end of incubation period, growth was obtained and re-inoculated into 50 mL "S. S." at pH 7, bacterial No./ml was resolute by plating on "CFU Agar Plate" for suspension tenfold Serial dilution. Broiler-chickens were inoculated by IP injection with 200 µl bacterial inoculum at a concentration of 6.7×10^{9} CFU/ ml[1]. After the inoculation, the chickens were placed under observation for pathological signs and symptoms which included severe diarrhea, loss of appetite, and ruffled feathers. Sample swabs were cultured on Beef Infusion Broth for a day at 37 ^{o}C after which they were inoculated on MacC agar. Agg test was carried out to confirm *E. coli* O78 strain via its antiserum.

Treatment and sample collection
For a single oral dose study (group A-F), an aliquot of 1 mL blood sample was collected from the jugulars, wing or leg veins of chickens at different time periods (0.25, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 24.00, 48.00, 72.00 and 96.00 h) after drug administration. Collection of blood was made into Na EDTA tubes, and then centrifuged at 1,500 gm for 10 min. Plasma were recovered at -20 °C. A similar procedure was carried out for the multiple oral dose study (group G- L). Three broiler-chickens were slaughtered at the first, third, fifth, seventh- and ninth-day post FF treatment. Muscle, liver, kidney, fat, and skin specimens were collected and stored at -20°C. For studying *E. coli* O78 CFU, faecal matter samples were taken at 24 hr post infection for 4 days from groups K-N.

Plasma and tissue extraction
One (1) g of tissue sample (0.5 ml of plasma) was weighed into a polypropylene centrifugal tube (4 mL). An aliquot of 4 mL of 6N HCl (2 ml for plasma) was added and mixed for few seconds. Then, it was placed in a shaking aquatic bath at 100 °C for 3 hr to complete digestion. The color of the tube content was dark brown to black. Immediately after hydrolysis, while still hot, it was extracted with 10 mL ethyl acetate (5 mL for plasma), then centrifuged for 5 min at 2,500 rpm. The ethyl acetate (upper) layer was discarded. Care was taken not to disturb or transfer the black tarry residue at the interface of the two layers. An aliquot of 4 mL NaOH, 30% (w/w) (2 mL for plasma) was added to the hydrolysate to adjust the pH to ≥ 12.5. The recovered basified hydrolysate was poured into a Varian ChemElut® CE1020 diatomaceous earth sorbent pillars (stopcock closed). The columns were allowed to stand for at least 45 min. The columns were eluted with 3X 10 mL of ethyl acetate (5 mL for plasma) slowly at a flow rate of ca 1 drop/s. The SPE eluates were vanished to aridity at 45^{o}C below nitrogen stream. The remaining dry particles were dissolved in 1 mL mobile stage (0.5 mL for plasma), using vortex, then filtered through a 0.2 µm Acrodisc filter.[11]

Chromatographic conditions
All plasma and tissue samples were measured by using a multi-wave detector at 223 nm and separated by Agilent column C18 (4.6 mm i.d, 250 mm, 5µm) at ambient temperature, with 100 µL injection volume. The mobile stage reagents were acetonitrile and water (1:2 v/v) field 0.1 % glacial acetic acid with flow degree 0.8 mL/min. The investigative technique was validated according to EU[12] (Figures 2-4). Standard of FFA at concentrations 0.0488–9.76 µg/mL (Table 2), were prepared in the mobile phase with a correlation coefficient, (r2) = 0.9999; equation regression, y = 65.792x - 0.0661; and accuracy = 99.3 ± 1.36. The range of FFA recovery from plasma and tissues ranged from 97-101.2%. Relative standard deviation (RSD%) of the repeatability and reproducibility were 0.032 %, and 1.2 %, respectively. The pooled RSD% for robustness did not exceed 2.8 %. Limit of detection (LOD) and limit of quantification (LOQ) remained 0.005, formerly 0.015 and System suitability (Table 3).

Determination of faecal CFU of E. coli O78
CFU of *E. coli* O78 was determined as described.[13] Test samples (faecal matters) were weighed, and Buffer Peptone Water (BPW) was then added at a ratio of (25:225). An aliquot of 1 mL of suspension was transferred by sterile pipette and 12-15 ml of VRBL media were added into each Petri Dish at 44^{o}C. The inoculum was mixed thoroughly and the mixture was allowed to solidify. A second layer of previous media was poured, allowed to cool and solidified. The cultures were inverted and placed in an incubator at 37 ^{o}C for 24 h, CFU of *E. coli* O78 was determined.

Statistical data analysis
All data were expressed as mean±SEM. One-way ANOVA (analysis of variance) was performed using the Statistical Package for Social Sciences (SPSS Inc., version 20.0, Chicago, IL, USA) to express the differences between groups.[14] Differences between the means of different parameters were considered significant at $p<0.05$. An add-in program for Microsoft Excel version 2 was used to calculate other parameters.[15]

Results and Discussion

The experimental broiler-chickens infected with *E. coli* O78 suffered from diarrhea 10 h after infection and 48 h post inoculation. Some of the chickens developed depression, loss of appetite, ruffled feathers, and diarrhea. They had necropsy, gross abrasions, pericardium fibrin deposits, swollen spleen and liver, air sac diffused thickening with a fibrinous exudate, and intestinal congestion. *E. coli* O78 was enhanced in the liver, spleen, and heart. The results of the oral pharmacokinetic parameters of FFA were presented in Tables 4 and 5. FF was rapidly absorbed after a single oral management at concentrations of 30 and 60 mg/kg BW. The first sample time was quantifiable at 15 min in all broiler-chickens. The C_{max} was 7.9 ± 0.21, and 7.04 ± 0.46 µg/mL which were obtained at 1.7 ± 0.01, and 1.65±0.04 hr (t_{max}) for the two doses (30 and 60 mg/kg BW). The C_{max} was higher than those earlier reported for broiler-chickens at a dose 30 mg/kg BW (5.82 ± 2.43, 4.83, and 3.20 ± 0.20 µg/mL),[16] and lesser than the values obtained for turkeys (12.25 ± 2.62 µg/mL) at a greater time, T_{max} of 2.0 ± 1.22 h.[17] The observed t_{max} of 1.7 h was similar to the values obtained for healthy broiler-chickens (1.4 h),[18] and geese (0.5 h).[4] Contrarily, the value obtained at (2 h) was smaller than those reported for turkey[17]

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when all species were administered with oral formulation. These differences might be attributed to anatomical variations between the different species, healthy status, and age. Also, the method of administering dosage in each case, could affect the protein binding degree of the drug. Thus, the systemic availability, and kinetic parameters, may vary widely among different species.[19] The removal half-life ($t_{1/2\beta}$) of FF after oral management at a dose of 30 mg/kg BW was 3.88±0.09, 3.92 ± 0.0003, and 3.85 ± 0.001 h for groups A, C, and E as shown in Table 4. There was no significant difference between the three groups, while FF at a dose of 60 mg/kg BW removal half-life ($t_{1/2\beta}$) remained as 3.94±0.001 hr for groups B, D, and F. In the study that compared fit, and *E. coli* infected poultry there was no variation in the cutting-edge $T_{1/2\beta}$[1] following IV administration of FF at 30 mg/kg BW. The $t_{1/2\beta}$ values obtained were for groups A, C, and E (Table 4) and were more than what was recorded for the healthy broiler-chickens (1.78 ± 0.19 h and 2.25 ± 0.53 h),[16] and that infected with *E. coli* (1.73 ± 0.25 h),[17] but similar to the data recorded for turkeys (3.76 hr)[17], on the same amount of 30 mg/kg BW. The average of C_{max}, $T_{1/2\infty}$, AUC_{0-12h}, $AUC_{0-\infty}$, and AUMC of group E was significantly decreased ($p<0.05$) compared with groups A and C. Meanwhile, CL_2/F, and V_2/F were significantly increased in relation to groups A, and C. C_{max}, AUC_{0-12h}, $AUC_{0-\infty}$, and AUMC of group F were significantly decreased ($p<0.05$) compared with groups B and D, but CL_2/F, and V_2/F were significantly increased with respect to groups B, and D. These results suggest that acidifier has no effect on kinetic parameters in healthy and infected broiler-chickens.

The results obtained for FF in infected broiler-chickens significantly lowered serum levels compared with apparently healthy broiler-chickens after oral administration of the drug alone and in combination with acidifier at different time intervals. This observation could be due to faster extravascular distribution, and high ability of FF to reach the diseased tissues. Our finding is similar to a study where cephradine concentrations in serum, showed a significant decrease in *Salmonella entretidis*-infected broiler chickens compared with the healthy control, following repeated oral administrations. These lower concentrations of serum in the infected experimental chickens might be attributed to a higher power of penetration of drug to the diseased tissues.[20] The FF residue in tissue samples, and FFA metabolite after oral dose of 30, and 60 mg/kg BW daily for three consecutive days were determined and the results are presented in Tables 6 and 7. Repeated oral administration of FF at a dose of 30 mg/kg BW for three consecutive days in normal, and *E. coli* O78-infected broiler-chickens showed that the drug could be found in breast muscles, fat, and skin till the 5th day from the first oral dose, but it was still detectable in the liver, and kidney till the 7th day post last oral dose. The results revealed that the liver contained the highest concentrations of drugs, while the lowest concentration of drug was located in the breast muscle. A progressive sequence of FF levels was observed in the muscles, fat, and skin. Liver and kidney, were observed with growing time in all the groups examined. A study on FF in broiler-chickens,[7] reported the liver, and kidney to have the highest concentration of FF residue. The present findings indicated that the drug was detectable in the liver and kidney until the 7th day after cessation of treatment. This observation suggests the drug had great penetration ability in these tissues, and FF could be an excellent medication for *E. coli* O78 treatment of gastrointestinal and urinary tract infections. The estimated withdrawal periods of FF at 30 mg/kg BW were 7, 7, 5, and 5 days for liver, kidney, skin, fat and muscle, while withdrawal period for FF at 60 mg/kg BW were 9 days for fat, muscle and skin. In Group K, infection was significantly decreased, and the levels of FF in breast muscle (1st, 3rd, and 5th day), skin, and fat (1st and 5th day), kidney (5th and 7th day), and liver (1st, 3rd, 5th and 7th) day post drug cessation. These indicated that the infection decreased FF levels of the different examined tissues in the presence of acidifier (Group I). On the 3rd day after treatment, FFA had spread to the liver reaching up to 6039.4 ± 140.55, 5995.13 ± 161.9, and 4053.13 ± 186.23 in Groups G, K, and I in 30 mg/kg BW treated groups and 10441.43 ± 70.11, 10298.48 ± 676.46, and 6799.49 ± 865.8 in Groups H, J, and L in 60 mg/kg BW treated groups. It was clear that the highest amount was found in liver tissue of all the groups.

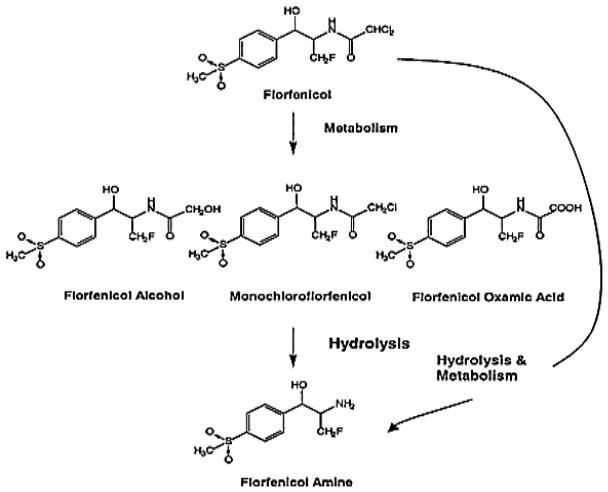

Figure 1: Metabolic and acid hydrolysis products of florfenicol

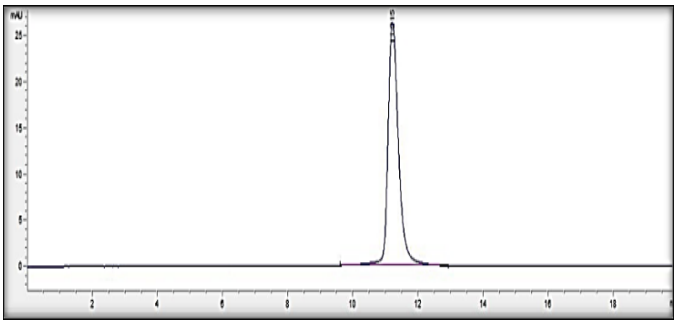

Figure 2: Chromatogram showing florfenicol amine standard at a concentration of 1 µg/mL

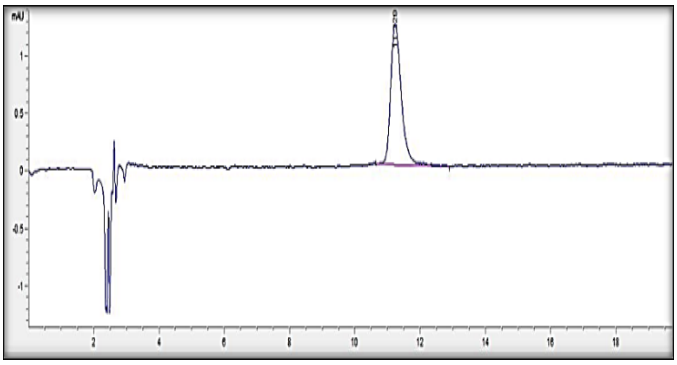

Figure 3: Chromatogram showing spiked tissue with florfenicol amine at a concentration of 10 ng/g

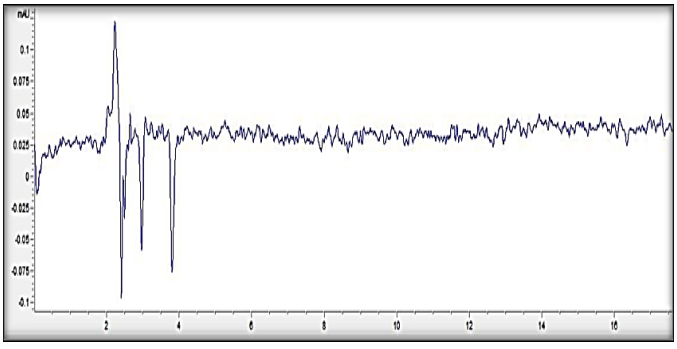

Figure 4: Chromatogram showing blank tissue sample

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Table 1: Grouping of experimental broiler-chickens

Group	No. of chicken	Drug supplement	Age	Administration route	Dose	Duration
A	5	FF	22 d	PO	30 mg/kg BW	Once
B	5	FF	22 d	PO	60 mg/kg BW	Once
C	5	Acidifier + FF	18 d	PO	0.4 g/kg feed	Till exp. end
			22 d		30 mg/kg BW	Once
D	5	Acidifier +FF	18 d	PO	0.4 g/kg feed	Till exp. end
			22 d		60 mg/kg BW	Once
E	5	Acidifier + *E. coli* O78 +FF	18 d	PO	0.4 g/kg feed	Till exp. end
			20 d	IP	0.2 mL (6.7×10^9 CFU/mL)	----------
			22 d	PO	30 mg/kg BW	Once
F	5	Acidifier + *E. coli* O78 +FF	18 d	PO	0.4 g/kg feed	Till exp. end
			20 d	IP	0.2 mL (6.7×10^9 CFU/mL)	----------
			22 d	PO	60 mg/kg BW	Once
G	15	FF	22 d	PO	30 mg/kg BW	3 days
H	15	FF	22 d	PO	60 mg/kg BW	3 days
I	15	Acidifier + FF	18 d	PO	0.4 g/kg feed	Till exp. end
			22 d		30 mg/kg BW	3 days
J	15	Acidifier +FF	18 d	PO	0.4 g/kg feed	Till exp. end
			22 d		60 mg/kg BW	3 days
K	15	Acidifier + *E. coli* O78 +FF	18 d	PO	0.4 g/kg feed	Till exp. end
			20 d	IP	0.2 ml (6.7×10^9 CFU/mL)	----------
			22 d	PO	30 mg/kg BW	3 days
L	15	Acidifier + *E. coli* O78 +FF	18 d	PO	0.4 g/kg feed	Till exp. end
			20 d	IP	0.2 mL (6.7×10^9 CFU/mL)	----------
			22 d	PO	60 mg/kg BW	3 days
M	5	Acidifier + *E. coli* O78	18 d	PO	0.4 g/kg feed	Till exp. end
			20 d	IP	0.2 mL (6.7×10^9 CFU/mL)	----------
N	5	*E. coli* O78	20 d	IP	0.2 mL (6.7×10^9 CFU/mL)	----------

Table 2: Concentration of florfenicol amine and their corresponding HPLC peak response

Concentration (µg/ml)	Area under peak*
0.0488	3.31
0.0976	6.494
0.488	33.1885
0.967	65.67
1.952	128.754
4.88	332.6
9.76	656.54

*Mean of three replicates

Table 3: System-suitability study on 0.488 µg/ml florfenicol amine

	Retention time	Tailing factor	Theoretical Plates	Area under peak
Mean (n = 6)	11.215	0.71	15612.167	33.203
RSD (%)	0.042	0.01	3.1	0.02

The observation of a substantial high level of FF in liver tissue has been previously reported in broiler-chickens.[6,15,21] In the 30 mg/kg BW treated groups, FFA was detected in the muscle, skin, and fat until the 5th day post drug cessation, while it was detected in the kidney and liver until the 7th day post drug cessation. Meanwhile, in the 60 mg/kg BW treated group, FFA was detected in the muscle, skin, and fat until the 7th day post drug cessation, but in the kidney and liver, it was detected until the 9th day post drug cessation. High FFA concentration in the kidney, muscle, and liver indicated FF was an appropriate drug for treating urinary, gastrointestinal tract infection associated with septicemia caused by susceptible organisms like *E. coli* O78. The amount of FFA in tissues of the 60 mg/kg treated group remained

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prolonged relative to the 30 mg/kg treated group. This observation suggests that the rate of elimination of FF from the poultry chickens was lower at higher dose. The multiple doses could result in the detection of drug residue for a longer period of time, thereby extending the removal time.[7] In the presence of acidifier (Group I), there was no significant reduction in the level of FFA in all the examined tissues, except for the muscle at the 3rd day; skin, fat, and kidney at the 1st day. The marker FF residue in broiler-chickens was the amount of FF and its metabolite was proposed to be FFA, according to the European Union. The MRLs of 100 µg/kg in muscle, 200 µg/kg in fat, and skin, kidney had 750 µg/kg, and liver 2500 µg/kg, respectively.[5] All tissues were considered lower than the MRL standard to be safe for human consumption on the 5th day for a dosage of 30 mg/kg BW, and at the 7th day for a dosage of 60 mg/kg BW after the last oral dose.

Table 8 revealed the results obtained for the effects of multiple oral doses of FF in broiler-chickens supplemented with acidifier and acidifier alone on total *E. coli* O78 population. There was a gradual decrease in the CFU from the different multiple oral doses (30 and 60 mg/kg BW) supplemented with mixed acidifiers compared with the infected non-treated control group. The *E. coli* O78-infected broiler-chickens that were administered orally with acidifier showed a remarkable gradual decrease in the CFU of *E. coli* O78. The level was 2.77 ± 0.015 CFU/g in the samples 4 days after infection compared with those treated with FF at a dose of 30 and 60 mg/kg BW; after which were supplemented with acidifier having values of 2.62±0.033 and 2.58±0.036 CFU/g, respectively, as well as the non-treated infected group with a value of 4.00 ± 0.008 log CFU/gm. The present results revealed that CFU of *E. coli* O78 decreased following oral administration of the mixed acidifiers in the experimental broiler-chickens. Our findings are in accordance with earlier report on supplementation of broiler-chicken ration with acidifiers where it was reported that it could prevent and/ or inhibit enteropathogenic *E. coli.*[7,13,24] The results from the present study showed that oral administration of FF with acidifier significantly decreased CFU compared to administration of acidifier only. It was revealed from these observations that addition of acidifiers to poultry feeds could protect the broiler-chickens against enteropathogenic *E. coli* O78, and or might improve the potential activity of amoxicillin against bacterial infection.[22]

Table 4: Kinetic parameters of administration of florfenicol amine to broiler-chickens followed by single florfenicol oral management at a dose of 30 mg/kg BW (n = 5)

Parameter	Unit	Group A	Group C	Group E
C_{max}	µg/ml	7.9 ± 0.21	7.04 ± 0.46	4.63 ± 0.13[ab]
T_{max}	h	1.7 ± 0.01	1.65 ± 0.04	1.68 ± 0.001
$T_{1/2\infty}$	h	0.47 ± 0.06	0.47 ± 0.01	0.74 ± 0.004[ab]
$T_{1/2\beta}$	h	3.88 ± 0.09	3.92 ± 0.0003	3.85 ± 0.001
CL_2/F	L/h/ kg	0.41 ± 0.02	0.47 ± 0.04	0.67 ± 0.02[ab]
V_2/F	L/kg	0.62 ± 0.09	0.71 ± 0.02	0.96 ± 0.02[ab]
AUC_{0-t}	µg h/mL	56.81 ± 1.5	50.72 ± 3.4	33.57 ± 0.94[ab]
AUC_{0-inf}	µg h/mL	56.81 ± 1.5	50.72 ± 3.4	33.57 ± 0.94[ab]
AUMC		348.98 ± 6.98	314.9 ± 18.94	206.86 ± 5.82[ab]
MRT	h	6.14 ± 0.05	6.19 ± 0.001	6.16 ± 0.001

a: Significant change at $p < 0.05$ with respect to group A using ANOVA test.
b: Significant change at $p < 0.05$ with respect to group C using ANOVA test.

Table 5: Kinetic parameters of florfenicol amine for broiler-chickens followed by a single florfenicol oral management at a dose of 60 mg/kg BW (n = 5)

Parameter	Unit	Group B	Group D	Group F
C_{max}	µg/mL	14.15 ± 0.24	12.92 ± 0.7	8.71 ± 0.3[ab]
T_{max}	h	1.69 ± 0.0001	1.69 ± 0.0006	1.69 ± 0.0001
$T_{1/2\infty}$	h	0.75 ± 0.002	0.75 ± 0.001	0.75 ± 0.004
$T_{1/2\beta}$	h	3.94 ± 0.001	3.94 ± 0.001	3.94 ± 0.001
CL_2/F	L/h/ kg	0.44 ± 0.01	0.48 ± 0.02	0.71 ± 0.03[ab]
V_2/F	L/kg	0.66 ± 0.01	0.71 ± 0.03	1.06 ± 0.04[ab]
AUC_{0-t}	µg h/mL	103.98 ± 1.78	94.96 ± 4.84	63.96 ± 1.8[ab]
AUC_{0-inf}	µg h/mL	103.98 ± 1.78	94.96 ± 4.84	63.96 ± 1.8[ab]
AUMC		651.94 ± 11.18	595.31 ± 32.8	400.95 ± 11.19[ab]
MRT	h	6.27 ± 0.0005	6.27 ± 0.0008	6.27 ± 0.001

a: Significant change at $p < 0.05$ with respect to group B using ANOVA test.
b: Significant change at $p < 0.05$ with respect to group D using ANOVA test.

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Table 6: Tissue residue after oral administration of florfenicol at a dose of 30 mg/kg BW, daily for 3 consecutive days (n = 3)

Tissue		Florfenicol concentration (ng/g) Mean ± SD				
		1st	3rd	5th	7th	9th
Muscle	Group G	127.67 ± 10.33	112.5 ± 9.21	82.7 ± 1.5	ND	ND
	Group I	125.1 ± 9.01	108.57 ± 6.43[a]	79.5 ± 1.1	ND	ND
	Group K	103.95 ± 3.5[ab]	91.05 ± 2.85[ab]	70.2 ± 2.6[ab]	ND	ND
Skin and fat	Group G	372.7 ± 12.9	258.7 ± 10.01	100.9 ± 6.61	ND	ND
	Group I	368.47 ± 15.11[a]	255.13 ± 14.26	98.84 ± 4.81	ND	ND
	Group K	312.67 ± 17.29[ab]	221.67 ± 9.32[a]	77.18 ± 2.48[ab]	ND	ND
Kidney	Group G	3102.33 ± 86.49	1129.67 ± 67.76	708.78 ± 13.95	80.8 ± 4.99	ND
	Group I	2986.8 ± 66.36[a]	1118.47 ± 89.29	699.67 ± 23.78	76.1 ± 6.00	ND
	Group K	2757.98 ± 89.61[a]	1042.24 ± 63.33	610.33 ± 19.82[ab]	51.33 ± 3.28[ab]	ND
Liver	Group G	10052.27 ± 211.75	6039.4 ± 140.55	2238.67 ± 119.1	297.67 ± 12.84	ND
	Group I	9940.13 ± 253.05	5995.13 ± 161.9	2193.84 ± 145.57	271.33 ± 18.67	ND
	Group K	9009.35 ± 313.22[ab]	4053.13 ± 186.23[ab]	1498.67 ± 211.29[ab]	200.7 ± 15.01[ab]	ND

a: Significant change at $p < 0.05$ with respect to group G using ANOVA test.
b: Significant change at $p < 0.05$ with respect to group I using ANOVA test.

Table 7: Tissue residue after oral florfenicol administration at a dose of 60 mg/kg BW, daily for 3 consecutive days (n = 3)

Tissue		Florfenicol concentration (ng/g) Mean ± SE				
		1st	3rd	5th	7th	9th
Muscle	Group H	214.48 ± 13.92	201.38 ± 12.17	123.27 ± 5.92	93.73 ± 6.56	ND
	Group J	198.52 ± 15.23	189.29 ± 10.71	119.71 ± 8.27	86.86 ± 4.85	ND
	Group L	122.57 ± 9.97[ab]	144.4 ± 10.73[ab]	89.5 ± 6.24[ab]	63.71 ± 4.51[ab]	ND
Skin and fat	Group H	599.14 ± 22.58	416.25 ± 21.96	162.3 ± 15.05	115.38 ± 7.56	ND
	Group J	569.22 ± 30.32	380.46 ± 29.55	132.67 ± 13.83	113.82 ± 5.25	ND
	Group L	461.83 ± 17.75[ab]	324.86 ± 17.57[a]	112.1 ± 9.81[a]	69.09 ± 4.97[ab]	ND
Kidney	Group H	4907.89 ± 110.27	1787.13 ± 143.92	1137.12 ± 109.51	217.83 ± 10.56	89.4 ± 2.71
	Group J	4724.87 ± 194.82	1759.9 ± 138.2	1094.69 ± 100.52	193.09 ± 13.11	86.03 ± 5.3
	Group L	4010.51 ± 137.49[ab]	1255.86 ± 146.38[ab]	654.27 ± 51.07[ab]	118.57 ± 8.67[ab]	52.82 ± 4.5[ab]
Liver	Group H	17381.06 ± 468.74	10441.43 ± 870.11	3870.65 ± 333.02	514.67 ± 31.92	304.33 ± 16.17
	Group J	16959.48 ± 766.67	10298.48 ± 676.46	3722.93 ± 230.91	488.05 ± 34.38	299.38 ± 15.67
	Group L	15091.03 ± 866.01[a]	6799.49 ± 865.8[ab]	2086.7 ± 330.6[ab]	453.47 ± 24.34	103.67 ± 13.48[ab]

a: Significant change at $p < 0.05$ with respect to group H using ANOVA test
b: Significant change at $p < 0.05$ with respect to group J using ANOVA test

Table 8: Effect of acidifier on colony forming unit of *Escherichia coli* O78 in faecal samples collected for 4 consecutive days

Days	1st day	2nd day	3rd day	4th day
Control group (N)	3.78 ± 0.006	3.87 ± 0.006	3.93 ± 0.006	4.00 ± 0.008
Acidifier only group (M)	3.78 ± 0.016	3.57 ± 0.014[a]	3.42 ± 0.015[a]	2.77 ± 0.015[a]
Group of FF, 30mg/kg BW + Acidifier (K)	3.66 ± 0.01[ab]	3.44 ± 0.01[ab]	3.29 ± 0.021[ab]	2.62 ± 0.033[ab]
Group of FF, 60mg/kg BW + Acidifier (L)	3.5 ± 0.013[abc]	2.98 ± 0.013[abc]	2.87 ± 0.013[abc]	2.58 ± 0.036[abc]

a: Significant change at $p < 0.05$ with respect to control group using ANOVA test.
b: Significant change at $p < 0.05$ with respect to group of acidifiers using ANOVA test.
c: Significant change at $p < 0.05$ with respect to group of acidifiers with florfenicol at 30 mg/kg BW using ANOVA test.

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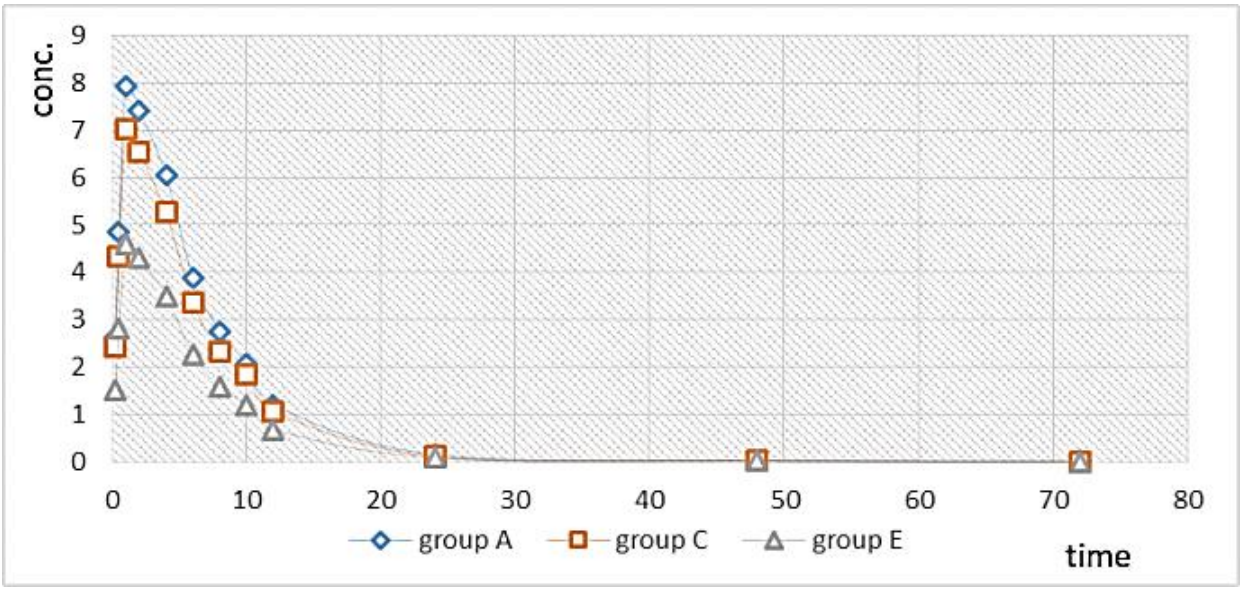

Figure 5: Mean plasma concentration versus time-course of florfenicol amine in plasma after a single oral dose of florfenicol at 30 mg/kg BW (N = 5)

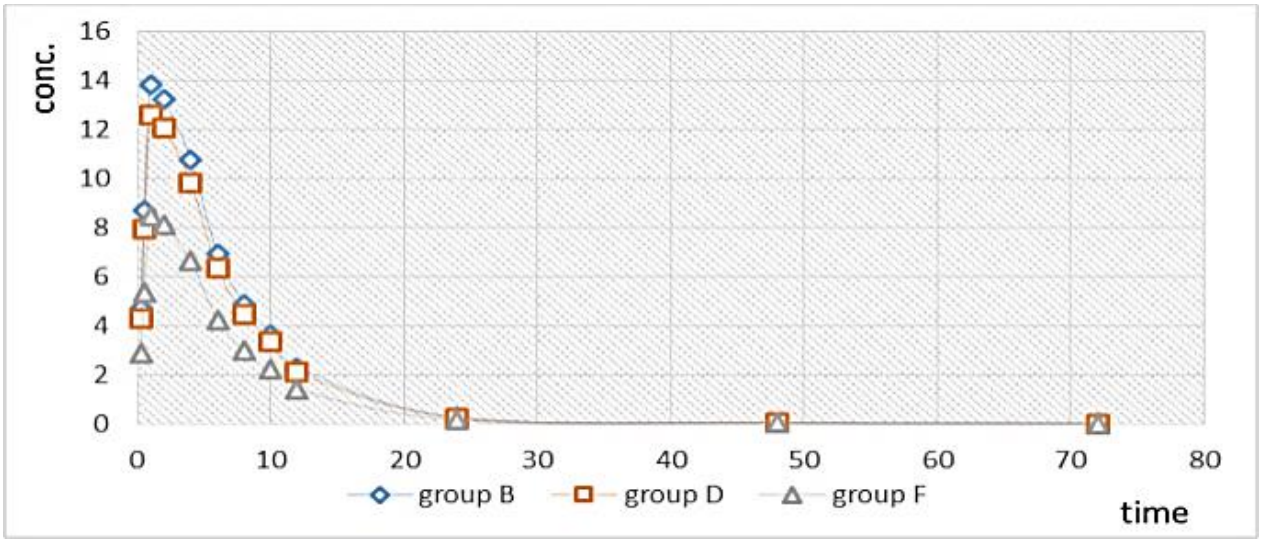

Figure 6: Mean plasma concentration versus time-course of florfenicol amine in plasma after a single oral dose of florfenicol at 60 mg/kg BW (n = 5)

Conclusion

The results obtained from this study revealed that addition of acidifiers to broiler-chicken ration did not affect pharmacokinetic pattern and tissue distribution of FF after oral administration. The acidifiers could act alone as protection against enteropathogenic *E. coli* O78 and or might improve the potential activity of FF against *E. coli* O78 infection. Also, the drug was detected until the 7th day in the liver and kidney after treatment cessation, an indication of the high penetration ability of the drug in these tissues. It was observed that FF might be a suitable drug for the treatment of gastrointestinal and urinary tract infections caused by *E. coli* O78. Finally, the study recommends the use of acidifier with FF at a dose of 30 mg/kg BW for the treatment of *E. coli* O78-infection in broiler-chickens.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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