**Allicin affects the pharmacokinetics of sulfadiazine and florfenicol by downregulating the expression of jejunum P-gp and BCRP in broilers**

Xiaoming Wang,* Yue Wang,* Ci Fang,* Qianmei Gong,* Jinhua Huang /***,* Yujuan Zhang,*† and Liping Wang*†

*MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Weigang, Nanjing 210095, China; and †Jiangsu Key Laboratory of Sericultural Biology and Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang, Jiangsu 212018, China

**ABSTRACT**

Allicin, one of the main bioactive compounds in garlic, is an excellent feed additive. It is unknown whether allicin affects the expression of P-gp and BCRP, 2 important ABC efflux transporters related to the pharmacokinetics of antimicrobials in chickens. In this study, by bidirectional transport test and broiler jejunum in situ perfusion, we found that allicin inhibited the efflux transport of P-gp and BCRP for their substrates sulfadiazine and florfenicol, 2 commonly used antimicrobials in broilers. Furthermore, we observed that allicin decreased the mRNA expression of chicken jejunum P-gp and BCRP. Pretreatment with allicin changed the pharmacokinetic behavior of orally administered sulfadiazine, by increasing AUC (41.85 vs. 31.24, \( P < 0.01 \)) and \( C_{\text{max}} \) values (9.82 vs. 8.40, \( P < 0.05 \)) and decreasing \( \text{CL}_{Z} \) (0.45 vs. 0.62, \( P < 0.01 \)). Similarly, pretreatment with allicin also altered pharmacokinetics of orally administered florfenicol, by increasing AUC (18.38 vs. 13.52, \( P < 0.01 \)) and decreasing \( \text{CL}_{Z} \). The results indicate that allicin could inhibit jejunum P-gp and BCRP expression and efflux function, thereby increasing the plasma concentrations of their substrates in broilers.

**Key words:** allicin, P-gp, BCRP, inhibition, broilers

2022 Poultry Science 101:101947

https://doi.org/10.1016/j.psj.2022.101947

**INTRODUCTION**

Due to the development of antimicrobial resistance (AMR), more and more antimicrobials are prohibited to be used in animal farms. It is urgently needed to identify new antimicrobials or alternative approaches for animal production. Allicin, a thioester of sulfenic acid, is one of the main bioactive compounds in garlic (Salehi et al., 2019; Shang et al., 2019), which has a broad spectrum of biological properties, such as anti-inflammatory, immunomodulatory, antibiotic, antifungal, antiparasitic, and antioxidant activities (Aziz and Ataa, 2018; Bhattacharyya et al., 2019; Chen et al., 2019; Zainal et al., 2020). Due to the above bioactivity, allicin has been considered as an alternative antibiotic and widely used to prevent and treat diarrhea in pigs and chickens (Alnassan et al., 2015; Zhao et al., 2016; Liu et al., 2017). Additionally, allicin has been demonstrated to be an excellent feed additive, which could improve piglet performance (Huang et al., 2011). Recent studies have observed that the herb-drug interaction (HDI) may result in clinical risk when an antibiotic is used in combination with a natural product, particularly for an antibiotic that has a narrow therapeutic window (Ezuruike et al., 2019; Wang et al., 2020). It is unknown whether allicin causes HDI when added into animal feeds for a long time.

Glycoprotein (P-gp, encoding gene Abcb1) and breast cancer resistance protein (BCRP, encoding gene Abcg2) are important members of the ATP-binding cassette (ABC) transporter class, which are usually co-localized in the excretory and barrier-function tissues, such as intestine, kidney, and liver (Villanueva et al., 2019). It has been confirmed that they are responsible for the systemic disposition of numerous structurally and pharmacologically unrelated drugs, carcinogens, toxins, and other xenobiotics (Virkel et al., 2018; Durmus et al., 2019). Pharmacologically, using herb to overcome the efflux of transporters is an efficient strategy to improve the bioavailability of P-gp or BCRP substrates (Virkel et al., 2018). The activities of P-gp and
BCRP could be regulated by xenobiotics (Virkel et al., 2018). The effects of allicin on the activities of P-gp and BCRP appear to be tissue/cell-dependent. For instance, on the one hand, allicin could inhibit P-gp-mediated efflux in liver and duodenum, leading to the enhanced cellular uptake of ritonavir (Patel et al., 2004). On the other hand, allicin could upregulate Abca1, promoting cholesterol efflux and reducing lipid accumulation via PPARγ/LXRα signaling in THP-1 macrophage-derived foam cells (Lin et al., 2017). Nevertheless, these observations suggest that allicin can regulate the expression of ABC transporters and consequently affect the pharmacokinetics of their substrates. Given the great potential of allicin in the management of antimicrobial resistance in poultry industry, it is necessary to comprehensively evaluate the effect of allicin on the expression and efflux function of ABC transporters in chickens.

This study was set to explore the effects of allicin on the expression and transport function of chicken jejunal P-gp and BCRP. Our results indicated that allicin could down-regulate the expression and activity of P-gp and BCRP in chickens, consequently altering the pharmacokinetic behavior of their respective substrates, sulfadiazine (substrate of P-gp) and florfenicol (substrate of BCRP). The findings should be informative for combined use of allicin and antimicrobial drugs (sulfadiazine and florfenicol) in the poultry industry, to increase the bioavailability and avoid possible adverse effects.

**MATERIALS AND METHODS**

**Reagents**

Sulfadiazine and florfenicol were obtained from the China Institute of Veterinary Drug Control (Beijing, China). Reverse transcription kit and SYBR green were purchased from Takara (Tokyo, Japan). All other chemicals were of analytical grade and obtained from standard suppliers unless mentioned otherwise.

Alliinase and alliin were obtained from Ailexin Drug Company (Xinjiang, China). It has been reported that 1 g of alliin reacts with alliinase to form 0.458 g of allicin (Liang et al., 2013). For the animal studies, allicin was prepared by incubating appropriate quantity of alliin with alliinase in water under magnetic stirring for 30 min at room temperature. Afterwards, the reaction product allicin was collected through a 10 kDa cut-off ultrafiltration tube, by centrifugation (12,000 r/min) for 10 min at 4°C. The concentration of allicin in filtrates is determined by high-performance liquid chromatography (HPLC) as described (Maitisha et al., 2021).

**Cell Lines**

Wild-type MDCK cells were purchased from Shanghai Institute of Cell Biology (Shanghai, China) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin at 37°C and 5% CO₂. MDCK-chAbcb1 and MDCK-chAbcg2 cell lines stably expressing chicken P-gp and BCRP were established as described (Zhang et al., 2019), and cultured in the above medium supplemented with 100 µg/mL hygromycin B.

**Animals and Groups**

Arbor Acres broilers (one-day-old) were purchased from a local commercial poultry farm (Nanjing, Jiangsu, China). Then the broilers were randomly divided into 3 groups. Group I chickens were administered a basal diet and water (control). Group II chickens were orally administered 32 mg/kg b.w. allicin. Group III chickens were orally administered 100 mg/kg b.w. allicin. All the broilers were provided the given diet and water ad libitum and managed under the recommended humidity and temperature for 2 to 6 weeks. Then some of the broilers were used to evaluate mRNA expression at different ages (2, 4, and 6 weeks). Some of the broilers at age of 4 wk were used to conduct in situ single-pass intestinal perfusion, while the rest of the broilers were used to conduct pharmacokinetics experiments. The design flowchart is illustrated in Figure 1. The treatment procedures were approved by the Science and Technology Agency of Jiangsu Province (approval no. 2017-0007, approval date: February 15, 2017) and performed in accordance with the guidelines of the Science and Technology Agency of Jiangsu Province and Nanjing Agricultural University.

**Bidirectional Transport Experiment**

Bidirectional transport experiment was carried out as described previously (Zhang et al., 2019) to confirm whether allicin affects the transport of sulfadiazine and florfenicol by altering the expression of P-gp and BCRP, respectively. Briefly, MDCK-chAbcb1 or MDCK-chAbcg2 cells were seeded in a 12-well transwell plate (Corning, Acton, MA) at a density of 1.0 × 10⁵ cells/well and cultured for 5 d until the transepithelial electrical resistance values were greater than 300 to 400 Ω cm². The bidirectional transport of sulfadiazine (25 µM) and florfenicol (20 µM) was carried out by replacing the donor buffer with transport buffer containing the above drugs with or without the preincubation of allicin for 2 h (5 µM, selected based on the cell viability of more than 90%). The samples were collected at different time points from the receiving sides and subjected to HPLC analysis. The apparent permeability coefficients ($P_{app}$), the efflux ratio ($ER$), and the net efflux ratio ($NER$) were calculated using the following equations:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A \times C_0}, \quad \text{ER} = \frac{P_{app(\text{BL} - \text{AP})}}{P_{app(\text{AP} - \text{BL})}}, \quad \text{NER} = \frac{\text{ER(MDCK - chAbcb1/chAbcg2)}}{\text{ER(MDCK)}}$$

where $A$ is the membrane surface area of the filter (1.12 cm²), $C_0$ is the initial concentration of the test drug, $dQ$ is the amount of transported drug, $dt$ is the
time elapsed, $P_{\text{app(AP-BL)}}$ and $P_{\text{app(BL-AP)}}$ are the apparent permeability coefficients of apical-to-basolateral and basolateral-to-apical directions, respectively. Each group has 5 cell monolayers, and the experiment was repeated three times.

**Abcb1 and Abcg2 mRNA Expression Determined by Real Time RT-PCR**

The mRNA expression levels in jejunum of broilers at the age of 2, 4, and 6 weeks (10 birds/group) were detected by real time RT-PCR. Total RNA was extracted using TRIZol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. RNA level was quantified using a photometer (Eppendorf, Hamburg, Germany) at 260/280 nm. RNA integrity was confirmed by 1% agarose gel electrophoresis and ethidium bromide staining. Then cDNAs were synthesized using Transcriptor first-strand cDNA synthesis kit (Vazyme, Nanjing, China) according to the manufacturer’s instructions. The primers for chicken Abcb1 and Abcg2 (Table 1) were designed according to the cloned sequences. β-actin was used as an internal control for each sample. Chicken Abcb1 and Abcg2 mRNAs were quantified according to the method of the recommended kit (SYBR Green, Toyobo, Osaka, Japan) on real time PCR detection system (Bio-Rad, Hercules, CA) and analyzed by the $2^{- \Delta \Delta Ct}$ method.

**In situ single-Pass Intestinal Perfusion of Sulfadiazine and Florfenicol in Allicin-Treated Broilers**

P-gp- and BCRP-mediated efflux were determined by in situ perfusion using their respective substrates. In brief, for P-gp-mediated transport of sulfadiazine, 30 broilers (one-day-old) were divided into 3 experimental groups (10 birds/group): control group, and orally administered allicin groups (32 and 100 mg/kg b.w.) for 4 wk. Broilers were fasted for 12 h with free access to water before the perfusion experiment. Then, the broilers were anesthetized with urethane intraperitoneally (0.5 mL/kg b.w.) and an incision was made through the abdominal cavity. A 10-cm intestinal segment was

![Flowchart for animal experimental design.](image)

**Table 1. Primer sequences used in real time RT-PCR.**

| Genes | Sequence (5’-3’) |
|-------|-----------------|
| Abcb1 | F: GCTGTTGTATTTGGTGCTATGG R: ACAAAACAGGGGCCGGCTGG |
| Abcg2 | F: CCTACCTTCCTGGGCTATGG | R: TCGGCCGCTATAGCTTGAAATC |
| β-actin | F: TGCGTGACATCAAGGAGAAG R: TGCCAGGGTACATTGTGGTA |
carefully exposed to air for intestinal perfusion. Two polyethylene cannulas were inserted through small slits at the proximal and distal ends (inlet and outlet). Initially, the intestinal segment was gently flushed with Krebs-Ringer buffer for about 30 min until a clear effluent flowed out. Then, sulfadiazine (100 μg/mL) solution was introduced into the loop at a constant flow rate of 0.2 mL/min. The intestinal fluid was collected in bottles at the outlet of the intestine at intervals of 10 min for up to 100 min. The lengths and internal radii of the intestinal segments were measured after all chickens were killed by cervical dislocation. Another 30 broilers (4 wk old) were selected to determine the BCRP-mediated transport with the same procedure, using florfenicol (100 μg/mL) as the probe.

All samples were weighed and centrifuged for 10 min at 4,500 rpm. The supernatants were filtered using a 0.22-μm filter (Millipore, Burlington, MA) and samples were collected for analysis by HPLC. The apparent permeability coefficient (P_app) and absorption rate constant (K_a) of sulfadiazine or florfenicol were calculated as follows:

\[ P_{app} = \frac{-Q_{in} \cdot (C_{out} - C_{in})}{2\pi rl} \]  
\[ K_a = \left(1 - \frac{C_{out} \cdot Q_{out}}{C_{in} \cdot Q_{in}}\right) \cdot \frac{Q}{V} \]

where \( Q_{in} / Q_{out} \) are the intestinal perfusate input and output volumes (mL), \( C_{in} \) and \( C_{out} \) represent the mass concentrations of the enteric importer and exporter perfusate (μg/mL), \( Q \) represents the perfusion rate (0.2 mL/min), \( V \) is the volume of bowel perfusion, and \( 2\pi rl \) is the area of the mass transfer surface (cm²).

**Pharmacokinetic Analyses of Sulfadiazine and Florfenicol in Broilers**

Forty 4-wk-old broilers were used in this study and divided into 4 groups (10 birds/group). Groups I/II and III/IV were used to study the influence of allicin-dependent P-gp and BCRP expression on the pharmacokinetics of their substrates (sulfadiazine and florfenicol), respectively. In brief, Groups I and III received a single dose of 20 mg/kg b.w. of sulfadiazine and florfenicol by gavage, respectively; Groups II and IV were first orally administered allicin with 32 mg/kg b.w. for 4 wk, and then orally administered sulfadiazine and florfenicol with a single dose of 20 mg/kg b.w. by gavage, respectively. For Groups I and II, blood samples were taken from the wing vein of each chicken and collected into heparin-coated tubes prior to sulfadiazine administration and at each time point of 0.167, 0.333, 0.5, 0.75, 1, 2, 4, 6, 8, 10, and 12 h following the final administration of sulfadiazine. For Groups III and IV, blood samples were taken in a similar way and at each time point of 0.083, 0.167, 0.25, 0.333, 0.5, 0.75, 1, 2, 4, 6, 8, 10, and 12 h following the final administration of florfenicol. Plasma was rapidly harvested by centrifugation at 5,000 g for 5 min and stored at −80°C until further assays.

**Detection of Plasma Sulfadiazine and Florfenicol by HPLC**

The extraction and HPLC procedures were described (Liu et al., 2016) with slight modification. In brief, all plasma samples were analyzed on Thermo Fisher U3000 HPLC system. Frozen plasma (0.2 mL) was thawed at 4°C and the plasma sample was extracted twice with 1 mL and 0.5 mL acetonitrile, respectively. The acetonitrile supernatants were combined, evaporated under nitrogen at 40°C, and dissolved in 0.2 mL of mobile phase, from which 20 μL was injected into the HPLC system. The detection conditions for sulfadiazine were: the mobile phase consisting of a mixture of acetonitrile-water at a ratio of 18:82 (v/v) wavelength at 277 nm, the column temperature at 35°C, and the flow rate at 1 mL/min. The detection conditions for florfenicol were: the mobile phase consisting of a mixture of acetonitrile-water at a ratio of 20:80 (v/v) wavelength at 224 nm, the column temperature at 35°C, and the flow rate at 1 mL/min. The calibration samples were prepared with 7 different concentrations of the respective drug using blank plasma. A linear relationship existed in the calibration curve from 0.1 to 100 μg/mL, which consistently yielded a correlation coefficient of >0.999. The limit of detection/quantification (LOD and LOQ) of sulfadiazine and florfenicol were both 0.05 μg/mL and 0.1 μg/mL, respectively. At concentrations of 0.1, 1, and 10 μg/mL, the recovery ranges of and florfenicol were 91.3 to 98.2% and 94.7 to 97.1%, respectively.

Pharmacokinetic calculations were performed on each individual set of data using Winnonlin 5.2 practical pharmacokinetic software (Version 5.2, Chinese Pharmacologic Association, Beijing, China).

**Statistical Analysis**

SPSS (Version 20.0, SPSSInc., Chicago, IL) was used for statistical analysis. The data were presented as the mean ± standard deviation (SD). One-way analysis of variance and post hoc Tukey’s tests were used to assess the statistical significance of differences. \( P < 0.05 \) was considered significant and \( P < 0.01 \) was considered extremely significant.

**RESULTS**

**Allicin Decreases the mRNA Levels of Abcb1 and Abcg2 in Broilers at Different Ages**

The mRNA levels of Abcb1 and Abcg2 were evaluated in the jejunum of broilers at ages from 2 to 6 wk after treatment with allicin (32 and 100 mg/kg) (Figure 2). Compared to the controls, 2 different concentrations of allicin significantly down-regulated the Abcb1 mRNA levels in the jejunum at all age groups (\( P < 0.01 \), \( P < 0.01 \), respectively).
Except at the age of 2 wk treated with 100 mg/kg of allicin (Figure 2A). Similarly, the expression of the \textit{Abcg2} mRNA was significantly decreased ($P < 0.01$, $P < 0.05$) at all age groups in allicin-treated broilers vs. untreated broilers, except at the age of 2 wk treated with 32 mg/kg of allicin (Figure 2B). However, there was no significant concentration-dependent changes of the mRNAs between allicin-treated groups, despite a slight fluctuation. The results indicate that long-time administration of allicin would decrease the mRNA expression of \textit{Abcb1} and \textit{Abcg2} in the jejunum of broilers.

\section*{Allicin Inhibits P-gp/BCRP-Mediated Transport of Sulfadiazine/Florfenicol}

According to the NER values shown in Figure 3, sulfadiazine and florfenicol are the substrates of P-gp and BCRP, respectively. The NER values of sulfadiazine and florfenicol were more than 2, as detected by a bidirectional transport assay in MDCK-ch\textit{Abcb1} and MDCK-ch\textit{Abcg2} cells, respectively. The NER values of sulfadiazine and florfenicol significantly decreased from 3.29 ± 0.31 to 1.92 ± 0.01, and 2.47 ± 0.11 to 1.52 ± 0.10 by allicin in MDCK-ch\textit{Abcb1} cells and MDCK-ch\textit{Abcg2} cells ($P < 0.05$), respectively. The above results indicate that allicin can reverse the efflux of sulfadiazine and florfenicol mediated by P-gp and BCRP, respectively.

\section*{Allicin Enhances the Uptake of Sulfadiazine and Florfenicol in the Jejunum of Broilers}

To further evaluate whether down-regulated expression of \textit{Abcb1} and \textit{Abcg2} mRNAs by allicin is accompanied by weaker efflux function in the small intestine, perfusion model in the jejunum of broilers was chosen to study the allicin-substrate interaction. $P_{\text{app}}$ values for sulfadiazine and florfenicol in the jejunum of broilers are presented in Figure 4. The results showed that the intestinal $P_{\text{app}}$ values of sulfadiazine and florfenicol distinctly increased after allicin (32 and 100 mg/kg) treatment for 4 wk. The $P_{\text{app}}$ values of sulfadiazine increased from 0.53 to 0.61 and 0.85 ($P < 0.05$, $P < 0.01$), respectively, while the $P_{\text{app}}$ values of florfenicol increased from 0.26 to 0.64 and 0.83 ($P < 0.01$), respectively. The increased $P_{\text{app}}$ suggest that allicin-induced downregulation of \textit{Abcb1} and \textit{Abcg2} mRNA expression enhances the permeability of their substrates in the small intestine.

\section*{Allicin Affects the Pharmacokinetics of Sulfadiazine and Florfenicol in Broilers}

The mean plasma concentration-time profiles of sulfadiazine and florfenicol in broilers following oral administration of allicin (32 mg/kg) and control are shown in Figure 5, and the relevant pharmacokinetic parameters are listed in Tables 2 and 3, respectively. Compared to the control treatment, allicin treatment significantly increased AUC and Cmax of sulfadiazine in broilers from 41.85 to 31.24 (µg/mL)$\cdot$h ($P < 0.01$) and 9.82 to 8.40 (µg/mL) ($P < 0.05$), respectively, and significantly decreased CLz ($P < 0.01$) from 0.45 to 0.62 (L/h/kg) (Table 2). In addition, allicin treatment significantly increased AUC (18.38 vs. 13.52 (µg/mL)$\cdot$h, $P < 0.05$) and significantly decreased CLz (1.13 vs. 1.51 (L/h/kg), $P < 0.01$) (Table 3). The results support that allicin-induced downregulation of \textit{Abcb1} and \textit{Abcg2} mRNA expression can affect the pharmacokinetic behaviors of sulfadiazine and florfenicol in healthy broilers.
DISCUSSION

The poultry industry has encountered increasing pressure to reduce the use of antimicrobial growth promoters due to the AMR and the pollution caused by their excretion to the environment (Wepking et al., 2017; Muaz et al., 2018). However, incidence rates of diarrhea caused by *Escherichia coli* and necrotizing enteritis caused by *Clostridium perfringens* are increasing with the reduced use of antimicrobials, which cost a huge loss to the poultry industry. In this sense, particular interest is now being paid to the phytogenic feed additives due to their plant-derived properties and growth-promoting effects (Lillehoj et al., 2018; Kholif et al., 2020; Saliu et al., 2020). A wide range of natural plants and/or their extracts have been used as feed additives to...

**Table 2.** Pharmacokinetic parameters of sulfadiazine orally administered in broilers at age of four weeks (mean ± S.D., \( n = 10 \)).

| Parameters | Control | Allicin (32 mg/kg) |
|------------|---------|-------------------|
| \( AUC_{0-24} \) (h×μg/mL) | 31.24 ± 4.70 | 41.85 ± 4.79** |
| \( C_{\text{max}} \) (μg/mL) | 8.40 ± 1.35 | 9.82 ± 0.44* |
| \( T_{\text{max}} \) (h) | 1.00 ± 0.43 | 1.04 ± 0.40 |
| \( T_{1/2,b} \) (h) | 2.06 ± 0.93 | 2.01 ± 0.58 |
| \( V_{a} \) (L/kg) | 1.62 ± 0.65 | 1.41 ± 0.50 |
| MRT (h) | 3.39 ± 0.28 | 3.60 ± 0.14 |
| CL\(_{z}\)/F (L/h/kg) | 0.62 ± 0.13 | 0.45 ± 0.06** |

Mean ± SD, \( n = 5 \), *\( P < 0.05 \), **\( P < 0.01 \), compared with control.

\( AUC_{0-24} \): area under the plasma concentration-time curves; \( C_{\text{max}} \): maximal plasma concentration; \( CL\(_{z}\)/F \): apparent clearance fraction of the dose absorbed; MRT: mean retention time; \( T_{\text{max}} \): time to obtain \( C_{\text{max}} \); \( T_{1/2,b} \): elimination half-life; \( V_{a} \)/F: apparent volume of distribution fraction of the dose absorbed.

**Table 3.** Pharmacokinetic parameters of florfenicol orally administered in broilers at age of four weeks (mean ± S.D., \( n = 10 \)).

| Parameters | Control | Allicin (32 mg/kg) |
|------------|---------|-------------------|
| \( AUC_{0-24} \) (h×μg/mL) | 13.52 ± 2.89 | 18.38 ± 3.70* |
| \( C_{\text{max}} \) (μg/mL) | 7.01 ± 1.61 | 7.61 ± 1.80 |
| \( T_{\text{max}} \) (h) | 0.71 ± 0.16 | 0.69 ± 0.18 |
| \( T_{1/2,b} \) (h) | 3.43 ± 0.81 | 4.63 ± 1.99 |
| \( V_{a} \) (L/kg) | 8.57 ± 3.90 | 7.61 ± 2.89 |
| MRT (h) | 3.74 ± 0.94 | 4.86 ± 1.43 |
| CL\(_{z}\) (L/h/kg) | 1.51 ± 0.40 | 1.13 ± 0.48* |

Mean ± SD, \( n = 5 \), *\( P < 0.05 \), compared with control.

\( AUC_{0-24} \): area under the plasma concentration-time curves; \( CL\(_{z}\) \): apparent clearance; \( C_{\text{max}} \): maximal plasma concentration; MRT: mean retention time; \( T_{\text{max}} \): time to obtain \( C_{\text{max}} \); \( T_{1/2,b} \): elimination half-life; \( V_{a} \): apparent distribution volume.
improve growth performance, digestibility, immune response and intestinal health in breeding industry (Alagawany et al., 2019; Lin et al., 2020). Of interest, allicin has emerged as a promising antibiotic alternatives because of its antibacterial, antiparasitic and antifungal activities (El-Saber Batiha et al., 2020).

The improved growth performance of livestock or poultry by herbs or natural products suggest that proper application of herbs or natural products may be a novel and effective approach for reducing misuse of antimicrobial agents. Alteration of P-gp and BCRP expression or activity by herbal constituents has implication for absorption and bioavailability of P-gp or BCRP substrates (Zhang et al., 2016; Lou et al., 2019; Patel et al., 2019). In this study, we observed that pretreatment with allicin affected the pharmacokinetics of sulfadiazine and florfenicol (P-gp and BCRP substrates), suggesting that allicin is capable of eliciting HDI if consumed in high quantities with concomitant use of conventional antimicrobials in broilers. Similar results have been seen in other herbal constituents. For example, anemoside B4, the major effective saponin in Pulsatillae radix, has been shown to reduce the plasma concentration of florfenicol, which is related to upregulated mRNA expression of Abcb1 and CYP450 (Li et al., 2019). In addition, other natural compounds like berberine, piperine, quercetin, genistein, naringin, sinomenine, glycyrrhizin, and nitrile glycoside have demonstrated the capacity to enhance the bioavailability of co-administered drugs by inhibiting drug efflux pumps or oxidative drug metabolism (Varma et al. 2003; Peng et al., 2006; Patel et al. 2019; Zhang et al., 2019). We have identified a large number of chemically unrelated drugs, including enrofloxacin, ciprofloxacin, tilmicosin, sulfadiazine, ampicillin, florfenicol, and clindamycin commonly used in veterinary clinic, as chicken P-gp or BCRP substrates (Zhang et al., 2018). Moreover, some of these drugs are also substrates of CYP3A4, and the situation becomes more complicated with the participation of CYP450 (Dunkoksung et al., 2019; Bai et al., 2020). Therefore, more attentions need to be paid when the substrates were coadministered to the animals with inhibitors or inducers of P-gp, BCRP, or CYP450. Similarly, when considering a natural plant and/or its extract used as a feed additive, its effect on P-gp, BCRP, or CYP450 should be comprehensively evaluated. It is a good strategy through inhibiting P-gp or BCRP to increase the plasma concentration of a drug with a low apparent permeability, but it may bear the risk of undesired side-effects if the drug has a small margin of safety.

Our previous study has shown that berberine, another natural product commonly used to treat diarrhea caused by bacteria in clinic, not only inhibits the mRNA expression of P-gp through the CXR pathway precipitated by molecular docking (Zhang et al., 2019), but also as a substrate of P-gp to compete the transport with the co-administered drugs. However, the mechanisms behind the inhibitory effect of allicin on chicken P-gp and BCRP mRNA expression, and the effect of allicin on chicken CYP450 are still unknown, which are worthy to be further studied.

FUNDING

This research was funded by the National Natural Science Foundation of China (31872517 and 32002330), the Natural Science Foundation of Jiangsu Province (BK20200535), the National Key Research and Development Program (2016YFD0501308), and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

ACKNOWLEDGMENTS

We are grateful to professor Shile Huang from Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center for kindly revising the manuscript.

DISCLOSURES

None.

REFERENCES

Alagawany, M., S. S. Elhessr, M. R. Farag, M. E. Abd El-Hack, A. F. Khafaga, A. E. Taha, R. Tiwari, M. I. Yatoo, P. Bhatt, G. Marappan, and K. Dhamn. 2019. Use of licorice (glycyrrhiza glabra) herb as a feed additive in poultry: current knowledge and prospects. Animale (Basel) 9:530.
Ahassan, A. A., A. Thabet, A. Drugaches, and B. Gangoura. 2015. In vitro efficacy of allicin on chicken eimeria tenellaporzoites. Parasitol. Res. 114:3913-3915.
Aziz, A., and A. M. Ataa. 2018. Use of enzyme lysozyme and antibiotic allicin for food preservation and the prevention of damage. Corresponding author. J. Biotechnol. 34:12.
Bai, J., L. Li, S. Zhao, X. Fan, J. Zhang, M. Hu, Y. Chen, Y. Sun, B. Wang, J. Jin, X. Wang, D. Zhang, J. Hu, and Y. Li. 2020. Heterotrophic activation of flavonoids on cytochrome P450 3A4: a case example of alleviating dromedaron-induced cytotoxicity. Toxicol. Lett. 319:187-196.
Bhattacharya, S., D. Sen, and C. Bhattacharjee. 2019. Growth inhibition characteristics of Escherichia coli in contrast to Bacillus subtilis by natural antibiotic ‘Allicin’ extracted from allium sativum bulbs. Proc. 106th Indian Sci Cong..
Chen, Y., Y. Wang, M. Yang, and M. Guo. 2019. Allicin inhibited Staphylococcus aureus-induced mastitis by reducing lipid raft stability via LxRs in mice. J. Agric. Food Chem 67:10863–10870.
Dunkoksung, W., N. Vardhanabhuti, P. Siripong, and S. Jianmongkol. 2019. Rhinacanthin-C mediated herb-drug interactions with drug transporters and phase I drug-metabolizing enzymes. Drug Metab. Dispos. 47:1040–1049.
Durmus, S., M. V. D. Valk, S. F. Tennissen, J. Y. Song, E. Wagenaar, J. H. Beijnen, and A. H. Schinkel. 2019. ABC transporters Mdr1a/1b, Bcrp1, MRP2 and MRP3 determine the sensitivity to PhilP/DSS-induced colon carcinogenesis and inflammation. Arch. Toxicol. 93:775–790.
El-Saber Batiha, G., A. Magdy Bedhiysh, G. W. L., Y. H. A. Elewa, A. A.-S. A. M. E. Abd El-Hack, A. E. Taha, M. A.-E. Y. and H. Prasad Devkota. 2020. Chemical constituents and pharmacological activities of garlic (Allium sativum L.): a review. Nutrients 12.
Liang, Y., J. J. Zhang, Q. B. Zhang, Z. X. Wang, Z. N. Yin, X. X. Li, Lin, X. L., H. J. Hu, Y. B. Liu, X. M. Hu, X. J. Fan, W. W. Zou, Lillehoj, H., Y. Liu, S. Calsamiglia, M. E. Fernandez-Miyakawa, Liu, S., J. Chen, B. Kang, Q. Jia, H. W. Xing, J. Y. Bai, and Z. Y. Ying. 2020. Chinese herb feed additives mixture enhances the lactational performance, feed utilization and ruminal fermentation of Friesian cows. Anim. Biotechnol. 32:708–718.

Li, S., X. Li, R. Yang, B. Wang, J. Li, L. Cao, S. Xiao, and W. Huang. 2019. Effects of anemode B4 on pharmacokinetics of florenchol and mRNA expression of CXR, MDR1, CYP3A37 and UGT1E in broilers. J. Vet. Med. Sci. 81:1804–1809.

Liang, Y., J. J. Zhang, Q. B. Zhang, Z. X. Wang, Z. N. Yin, X. X. Li, J. Chen, and L. M. Ye. 2013. Release test of alliin/allinase double-layer tablet by HPLC-Allicin determination. J. Pharm. Anal. 3:187–192.

Lillehoj, H., Y. Liu, S. Calsamiglia, M. E. Fernandez-Miyakawa, F. Chi, R. L. Cravens, S. Oh, and C. G. Gay. 2018. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet. Res. 49:76.

Lin, X. L., H. J. Hu, Y. B. Liu, X. M. Hu, X. J. Fan, W. W. Zou, Y. Q. Pan, W. Q. Zhou, M. W. Peng, and C. H. Gu. 2017. Allicin induces the upregulation of ABCA1 expression via PPARgamma/LXRalpha signaling in THP-1 macrophage-derived foam cells. Int J. Mol. Med. 39:1452–1460.

Lin, Z. N., L. Ye, Z. W. Li, X. S. Huang, Z. Lu, Y. Q. Yang, H. W. Xing, J. Y. Bai, and Z. Y. Ying. 2020. Chinese herb feed additives improved the growth performance, meat quality, and nutrient digestibility parameters of pigs. Anim. Model Exp. Med. 3:47–54.

Liu, S., J. Chen, B. Kang, Q. Jiang, H. E. Liuqin, W. U. Fei, N. Huang, L. I. Huan, Y. Yin, and K. Yao. 2017. Effects of dietary α-ketoglutarate and allicin on growing development and nutrient apparent digestibility of growing pigs. Chinese J. Anim. Nutr.

Liu, Y., L. Guo, M. Zloh, Y. Zhang, and J. Huang. 2016. Relevance of breast cancer resistance protein to pharmacokinetics of florenchol in chickens: a perspective from in vivo and in vitro studies. Int. J. Mol. Sci. 19:3165.

Lou, Y., Z. Guo, Y. Zhu, G. Zhang, Y. Wang, X. Qi, L. Lu, Z. Liu, and J. Wu. 2019. Astragal radix and its main bioactive compounds activate the Nr2-mediated signaling pathway to induce P-glycoprotein and breast cancer resistance protein. J. Ethnopharmacol. 228:82–91.

Maitisha, G., M. Aimaiti, Z. An, and X. Li. 2021. Allicin induces cell cycle arrest and apoptosis of breast cancer cells in vitro via modulating the p53 pathway. Mol. Biol. Rep. 48:7261–7272.

Muz, K., M. Riaz, S. Akhtar, S. Park, and A. Ismail. 2018. Antibiotic residues in chicken meat: global prevalence, threats, and decontamination strategies: a review. J. Food Prot. 81:619–627.

Patel, H. B., U. D. Patel, B. S. Mathapati, and C. M. Modi. 2019. Effect of piperine and quercetin alone or in combination with marbofloxac in CYP3A37 and MDR1 mRNA expression levels in broiler chickens. Res. Vet. Sci. 126:178–183.

Patel, J., B. Buddha, S. Dey, D. Pal, and A. K. Mitra. 2004. In vitro interaction of the HIIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity. Am. J. Ther. 11:262–277.

Peng, S. X., D. M. Ritchie, M. Cousineau, E. Danser, R. Dewire, and J. Floden. 2006. Altered oral bioavailability and pharmacokinetics of P-glycoprotein substrates by co-administration of biochanin A. J. Pharm. Sci. 95:1984–1993.

Salehi, B., P. Zucca, I. E. Orhan, E. Azzini, C. O. Adetunji, S. A. Mohammad, S. K. Banerjee, F. Sharopov, D. Rigano, and J. Sharib-Rad. 2019. Allicin and health: a comprehensive review. Trends Food Sci. Technol.

Salu, E. M., H. Ren, F. G. Borojojeni, J. Zentek, and W. Vahjen. 2020. The impact of direct-fed microbials and phyto-genic feed additives on prevalence and transfer of extended-spectrum beta-lactamase genes in broiler chicken. Microorganisms 8.

Shang, A., S. Y. Cao, X. Y. Xu, R. Y. Gan, G. Y. Tang, H. Corke, Y. Mavumengwana, and H. B. Li. 2019. Bioactive compounds and biological functions of garlic (allium sativum L.). Foods 8:246.

Varma, M. V. S., Y. Ashokraj, C. S. Dey, and R. Panchagnula. 2003. P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. Pharmacol. Res. 48:347–359.

Villanueva, S., W. Zhang, F. Zechinatti, A. Mottino, and M. Vore. 2019. ABC Transporters in extrahaepatic tissues: pharmacological regulation in heart and intestine. Curr. Med. Chem. 26:1155–1184.

Virkel, G., M. Ballent, C. Lanusse, and A. Lifschitz. 2018. Role of ABC transporters in veterinarian medicine: pharmacaco-toxicological implications. Curr. Med. Chem. 26:1251–1269.

Wang, K., Q. Gao, T. Zhang, J. Ruo, L. Ding, and F. Qiu. 2020. Inhibition of CYP2C9 by natural products: insight into the potential risk of herb-drug interactions. Drug Metab. Rev. 52:235–257.

Wepping, C., B. Avera, B. Budgley, J. E. Barrett, J. Franklin, K. F. Knowlton, P. P. Ray, C. Smitherman, and M. S. Strickland. 2017. Exposure to dairy manure leads to greater antibiotic resistance and increased mass-specific respiration in soil microbial communities. Proc. Biol. Sci. 284.

Zainal, M., N. Zain, I. M. Amin, and V. N. Ahmad. 2020. The antimicrobial and antibiofilm properties of allicin against Candida albicans and Staphylococcus aureus-a therapeutic potential for denture stomatitis. Saudi Dental J 33:105–111.

Zhang, G., R. Ou, F. Li, J. Wu, L. Zheng, Y. Tong, Y. Liu, Z. Liu, and L. Lu. 2016. Regulation of drug-metabolizing enzymes and efflux transporters by Astragal radix decoction and its main bioactive compounds: Implication for clinical drug-drug interactions. J. Ethnopharmacol. 180:104–113.

Zhang, Y., L. Guo, J. Huang, Y. Sun, F. He, M. Zloh, and L. Wang. 2019. Inhibitory effect of berberine on broiler P-glycoprotein expression and function: in situ and in vitro studies. Int. J. Mol. Sci. 20:1966.

Zhang, Y., J. Huang, Y. Liu, T. Guo, and L. Wang. 2018. Using the lentiviral vector system to stably express chicken P-gp and BCRP in MDCK cells for screening the substrates and studying the interplay of both transporters. Arch. Toxicol. 92:2927–2942.

Zhao, J., K. Yang, Q. Zhang, and L. I. Chuanwei. 2016. Effect of allicin on weight gain and diarrhea rate of Hu sheep during the growth period. Heilongjiang Anim. Sci. Vet. Med 6:154–155.