Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 1, 8-16

Comparing the effect of nitazoxanide and tylosin against necrotic enteritis in broilers

Eman M.El. El-Sherbeny[1*], Doaa M.R. Sharaf[2]

[1]*Pharmacology unit, Animal Health Research Institute (AHRI), Tanta lab. Agricultural Research Center (ARC), Giza, Egypt.*
[2]*Bacteriology unit, Animal Health Research Institute (AHRI), Tanta lab. Agricultural Research Center (ARC), Giza, Egypt.*

ARTICLE INFO

Recieved: 30 September 2023

Accepted: 05 November 2023

***Correspondence:**

Corresponding author: Eman M.El. El-Sherbeny
E-mail address: myvetaddress@gmail.com

Keywords:

Antibiotic resistance
Broilers
Clostridium perfringens
Nitazoxanide
Tylosin
Necrotic enteritis.

ABSTRACT

This work compared the antibacterial activity and in vivo effects of nitazoxanide and tylosin against infected broilers with antibiotic resistant *Clostridium perfringens* field strain to control necrotic enteritis disease. Both in vitro and in vivo assessment studies were used. Firstly, *C. perfringens* was isolated with a total rate of 46% from liver and intestine samples of diseased broilers. By using PCR molecular typing all tested isolates were type A (produced only alpha-toxin (*cpa* virulence gene)) and included *Bla* and *tetK* (resistant genes). Using antibiotic sensitivity test they showed multi-drugs resistance against amoxiclave, tetracycline, gentamicin, clindamycin, sulphamethoxazole-trimethoprim and cefoxitin. Minimal inhibitory and bactericidal concentrations (µg/ml) were 0.4 and 12 for nitazoxanide and 0.7 and 49 for tylosin respectively. Then, a total of 90 one-day-old-chicks were divided into 6 groups; G1: negative-control, G2: infected-control, G3: non-infected (nitazoxanide 15.4 mg/kg body weight), G4: non-infected (tylosin 20 mg/kg body weight), G5: infected-nitazoxanide (15.4 mg/kg), and G6: infected-tylosin (20 mg/kg). Treatments lasted for 5 days in drinking water. No adverse effects on liver or kidney parameters were recorded in non-infected treated groups. Both treatments overcome the infection signs, *C. perfringens* count and revealed a significant improvement in most of inflammatory and biochemical parameters to their normal levels especially, G5 reflected a significant increase in total protein, albumin, globulin while reduced alanine and aspartate aminotransferase and gamma-glutamyl transferase activities, C-reactive protein, uric acid, and creatinine levels than G2. Finally, nitazoxanide revealed a significant anti-clostridial activity as tylosin for the control of necrotic enteritis in broilers.

Introduction

Necrotic enteritis (NE), one of chief wide-reaching bacterial enteric diseases of chickens, caused a critical economic sufferer that threat the poultry production through medication cost, poor growth performances or sudden death with mortality rates up to 50% (Salem *et al*., 2021; Abd El-Hack *et al*., 2022). Clinical cases of NE have been reported in variable frequencies in commercial chickens either reared in cages or floor pens. It observed primarily in broiler chicks (2 to 5 weeks of age), besides older pullets and layers (Elkomy *et al*., 2019). Multi-predisposing factors promoted its outbreaks incidence as; coccidiosis, interdiction the antibiotics prophylactic use in feeds besides ration components factors that increased the intestinal viscosity (Kasab-Bachi, 2017). NE caused by excessive growth of commensally colonized intestinal *Clostridium perfringens* bacteria, anaerobic Gram-positive spore-forming non motile bacilli, that usually types A or C and rarely type D. It was also established in soil and sewage. *C. perfringens* is associated with different clinical infestations in animals and humans. Its types, virulence and pathogenicity depend mainly on its produced potent protein toxins and extracellular enzymes which proliferate in intestinal tract causing neurologic, histotoxic and intestinal infections ranging from subclinical signs to serious disease that threat life (Gohari *et al*., 2021; Kronfold *et al*., 2022). These virulence genes are responsible for hydrolysis of membrane phospholipids and restrain leukocytes from entering to the infected tissues and suppress the immune response (Fu *et al*., 2022). *C. perfringens* is categorized into five types (A, B, C, D, and E) mainly according to their produced major lethal toxins (Alpha, beta, epsilon and iota) which are encoded by *cpa*, *cpb*, *etx* and *itx* genes respectively (Rood *et al*., 2018). *C. perfringens* uses chromosomally encoded alpha toxin and perfrinolysin to induce its histotoxic and intestinal disease form. As well, some of *C. perfringens* strains type A produce an enterotoxin that induced the food poisoning symptoms and gastrointestinal illness in humans (Uzal *et al*., 2014).

Recently, the excessive and uncontrolled uses of various antibiotic classes lead to resistance in the normal enteric flora including *C. perfringens* resistance. Hence, some clinical outbreaks revealed no respond to certain treatments and there are many concerns about the potential risk of drug-resistant *C. perfringens* transmission from animals to humans. By increasing awareness of banning antibiotic growth promoters, treatment failure and rising of antimicrobial resistance (AMR); monitoring of *C. perfringens* isolates (El-Nagar *et al*., 2022) besides, penetrating efficient therapeutic alternatives were considered as a critical need in controlling of NE (Nasr El-Deen *et al*., 2019; Hussein *et al*., 2020).

Nitazoxanide (NTZ), approved drug from the Food and Drug Administration (FDA), originally developed as an anti-protozoal, was subsequently found to be effective with a good safety profile broad anti-infective agent with diverse pharmacological properties (Odingo *et al*., 2017; Ahmed *et al*., 2021). Hence, it has received ample attention in the field of drug discovery and drug development. NTZ has a lethal effect on anaerobic bacteria via blocks critical components of their energy metabolism. It inhibits the bacterial pyruvate ferredoxin/flavodoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction, besides reduction of the nitro group (Hoffman *et al*., 2007). It also inhibits *C. difficile* vitamin cofactor of PFOR (Devasahayam *et al*., 2010).

Subsequent oral uptake of the pro-drug, NTZ, is absorbed from

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
ISSN: 2090-6277/2090-6269/ © 2011-2024 Journal of Advanced Veterinary Research. All rights reserved.

the gastrointestinal tract and revealed an excellent bioavailability (Balderas-Acata *et al*., 2011), with double absorption when taken with food (Stockis *et al*., 2002a). Resistance against NTZ is delayed and it renders as a good alternative to metronidazole and benzmidazoles to avoid their mutagenicity and resistance that could become a problem especially with the prospective zoonotic pathogens (Hemphill *et al*., 2006). As well, in vitro efficacy of NTZ and tizoxanide is much greater than metronidazole. Regarding to NTZ uses and administration in chickens, Antonya *et al*., (2020) recorded its positive anti-newcastle viral effect in two-week old chickens given intramuscularly at 12.5 µM post 72 h, besides its immune-modulatory effects.

Macrolide antimicrobials are a group of structurally similar compounds where tylosin is considered as one of extensive clinically used member of this class (Wijayanti *et al*., 2022). They may be bactericidal or bacteriostatic, rely on its concentration and type of treated bacteria. Their actions induced through reservation of bacterial protein translation and synthesis by means of reversible binding to their 23S rRNA of the 50S ribosomal subunit and induce the dissociation of peptidyl-tRNA from the ribosome; consequently, this action interfere with the translocation reaction necessary for RNA-dependent protein synthesis in treated bacteria (Gaynor and Mankin, 2005). Tylosin lessened the prevalence of *C. perfringens* enterotoxaemia mortalities (Vissiennon *et al*., 2000). Post banned its use as growth promoter in 1999 by the European Union and national legislation, NE outbreaks and therapeutic antimicrobials usage raises (Casewell *et al*., 2003). Therefore, tylosin tartrate, a drinking water form, is used as a one of vital antimicrobials (OIE, 2004). Various oral bioavailability were reported for tylosin depending on its form, to be relatively low (8-12%) in turkey (Po ´zniak *et al*., 2021), (13.74 -27.0%) (Ji *et al*., 2014), (30.7-34%) (Kowalski *et al*., 2002) in chickens, (35.4 - 40.6%) (Abu-Basha *et al*., 2012), 89.2% (Aboubakr and Elbadawy, 2017) and 90.29% (Soliman and Sedeik, 2016) in broilers.

Nowadays, macrolide resistance has also remained an escalating problem in food animals (Das *et al*., 2020). Using of tylosin was stated to raise revival of erythromycin-resistant enterococci (Samanta and Bandyopadhyay, 2020), macrolide-resistant *Campylobacter* spp. in animals, especially *C. jejuni* in poultry (Burton *et al*., 2016), besides the acquired resistance that latently amplified in human pathogenic strains isolated from animals. Also, cross-resistance among macrolides, lincosamids and streptogramin group B antibiotics is known to occur (Schwarz *et al*., 2006).

To the best of our knowledge, there was a scarcity in studies conducted on the in vivo effect of NTZ on broilers against experimental infection of *C. perfringens* caused necrotic enteritis. Therefore, the current trial was carried out to assess its efficacy through a comparative study of the antibacterial activity of NTZ and tylosin against antibiotic resistant field strains of *C. perfringens* type A that previously isolated from apparently diseased broilers and identified their virulence and resistance genes using PCR molecular typing. Also, this study detected the antimicrobial resistance pattern of some of the obtained isolates to commonly used antimicrobials and defined NTZ and tylosin minimal inhibitory and bactericidal concentrations (MIC and MBC). Furthermore, this study evaluated the in vivo effects of NTZ and tylosin on clinical symptoms, mortalities, *C. perfringens* count, biochemical alterations; C-reactive protein, non-specific immunity, besides some of hepatic and renal function profiles of non-infected and infected broilers.

Materials and methods

Sample collection, isolation and identification

Samples were collected from the liver and small intestine (n = 50) of apparently diseased broilers that showed NE signs (depression, reduced mobility, and diarrhea) at age of 2-3 weeks from different poultry farms in El-Gharbia Governorate, Egypt. Suspected diseased broilers were transferred under aseptic condition in an ice box to eviscerate in the Animal Health Research Institute (Tanta branch) where required organs were collected from cases of NE post mortem lesions (distended small intestine by gases with degenerated mucosa).

Five grams of each sample were added to 5ml of PBS (Phosphate buffer saline) in sterile mortar and this suspension was added to a tube that contained cooked meat broth and heated to 65ºC for 10 min in order to select bacterial spores. Then the tubes were incubated for 24 h/37ºC in anaerobic CO_2 condition. In the next step each sample was streaked by sterile loopful on already prepared blood agar supplemented with sheep blood 10% and incubated for 18 h/37ºC under anaerobic condition. The circular disordered flat and bright colonies were indicative for presence of *Clostridium* (Doosti and Mokhtari-Farsani, 2014).

Gram Stain was used as a method of differentiating bacterial species into two large groups Gram-positive and Gram-negative. Films from suspected pure cultures were stained with Gram stain (Cruickshank *et al*., 1975) and examined microscopically.

Suspected colonies were chemically identified by sugar fermentation, gelatin liquefaction, indol, oxidase tests and action on litmus milk (Macfaddin, 2000).

Molecular typing of C. perfringens isolates using multiplex PCR

Two pure isolates of *C. perfringens* were randomly selected and typed in the Reference Laboratory for veterinary Quality control on Poultry production (RLQP), at the Animal Health Research Institute using multiplex PCR targeting alpha, beta, epsilon and iota toxins genes (Table 1), by using Midland Certified Reagent Company-oilgos (USA).

The used drugs

Nitazoxanide antibiotic (Nitazode, each 5ml contain 100mg nitazoxanide base), manufactured by Al Andalous for Pharmaceutical Ind., Egypt

Table 1. Target genes, oligonucleotide primers sequences, amplicon sizes and cycling conditions for molecular typing of *C. perfringens* isolates using multiplex PCR.

Toxin	Sequence	Amplified product	Reference
Alpha toxin	GTTGATAGCGCAGGACATGTTAAG CATGTAGTCATCTGTTCCAGCATC	402 bp	YOO *et al*. (1997)
Beta toxin	ACTATACAGACAGATCATTCAACC TTAGGAGCAGTTAGAACTACAGAC	236 bp	
Epsilon toxin	ACTGCAACTACTACTCATACTGTG CTGGTGCCTTAATAGAAAGACTCC	541 bp	
Iota toxin	GCGATGAAAAGCCTACACCACTAC GGTATATCCTCCACGCATATAGTC	317 bp	
bla	ATGAAAGAAGTTCAAAAATATTTAGAG TTAGTGCCAATTGTTCATGATGG	780 bp	Catalán *et al*. (2010)
tetK	TTATGGTGGTTGTAGCTAGAAA AAAGGGTTAGAAACTCTTGAAA	382 bp	Gholamiandehkordi *et al*. (2009)

and tylosin antibiotic (TyloGerm, W.S.P. each 1 g contains 540.96 mg of tylosin tartrate equivalent to 500 mg of tylosin base) manufactured by Royal Link Pharma for FG for Trading and Veterinary supplies were used in this study.

In vitro antibacterial activity tests

Antimicrobial susceptibility test (AST) assay

AST of isolates were applied using the disk diffusion method on Mueller Hilton agar according to the guidelines of CLSI (2018) for anaerobic sp. From six different families of commonly used antibiotics in the field; β-lactamases-inhibitors, tetracyclines, aminoglycosides, lincosamides, folates-inhibitors, and cephalosporin; 6 standard discs (Oxoid Basingstone, UK) namely amoxiclav AMC (30µg), tetracycline TE (30µg), gentamicin GEN (10µg), clindamycin DA (2µg), sulphamethoxazole-trimethoprim (Cotrimoxazole) COT (25µg) and cefoxitin FOX (30µg) were utilized. Results of antibiogram resistance patterns of isolates were categorized by measuring the inhibition zone diameter (IZD) in millimeters (mm) according to Alimolaei *et al.* (2015). Multiclass resistance was defined as resistance to three or more antimicrobial classes.

The antibacterial activity of NTZ and tylosin against *C. perfringens* was assessed using well diffusion method (CLSI, 2018; 2021), where drugs were placed into *C. perfringens* inoculated agar. After anaerobic incubation at 37ºC/24h, the inhibition zones diameters (IZD) were determined in (mm). Each antimicrobial assay was performed simultaneously in triplicate.

MIC and MBC values of NTZ and tylosin

The MIC as a quantitative bioassay was determined by using a micro-broth dilution test in a 96-well microplate using standard procedures (Rotilie *et al*., 1975; CLSI, 2018) for anaerobes. A solution of 25 mg/ml concentration for NTZ and tylosin were twofold serially diluted throughout wells till 0.00019 mg/ml. Both negative (sterile well, only drug without bacteria) and positive (growth well, only bacteria without drug) controls were included. Referring to the results of the MIC assay a loopful, from each clear well was subcultured on specific agar plates, then observed for growth. The lowest concentration that inhibited visible growth and showed no turbidity was recorded as the MIC and the lowest concentration showing no growth was recorded as the minimum bactericidal concentration (MBC). The experiment was performed in duplicate. Standardized guidelines to interpret tylosin MIC values for *C. perfringens* are not well established (Kasab-Bachi, 2017). Hence, we used, where available, the interpretive criteria and breakpoints available in the published literature (Giguere, 2006); tylosin tartrate (S ≤ 0.5, I = 1 - 4, R ≥ 8).

In vivo assessment of the used drugs

Experimental design

A total of 100 one-day-old broiler chicks were purchased from a private farm. Then, the livers of 10 chicks were tested bacteriologically to ensure that they are free from any systemic *C. perfringens* infection. The remaining 90 chicks were divided into 6 groups (15 birds/group); G1: negative control, G2: positive control infected orally with *C. perfringens* type A (1×10^8 CFU/ml/chick), G3: non-infected treated with (nitazoxanide 15.4 mg/kg body weight) and G4: non-infected treated with (tylosin 20 mg/kg b.wt), G5: infected and treated with (nitazoxanide 15.4 mg/kg b. wt) and G6: infected and treated with (tylosin 20 mg/kg b. w). All groups had the same management and vaccination with free access to rations and water. At the 15th day of age, broilers were inoculated orally with one of our PCR confirmed field strain isolated in the current study, *C. perfringens* type A, at a dose equivalent to 2ml of 10^8 CFU (Eid *et al*., 2020); the dose adjusted with reference to 0.5 McFarland Standard with approximate cell density of 1.5 x 10^8 CFU/ml. Broilers were observed daily for morbidity, mortality, clinical signs, and necropsy finding. Post the appearance of NE clinical symptoms in the infected groups, at the 18th day of age (three days post inoculation), daily freshly prepared medications were given to treated groups in the morning hour of each day for 5 consecutive days till the 22nd day of age using the pulse dosing technique, where birds were deprived from water for one hour then therapeutic drugs dissolved in adequate amount of drinking water that enough to 2-3 h of consumption, as one shot dose, to insure the consumption of the whole treatment dose. NTZ was used at a dose of 50 µM equal to (15.4 mg/kg of body weight/day) according to Antonya *et al.* (2020). Meanwhile, tylosin was used at a dose of (20 mg/Kg of body weight/day) according to Ribeiro (2020) and the recommended commercial dose. The experiment lasted until the 32nd day of age. The experiment was carried out in accordance with the guidelines set by the Egyptian Ethics Committee and the NIH of Research Ethics Committee for environmental and clinical studies at Animal Health Research Institute (AHRI) for the Care and Use of Laboratory Animals.

Sampling

Blood samples

On the 23rd and 32nd day of age corresponding to post-treatment (PT) period and at the end of the study respectively blood samples were collected from the wing vein (5 bird/group) for serum in a clean dry centrifuge tube without anticoagulant for biochemical analysis which included alanine and aspartate aminotransferase (ALT and AST) (Reitman and Frankel, 1957) and Gamma-glutamyl transferase (GGT) activities (Szasz *et al*., 1974), total protein (TP) (Gornall *et al*., 1949), and albumin (Doumas *et al*., 1971) levels. Globulin was detected by subtraction of albumin value from TP. C reactive protein as one of the anti-inflammatory parameters was determined (Macleod and Avery, 1941). For kidney function evaluation, uric acid (Fossati *et al*., 1980) and creatinine (DI Giorgio, 1974) were estimated. All tests were determined using commercial kits (Spectrum and Egy Chem, Egypt).

Liver and intestine samples for C. perfringens count

Post treatment, for *C. perfringens* re-isolation and count; 1 g of small intestine and liver from all groups (3 broilers/group) were collected under aseptic conditions and followed the guidelines and recommendations of ISO (1997).

Statistical analysis

Experimental data were assessed by One-way analysis of variance (ANOVA), Duncan Multiple Range post-hoc analysis test using IBM SPSS software statistical program (version 23.0) for comparison of means at significance level ($P < 0.05$). Data were expressed as mean±SE (standard error) when (n=5).

Results

Identification of pure suspected colonies of *C. perfringens* isolates

On sheep blood agar colonies appeared raised, rounded, smooth, glistening and showed double zones of hemolysis. Microscopically, Gram stained smears from these colonies, revealed gram positive, short bacilli, straight with rounded ends and parallel sides. Biochemically, isolates were negative for catalase, indole, urease tests while positive with gelatinase activity, lecithinase reaction, sugar fermentation tests (lactose, glucose, sucrose and maltose).

C. perfringens isolation incidence

From all tested samples 12 isolates were positive from the liver and 34 isolates from the intestine were identified phenotypically and biochemically by bacteriological methods.

Molecular typing of C. perfringens strains by multiplex PCR (PCR amplification)

Results showed that the tested two *C. perfringens* isolates were positive for alpha toxin virulence gene (*cpa*) only and produced amplicons at 402 bp, none of the isolates possessed beta, epsilon or iota toxin gene, so they were considered *C. perfringens* type A (Fig. 1). Post detection of antibiotic phenotypic resistance results of our AST, *Bla* and *tetK* antibiotic resistant genes were detected in the tested samples at 780bp and 382bp respectively (Fig. 2).

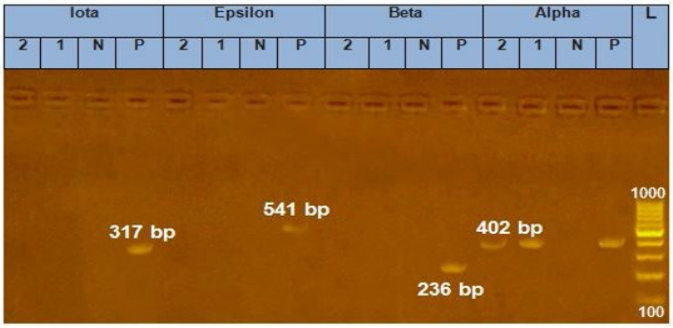

Fig. 1. Agarose gel electrophoresis of alpha, beta, epsilon or iota toxin gene of *C. perfringens*. Lane (L): 100-1000bp DNA Ladder, Lane P: positive control sample of *C. perfringens*, Lane N: negative control sample, Lane: 1-2: positive amplification of alpha toxin at 402bp of tested sample but negative for beta, epsilon and iota toxin.

Antimicrobial sensitivity test

AST of the all tested isolates of *C. perfringens* type A showed a multi-drugs resistance against all tested antimicrobial discs; amoxiclave, tetracycline, gentamicin, clindamycin, sulphamethoxazole-trimethoprim (Cotrimoxazole) and cefoxitin (Fig. 3 a). Therefore, oxytetracycline and amoxicillin-clavulinic acid antimicrobial resistant genes were tested to confirm these phenotypic results where their resistant genes (*Bla* and *tetK*) were confirmed.

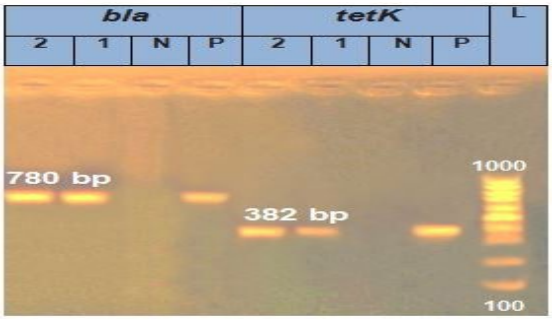

Fig. 2. Agarose gel electrophoresis of *bla* and *tetK* genes of resistant strain of *C. perfringens*. Lane (L): 100-1000bp DNA Ladder, Lane P: positive control sample of *C. perfringens*, Lane N: negative control, Lane: 1-2 : positive amplification of *Bla* gene at 780bp and *tetK* gene at 382bp for the two samples of *C. perfringens* isolates.

AST of NTZ was 2.73±0.08 while for tylosin IZD was 2.39±0.05 (Fig. 3 b and c). MIC and MBC were 0.4 and 12 µg/ml for NTZ and 0.7 and 49 µg/ml for tylosin respectively.

Clinical signs and mortality rate

Infected control G2 post inoculation by the *C. perfringens* infection showed various signs began with depression, reduced appetite and reluctance to move then followed by frothy fluid diarrhea and dehydration besides, recorded 20% (3/15) mortality rate. Most of these findings were relieved obviously by various treatments with no recorded mortalities either in non-infected or infected treated groups.

Table 2. Effect of NTZ and tylosin on *C. perfringens* count post treatment period (Mean±SE) n=3.

Items		*C. perfringens* count (x 10^2) CFU/g					
		G1	G2	G3	G4	G5	G6
Post treatment	Liver	NDa	15. 33±0.33d	NDa	NDa	1.26±0.33b	3.33±0.67c
	Small intestine	6.00±1.00ab	182.33±3.93c	2.67±0.67a	4.67±0.88a	4.33±0.33a	10.33±0.33b

The various letters in the same raw indicate statistically significant differences when (P<0.05).
G1: negative-control; G2: infected-control; G3: non-inf-(NTZ); G4: non-inf-(tylosin); G5: inf-(NTZ); G6: inf-(tylosin); ND: not detected.

Table 3. Effect of NTZ and tylosin on some of serum biochemical parameters (Mean±SE) n=5.

	G	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALT (u/l)	AST (u/l)	GGT (u/l)
Post treatment	1	4.11±0.22 c	1.80±0.06 b	2.31±0.26 b	42.38±0.71 a	57.60±1.27 a	21.37±0.54 a
	2	2.82±0.12 a	1.36±0.10 a	1.45±0.20 a	86.79±1.65 e	75.95±1.83 c	41.85±0.83 d
	3	3.95±0.19 bc	1.85±0.06 b	2.10±0.25 ab	47.62±1.03 b	62.16±0.82 b	24.59±0.81 b
	4	3.87±0.24 bc	1.76±0.07 b	2.11±0.24 ab	53.75±0.99 c	65.21±0.95 b	26.58±0.77 b
	5	3.44±0.04 b	1.78±0.06 b	1.65±0.07 ab	67.70±1.04 d	66.07±1.99 b	32.48±0.85 c
	6	3.42±0.18 b	1.51±0.08 a	1.91±0.23 ab	52.35±1.49 c	71.82±0.81 c	34.63±1.36 c
At the end of the experiment	1	4.32±0.19 b	2.11±0.06 a	2.22±0.16 a	44.88±1.31 a	61.48±1.24 a	18.62±0.52 a
	2	3.28±0.21 a	1.61±0.09 b	1.67±0.13 a	62.86±1.47 d	72.27±0.45 b	28.98±1.13 c
	3	4.13±0.19 b	1.94±0.08 b	2.19±0.20 a	46.99±0.98 ab	60.45±0.79 a	15.97±0.41 a
	4	4.07±0.18 b	2.04±0.13 b	2.03±0.25 a	50.54±0.50 bc	62.39±1.18 a	18.99±0.98 a
	5	4.01±0.14 b	1.96±0.09 b	2.05±0.14 a	47.06±1.17 ab	62.29±2.06 a	23.11±1.01 b
	6	4.06±0.04 b	2.09±0.07 b	1.98±0.06 a	52.64±1.22 c	68.74±0.81 b	23.12±1.57 b

The various letters in the same colon of the same period indicate statistically significant differences when (P<0.05).
G1: negative-control; G2: infected-control; G3: non-inf-(NTZ); G4: non-inf-(tylosin); G5: inf-(NTZ); G6: inf-(tylosin).

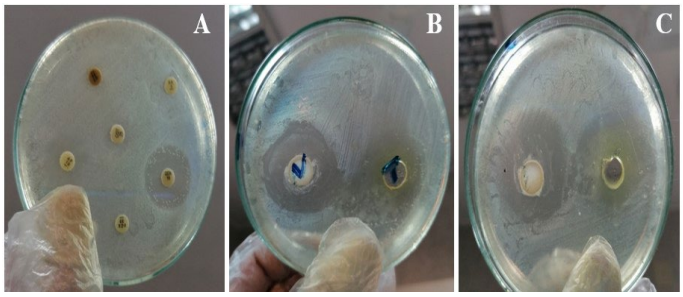

Fig 3. Showed the sensitivity test results of the tested *C. perfringens* type A isolates, a: sensitivity plate revealed one of *C. perfringens* isolate phenotypic resistance to various used commercial antibiotics, b: showed the sensitivity test of both nitazoxanide (the left well) and tylosin (the right well) against one of the tested *C. perfringens* type A isolate, c: represented sensitivity test of both nitazoxanide (the left well) and tylosin (the right well) against another tested isolate of *C. perfringens* type A.

C. perfringens count

Post treatment, *C. perfringens* count (Table 2) revealed a significant decrease in its count in liver and small intestine samples of infected treated G5 and G6 than the infected-control G2 that subsequently reduced liver infection and intestinal colonization. Meanwhile, non-infected treated G3 and G4 recorded a non-significant decrease in *C. perfringens* count than negative-control G1 in intestinal samples where it not detected in their liver tissue samples.

Serum biochemical parameters of liver

In Table 3 results of TP, albumin, globulin, activities of liver enzymes ALT, AST and GGT post treatment and at the end of the experiment showed that *C. perfringens* infected-control G2 revealed an observed impairment of liver biomarkers expressed as a significant decrease in total serum protein, albumin, and globulins besides a significant elevation in activities of ALT, AST and GGT when compared with negative-control G1. Regarding to non-infected treated G3 and G4, they revealed non-significant changes in TP, albumin and globulin levels, while they showed a various degree of elevation in our detected enzymes activities only post treatment period when compared with G1, where most of these values returned to its normal levels at the end of the study that indicated their non-permanent changes in liver cells. Infected treated G5 and G6 recorded a significant increase in most of TP, albumin and globulin values, meanwhile they showed a significant decrease in ALT, AST and GGT activities than G2 to convert mostly in various degrees toward G1. Generally, NTZ treated G3 and G5 showed less increase in enzymes activities after treatment and at the end of the experiment than tylosin treated G4 and G6 when compared with either control G1 or G2 respectively.

Effect on C-reactive protein and kidney biochemical markers

According to Table 4, post treatment and at the end of the experiment, serum analysis of C-reactive protein and some of kidney function parameters revealed that *C. perfringens* infection induced a significant elevation in C-reactive protein (as one of inflammation markers), serum uric acid and creatinine levels when compared with the negative-control G1. Non-infected treated G3 and G4 revealed non-significant changes in C-reactive protein and uric acid values when compared with G1, while tylosin treated G4 increased creatinine levels after treatment and at the end of the study. Infected NTZ treated G5 followed by tylosin treated G6 showed a significant decrease in C-reactive protein levels than G2. As well, both groups reflected great improvement in general kidney functions through induced a significant decrease in uric acid and creatinine levels in various degrees when compared with G2. However, G5 revealed more improvement in uric acid and creatinine levels toward normal values of G1 than G6.

Discussion

Since, *C. perfringens* secretes toxins that strengthen the inflammatory processes, induce immense damage in host metabolic process and facilitate bacterial invasion and dissemination besides accelerate the progression of diseases; therefore, obtaining an efficient antibacterial activity seems to be the main way for preventing or curing bacterial pathogen, reducing clinical signs, pathological changes, morbidity, and mortality rates consequently improving production efficiency. NTZ has a broad spectrum of anti-infective activity (Stachulski *et al*., 2020). Concerning to finding of alternative antibacterial drugs, in vivo animal trials are needed to evaluate the clinical efficacy of NTZ antimicrobial against *C. perfringens* infection in broilers. NTZ considered as a good alternative especially post increase the rates of clostridial resistance to metronidazole, substantial rate of treatment failure and infection recurrence, where some metronidazole-resistant strains were sensitive to NTZ (Freeman *et al*., 2011).

In the current study, *C. perfringens* isolation rate was 46 % from overall tested samples (100) (12 from liver and 34 from intestine) respectively. These results were similar to Younes (2005) and El-Rash (2012) that was 47.7%, but in another study (Abd El-Tawab *et al*., 2020), it was 70% (84% from intestine and 56% from liver) but lower percentage of 20.3% was reported by Eraky and Abd El-Ghany (2021), these variation in results may be attributed to the management differences, different season for collection of samples and may be due to using antibiotics in the rearing systems.

Multiplex PCR is a reliable technique for genotyping of *C. perfringens* strain and as a specific test for determination of its toxin genes. So, in the current study we used this technique as a test for serotyping of *C.*

Table 4. Effect of NTZ and tylosin on C-reactive protein, uric acid and creatinine (Mean±SE) n=5.

	G	C-reactive protein (mg/l)	Uric acid (mg/dl)	Creatinine (mg/dl)
Post treatment	1	7.30±0.23 a	4.61±0.15 a	0.39±0.01 a
	2	18.72±0.41 d	5.80±0.17 c	0.77±0.02 e
	3	7.41±0.27 a	4.49±0.13 a	0.38±0.01 a
	4	6.85±0.12 a	4.38±0.15 a	0.45±0.01 b
	5	11.39±0.25 b	5.12±0.10 b	0.67±0.02 d
	6	15.54±0.17 c	5.13±0.19 b	0.56±0.01 c
At the end of the experiment	1	9.12±0.16 ab	5.62±0.22 a	0.43±0.01 ab
	2	15.49±0.14 d	6.72±0.09 c	0.60±0.02 c
	3	8.73±0.12 a	5.58±0.17 a	0.42±0.01 ab
	4	9.25±0.09 ab	5.47±0.14 a	0.46±0.02 b
	5	9.63±0.24 b	5.41±0.12 a	0.40±0.02 a
	6	12.72±0.26 c	6.16±0.18 b	0.46±0.00 b

The various letters in the same colon of the same period indicate statistically significant differences when (P<0.05).
G1= negative-control, G2= infected-control, G3= non-inf-(NTZ), G4= non-inf-(tylosin), G5= inf-(NTZ), G6= inf-(tylosin).

perfringens isolates. Our molecular typing (Fig. 1) revealed that all the examined strains were *C. perfringens* type A by detecting *cpa* gene which was found in the tested isolates, but beta, epsilon and iota genes (*cpb*, *etx* and *itx*) were not detected in these isolates. This result agreed with Asal *et al*. (2023) as the only detected gene was *cpa* which is responsible for gas gangrene in case of animal and human severe infection. Another study by Bendary *et al*. (2022) detected enterotoxin *C. perfringens* cpe and beta toxin (*cpb2*) in their tested isolates. Alpha toxin is encoded by (*cpa*) gene and found in a stable area of chromosome which is responsible mainly of lethal toxin promoting cell lysis by hydrolysing of membrane phospholipids (Uzal *et al*., 2010). As well our detected virulence (*cpa*) gene and absence of other genes was agreed with Hassani *et al*. (2022). In the current study, the detection of resistance to beta lactam (*bla*) gene and tetracycline (*tetK*) gene was confirmed by detection of *bla* and *tetK* genes as present in (Fig. 2) which reflects bad and misuse of these antibiotics in wide range. These results agreed with that detected by Bendary *et al*. (2022).

There were few studies that looked into the *C. perfringens* AMR pattern, in spite of its harmful consequences on poultry healthiness (Mak *et al*., 2022). In our AST (Fig. 3-a), *C. perfringens* isolates showed multidrug-resistant profile to amoxiclav, tetracycline, gentamicin, clindamycin, sulphamethoxazole-trimethoprim and cefoxitin. In the same context, various antibiotic resistance patterns to commonly used antimicrobials were reported among *C. perfringens* isolates of broilers in Egypt. El-Nagar *et al*. (2022) recorded high resistance (100%) to Erythromycin, Metronidazole, Penicillin, Ampicillin, Amoxicillin, Amoxicillin-clavulanic acid, Lincomycin, Neomycin, Oxytetracycline, Spectinomycin, Streptomycin; aligned with 25% or low sensitivity to Colistin sulphate, Doxycyclin, Sulfamethoxazole-trimethoprim, Enrofloxacin, and Norfloxacin. In Eid *et al*. (2020) antibiogram various phenotypic resistances to lincomycin (82.26%), ampicillin (72.58%), nalidixic acid (69.35%), and spectinomycin (69.35%), in addition to (79.03%) of these isolates verified MDR phenotypes were recorded. As well, Osman and Elhariri (2013) found that all of 125 of *C. perfringens* isolates were completely resistant to gentamicin, erythromycin, streptomycin, lincomycin, neomycin, colistin, pefoxacin, trimethoprim-sulfamethoxazole, oxalinic acid and spiramycin. Our AST results were also similar to Bendary *et al*. (2022) as most of isolates showed multidrug resistant patterns. In another study, Park *et al*. (2015) reported a resistance to gentamicin, neomycin, streptomycin, colistin and intermediate resistance to bacitracin, tetracycline, clindamycin erythromycin, trimethoprim-sulamethoxazole, and sulfisoxazole. Where, Kovanda *et al*. (2019) determined resistant to tetracycline, chloramphenicol, and penicillin. Furthermore, Anju *et al*. (2020) reported that 44, 40, 40 and 26.6% of *C. perfringens* were resistant to gentamicin, bacitracin, erythromycin, and tetracycline, respectively. Meanwhile, Jang *et al*. (2020) found a 100% resistance to tetracycline. Also, Archambault and Rubin (2020) reviewed the AMR in *C. perfringens* and other anaerobes. In our research the examined strains showed presence of *Bla* and *tetK* resistant gene; these results confirmed the obtained results of disc diffusion results of our multidrug-resistant strains. AST of Mwangi *et al*. (2019) showed that 53% of the isolates had multidrug resistant profile for streptomycin, gentamycin, erythromycin tetracycline and bacitracin. Similarly, El-Nagar *et al*. (2022) found 100%, 75% and 25% antimicrobial-resistant genes among various isolates to (*tetK*) tetracyclines, (*Bla*) B. lactamase and macrolide (ermB) respectively. Contrary, most isolates showed high sensitivity to tested antibiotics (Wei *et al*., 2020) or sensitive to amoxicillin-clavulanic acid, cefclor and ofloxacin (Asal *et al*., 2023).

Our detected anti-clostridial effect of NTZ (Fig. 3 b and c) on *C. perfringens* could be attributed to its activity as a biologically active anion that inhibits the early step of the PFOR reaction by a non-competitive inhibition of the interaction of pyruvate with the thiamine pyrophosphate (TPP) cofactor. Inhibition of PFOR by NTZ stops the conversion of bacterial pyruvate to acetate, a key component of fatty acid biosynthesis, amino acid biosynthesis and energy production (Devasahayam *et al*., 2010). Regarding to NTZ structure-antimicrobial efficacy relationship, it considered a synthetic nitrothiazolyl-salicylamide derivative that has both a nitrothiazole part and a salicylic acid moiety connected together by a peptide bond, whereas the nitrothiazolyl moiety resembles that of the nitroimidazole drugs, tinidazole and metronidazole. Concerning to tylosin sensitivity, Kasab-Bachi (2017) found that most of 629 of *C. perfringens* and netB isolates revealed moderate resistant to tylosin tartrate, ceftiofur, and clindamycin; despite the absence of tylosin in their feed, besides a resistant to bacitracin, oxytetracycline, tetracycline and erythromycin. On the other side, they were sensitive to amoxicillin, florfenicol, and enrofloxacin. Also, Martel *et al*. (2004) found that *C. perfringens* strains were sensitive to tylosin, avilamycin, amoxicillin, chlortetracycline and oxytetracycline while flavomycin (bambermycin) showed low or no activity. These differences among sensitivity tests may be attributed to differences of the tested isolates, collected places, besides the routine treatment program of these farms or micro-organism exposure to various doses or periods of treated antibiotics. Therefore, increased *C. perfringens* resistance genes in Egypt indicate the disastrous of antibiotics misusing and so high economic losses due to controlling measures.

Our MIC and MBC (µg/ml) results recorded 0.4 and 12 for NTZ and 0.7 and 49 for tylosin respectively. Regarding in vitro antibacterial activity of NTZ, it was effective against both *C. difficile* and *C. perfringens* (Di Santo and Ehrisman, 2014). *C. difficile* MIC ranged from 0.06-0.5 ug/ml (CLSI, 2020). While, Pankuch and Appelbaum (2006) found that in vitro MIC range, MIC50 and MIC90 (ug/ml) of NTZ against *C. perfringens* (19 strain) were (0.25-4.0, 2.0, 4.0) and for tizoxanide (the primary metabolite of NTZ) were (0.25-2.0, 1.0, 2.0) respectively that considered similar to metronidazole (0.25-2.0, 0.5, 2.0) and higher than amoxicillin-clavulanate (0.015-0.12, 0.03, 0.12) while most of strains were resistance to clindamycin; that suggested the potential use of NTZ in treatment of clostridial infections. Furthermore, NTZ and tizoxanide showed equivalent MIC50 and MIC90 (ug/ml) values 0.5 and 1.0 compared to 0.5 and 2.0 respectively for metronidazole (Musher *et al*., 2006), where NTZ reported < 2 µg/ml compared to vancomycin < 8 µg/ml in another study (Hecht *et al*., 2007). As well, Dubreuil *et al*. (1996) estimated that MIC90s of NTZ = 1 mg/l for *C. perfringens* compared to 1, 0.5, 0.5, 0.125, 0.06, 0.125, 0.5, 0.06 and 16 mg/l for tizoxanide, NTZ-tizoxanide, metronidazole, amoxicillin, amoxicillin-clavulanic acid, piperacillin, cefoxitin, imipenem, and clindamycin respectively. Hence, NTZ has comparable efficacy to metronidazole and comparator drugs. In another study, NTZ, metronidazole and vancomycin showed equal MIC50= 0.25 and MIC90= 0.50 ug/ml against the 15 toxigenic *C. difficile* strains while, NTZ in 5% cecal contents, as a preliminary examination of its stability, revealed MIC50=4 and MIC90= 32 ug/ml compared to twofold increased for vancomycin and metronidazole. The MBCs (ug/ml) for NTZ, metronidazole, and vancomycin were 0.48±0.47, 0.37±0.21, and 0.82±0.25 and in 5% cecal contents they were 13.6±13, 0.49±0.34 and 1.0±36 respectively. Where, all of them had the same potencies in inhibiting the *C. difficile* strains growth (Mcvay and Rolfe, 2000).

Concerning tylosin, various MICs against *C. perfringens* strains were reported, since there is no standard interpretative criterion for this bacterium/antimicrobial combination currently available. Sensitive strains to tylosin tartrate showed 0.25-32 mg/L MIC range (Kovanda *et al*., 2019). While against 51 *C. perfringens* strains it ranged between 0.5 and 1 ug/ml (Lanckriet *et al*., 2010). Also, Martel *et al*. (2004) found a range of (0.03-1) ug/ml where MIC50 was 0.25. Kasab-Bachi (2017) found that tylosin reached MIC=18.4 ug/ml. Meanwhile, the mean MIC of tylosin tartrate of 629 *C. perfringens* isolates was 2.09±7.27, where MIC50 ≤ 1.25, MIC90 >10. Isolates in the higher range of the MICs were considered to have acquired resistance, while high resistance to tylosin were occurred when MIC > 32 µg/ml. Gharaibeh *et al*. (2010) found that tylosin MIC50 (µg/ml) was 64 for *C. perfringens*, where MIC90 was 256. Meanwhile, tylosin MIC = 1 for *C. perfringens* type A (NCTC 8798) reference strain. There is very limited information regarding tylosin MIC breakpoints against *C. perfringens* to specifically classify isolates into susceptible, intermediate, or resistant. Differences in MICs assays between studies may reflect the various degrees of sensitivity/resistance of *C. perfringens* strains towards our drugs depending on the good/misuse in poultry farms.

Regarding to our recorded signs and mortalities, non-infected G1, G3 and G4 showed no signs or mortalities. Meanwhile, depression, diarrhea, dehydration, reduced mobility, and appetite were the most visibly obvious symptom, where mortality rate was 20% in infected control G2. Infected medicated groups (G5 and G6) revealed improved clinical signs, birds were healthy and viable with no recorded mortalities, besides stopped the development of NE lesions in intestine and liver. Our results may be considered as a reflection of their antimicrobial activity that prevented the bacterial toxins degenerative changes. Similar, bad impacts and considerable mortalities post infection were reported (Elkomy *et al*., 2019; Nasr El-Deen *et al*., 2019; Hussein *et al*., 2020), where mortality rate was ranged from 25% (Nasr El-Deen *et al*., 2019) and 34% (Elkomy *et al*., 2019). In the same context, similar post-mortem lesions of dead or sacrificed birds as hyperemia, multi-foccal hemorrhages, inflamed or mild degenerated mucosa with gaseous contents in the jejunum, ileum and ceca of infected group were also recorded (Hussein *et al*., 2020). In the same context, nearly similar clinical efficacy trials using NTZ against *C. difficile* infection with its diarrhoea and enteritis were observed in rats (McDonald *et al*., 2018). Where, both NTZ and its metabolite tizoxanide inhibited and treated the infection to be equivalent to standard anticlostridial drug; metronidazole and vancomycin (Musher *et al*., 2006; 2009) with a 89.5% response rate and a lower infection recurrence rate 12.5% post NTZ-treatment compared with 82.4% and 28.6% for metronidazole respectively (Hemphill *et al*., 2006; Musher *et al*., 2006). Also, excellent in vitro and in vivo NTZ activities were noticed as prevented manifestations and decreased mortalities against 15 toxigenic strains of *C. difficile* in hamster at a therapeutic dose of 7.5 mg/100 g body weight compared to those of metronidazole (15 mg) and vancomycin (5 mg) (Mcvay and Rolfe, 2000). As well, when used as a prophylactic treatment all hamsters

appeared normal without gross signs of toxicity or detection of *C. difficile* or its toxin A in the cecal culture and all animals survived the tested 15 day post-infection period. By contrast, vancomycin and metronidazole groups induced fatal *C. difficile* intestinal disease in 20 and 70% of recipients, respectively. Similar tylosin efficacy against *C. perfringens* was reported (Collier *et al*., 2003; Lanckriet *et al*., 2010) at 100 and 200 mg/L/5 days that abolished the development of gut necrotic lesions and subsequent mortalities of the virulent strain. Also, Ellakany *et al*. (2009) used tylosin at 35 mg/kg b.wt/12 h daily/3 days from 14th-16th day, where its effect still recorded till 1 week post-treatment. Tylosin is used in the Egyptian poultry market for over 30 years. It has no harsh effect on Lactobacillus sp. count in the duodenum of broilers (Ellakany *et al*., 2008). These bacteria enhance the broiler health through sustaining absorption and digestion of nutrients, enhancing immunity, supporting resistance against various infections, inversely related to *C. perfringens*, competitively exclude pathogenic bacteria via the production of bacteriocins and its ability to compete for limiting nutrients (Shah *et al*., 2022).

Regarding to *C. perfringens* count post-treatment (Table 2), our results revealed a significant decrease in both liver and small intestine samples of infected treated G6 and G6 than the infected-control G2 that subsequently reduced liver infection and intestinal colonization. Meanwhile non-infected treated G3 and G4 recorded a non-significant decrease in *C. perfringens* count than negative-control G1. Our in vivo results confirmed our in vitro anticlostridial activity of NTZ and tylosin. In the same context, *C. perfringens* count post 24 hrs of tylosin treatment was 4x103, meanwhile post 48, 72 h or 7 days no *C. perfringens* was found while other *Clostridium* sp. were 25x102 at 72 h and non-countable >150 cfu x 105 post 7 days) (Ellakany *et al*., 2009). Additionally, post adding of 50 or 100 mg/kg/14 days of tylosin phosphate significant impairment in *C. perfringens* growth was recorded in this ascending order in duodenum (7.14±0.20) where a major part of tylosin is absorbed, jejunum (7.61±0.20) then caeca (9.00±0.23). It decreased the *C. perfringens* count (log10 CFU/g) than infected-control group (Shah *et al*., 2022). From another point of view, tylosin controlled NE through its modulation of *C. perfringens* colonization besides reduction of endogenous mucolytic microbiota activity that serve as an initiating step of *C. perfringens* virulence, provide pro-inflammatory mediated condition, affect other chick commensal bacteria and compromise barrier function that prevent further transport of antigenic or toxic substances across the mucosa (Collier *et al*., 2003).

About our serum biochemical parameters (Tables 3 and 4), infected-control G2 induced a significant decrease in TP, albumin and globulins besides an increase of liver enzymes activities (ALT, AST and GGT), C-reactive protein, uric acid, and creatinine levels compared with negative-control G1. Non infected treated G3 and G4 revealed a non significant changes in TP, albumin, globulin, C reactive protein, uric acid and creatinine, while they showed various degrees of elevation in our detected enzymes activities only post-treatment period when compared with G1, where most of these values returned to its normal levels at the end of the study that indicated their non-permanent changes in liver cells. Hence, no adverse effect on liver or kidney cells as vital organs of drug metabolism and elimination were recorded post NTZ or tylosin treatment. Meanwhile, infected treated G5 and G6 revealed a significant improvement in C-reactive protein levels, most of hepatic and renal detected markers than G2 with non-significant difference than G1 in most of these items. Generally, NTZ treated G3 and G5 showed less increase in enzymes activities after treatment and at the end of the experiment than tylosin treated G4 and G6 when compared with either control G1 or G2 respectively. Both drugs decreased loss of protein, tissue damage, liberating enzymes and inflammation that indicates the effectiveness of NTZ as tylosin in counteracting *C. perfringens* infection. Similar biochemical alterations for infected-control G2 were recorded by Allam *et al*. (2013); Abdel Ziz *et al*. (2016); Mabrouk (2016); Elkomy *et al*. (2019) and Nasr El-Deen *et al*. (2019). Elevation of hepatic tissue indicators may be attributed to the inflammation that resulted post-infection expansion as a part of body defense reaction (ElKomy *et al*., 2019). Even during subclinical infection, bacteria can reach the bile duct and portal blood stream through the damaged intestinal cells, sloughed mucosa and colonized in liver causing cholangiohepatitis and liberation of its intracellular enzymes. Also, clostridial toxins that confirmed in our PCR detection, α toxin, destructed the host cell membranes by hydrolysis and oxidation of its phospholipids, disseminated to the blood stream followed by systemic symptoms or death (Hussein *et al*., 2020) besides their ability to disturb the metabolic activity (ElKomy *et al*., 2019). Moreover, reduction in proteinogram of G2 that expressed by hypoproteinemia and hypoalbuminemia could be considered as a consequence to anorexia, mal absorption of nutrients from inflamed or damaged intestinal mucosa, impaired protein synthesis in the liver or increased its loss through kidney. Furthermore, bacteria compete with the host for uptake of amino acids and reduced nitrogen utilization (Ellakany *et al*., 2009). Likewise, bacteria produced toxic catabolic amino acid and increases gut epithelial cell turnover, which accompanied by an extremely high rate of metabolism, energy/protein consumption, and suppressed immunity. Also, El-Sheikh *et al*. (2018) indicated a significant decrease in levels of total globulins in *C. perfringens*-infected chickens. Contrary, hyperglobulinemia was recorded (Allam *et al*., 2013; Salah *et al*., 2015). Discrepancies among authors may be due to variation of sampling time, infection stage or bird immune response. For kidney function markers, significant renal disturbance could be attributed to tubular cell toxicity or inflammation (Ferguson *et al*., 2008). Similar increases in uric acid and creatinine levels post infection were reported by ElKomy *et al*. (2019) who noticed also pathological damaged changes in the kidneys induced by the bacterial toxins. These degenerative changes in renal tubules prevent the final metabolites of protein breakdown; uric acid and creatinine excretion leading to their increase in the serum.

Regarding NTZ use, for non-infected NTZ-treated G3, there was a scarcity about NTZ effect on broilers biochemical parameters but in other species some researches were applied. Antonya *et al*. (2020) recorded that NTZ on two-week old chickens had no gross pathological lesions on organs and it was considered safe. It is extremely well tolerated, even up to 4 g in a single dose or one multiple dosing regimen (0.5 g, bid for 7 days) with no abnormalities in blood chemistry (Stockis *et al*., 2002a; b). Elsayad and Al-Kazzaz (2018) reviewed that in acute studies on rodents and dogs, the oral LD50 was higher than 10 g/kg where there are neither systemic effects nor blood-related anomalies at the selected doses in rats. Also, Moron-Soto *et al*. (2017) treated dogs with a single oral dose of 37.5, 75 and 150 mg/kg for 14 days, and found that ALT and AST enzymes remained within physiological ranges. In another study, vital signs, clinical chemistry and urinalysis parameters remain unchanged post NTZ treatment (Fox and Saravolatz, 2005). As well, post many clinical studies, NTZ revealed a low toxicity and an excellent safety profile over 24 weeks of treatment (Hemphill *et al*., 2006). On the contrary, Shams *et al*. (2018) found that oral NTZ (18 mg/kg b.wt/day/14 days) in rats revealed some histopathological changes on liver and kidney expressed by a significant increase in ALT, AST, ALP, creatinine and urea and decrease in TP and albumin, after one-day post-treatment when compared with the control group, but these changes improved gradually by time. As well, Pepperrell *et al*. (2020) mentioned that two studies reported elevations in liver function tests and higher rates of chromaturia (yellow urine) in participants receiving NTZ compared with control, but all of these cases were mild and clinically insignificant. Regarding to infected-NTZ treated G5, Elsayad and Al-Kazzaz (2018) used NTZ orally at 100 mg/kg/day/7 days to treat S. mansoni-infected mice, where NTZ significantly decreased the elevated levels of liver enzymes (ALT, AST and ALP), urea and creatinine at 1-, 2- and 4-weeks post-treatment, besides induced some corrective action in other biochemical parameters. No alteration in the liver enzymes in non-infected NTZ-treated mice than the control. Also, it was found that NTZ treatment of infected calves with cryptosporidiosis resulted in a significant decrease in TP, albumin and globulin compared with the infected group (Abdel Megeed *et al*., 2015). Soufy *et al*. (2017) found that β-Globulin level markedly increased in the rats administered NTZ orally (100 mg/kg b. wt), which further indicated its role in host innate immune-modulation responses to impart pathogen clearance (Trabattoni *et al*., 2016). Meanwhile, Fahmy *et al*. (2021) found that NTZ exhibits limited immune-dependent efficacy; it showed non-significant elevation in CD4 expression level that considers as an important immunomodulation status marker that response to the intrinsic immune system homeostasis.

Concerning the NTZ effect on C-reactive protein, it has a marked anti-inflammatory property, that signifying its clinical use in treatment of enteritis cases. Similar anti-inflammatory effect was reported (Hong *et al*., 2012) through suppression the production of interleukins (IL) such as IL-6. Also as reported by Singh and Narayan (2011), NTZ was considered a very safe drug to use with no significant serious adverse effects reported in any study with no significant drug interactions. NTZ and other newer thiazolides may represent a significant advancement in the treatment of enteric infections and metronidazole resistant *C. difficile* infection.

For tylosin treated groups, in non-infected tylosin treated G4; also, El-Komy *et al*. (2019) concluded that special attention should be taken with tylosin due to the adverse effect on liver and kidney in broilers, which may be explained by Das *et al*. (2020) who noticed that among the poultry tissues, liver and kidney had the highest level of tylosin tartrate residues even post a week of withdrawal period. Meanwhile for infected tylosin treated G6, El-Komy *et al*. (2019) found that tylosin (100 ppm/7 days) in broilers feed challenged with subclinical *C. perfringens* type A induced significant increase in liver enzyme activities (AST, ALT and ALP), uric acid and creatinine and significant decrease in protein picture (TP and albumin) compared to negative control group. While it induced significant improvement in most of these items when compared with infected control chickens. Positive effect of tylosin might be due to the antibacterial action against *C. perfringens*, which suppressed the bacterial invasion, increased the integrity of the intestinal walls, promoted the absorption of essential nutrients and secretion of digestive enzymes and enhanced the

nutrient digestibility leading to improved protein profile.

Conclusion

All isolated field strains have type A (produced only alpha-toxin (*cpa* virulence gene)) and include *Bla* and *tetK* (resistant genes). Also, NTZ has no adverse biochemical insults on liver or kidney functions with a therapeutic effect on antibiotic resistant *C. perfringens* infected chickens. Further studies are needed to detect the NTZ pharmacokinetics/pharmacodynamic in broilers and its species-specific differences.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abd El-Hack, M.E., El-Saadony, M.T., Elbestawy, A.R., El-Shall, N.A., Ahmed, M., Saad, A.M., Salem, H.M., El-Tahan, A.M., Khafaga, A.F., Taha, A.E., AbuQamar, S.F, El-Tarabily, K.A., 2022. Necrotic enteritis in broiler chickens: disease characteristics and prevention using organic antibiotic alternatives- a comprehensive review. Poult. Sci. 101, 101590.

Abd El-Tawab, A., Rizk, A., El-Bardisy, M., Mayouf, S., 2020. Prevalence of Colistridium perfringens infection and virulence genes detection in broiler chicken, Benha Vet. Med. J. 38, 128-132.

Abdel Ziz, S.A., Abdel Motaal, S.M.A., Abd-Allah, O.E., Sarhan, M.M.I., 2016. Concurrent use of ciprofloxacin and metronidazole for controlling of some bacterial infections in broiler chickens. BVMJ. 31, 83-92.

Abdel Megeed, K.N., Hammam, A.M., Morsy, G.H., Khalil, F.A.M., Seliem, M.M.E., Aboelsoued, D., 2015. Control of cryptosporidiosis in buffalo calves using garlic (*Allium sativum*) and nitazoxanide with special reference to some biochemical parameters. Global Veterinaria, 14, 646-655.

Aboubakr, M., Elbadawy, M., 2017. Pharmacokinetics, tissue residues and efficacy of D-Tylo50/25R (tylosin-doxycycline combination) in broiler chickens. Int. J. Basic. Clin. Pharmacol. 6, 383-8. doi: 10.18203/2319-2003.ijbcp20170334.

Abu-Basha, E.A., Al-Shunnaq, A.F., Gehring, R., 2012. Comparative pharmacokinetics and bioavailability of two tylosin formulations in chickens after oral administration. J. Hell. Vet. Med. Soc., 63, 159-66. doi: 10.12681/jhvms.15431.

Ahmed, T., Rahman, S.M.A., Asaduzzaman, M., Islam, A.B.M.M.K., Chowdhury, A.K.A., 2021. Synthesis, in vitro bioassays, and computational study of heteroaryl nitazoxanide analogs. Pharmacol. Res. Perspect. 9, e00800. https://doi.org/10.1002/prp2.800.

Alimolaei, M., Ezatkhah, M., Bafti, M.S., Amini, M., 2015. Antibiotic susceptibility of *Clostridium perfringens* from organic broiler chickens. Onl. J. Vet. Res. 19, 465-470.

Allam, H.H., Nahad, A.G., Abdullaha, S.H., Dina, M.M., 2013. Immuno-Biochemical and pathological studies on necrotic enteritis in pekin duckling with trial of treatment. J Mansoura Vet. Med. XV, 211-226.

Anju, K., Karthik, K., Divya, V., Mala-Priyadharshini, M.L., Sharma, R.K., Manoharan, S., 2020. Toxinotyping and molecular characterization of antimicrobial resistance in *Clostridium perfringens* isolated from different sources of livestock and poultry. Anaerobe 67, 102298. https:// doi. org/ 10. 1016/j. anaerobe. 2020. 102298.

Antonya, F., Vashia, Y., Morlaa, S., Vandnab, Mohanb, H., Kumara, S., 2020. Therapeutic potential of Nitazoxanide against Newcastle disease virus: A possible modulation of host cytokines. Cytokine 131, 155115. https://doi.org/10.1016/j.cyto.2020.155115.

Archambault, M., Rubin, J.E., 2020. Antimicrobial resistance in *Clostridium* and Brachyspiraspp. and other anaerobes. Microbiol. Spectr. 8, ARBA- 0020- 2017. https:// doi. org/ 10. 1128/ micro biols pec. arba- 0020- 2017.

Asal, A.M.A., Abd El-Tawab, A.A., Hamouda, S.N.M., Rizk, A.M., 2023. Antibiogram pattern and molecular characterization of Colistridium Perfringens isolated from different species. BVMJ. 43, 69-74. doi: 10.21608/bvmj.2022.166104.1600.

Balderas-Acata, J.I., Ríos-Rogríguez, Bueno E.P., Pérez-Becerril, F., Espinosa-Martínez, C., Burke-Fraga, V., González-de la Parra, M., 2011. Bioavailability of Two Oral-Suspension Formulations of a Single Dose of Nitazoxanide 500 mg: An Open-Label, Randomized-Sequence, Two-Period Crossover, Comparison in Healthy Fasted Mexican Adult Volunteers. J. Bioequiv. Bioavailab. 3, 043-047. doi:10.4172/jbb.1000056.

Bendary, M.M., Abd El-Hamid, M., Algendy, R.M., Alzohairy, N., Ghoneim, M.M., Al-Sanea, M.M., Nahari, M.H., Moustafa, W.H., 2022. *Clostridium perfringens* associated with food borne infection of animal origins:insights into prevalence, antimicrobial resistance, toxin genes profiles and toxinotypes. Biology (Basal) 11, 551. DOI:10.3390/biology 11040551.

Burton, E., Gatcliffe, J., O'Neill, H.M., Scholey, D., 2016. Sustainable Poultry Production in Europe. In: Poultry Science Symposium Series, Thirty-One Ed . British Library, London, UK. P. 163.

Casewell, M., Friis, C., Marco, E., McMullin, P., Phillips, I., 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J. Antimicrob. Chemother. 52, 159-161.

Catalán, A., Espoz, M.C., Cortés, W., Sagua, H., González, J., Araya, J.E., 2010. Tetracycline and penicillin resistant *Clostridium perfringens* isolated from the fangs and venom glands of Loxosceles laeta: Its implications in loxoscelism treatment. Toxicon. 56, 890-896.

CLSI, 2018. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. M11, ED9. Wayne, PA: Clinical and Laboratory Standards Institute. p. 96

CLSI, 2020. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. Clinical and Laboratory Standars Institute Supplement M 100, Wayne, Pa, USA. p.100

CLSI, 2021. Performance Standards for Antimicrobi-al Susceptibility Testing; Clinical and Laboratory Standards Institute, Wayne, PA, USA. p. 31

Collier, C.T., Van der Klis, J.D., Deplancke, B., Anderson, D.B., Gaskins, H.R., 2003. Effects of tylosin on bacterial mucolysis, *Clostridium perfringens* colonization, and intestinal barrier function in a chick model of necrotic enteritis. Antimicrobial Agents and Chemotherapy 47, 3311-3317. DOI: 10.1128/AAC.47.10.3311–3317.2003.

Cruickshank, R., Dugaid, J. Marmion, B., Swain, R., 1975. Medical Microbiology: twelfth Ed, Edenburg, London and New York.

Das, D., Islam, M.S., Sikder, M.M.H., Alom, F., Khatun, M.S., Faruk, M.A.Z., 2020. Presence of antibiotic residue and residual effect of tylosin tartrate in broiler. International Journal of Natural and Social Sciences, 7, 29-35. https://doi.org/10.5281/zenodo.3946801.

Devasahayam, G., Scheld, W.M., Hoffman, P.S., 2010. Newer Antibacterial Drugs for a New Century. Expert Opin Investig Drugs 19, 215-234. doi:10.1517/13543780903505092.

DI Giorgio, J., 1974. Nonprotein nitrogenous constituents. In: clinical chemistry – principles and technics, Second Ed. Henry, R.J., Cannon, D.C., Winkelman, J.W. editors, Harper and Row, Hagerstown (MD), pp. 541-553.

Di Santo, N., Ehrisman, J., 2014. A functional perspective of nitazoxanide as a potential anticancer drug. Mutat Res. 768, 16-21.

Doosti,A., Mokhtari-Farsani, A., 2014. Study of frequency of Colistridium dificile tcdA, tcdB, cdtA and cdtB genes in feces of calves in south west of Iran. Annals of Clinical Microbiology Antimicribials 13, 21. http://www.ann-clinmicrob.com/content/13/1/21.

Doumas, B.T., Watson, W.A., Biggs, H.G., 1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta. 31, 87-96.

Dubreuil, L., Houcke, I., Mouton, Y., Rossignol, J.F., 1996. In vitro evaluation of activities of nitazoxanide and tizoxanide against anaerobes and aerobic organisms. Antimicrob. Agents Chemother. 40, 2266-2270.

Eid, S., El Atfehy, N.M., Amer, F., Tolba, H.N., Hamed, R.I. 2020. Prevention of Necrotic Enteritis in Broiler Chickens by Prebiotics and Probiotics VS Control by Antibiotics, in Vivo Study. AJVS. 64, 143-153. DOI: 10.5455/ajvs.76994.

Elkomy ,A.A., Farag, E., Shahat, I., Gharbawy, E.L., Elbadawy, M., 2019. Comparative studies on the efficacy of lincomycin and bacitracin for the control of necrotic enteritis in broiler chickens. Int. J. Basic Clin. Pharmacol. 8, 1153-1158. http://dx.doi.org/10.18203/2319-2003.ijbcp20192177.

El-Komy, A.A., Farag, E.A.H., El-Ghazaly, A.M.A., 2019. Effect of tylosin and salinomycin on some biochemical and haematological parameters in broiler chickens challenged with *Clostridium perfringens*. IJMPR. 3, 70-80.

Ellakany, H.F., Rayhan, E.A., Awad, A.M., Abd El-Hamid, H.S., 2009. The effect of antibiotics and acidifier treatment on the count of lactobacillus sp., E. coli and *Clostridium* sp. in the intestine of chickens. Kafrelsheikh, Vet. Med. J., 3rd Sci. Congress. 10-12 May, 7, 406-429.

El-Nagar, S., Shalaby, A.G., Badr, H., 2022. Antibiotic resistance pattern of *Clostridium perfringens* isolated from necrotic enteritis cases of broiler chickens in Luxor city. Egyptian Journal of Animal Health 2, 63-74.

El-Rash, A.N.A., 2012. Studies on Colistridium perfringens in laying hens; M.V.Sc. Thesis. Fac. Vet. Med. Cairo Univ. Ancestral chymase. Science 271, 502-516.

Elsayad, M.H., Al-Kazzaz, M.A., 2018. Possible ameliorative effects of nitazoxanide against Schistosoma mansoni-induced biochemical insults in mice. Pharmacologia, 9, 13-17. DOI: 10.5567/pharmacologia.2018.13.17.

El-Sheikh, S.M., Khairy, M.H., Eleiwa, N.Z.E., Osama, E.A., Abd El-Monsef, A.G., 2018. Effect of sanguinarine phytobiotic, sodium butyrate compared to ampicillin on controlling necrotic enteritis in broiler chickens. Slov. Vet. Res. 55, 405-514.

Eraky, R.D., Abd El-Ghany, W.A., 2021. Genetic characterization, antibiogram pattern and pathogenesity of Colistridium perfringens isolated from broilerchicken with necrotic enteritis; Journal of the Indonesian Tropical animal Agriculture 47, 1-16. DOI :10.14710/jitaa.47.1.1-16.

Fahmy, M.E.A., Abdelaal, A.A., Hassan, S.I., Shalaby, M.A., Ismail, M.A.M., Khairy, R.A., Badawi, M.A., Afife, A.A., Fadl, H.O., 2021. Antiparasitic and immunomodulating effects of nitazoxanide, ivermectin and selenium on Cryptosporidium infection in diabetic mice. Braz. J. Vet. Parasitol. 30, e012121. https://doi.org/10.1590/S1984-29612021087.

Ferguson, M.A., Vaigya, V. S., Bonventre, J.V., 2008. Biomarkers of nephrotoxic acute kidney injury. Toxicology 245, 182-193.

Fossati, P., Prencipe, L., Betri, G., 1980. Colorimetric method for determination of serum uric acid. Clin. Chem. 26, 227-273.

Fox, I.M., Saravolatz, L.D., 2005. Nitazoxanide: a new thiazolide antiparasitic agent. Reviews of anti-infective agents, Louis D. Saravolatz, Section Edito. Clin. Inf. Dis. 40, 1173-1180.

Freeman, J., Baines, S.D., Todhunter, S.L., Huscroft, G.S., Wilcox, M.H., 2011. Nitazoxanide is active against *Clostridium* difficile strains with reduced susceptibility to metronidazole. J Antimicrob Chemother 66, 1407-1408.

Fu. Y., Alenezi, T., Sun, X., 2022. *Clostridium perfringens* –Induced Necrotic Diseases: An Overview. Immuno. 2, 387-407. Doi:10.3390/immune 2020024.

Gaynor, M., Mankin, A.S., 2005. Macrolide Antibiotics: Binding Site, Mechanism of Action, Resistance. Frontiers in Medicinal Chemistry 2, 21-35. http://dx.doi.org/10.2174/1567204052931113.

Gharaibeh, S., Al Rifai, R., Al-Majali, A., 2010. Molecular typing and antimicrobial susceptibility of *Clostridium perfringens* from broiler chickens. Anaerobe 16, 586-589.

Gholamiandehkordi, A., Eeckhaut, V., Lanckriet, A., Timbermont, L., Bjerrum, L., Ducatelle, R., Haesebrouck, F., Van Immerseel, F., 2009. Antimicrobial resistance in *Clostridium perfringens* isolates from broilers in Belgium. Vet. Res. Commun. 33, 1031-1037.

Giguere, S., 2006. Lincosamindes, Macrolides, and Pleuromutilins. In: Antimicrobial Therapy in Veterinary Medicine, fourth Ed, Wiley Blackwell, Ames, p. 179-190.

Gohari, I.M., Navarro, M.A., Jihong, L., Shrestha, A., Uzal, F., McClane, B.A., 2021. Pathogenicity and virulence of *Clostridium perfringens*. Virulence 12, 723-753.

Gornall, A.G., Bardawill, C.J., David, M.M., 1949. Determination of serum protein by means of the biuret reagent. J. Biol. Chem. 177, 751.

Hassani, S., Pakbin, B., Bru"ck, W.M., Mahmoudi, R., Mousavi, S., 2022. Prevalence Antibiotic Resistance ,Toxin-Typing and Genotyping of *Clostridium* perfringens in Raw beef meats obtained from Qazvin city, Iran: Antibiotics 11, 340. Doi:10.3390/antibiotics 11030340.

Hecht, D.W., Galang, M.A., Sambol, S.P., Osmolski, J.R., Johnson, S., Gerding, D.N., 2007. In vitro activities of 15 antimicrobial agents against 110 toxigenic *Clostridium* difficile clinical isolates collected from 1983 to 2004. Antimicrob. Agents Chemother. 51, 2716-9.

Hemphill, A., Mueller, J., Esposito, M., 2006. Nitazoxanide, a broad-spectrum thiazolide anti-infective agent for the treatment of gastrointestinal infections. Expert Opin. Pharmacother. 7, 953-964.

Hoffman, P.S., Sisson, G., Croxen, M.A., Welch, K., Harman, W.D., Cremades, N., Morash, M.G., 2007. Antiparasitic drug nitazoxanide inhibits the pyruvate oxidoreductases of Helicobacter pylori, selected anaerobic bacteria and parasites, and *Campylobacter* jejuni. Antimicrob. Agents Chemother. 51, 868-876.

Hong, S.K., Kim, H.J., Song, C.S., Choi, I.S., Lee, J.B., Park, S.Y., 2012. Nitazoxanide suppresses IL-6 production in LPS-stimulated mouse macrophages and TG-injected mice. Int. Immunopharmacol. 13, 23-27.

Hussein, E.O.S., Ahmed, S.H., Abudabos, A.M., Suliman, G.M., Abd El-Hack, M.E., Swelum, A.A., Alowaimer, A.N., 2020. Ameliorative Effects of Antibiotic-, Probiotic- and Phytobiotic-Supplemented Diets on the Performance, Intestinal Health, Carcass Traits, and Meat Quality of *Clostridium perfringens*-Infected Broilers. Animals 10, 669.

ISO, 1997. Standard ISO 7937/1997. Directiva general para el recuento de *Clostridium perfringens*. Métodoporrecuento de colonias. [In Spanish]. http://www.iso.org/iso/iso-catalogue/catalogue ics/ catalogue detail ics.htm? csnumber=14908&ICS1=07&ICS2=100&ICS3=30.

Jang, Y.S., Kim, D.H., Bae, D., Kim, S.H., Kim, H., Moon, J.S., Song, K.Y., Chon, J-W., Seo, K-H., 2020. Prevalence, toxin-typing, and antimicrobial susceptibility of *Clostridium perfringens* from retail meats in Seoul, Korea. Anaerobe 64, 102-235.

Ji, L.W., Dong, L.L., Ji, H., Feng, X.W., Li, D., Ding, R.L., *et al.* 2014. Comparative pharmacokinetics and bioavailability of tylosin tartrate and tylosin phosphate after a single oral and i.v. administration in chickens. J. Vet. Pharmacol. Ther. 37, 312-5. doi: 10.1111/jvp.12092.

Kasab-Bachi H., 2017. Epidemiology of *Clostridium perfringens* and *Clostridium* difficile among Ontario broiler chicken flocks. Thesis, PhD of Population Medicine, University of Guelph, Guelph, Ontario, Canada.

Kovanda, L., Zhang, W., Wei, X., Luo, J., Wu, X., Atwill, E.R., Vaessen, S., Li, X., Liu, Y., 2019. In Vitro Antimicrobial Activities of Organic Acids and Their Derivatives on Several Species of Gram-Negative and Gram-Positive Bacteria. Molecules 24, 3770. doi:10.3390/molecules24203770.

Kowalski, C., Rolinski, Z., Zan, R., Wawron, W., 2002. Pharmacokinetics of tylosin in broiler chickens. Pol. J. Vet. Sci. 5, 127-30.

Kronfold, H., Kemper, N., Holzel, C.S., 2022. Phenotypic and genorypic characterization of *C. perfringens* isolated from dairy cows with a pathological puerperium, Vet. Sci. 9, 173. Doi:10.339/vet sci 9040173.

Lanckriet, A., Timbermont, L., De Gussem, M., Marien, M., Vancraeynest, D., Haesebrouck, F., Ducatelle, R., Van Immerseel, F., 2010. The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model, Avian Pathology 39, 63-68. https://doi.org/10.1080/03079450903505771.

Mabrouk, M.S., 2016. Concurrent use of ciprofloxacin and metronidazole for controlling of some bacterial infections in broiler chickens. Thesis, Ph.D. of Vet. Med. (Pharmacology) Zagazig University.

Macfaddin, J.F., 2000. Biochemical test for identification of medical bacteria, third Ed, Lippin Cott Willians and wilkins, Philadelphia, Pa.

Macleod, C.M., Avery, O.T., 1941. The occurrence during acute infections of a protein not normally present in the blood. III. Immunological properties of the C-reactive protein and its differentiation from normal blood proteins. J. Exper. Med. 73, 191.

Mak, P.H.W., Rehman, M.A., Kiarie, G.E., Topp, E., Diarra, M.S. 2022. Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review. Journal of Animal Science and Biotechnology 13, 148. https://doi.org/10.1186/s40104-022-00786-.

Martel, A., Devriese, L.A., Cauwerts, K., De Gussem, K., Decostere, A., Haesebrouck, F., 2004. Susceptibility of *Clostridium perfringens* strains from broiler chickens to antibiotics and anticoccidials, Avian Pathology 33, 3-7. https://doi.org/10.1080/0307945031000163291.

McDonald, L.C., Gerding, D.N., Johnson, S., Bakken, J.S., Carroll, K.C., Coffin, S.E., Dubberke, E.R., Garey, K.W., Gould, C.V., Kelly, C., Loo, V., Shaklee Sammons, J., Sandora, T.J., Wilcox, M.H., 2018. Clinical Practice Guidelines for *Clostridium* difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin. Infect. Dis. 66, e1–e48.

Mcvay, C.S., Rolfe, R.D., 2000. In vitro and in vivo activities of nitazoxanide against *Clostridium* difficile. Antimicrob. Agents Chemother. 44, 2254-2258. https://doi.org/10.1128/aac.44.9.2254-2258.2000.

Moron-Soto, M., Gutierrez, L., Sumano, H., Tapia, G., Alcala-Canto, Y., 2017. Efficacy of nitazoxanide to treat natural Giardia infections in dogs. Parasites and Vectors 10, 52. DOI 10.1186/s13071-017-19952.

Musher, D.M., Logan, N., Bressler, A.M., Johnson, D.P., Rossigno, J-F., 2009. Nitazoxanide versus Vancomycin in *Clostridium* difficile Infection: A Randomized, Double-Blind StudyClinical Infectious Diseases, 48, e41-6.

Musher, D.M., Logan, N., Hamill, R.J., DuPont, H.L. Lentnek, A., Gupta, A., Rossigno, J-F., 2006. Nitazoxanide for the Treatment of *Clostridium* difficile Colitis. Clin. Infect. Dis. 43, 421-7.

Mwangi, S., Timmons, J., Fity-coy, S., Parveen, S., 2019. Characterization of *Clostridium perfringens* recovered from broiler chicken affected by necrotic enteritis .Poult. Sci. 98 128-135.

Nasr El-Deen, N.A.M., Gamal El-Din, I.M., Khodary, M.R., 2019. Effect of Experimental *Clostridium* ringens Infection on Some Immunological, Hematological and Biochemical Values in Broiler Chickens. Zag. Vet. J. 47, 222-233. DOI: 10.21608/zvjz.2019.12216.1036.

Odingo, J., Bailey, M.A., Files, M., Early, J.V., Alling, T., Dennison, D., Bowman, J., Dalaiv, S., Kumar, N., Cramer, J., Masquelin, T., Hipskind, P.A., Parish, T., 2017. In Vitro Evaluation of Novel Nitazoxanide Derivatives against Mycobacterium tuberculosis. ACS Omega 2, 5873-5890. DOI: 10.1021/acsomega.7b00892.

OIE, 2004. World Organization for Animal Health (OIE) List of Antimicrobials of Veterinary Importance. www.oie.int (accessed 2.1.14).

Osman, K.M., Elhariri, M. 2013. Antibiotic resistance of *Clostridium perfringens* isolates from broiler chickens in Egypt. OIE Rev. Sci. Tech. 32, 841-850. https:// doi. org/ 10. 20506/ rst. 32.2. 2212.

Pankuch, G.A., Appelbaum, P.C., 2006. Activities of tizoxanide and nitazoxanide compared to those of five other thiazolides and three other agents against anaerobic species. Antimicrob. Agents Chemother. 50, 1112-1117. doi:10.1128/AAC.50.3.1112–1117.2006.

Park, J.Y., Kim, S., Oh, J.Y., Kim, H.R., Jang, I., Lee, H.S., Kwon, Y.K., 2015. Characterization of *Clostridium perfringens* isolates obtained from 2010 to 2012 from chickens with necrotic enteritis in Korea. Poult. Sci. 94, 1158-1164.

Pepperrell, T., Pilkington, V., Owen, A., Wang, J., Hil, A.M., 2020. Review of safety and minimum pricing of nitazoxanide for potential treatment of COVID-19. Journal of Virus Eradication, 6, 52-60.

Po ´zniak, B., Tikhomirov, M., Bobrek, K., Jajor, P., Switała, M., 2021. Tylosin Dosage Adjustment Based on Allometric Scaling in Male Turkeys. Antibiotics 10, 1057. https://doi.org/10.3390/antibiotics10091057.

Reitman, S., Frankel, S., 1957. A colorimetric method for determination of serum Glutamic oxaloacetic transaminase and serum Glutamic pyruvic transaminase. Am .J. clin. Path. 25, 65.

Ribeiro, S.G., 2020. Microbiological assessment of chicken meat with focus on non-typhoidal Salmonella and mcr-mediated colistin resistance in Enterobacteriaceae. Thesis, Mestrado em controlo de qualidad. Faculdade de Farmácia, Universidade do Porto, Portugal.

Rood, J.I., Adams, V., Lacey, D., McClane, B.A., Melville. S.B., Moore, R.J., Popoff, M.R., Sarker, M.R., Songer, J.J., 2018. Expansion of the *Clostridium perfringens* toxin-based typing scheme; Anaerobe 53, 5-10. Doi: 10.1016/j.anaerobe. 2018.04.011.

Rotilie, C.A., Fass, R.J., Prior, R.B., Perkins, R.L., 1975. Microdilution technique for antimicrobial susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. 7, 311-5.

Salah, H., Mansour, E., Reham, R.R., Abd El Hamid, E.S., 2015. Study on the effect of humic acid on growth performance, immunological, some blood parameters and control intestinal Closterdium in broiler chickens. Zag. Vet. J. 43, 102-109.

Salem, H.M., Ismael, E., Shaalan, M., 2021. Evaluation of the effects of silver nanoparticles against experimentally-induced necrotic enteritis in broiler chickens. Int. J. Nanomed. 16, 6783-6796.

Samanta, I., Bandyopadhyay, S., 2020. The emergence of antimicrobial-resistant bacteria in livestock, poultry and agriculture, chapter. In Antimicrobial Resistance in Agriculture. pp. 19-27. DOI:10.1016/B978-0-12-815770-1.00003-1.

Schwarz, S., Cloeckaert, A., Roberts, M.C., 2006. Mechanisms and spread of bacterial resistance to antimicrobial agents. In F.M. Aarestrup (Ed.), Antimicrobial Resistance in Bacteria of Animal Origin 1st edn. Washington, DC: ASM Press. pp. 73-99.

Shah, S.H., Sheikhb, I.S., Kakarb, N., Sumairaa, Afzala, S., Mehmooda, K., Rehman, H.U., 2022. In vivo analysis the effect of antibiotic growth promoters (AGPs), Oxytetracycline di-hydrate and Tylosin phosphate on the intestinal microflora in broiler chicken. Brazilian Journal of Biology 84, e258114. https://doi.org/10.1590/1519-6984.25811.

Shams, G-E., Fouad, A-E., Naiem, N., 2018. Nitazoxanide Adverse Effects on Biochemical Markers of Liver &Kidney Injury and Antioxidant Enzymes on Rats. Int. J. Pharm. Res. Allied Sci. 7, 1-6.

Singh, N., Narayan, S., 2011. Nitazoxanide: A Broad-Spectrum Antimicrobial. Medical Journal Armed Forces India 67, 67-68.

Soliman, A.M., Sedeik, M., 2016. Pharmacokinetics and tissue residues of tylosin in broiler chickens. Pharmacol. Pharm.7, 36-42. doi: 10.4236/pp.2016.71006.

Soufy, H., El-Beih, N.M., Nasr, S.M., Abd El-Aziz, T.H., Khalil, F.A.M., Ahmed, Y.F., Abou Zeina, H.A.A., 2017. Effect of Egyptian propolis on cryptosporidiosis in immunosuppressed rats with special emphasis on oocysts shedding, leukogram, protein profile and ileum histopathology. Asian Pacific Journal of Tropical Medicine 10, 253-262. https://doi.org/10.1016/j.apjtm.2017.03.004.

Stachulski, A.V., Taujanskas, J., Pate, S.L., Rajoli, R.K.R., Aljayyoussi, G., Pennington, S.H., Ward, S.A., Hong, W.D., Biagini, G.A., Owen, A., Nixon, G.L., Leung, S.C., O'Neill, P.M., 2020. Therapeutic Potential of Nitazoxanide: An Appropriate Choice for Repurposing versus SARS-CoV-2? Review. ACS Infect. Dis. 7, 1317-1331.

Stockis, A., Allemon, A.M., De Bruyn, S., Gengler, C., 2002 a. Nitazoxanide pharmacokinetics and tolerability in man using single ascending oral doses. Int. J. Clin. Pharmacol. Ther. 40, 213-20.

Stockis, A., De Bruyn, S., Gengler, C., Rosillon, D., 2002 b. Nitazoxanide pharmacokinetics and tolerability in man during 7 days dosing with 0.5 and 1 g b.i.d. Int. J. Clin. Pharmacol. Ther. 40, 221-7.

Szasz, G., Weimann, G., St~ihler, F., Wahlefeld, A.W., Persijn, J.P., 1974. New substrates for measuring y-glutamyl transpeptidase activity. Clin. Chem. Clin. Biochem. 12, 228.

Trabattoni, D., Gnudi, F., Ibba, S.V., Saulle, I., Agostini, S., Masetti, M., Biasin, M., Rossignol, J.F., Clerici, M., 2016. Thiazolides Elicit Anti-Viral Innate Immunity and Reduce HIV Replication. Sci. Rep. 6, 27148.

Uzal, F.A., Freedman, J.C., Shrestha, A., Theoret, J.R., Garcia, J., Awad, M.M., 2014. Towards an understanding of the role of *Clostridium perfringens* toxins in human and animal disease. Fut. Microbiol. 9, 361-377.

Uzal, F.A., McClane, B.A., Vidal, J.E., Gurjar, A.A., 2010. Colistridium perfringens Toxins Involved in Mammalian veterinary Diseases. Open Toxino. J. 2, 24-42. Doi:10.2174/187541470 1003020024.

Vissiennon, T., Kro¨ ger, H., Ko¨ hler, T., Kliche, R., 2000. Effect of avilamycin, tylosin and ionophore anticoccidials on *Clostridium perfringens* enterotoxaemia in chickens. Berl. Munch. Tier-a¨rztl. Wschr, 113, 9-13.

Wei, B., Cha, S.Y., Zhang, J.F., Shang, K., Park, H.C., Kang, J., Lee, K.J., Kang, M. Jang, H.K., 2020. Antimicrobial susceptibility and association with toxin determinants in Colistridium perfringens isolates from chickens. Microorganisms 8, 1825. DOI:10.3390/microorganisms 8111825.

Wijayanti, A.D., Ardiansyah, R.D., Pratama, A.M., Haryanto, A., Fitriana, I., 2022. Validation method for determining enrofloxacin and tylosin levels in broiler liver, kidney, and muscle using high-performance liquid chromatography. Veterinary World 15, 268-274. doi: www.doi.org/10.14202/vetworld.2022.268-274.

Yoo, H.S., Lee, S.U., Park, K.Y., Park, Y.H., 1997. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by Multiplex PCR. Journal of Clinical Microbiology 35, 228-232. Doi: 10.1128/jcm.35.1.228-232.1997.

Younes, A.M., 2005. Use of polymerase chain reaction (PCR) for diagnosis of *Clostridium perfringens* in chickens, Thesis, Master of Microbiology, Fac. Vet. Med. Cairo University, Egypt.