Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 47, Number 2, p. 222-233, June 2019
DOI: 10.21608/zvjz.2019.12216.1036

RESEARCH ARTICLE

Effect of Experimental *Clostridium perfringens* Infection on Some Immunological, Hematological and Biochemical Values in Broiler Chickens

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Article History: Received: 23/04/2019 Received in revised form: 08/05/2019 Accepted: 11/05 /2019

Abstract

This study aimed to demonstrate the effect of *Clostridium perfringens* (*Cl. perfringens*) infection on broiler chickens. Also, to compare between the effect of amoxicillin and / or organic acids on *Cl. perfringens* infection through the evaluation of hemogram, blood chemistry, hepato-renal functions and immune response using ELISA technique. One hundred, one-day old Hubbard chicks were divided into five equal groups. Group 1: negative control, Group 2: infected broilers with *Cl. perfringens* type A (1.9×10^9 organism/mL), Group 3: infected chickens and treated with amoxicillin [15 mg/kg body weight (B.W.)] for 5 successive days, Group 4: chickens were administered organic acids (1 mL /L water) then were infected and Group 5: infected chickens then treated with both amoxicillin and organic acids. *Cl. perfringens* infection resulted in decreased appetite, ruffled feathers and brownish diarrhea with sudden death in some cases with a mortality rate up to 25%. Birds infected and treated with amoxicillin showed mild clinical signs with 15% mortalities. Majority of chickens supplemented with organic acids or with organic acids and amoxicillin followed by *Cl. perfringens* infection showed depression with a mild diarrhea and 10% mortality rate. Chickens infected with *Cl. perfringens* had macrocytic hypochromic anemia, leukocytosis, heterophilia and monocytosis. In addition, a significant decrease of total protein, albumin, phagocytic % and phagocytic index with a significant increase of total golbulins, liver enzymes activities, serum uric acid, creatinine and glucose levels were reported. Treatment of *Cl. perfringens* infection with amoxicillin, organic acids alone or their combination resulted in a positive effect in treatment, ameliorating the severity of infection and a significant improvement in some immunological and biochemical parameters. In conclusion, the combination of amoxicillin and organic acids showed the best results as it returned all hematological, biochemical and immunological parameters to their normal levels.

Keywords: *Clostridium perfringens,* Amoxicillin, Organic acids, Chickens, Phagocytic activity.

Introduction

Clostridium perfringens (*Cl. perfringens*) is an anaerobic spore forming Gram positive bacilli found in the intestinal tract of birds as a part of normal microflora [1]. Necrotic enteritis is an important disease caused by *Cl. perfringens* type A and C in broiler chickens [2]. *Cl. perfringens* type A produces α-toxin that destroys the host cell membranes by hydrolysis and oxidation of membrane phospholipids and reaches the blood stream, causing systemic symptoms and death [3]**.** The disease is reported all over the world and causes severe economic losses reflected by reduction of body weight and increase of feed conversion rate, while the mortality rate may reach up to 50% [4,5].

Necrotic enteritis can be prevented by the use of antimicrobials, among these; amoxicillin and metronidazole [6]. Amoxicillin is a broad spectrum β-lactam antibiotic that provides bactericidal activity against Gram+ve bacteria. It is given

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parentrally in the form of amoxicillin sodium and orally in the form of amoxicillin trihydrate [7]. Amoxicillin acts during bacterial multiplication by inhibition of cell wall mucopeptide biosynthesis [8].

Increasing awareness of treatment failure and application of antibiotic alternatives can be effective in controlling of necrotic enteritis [9]. Alternative preventive agents as organic acids are widely used [10]. Organic acids inhibit bacterial growth by lowering intestinal pH or by crossing the bacterial cell membrane resulting in the decrease of bacterial intracellular pH and inhibition of metabolic reactions reducing bacterial growth [11].The long treatment period of organic acids is preferable as it is used as growth promotor either by competitive inhibition of colonization of pathogenic bacteria or by enhancing the digestibility [12].

The present study was conducted to gain more information about the effect of *Cl. perfringens* infection on hematological, biochemical and some immunological parameters in broilers. Moreover, it clarifies the effect of amoxicillin and organic acids on *Cl. perfringens* infection and the fore-mentioned parameters.

Materials and Methods

Bacterial strains

Cl. perfringens type "A" (1.9×10^9 organism/mL) was obtained from the Anaerobes Unit, Animal Health Research Institute, Dokki, Giza, Egypt to be used for experimental infection.

Candida albicans and *Staphylococcus aureus* (1.7×10^8 CFU) strains were obtained from Department of Bacteriology, Mycology and Immunology, Animal Health Research Institute, Zagazig branch to be used for the phagocytic assay.

Drugs

a) Amoxicillin trihydrate (697 mg/g powder) was obtained from Dechra Veterinary Products Co., LLC, Kansas, United States.

b) Organic acids (Mix acid® Nanjing Weite Veterinary Co., Ltd, China). Each 1 liter contains: formic acid 85% (150 g), tartaric acid 99% (15 g), acetic acid 80% (40 g), propionic acid 99% (250 g), lactic acid 80% (50 g), phosphoric acid 75% (55 g), fumeric acid 99% (80 g), Sorbic acid 99% (15 g), Malic acid 99% (12 g), Benzoic acid 99% (22 g), Ammonium formate (90 g), Calcium lactate (10 g), Copper pentasulphate (10 g) and distilled water up to 1 liter.

Experimental chicks

One hundred, one-day old, commercial Hubbard chicks from Al-Kahira Poultry Company, 10th of Ramadan city, Egypt were used for this investigation. The chicks were reared under standard environmental and hygienic conditions and food added ad libitum. All chicks were vaccinated against Newcastle disease at 7 and 18 days old using Hitchner B1 and LaSota live virus vaccines (Intervet Boxmeer Company, Boxmeer, Netherlands); the vial contains 106 EID50 Newcastle disease virus, dissolved in physiological saline (30 mL per 1000 doses) as eye drops). Vaccination of all chicks against Gumboro disease was done at 15 days old using Holland. Gumboro vaccine (Rhone-Merieau Company, France); the Vial was dissolved in 50 mL physiological salin / 1000 bird as eye drops. [13].

Experimental design

One hundred, one-day old Hubbard chicks were divided into five equal groups. Group 1 was kept as a negative control. Group 2 was infected with 2mL broth culture (1.9×10^9 organism/mL) 3 times orally at 19, 21 and 23 days old [14]. Group 3 was infected with *Cl. perfringens* at the same pattern in group 2 then treated with amoxicillin (15 mg/kg B.W.) after the appearance of the clinical signs [15] for 5 successive days in drinking water. Group 4 was given organic acids (1 mL / L water) [16] from the 1st day old till the end of the experiment then infected with *Cl. perfringens* as in the 2nd group. Group 5 was infected with *Cl. perfringens* as in group 2 then treated with amoxicillin and organic acids for the same days. This study was approved by the committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine Zagazig University.

Sampling

Five birds, from each group, were used for collecting blood samples at the age of 32nd and 39th days old. Each blood sample was subdivided into three parts. The 1st part was taken on dipotassium salt of EDTA (1mg/1mL blood) for hematological examination. The 2nd part was collected into clean centrifuge tube for obtaining serum by centrifugation for biochemical studies. The third one (2 mL of blood) was placed in a sterile plastic centrifuge tube containing heparin (50 I.U /mL) to be used for phagocytic activity [17].

Hematological studies

Erythrocytic count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and total and differential leukocytic count were measured by using the automatic cell counter sysmex XT (2000 IV; IDEXX Laboratories, Inc., United States).

Biochemical studies

The biochemical tests were performed using test kits of Diamond- Egypt. Determination of serum total proteins [18], serum albumin [19], serum globulins [20], serum alkaline phosphatase (ALP) [21], serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) [22] were applied. Electrophoretic analysis of serum proteins was carried out for determination of serum alpha, beta and gamma globulins [23]. Determination of serum levels of sodium, potassium [24], serum uric acid [25] and serum creatinine [26] were conducted.

Immunological studies

Serum IgM and IgG were measured based on sandwich ELISA [27] using chicken ELISA kits of Bethyl Laboratories Inc., United States. Measurement of phagocytic activity and phagocytic index was applied by separation of mononuclear leukocytic cells using lymphocytic separation medium "Ficoll-Hypaque" density gradient [17]. Smears were prepared from the deposit, dried in air and stained with Leishman's stain (Lab supply co., Egypt). Finally, a total number of 100 phagocytic cells were counted randomly in about ten microscopic fields under a light microscope (Nikon, United States) using oil immersion lens [28]. Phagocytic% and phagocytic index were recorded using the following equations:

$$\text{Phagocytic \%} = \frac{\textit{Total number of phagocytes containing C.albicans}}{\textit{Total number of phagocytic cells}} \times 100$$

$$\text{Phagocytic index} = \frac{\textit{Total number of ingested C.albicans}}{\textit{Total number of phagocytic cells containing C.albicans}}$$

Statistical analysis

The obtained data were statistically analysed by PASW Statistics (SPSS version 18.0 for Windows) using one way ANOVA. The comparison of means was carried out with Duncan's multiple range test.

Results

In the presented study, *Cl. perfringens* infection in chickens of Group 2 resulted in decreased appetite, depression, emaciation, ruffled feather and brownish diarrhea with mortality rate 25%. Birds infected with *Cl. perfringens* and treated with amoxicillin (Group 3) showed mild clinical signs with 15% mortalities. Majority of chickens, which were contentiously supplemented with organic acids (Group 4) or treated simultaneously with organic acid and amoxicillin (Group 5), showed depression with a mild diarrhea and 10% mortality rate.

Chickens infected with *Cl. perfringens* (Group 2) showed macrocytic hypochromic anemia (Table 1), significant leukocytosis, heterophilia, monocytosis and lymphopenia (Table 2), a significant decrease of total

protein and albumin with a significant increase of total golbulins (Table 3). Moreover, a significant increase in serum AST, ALT and ALP activities as well as serum potassium, uric acid and creatinine levels with a significant decrease in serum sodium level were detected (Table 4).A significant decrease of phagocytic % and phagocytic index with a significant increase of IgG and IgM were recorded in Group 2 when compared with normal control (Table 5). Treatment of *Cl. perfringens* infection with amoxicillin (group 3) or organic acids (group 4) resulted in a positive effect in treatment, ameliorating the severity of infection and returning of some immunological, hematological and biochemical parameters towards their normal levels specially in *Cl. perfringens*-infected chickens treated with amoxicillin and organic acids at 39 days old (Group 5).

Table 1: Erythrogram (mean values ± S.E) of *Cl. perfringens* infected chickens at 14 and 21 days of the infection (n=5).

Parameters	Age	Gp (1) Control	Gp (2) Clostridium	Gp (3) Clostridium → Amoxicillin	Gp (4) Organic acids → Clostridium	Gp (5) Clostridium → Amoxicillin + Organic acids
RBCs x10^6/µL	32 days	2.42 ± 0.04 ^{a}	1.83 ± 0.06 ^{d}	2.15 ± 0.02 ^{c}	2.01 ± 0.01 ^{c}	2.20 ± 0.01 ^{b}
	39 days	2.54 ± 0.05 ^{a}	1.75 ± 0.04 ^{c}	2.30 ± 0.04 ^{b}	2.15 ± 0.03 ^{b}	2.47 ± 0.03 ^{a}
Hb g/dL	32 days	8.88 ± 0.03 ^{a}	6.85 ± 0.05 ^{d}	8.02 ± 0.02 ^{b}	7.52 ± 0.04 ^{c}	8.20 ± 0.06 ^{b}
	39 days	9.14 ± 0.04 ^{a}	6.50 ± 0.03 ^{d}	8.35 ± 0.05 ^{b}	8.00 ± 0.08 ^{c}	8.89 ± 0.05 ^{a}
PCV %	32 days	34.65 ± 0.53 ^{a}	28.77 ± 0.12 ^{e}	32.44 ± 0.10 ^{c}	31.28 ± 0.25 ^{d}	33.59 ± 0.21 $_b$
	39 days	35.55 ± 0.25 $_{ab}$	27.11 ± 0.38 ^{e}	33.81 ± 0.26 ^{c}	$_d$ 32.05 ± 0.23	35.35 ± 0.28 $_{ab}$
MCV fL	32 days	143.32 ± 0.53 $_c$	157.21 ± 4.01 $_a$	150.88 ± 1.67 $_a$	155.62 ± 0.59 $_a$	152.68 ±1.70 $_{ab}$
	39 days	140.0 ± 2.11 ^{d}	154.91 ± 1.37 $_a$	147.00 ±1.92 $_{bc}$	149.07 ±1.19 $_{bc}$	143.12 ±1.44 $_{cd}$
MCH pg	32 days	36.69 ± 1.13 $_{ab}$	37.43 ± 0.96 ^{a}	37.30 ± 0.40 ^{a}	$_a$ 37.41± 0.02	37.27 ± 0.05 $_{ab}$
	39 days	35.98 ± 0.62 $_{abc}$	37.14 ± 0.86 $_{ab}$	36.30 ± 0.50 $_{ab}$	$_{ab}$ 37.21 ± 0.09	35.00 ± 0.30 $_{abc}$
MCHC %	32 days	25.62 ± 0.11 ^{a}	23.81 ± 0.09 ^{d}	24.72 ± 0.02 ^{b}	$_c$ 24.04 ± 0.10	24.41 ± 0.025 $_b$
	39 days	25.71 ± 0.11 ^{a}	23.98 ± 0.25 ^{c}	24.70 ± 0.26 $_{bc}$	$_{bc}$ 24.96 ± 0.29	25.15 ± 0.10 $_{ab}$

Different letters in the same column indicate significant changes (P <0.05).
Gp: Group

Table 2: Leukogram x10^3/µL (mean values ± S.E) of *Cl. perfringens* infected chickens at 14 and 21 days of the infection (n=5)

Groups / Parameters		Gp (1) Control	Gp (2) Clostridium	Gp (3) Clostridium → Amoxicillin	Gp (4) Organic acids → Clostridium	Gp (5) Clostridium → Amoxicillin + Organic acids
T.L.C	32 days	21.08 ± 0.11 ^{b}	23.26 ± 0.55 ^{a}	23.09 ± 0.98 ^{a}	23.15 ± 0.80 ^{a}	22.56 ± 0.19 ^{a}
	39 days	21.22 ± 0.17 ^{c}	23.43 ± 0.15 ^{a}	22.34 ± 0.46 ^{b}	22.89 ± 0.23 ^{b}	21.45 ± 0.43 ^{c}
Heterophils	32 days	4.41 ± 0.053 ^{c}	7.10 ± 0.198 ^{a}	6.68 ± 0.44 ^{a}	6.92 ± 0.36 ^{a}	5.86 ± 0.05 ^{b}
	39 days	4.50 ± 0.04 ^{c}	7.28 ± 0.405 ^{a}	5.80 ± 0.19 ^{b}	6.40 ± 0.35 ^{b}	5.00 ± 0.26 ^{c}
Eosinophils	32 days	1.07 ± 0.01 ^{a}	1.08 ± 0.02 ^{a}	1.11 ± 0.035 ^{a}	1.10 ± 0.02 ^{a}	1.08 ± 0.01 ^{a}
	39 days	1.08 ± 0.01 ^{a}	1.09 ± 0.01 ^{a}	1.08 ± 0.004 ^{a}	1.09 ± 0.01 ^{a}	1.086 ± 0.01 ^{a}
Lymphocytes	32 days	12.80 ± 0.06 ^{a}	10.54 ± 0.36 ^{c}	11.73 ± 0.35 ^{b}	11.68 ± 0.32 ^{b}	12.35 ± 0.11 ^{b}
	39 days	12.82 ± 0.08 ^{a}	10.86 ± 0.16 ^{c}	11.82 ± 0.19 ^{b}	11.44 ± 0.26 ^{b}	12.45 ± 0.20 ^{a}
Monocytes	32 days	2.20 ± 0.01 ^{c}	3.95 ± 0.10 ^{a}	3.00 ± 0.20 ^{b}	2.85 ± 0.11 ^{b}	2.77 ± 0.08 ^{b}
	39 days	2.30 ± 0.01 ^{c}	3.57± 0.14 ^{a}	3.04 ± 0.11 ^{b}	3.06 ± 0.04 ^{b}	2.31 ± 0.07 ^{c}
Basophils	32 days	0.59 ± 0.01 ^{a}	0.58 ± 0.02 ^{a}	0.57 ± 0.03 ^{a}	0.60 ± 0.01 ^{a}	0.59 ± 0.01 ^{a}
	39 days	0.63 ± 0.02 ^{ab}	0.63 ± 0.01 ^{ab}	0.60± 0.01 ^{a}	0.60 ± 0.01 ^{b}	0.60 ± 0.01 ^{b}

Different letters in the same column indicate significant changes (P <0.05).
Gp: Group, T.L.C: Total leukocytic count

Table 3: Proteinogram (mean values ± S.E) of *Cl. perfringens* infected chickens 14 and 21 days of the infection (n=5).

Parameters	Age	Gp (1) Control	Gp (2) Clostridium	Gp (3) Clostridium → Amoxicillin	Gp (4) Organic acids → Clostridium	Gp (5) Clostridium → Amoxicillin + Organic acids
Total protein g/ dL	32 days	4.26 ± 0.02 ^{ab}	3.96 ± 0.03 ^{d}	4.08 ± 0.03 ^{c}	4.14 ± 0.01 ^{c}	4.19 ± 0.01 ^{c}
	39 days	4.49 ± 0.01 ^{a}	3.92 ± 0.02 ^{d}	4.41 ± 0.01 ^{b}	4.35 ± 0.02 ^{c}	4.50 ± 0.01 ^{a}
Albumin g/ dL	32 days	1.44 ± 0.01 ^{a}	1.01 ± 0.01 ^{d}	1.10 ± 0.01 ^{c}	1.20 ± 0.01 ^{b}	1.24 ± 0.01 ^{b}
	39 days	1.55 ± 0.02 ^{a}	0.90 ± 0.02 ^{d}	1.45 ± 0.01 ^{b}	1.37 ± 0.02 ^{c}	1.56 ± 0.01 ^{a}
α- globulin g/ dL	32 days	0.84 ± 0.01 ^{ab}	0.84 ± 0.01 ^{ab}	0.87 ± 0.02 ^{a}	0.82 ± 0.01 ^{b}	0.84 ± 0.01 ^{ab}
	39 days	0.92 ± 0.004 ^{a}	0.91 ± 0.01 ^{a}	0.92 ± 0.003 ^{a}	0.93 ± 0.004 ^{a}	0.93 ± 0.003 ^{a}
β- globulin g/ dL	32 days	0.84 ± 0.01 ^{abc}	0.81 ± 0.02 ^{bc}	0.86 ± 0.01 ^{a}	0.85 ± 0.02 ^{ab}	0.86 ± 0.01 ^{a}
	39 days	0.84 ± 0.01 ^{abc}	0.87 ± 0.01 ^{a}	0.82 ± 0.01 ^{c}	0.81 ± 0.01 ^{c}	0.83 ± 0.01 ^{bc}
γ- globulin g/ dL	32 days	1.14 ± 0.01 ^{b}	1.30 ± 0.01 ^{a}	1.25 ± 0.004 ^{a}	1.27 ± 0.01 ^{a}	1.25 ± 0.004 ^{a}
	39 days	1.18 ± 0.01 ^{b}	1.24 ± 0.01 ^{a}	1.22 ± 0.003 ^{a}	1.24 ± 0.01 ^{a}	1.18 ± 0.01 ^{c}
Total globulins g/ dL	32 days	2.82 ± 0.01 ^{c}	2.95 ± 0.03 ^{ab}	2.98 ± 0.02 ^{a}	2.94 ± 0.006 ^{ab}	2.95 ± 0.005 ^{ab}
	39 days	2.94 ± 0.01 ^{c}	3.02 ± 0.02 ^{a}	2.96 ± 0.01 ^{b}	2.98 ± 0.01 ^{b}	2.94 ± 0.01 ^{c}

Different letters in the same column indicate significant changes (P <0.05).
Gp: Group

Table 4: Some biochemical values (mean values + S.E) of *Cl. perfringens* infected chickens at 14 and 21 days of the infection (n=5).

Parameters	Age	Gp (1) Control	Gp (2) Clostridium	Gp (3) Clostridium → Amoxicillin	Gp (4) Organic acids → Clostridium	Gp (5) Clostridium → Amoxicillin + Organic acids
AST U/L	32 days	48.60 ± 1.72 ^{d}	85.00 ± 2.00 ^{a}	70.60 ± 2.50 ^{b}	72.00 ± 1.14 ^{b}	65.00 ± 2.28 ^{c}
	39 days	50.80 ± 1.02 ^{d}	88.00 ± 1.84 ^{a}	58.80 ± 0.86 ^{c}	62.40 ± 1.63 ^{b}	53.40 ± 1.08 ^{d}
ALT U/L	32 days	9.22 ± 0.20 ^{d}	20.16 ± 0.46 ^{a}	15.94 ± 0.25 ^{b}	16.62 ± 0.21 ^{b}	15.16 ± 0.14 ^{c}
	39 days	9.16 ± 0.27 ^{d}	20.68 ± 0.30 ^{a}	11.20 ± 0.22 ^{c}	13.38 ± 0.18 ^{b}	9.84 ± 0.32 ^{d}
ALP IU/L	32 days	52.14 ± 0.19 ^{e}	90.76 ± 0.22 ^{a}	70.88 ± 0.86 ^{c}	74.74 ± 0.16 ^{b}	62.24 ± 1.21 ^{d}
	39 days	52.62 ± 0.24 ^{d}	90.48 ± 1.54 ^{a}	60.56 ±1.60 ^{c}	65.30 ± 1.31 ^{b}	53.94 ± 1.05 ^{d}
Sodium m.Eq/L	32 days	133.6 ± 1.21 ^{a}	110.6 ± 1.08 $_{e}$	120.2 ± 1.07 ^{c}	114.4 ± 1.21 ^{d}	124.0 ± 1.41 ^{b}
	39 days	135.80 ± 1.28 $_{ab}$	105.20 ± 1.24 $_{e}$	128.00 ± 1.00 $_{c}$	123.40 ± 1.50 $_{d}$	132.60 ± 1.54 $_{b}$
Potassium m.Eq /L	32 days	3.84 ± 0.09 ^{de}	4.50 ± 0.07 ^{a}	4.26 ± 0.05 ^{b}	4.34 ± 0.05 ^{ab}	4.14 ± 0.08 ^{bc}
	39 days	3.88 ± 0.11 ^{c}	4.38 ± 0.11 ^{a}	4.18 ± 0.11 ^{b}	4.20 ± 0.08 ^{b}	3.98 ± 0.09 ^{c}
Uric Acid mg/ dL	32 days	4.80± 0.26 ^{d}	10.50 ± 0.57 ^{a}	7.40 ± 0.27 ^{b}	7.60 ± 0.21 ^{b}	6.70 ± 0.22 ^{c}
	39 days	5.20 ± 0.18 ^{c}	11.40 ± 0.41 ^{a}	6.50 ± 0.14 ^{b}	6.92 ± 0.31 ^{b}	5.50 ± 0.21 ^{c}
Creatinine mg dL	32 days	0.80 ± 0.02 ^{d}	1.80 ± 0.16 ^{a}	1.10 ± 0.04 ^{bc}	1.30 ± 0.07 ^{b}	1.01 ± 0.02 ^{c}
	39 days	0.83 ± 0.10 ^{d}	1.96 ± 0.12 ^{a}	0.95 ± 0.03 ^{c}	1.25 ± 0.07 ^{b}	0.86 ± 0.05 ^{d}

Different letters in the same column indicate significant changes (P <0.05).
Gp: Group

Table 5: Some immunological parameters (mean + S.E) of *Cl. perfringens* infected chickens at 14 and 21 days of the infection (n=5).

Parameters	Age	Gp (1) Control	Gp (2) Clostridium	Gp (3) Clostridium → Amoxicillin	Gp (4) Organic acids → Clostridium	Gp (5) Clostridium → Amoxicillin + Organic acids
Phagocytic %	32 days	76.00 ± 1.30 ^{a}	36.00± 0.71 ^{d}	60.00 ± 1.14 ^{c}	64.00± 1.23 ^{c}	68.00 ± 2.00 ^{b}
	39 days	77.00 ± 0.95 ^{a}	40.00 ± 0.71 ^{c}	68.0 ± 1.70 ^{b}	66.00 ± 1.23 ^{b}	74.00 ± 1.41 ^{a}
Phagocytic ndex	32 days	4.60 ± 0.13 ^{a}	1.00 ± 0.07 ^{d}	2.80 ± 0.14 ^{c}	3.10 ± 0.12 ^{c}	3.30 ± 0.14 ^{b}
	39 days	4.70 ± 0.17 ^{a}	1.80 ± 0.11 ^{c}	3.20 ± 0.07 ^{b}	3.00 ± 0.17 ^{b}	4.40 ± 0.14 ^{a}
Ig M mg/dL	32 days	224.00 ± 1.52 $_{c}$	245.00 ± 4.30 $_{a}$	238.00 ± 1.70 $_{b}$	241.00 ± 1.58 $_{a}$	232.00 ± 2.28 $_{b}$
	39 days	228.00 ± 1.23 $_{c}$	241.00 ± 2.61 $_{a}$	235.00± 1.00 $_{b}$	238.00 ± 2.12 $_{b}$	230.00 ± 2.35 $_{c}$
Ig G mg/dL	32 days	912.00 ± 2.12 $_{c}$	930.00 ± 3.21 $_{a}$	924.80 ± 1.72 $_{b}$	926.00 ± 2.26 $_{a}$	923.00 ± 1.41 $_{b}$
	39 days	918.00 ± 2.00 $_{c}$	938.40 ± 3.67 $_{a}$	932.60 ± 1.21 $_{b}$	929.60 ± 1.57 $_{b}$	918.20 ± 1.99 $_{c}$

Different letters in the same column indicate significant changes (P <0.05).
Gp: Group

Discussion

The present study was designed to investigate the efficacy of amoxicillin and/or organic acids in treatment of *Cl. perfringens* infection in broilers and their effects on hemato-biochemical, proteinogram and immunological parameters. *Cl. perfringens*-infected chickens showed depression, emaciation, ruffled feather and brownish diarrhea in addition to sudden death. These clinical signs may be due to the effect of *Clostridium* toxins [29], which may reach the liver and lead to disturbance in the metabolic activity [30]. The recorded clinical signs matched with those published previously [31,32]. Medication of chickens infected with *Cl. perfringens* using amoxicillin and/or organic acids was effective and resulted in milder clinical symptoms and decrease of the mortality rate. These results may be due to bactericidal activity of amoxicillin [8], and the inhibitory effect of organic acids on microbial flora colonization due to lowering the pH of intestine [11]. Sarkar *et al.*, [33] and Lensing *et al*., [34] proved the efficacy of amoxicillin or organic acids in controlling of necrotic enteritis disease, respectively.

Regarding to the erythrogram and leukogram, chickens infected with *Cl. perfringens* (Group 2) showed macrocytic hypochromic anaemia, which may be due to destruction of RBCs by *Cl. perfringens* toxins inducing hemolytic anaemia. Our results agree with El-Boraay [35] who stated that the hemolysis induced by clostridial toxins was occurred through breakdown of phospholipids of erythrocytic membranes. *Cl. perfringens*-infected broilers then treated with amoxicillin and/or organic acids showed a significant improvement in RBCs count, Hb content and PCV compared to the infected untreated group. Nagaralli *et al.*, [8] indicated that amoxicillin act by inhibition of cell wall mucopeptide biosynthesis during bacterial multiplication. Mikkelsen *et al*., [36] mentioned that *Cl. perfringens* growth was suppressed by organic acids supplementation. Our results were in consistence with previously published papers [37,38], in which an improvement of erythrogram parameters in *Cl. perfringens*-infected broilers then treated with amoxicillin or organic acids were detected.

Regarding leukogram, *Cl. perfringens* infection induced significant leukocytosis, heterophilia, monocytosis with a significant lymphopenia. Alterations of leukogram in the present study may be due to the bacterial infection and inflammation that lead to leukocytosis, heterophilia and monocytosis which are responsible for phagocytosis of the infective microorganism and damaged cells [39]. Moreover, leukocytosis is a characteristic feature of bacterial infection [40]. Many authors reported a leukocytosis and heterophilia in *Cl. perfringens*-infected broilers [41]. On the other hand, variation in leukogram of *Cl. perfringens*-infected birds was reported elsewhere [32]. This variation may be related to the stage of infection or immune response of the birds. We stated that treated birds with amoxicillin or organic acids showed improvement in the leukogram compared to non-treated group suggesting the efficacy of treatment. Similar results evidenced an improvement in the leuocytic parameters in *Cl. perfringens*- infected broilers treated with amoxicillin or organic acids [37,38].

Concerning proteingram, *Cl. perfringens* infection in broilers produced significant hypoproteinemia and hypoalbuminemia with a significant increase of total and gamma globulins. The hypoalbuminemia may be due to the decreased feed intake, the loss through the intestine and the kidneys, liver failure to synthesize albumin or may be due to liver damage by clostridial toxins [30]. The recorded hyperglobulinemia is due to bacterial septicemia [42]. Bacteria cause lymphocyte stimulation and its differentiation into T and B lymphocytes resulting in increase of globulins levels and acquired immune response. These findings agree with previously published papers [43, 44] in which a significant increase in serum levels of γ and total globulins in *Cl. perfringens*-infected chickens was noticed. In contrast, a previous study [38] indicated a significant decrease in serum levels of α, β, γ-globulins and total globulins in *Cl. perfringens*-infected chickens. Treatment of *Cl. perfringens* infection in broilers with amoxcillin and/or organic acids induced a

significant improvement of serum total proteins and albumin coupled with a significant decrease of γ and total globulins suggesting the efficacy of treatment and the potent immunostimulant effect of organic acids. Our results agree with previous studies [38,45] in which a significant increase of serum total proteins and albumin were found in *Cl. perfringens*-infected chickens and treated by ampicillin or organic acids, respectively compared with the infected group.

Determination of serum liver enzymes (AST, ALT and ALP) is used as standard tests for liver damage [46]. Our results revealed that infection of broiler chickens with *Cl. perfringens* resulted in a significant increase in the activity levels of AST, ALT and ALP denoting hepatic damage and biliary stasis caused by clostridial toxins [40]. The recorded increase of serum potassium, uric acid and creatinine levels with a significant hyponatremia in *Cl. perfringens*-infected chickens may be due to clostridial toxins, cellular necrosis and damage of the kidneys. The degeneration of renal tubules prevented excretion of uric acid and creatinine leading to increase of their levels in serum of infected birds [47]. The improvement of liver and kidney function tests in *Cl. perfringens*-infected chickens treated with amoxicillin or organic acids or both may be due to amoxicillin and organic acids ameliorated the deleterious effect of *Cl. perfringens* on the kidneys. Our results agree with Allam and Co-authors [43] who reported a significant increase in serum levels of AST, ALT, ALP, uric acid and creatinine.

In the present study, broilers infected with *Cl. perfringens* showed a significant decrease of phagocytic % and phagocytic index coupled with a significant increase of IgM and IgG. This reduction in phagocytic activity may be due to the infection's negative effect on immune system. IgM and IgG isotypes are indicators for resistance of diseases in poultry [48]. Our results agree with Salah *et al* .,[44] who proved that *Cl. perfringens* infection in chicken resulted in a decrease of phagocytic index and phagocytic %. Treatment of infected chickens with amoxicillin, organic acids or both showed improvement of the examined immunological parameters.

Conclusion

In conclusion, infection of broilers with *Cl. perfringens* resulted in clinical bad impact and considerable mortalities beside alterations of hematological, biochemical and immunological values. However, treatment of infected birds with amoxicillin or organic acids had a beneficial effect in control of the infection. The best results were obtained by treatment with a combination of amoxicillin and organic acids.

Conflict of interest

None of the authors have any conflict of interest to declare.

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الملخص العربي

تأثير الاصابة التجريبية بعدوي الكلوستريديم بيرفرنجنز علي بعض القيم المناعية والدم والبيوكيميائية في دجاج التسمين

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يهدف هذا العمل إلى دراسة تأثير العدوي بالكلوستريديم بيرفرنجنز علي دجاج التسمين و مقارنة تأثير الأموكسي سيلين و/ أو الأحماض العضوية علي العدوي بالكلوستريديم بيرفرنجنز من خلال فحص الاعراض الاكلينيكية على الطيور ودراسة صورة وكيمياء الدم وكذلك وظائف الكبد و الكلي و الاستجابة المناعية باستخدام الاليزا.

تم تقسيم مائة كتكوت عمر يوم واحد الي خمس مجموعات متساوية :مجموعة (١): مجموعة ضابطة. مجموعة (٢): معداة بالكلوستريديم بيرفرنجنز نوع (أ) بجرعة ١.٩ x $١٠^{٩}$ ميكروب لكل مل. مجموعة (٣): معداة بالكلوستريديم بيرفرنجنز نوع (أ) و معالجة بالاموكسي سيللين (١٥ مجم/ كجم وزن حي) لمدة ٥ أيام متتالية في ماء الشرب. مجموعة (٤): تم اعطائها أحماض عضوية (١ مل/ لتر ماء شرب) منذ اليوم الأول و حتي انتهاء التجربة و تمت اصابتها بعدوى الكلوستريديم بيرفرنجنز نوع (أ). مجموعة (٥): معداة بالكلوستريديم بيرفرنجنز نوع (أ) و معالجة بالاموكسي سيللين و الأحماض عضوية لنفس ذات المدة.العدوي بالكلوستريديم بيرفرنجنز نتج عنها نقص الشهية و هيشان الريش مع اسهال بني و الموت المفاجئ في بعض الحالات و كانت نسبة النفوق ٢٥%. الطيور المصابة و المعالجة بالأموكسي سيللين ظهر عليها أعراض اكلينيكية خفيفة مع نسبة نفوق ١٥ % بينما المعالجة بالأحماض العضوية أو الأحماض العضوية مع الاموكسي سيلين كانت تعاني من انكماش او اسهال خفيف و نسبة نفوق ١٠%. بدارى التسمين المصابة بالكلوستريديم بيرفرنجنز كانت تعاني من الأنيميا و زيادة عدد كرات الدم البيضاء مع نقص معنوى البروتين الكلي و الألبيومين ومستوي الصوديوم و زيادة معنوية للجلوبيولينات الكلية بالاضافة الي زيادة أنشطة انزيمات الكبد والبوتاسيوم و حمض البوليك و الكرياتينين في الدم. كما لوحظ وجود نقص معنوي في نسبة معامل الخلايا المتبلعمة. أظهرت الدراسة ان علاج الدجاج المصاب بالكلوستريديم بيرفرنجنزباستخدام المضاد الحيوي أموسي سيللين أو الاحماض العضوية و خاصة الاثنين معا له نتائج ايجابية في علاج و تخفيف من حدة الاصابة و نتج عن ذلك تحسن معنوي في القيم المناعية و البيوكيميائية. ونستخلص من الدراسة بأن استخدام المضاد الحيوي أموكسي سيللين و الاحماض العضوية أعطى أفضل نتائج حيث انه اعاد القيم المناعية و البيوكيميائية والدم الى معدلاتها الطبيعية.