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Original Research Article

EFFECT OF SANGUINARINE PHYTOBIOTIC, SODIUM BUTYRATE COMPARED TO AMPICILLIN ON CONTROLLING NECROTIC ENTERITIS IN BROILER CHICKENS

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Abstract: This study aimed to investigate the efficacy of Sanguinarine phytobiotic, Sodium butyrate compared to ampicillin in treatment of *Clostridium perfringens* infection in broiler chickens with a special reference to their effects on growth performance, hematobiochemical and immunological profile. A total of 150 one-day old Cobb broiler chicks were used in this study. On day 14th of age all chicks were divided into 5 equal groups (30 each). Group (G1) non-infected, non-treated (control), G2 infected with *C. perfringens*, non treated, G3 infected, treated with Sodium butyrate, G4 infected and treated with Sanguinarine phytobiotic and G5 infected and treated with ampicillin. Administration of drugs in drinking water was continued for 5 days from 18-23 day of age. The results revealed that the infected broiler chickens with *C. perfringens* and non treated(G2) showed clinical signs of necrotic enteritis represented by loss of appetite, diarrhea, dehydration, anorexia in addition to a recorded mortality rate 30% and lesion score 80%. Moreover, significant reduction ($P<0.05$) in body weight, weight gain, erythrogram, phagocytosis and phagocytic index, Nitric oxide, HI titers, total protein, albumin and total globulin beside significant increase ($P<0.05$) in feed conversion rate (FCR), leukogram, liver enzymes, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (AST-ALT and ALP), uric acid and creatinine associated with non-significant increase ($P<0.05$) in globulin fractions were observed. Treatment of the infected chickens with these drugs led to improvement in clinical signs, mortality rate, lesion score, growth performance, hematobiochemical and immunological profile compared with infected non treated birds (G2), but ampicillin had superior effect against *Costridium perfringes* infection. It could be concluded that *C. perfringens* infection in broiler chickens induced adverse effects on growth performance, hemato-biochemical and immumological profile of birds which could be reversed or ameliorated by using Sanguinarine phytobiotic, Sodium butyrate or ampicillin. So, the study recommended the use of such treatments hand in hand with antibiotics for controlling necrotic enteritis in broilers.

Key words: necrotic enteritis; Sodium butyrate; phytobiotic; ampicillin

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Introduction

Chicken meat is considered one of a high quality meat due to low its fat content (1). Therefore, poultry industry is one of the most important tool providing high quality protein for human consumption all over the world (2).

*Clostridium perfringens*is a spore-forming gram positive anaerobic rod which is a common inhabitant in the intestine of healthy broiler chickens (3). This microorganism with some predisposing factors as dietary ingredients or changes in the diet, severe stress, coccidiosis, or immunosuppressive affections can help in the overgrowth of *C. perfringens* , subsequent toxin production and development of necrotic enteritis (NE) (4). NE is an enterotoxemia of poultry which causes an important economic loss, about of two billions dollars/year in USA (5). *C. perfringens* toxogenic types (A and C) are the etiological agent associated with necrotic enteritis (6). It occurs in broilers aging between 2-6 weeks (7). The incidence of *C. perfringens* causing NE has increased since the ban of in-feed antibiotic growth promoters (AGP) (8).

Sodium butyrate is an organic acid added to poultry diet to enhance the performance and the immune response of birds (9). It is known as acidifiers and used a valuable tool in maintaining the gut health (10). It has the potential to minimize the enteric pathogens load and minimizing the pathogenic microorganism by reducing the intestinal pH (11). Also, organic acids do not require withdrawal period, increase shelf-life of poultry products they can make a valuable contribution to flock health and safety of food that might provide a significant tool for the poultry industry in combating occurrence of intestinal diseases and in reduction of food borne pathogens (12).

Phytobiotics are defined as natural feed additives that are safe to animal and poultry (13). Phytobiotics are different substances, mainly plant materials extracts as leaves, flowers, seeds, buds, fruits, twigs, root, bark, wood, and herbs (14). The active materials have many various secondary plant metabolites with wide range of physiological effects (15). Those were reported to improve broiler performance (16).

Ampicillin is semi-synthetic, broad-spectrum penicillin; it belongs to β-lactame group of antibiotic. Its only difference from penicillin is the existence of an amino group which enhances its penetration via the outer membrane of bacteria. Its effect is either bactericidal or bacteriolytic and its action is mostly marked during the active growth phase (17). It is stable in acid and well absorbed from the gastrointestinal tract, used as oral medication against systemic infections (18). However, the excessive use of antibiotics growth promotes (AGP) has resulted in the development of drug-resistant bacteria, antibiotic residues in the body of the birds and imbalance of normal microbiota (19). So, there was an increasing interest in searching for growth-promoting and immune system-strengthening alternatives.

The aim of this study was to evaluate the efficacy of Sanguinarine phytobiotic, Sodium butyrate compared to ampicillin in treatment of *C. perfringens* infection in broiler chickens, with regard to their effects on growth performance, heamatobiochemical and immunological profile.

Material and methods

Drugs and treatment

Sodium butyrate (Admix)® 30: It is water soluble formulation. It is produced by EGAVET Company (Reg. No: 1/9995-22/9/2014) and it was added in drinking water for 5 successive days. Sanguinarine (San-grovit)® It is Phytobiotics powder, natural herbal extract anti-Clostridial growth promoter, extracted from Macleaya Cordata. Obtained from Delta Vet Center, Egypt (Batch No: 1703a031) and it was added in drinking water for 5 successive days. Ampicillin trihydrate (Ampicure)®: It is water soluble powder antibiotic. It is a broad-spectrum semisynthetic antibiotic inactivated by beta-lactamases. It is relatively stable in gastric acid. It was obtained from Pharma Sweed–Egypt Company (Reg. No: 3618/2014) and was added in drinking water for 5 successive days.

Chickens

A total of 150, one-day old commercial mixed sex Cobb broiler chicks were purshased from El-Kahera poultry Company and were kept in wire floor batteries under hygienic measures. Chicks were fed commertial ready made ration obtained from Feed Mix Company.

Vaccines and vaccination

All chicks were vaccinated with Newcastle disease virus (NDV) (HitchnerB1 on 7 days and LaSota on 18 days of age) and Infectious bursal disease (IBD) vaccine on 14 days.

- ND vaccines: Hitchner B1 and LaSota live virus vaccines were obtained from Intervet Boxmeer Company. Vial contain 10^6 EID_{50} Newcastle disease virus, dissolved in physiological saline (30 mL per 1000 doses) as eye drops.
- Holland. Gumboro vaccine was obtained from Rhone-Merieau Company, France Vial dissolved in 50 ml physiological salin / 1000 bird as eye drops.

Clostridium infection

Toxigenic *C. perferingens* type A strain was obtained from Animal Health Research Institute, Dokki,Giza.On 14th day of age chicks were orally inoculated with 0.5mL of *C. perfringens* type A broth culture ($1x10^9$CFU /mL) (20).

Experimental design

On day 14 of age all chicks were randomly divided to 5 groups (30 each). Group (G)1 non-infected,non-treated (control), G2 infected orally with *C. perfringens*, non-treated, G3 infected and treated with Na-butyrate (2 ml/liter), G4 infected and treated with Phytobiotics (Sangrovit) (1 gm/10 litre) and G5 infected and treated with ampicillin trihydrate (Ampicure) (1.25 gm/litre). Treatment was started in all groups after appearance of clinical signs and was given in drinking water for 5 successive days. Chicks were weekly weighed to determine body weight, weight gain, feed consumption and feed conversion rate (FCR).

Sampling

Two blood samples from each bird at one day post treatment were collected. 1st sample was taken and divided into 2 parts, the first part was put in a tube contain EDTA as anticoagulant for the haematological studies to determine erythrogram and leukogam (total erythrocytic, leukocytic and differential leucocytic count) (21). (PCV) (22), haemoglobin content (23). The second part was put in a tube containing heparin for determination of phagocytic activity (24).

The 2nd blood sample was collected without anticoaggulant to obtain clear serum for measuring transaminases aspartate aminotransferase and alanine aminotransferase (AST-ALT) (25), Alkaline phosphatase (ALP) (26), serum total protein (27). Serum protein fractions were performed using cellulose acetate electropheresis test (28), serum uric acid (29) and creatinine (30). Determination of nitric oxide (31) and hemagglutination inhibition (HI) test for estimation titer of ND (32).

Statistical analysis

The obtained data was analyzed by using computerized SPSS program version 16 using one way ANOVA (33).

Results

The broiler chickens infected with *C. perfringens* showed clinical signs represented by loss of appetite, depression, polydipsia, ruffled feathers, diarrhea, dehydr-ation, emaciation, in addition to, a recorded mortality rate was 30% and lesion score 80%. The broiler chickens infected with *C. perfri-ngens* (G2) showed a significant reduction ($P<0.05$) in body weight, weight gain, feed consumption beside increase in feed conversion rate. Growth performance of infected broiler chicks was improved with sanguinarine phytobiotic, sodium butyrate and ampicillin treatment (Table1).

G2 showed a significant ($P<0.05$) reduction in erythrocytic count, haemoglobin content, PCV, beside a significant increase in total leukocytic count, heterophile, lymphocyte, monocyte, eosinophile, basophile compared with G1 (Table 2).

Table 1: Effect of Sodium butyrate, Sanguinarine Phytobiotic and Ampicillin on growth performance at 1st, 7th and 14th post treatment of broiler chickens infected with *C. Perfringens*

Groups	Initial body Weight (14 day of age, g)	1st day post treatment				7th day post treatment				14th day post treatment			
		Body weight (g)	Weight gain (g)	FC	FCR	Body weight (g)	Weight gain (g)	FC	FCR	Body weight (g)	Weight gain (g)	FC	FCR
G1	455.68±0.22^{a}	791.87±2.44^{a}	336.19±1.55^{a}	487.48	1.45	1146.68±2.67^{a}	354.81±2.78^{a}	592.89	1.67	1799.67±5.22^{a}	652.99±2.98^{a}	1142.73	1.75
G2	459.96±3.86a	680.89±2.53^{c}	220.90±4.98^{c}	386.58	1.75	994.80±3.56^{c}	313.91±3.86^{c}	574.54	1.83	1563.24±4.10^{c}	568.44±4.76^{c}	1080.72	1.90
G3	459.47±0.69^{a}	740.98±2.44^{b}	281.51±1.57^{b}	470.12	1.67	1076.48±0.22^{b}	335.50±3.13^{b}	580.42	1.73	1689.76±2.32^{b}	613.28±3.89^{b}	1103.90	1.80
G4	457.98±0.49^{a}	738.79±2.44^{b}	280.81±1.69^{b}	474.57	1.69	1079.40±0.22^{b}	340.61±3.37^{b}	579.04	1.70	1680.24±2.22^{b}	600.84±1.65^{b}	1093.53	1.82
G5	461.47±0.38^{a}	780.98±0.22^{a}	319.59±1.47^{a}	485.78	1.52	1134.86±1.67^{a}	353.88±6.36^{a}	583.90	1.65	1781.87±3.52^{a}	647.01±3.67^{a}	1138.37	1.76

G1: Control; G2: Infected non treated, G3: Infected+Na But; G4: Infected +Phyto; G5: Infected + Amp. Values were means ± standard error. Means within the same column with different superscripts were significantly different (P<0.05) FCR: feed conversion rate, FC: feed consumption.

Table 2: Effect of Sodium butyrate, Sanguinarine Phytobiotic and Ampicillin on erythrogram and leukogram in broiler chickens infected with C. perfingens

Groups	RBCs (106/ mm3)	Hb (gm/dl)	PCV (%)	Total WBCs X10^{3}/µl	Deferential leukoctic count X10^{3}/µl				
					heterophils	lymphocyte	monocyte	eosinophils	Basophils
G1	4.27±0.22^{a}	10.08±0.35^{a}	30.15±0.30a	11.13±0.42^{c}	2.67±0.15^{c}	3.61±0.29^{c}	1.38±0.15^{c}	1.73±0.13^{c}	1.74±0.07^{c}
G2	2.79±0.22^{c}	7.06±0.36^{c}	26.21±0.51^{c}	15.22±0.90^{a}	3.73±0.12^{a}	4.99±0.25^{a}	2.08±0.11^{a}	2.43±0.10^{a}	1.99±0.05^{a}
G3	3.41±0.16^{b}	8.17±0.57^{b}	28.79±0.64^{b}	13.28±0.99^{b}	3.21±0.20^{b}	4.42±0.30^{b}	1.74±0.13^{b}	2.05±0.10^{b}	1.86±0.06^{c}
G4	3.51±0.24^{b}	8.19±0.54^{b}	28.80±0.52^{b}	13.30±0.91^{b}	3.29±0.18^{b}	4.36±0.19^{b}	1.73±0.15^{b}	2.07±0.11^{b}	1.85±0.08^{c}
G5	3.94±0.36^{a}	9.81±0.45^{a}	29.87±0.71^{a}	11.59±0.91^{c}	2.89±0.15^{c}	3.72±0.48^{c}	1.44±0.17^{c}	1.79±0.10^{c}	1.75±0.06^{c}

G1: Control; G2: Infected non treated, G3: Infected+Na But; G4: Infected +Phyto; G5: Infected + Amp. Values were means ± standard error. Means within the same column with different superscripts were significantly different (P<0.05).

Phagocytosis and phagocytic index were significantly decreased in infected broiler chickens compared with non-infected broiler chickens (Table 3). Similarly, nitric oxide and HI titer against Newcastle were inhibited. In contrast, the treated broiler chickens with Sanguinarine phytobiotic, Sodium butyrate and ampicillin showed enhancing in phagocytosis and phagocytic index, Nitric oxide and HI titers against Newcastle (Table 3).

Total protein and albumin contents were significantly decreased (P<0.05) in infected broiler chickens compared with G1 (Table 4). Infected broiler chickens treated with ampicillin showed increasing in total protein and albumin contents compared with other treatments (Table 4). While α, β, and γ globulin contents were non-significantly inhibited under ampicillin treatment. Liver enzymes and kidney function were increased in broiler chickens infected with *C. perfringens* compared with non-infected non treated group (Table 5).

Table 3: Effect of Sodium butyrate, Sanguinarine Phytobiotic and Ampicillin on phagocytic activity %, phagocytuc index, Nitric oxide and HI titers against ND in broiler chickens infected with *C. perfringens*

Groups	Phagocytosis	Phagocytic index	Nitric oxide	HI titers against Newcastle
G1	58.68±2.81^{a}	2.80±1.30^{a}	19.05±1.32^{a}	2.43±1.32^{a}
G2	54.32±2.92^{c}	2.00±0.32^{c}	14.67±0.17^{c}	2.00±0.39^{b}
G3	56.36±3.84^{b}	2.61±0.31^{b}	16.85±0.33^{b}	2.25±0.18^{ab}
G4	56.42±2.85^{b}	2.62±0.25^{b}	16.99±0.52^{b}	2.40±0.31^{ab}
G5	57.75±1.90^{a}	2.60±0.37^{b}	19.65±0.46^{a}	2.25±0.18^{ab}

G1: Control; G2: Infected non treated, G3: Infected+Na But; G4: Infected +Phyto; G5: Infected + Amp. Values were means ± standard error. Means within the same column with different superscripts were significantly different (P<0.05).

Table 4: Effect of Sodium butyrate, Sanguinarine Phytobiotic and Ampicillin on serum total protein, albumin and globulin fraction in broiler chickens infected with *C. perfringens*

Group	T.P	Alb	Globulin(Gm/dl)				A/G ratio
			α	β	γ	Total	
G1	5.67±0.10^{a}	2.95±0.15^{a}	0.75±0.10^{a}	0.93±0.18^{a}	1.04±0.14^{a}	2.72±0.05^{a}	1.08±0.19^{a}
G2	4.07±0.11^{b}	2.05±0.10^{b}	0.84±0.12^{a}	0.92±0.14^{a}	0.92±0.16^{a}	2.02±0.11^{c}	1.01±0.14^{a}
G3	5.03±0.25^{ab}	2.53±0.12^{ab}	0.80±0.16^{a}	0.90±0.12^{a}	0.90±0.11^{a}	2.60±0.10^{ab}	1.01±0.10^{a}
G4	5.24±0.16^{a}	2.55±0.15^{a}	0.79±0.13^{a}	0.91±0.14^{a}	0.89±0.11^{a}	2.59±0.02^{ab}	1.02±0.09^{a}
G5	5.32±0.25^{a}	2.75±0.06^{a}	0.74±0.16^{a}	0.85±0.14^{a}	0.88±0.15^{a}	2.47±0.09^{b}	1.07±0.06^{a}

G1: Control; G2: Infected non treated, G3: Infected+Na But; G4: Infected +Phyto; G5: Infected + Amp. Values were means ± standard error. Means within the same column with different superscripts were significantly different (P<0.05). TP: total protein; Alb: albumin and A/G ratio: albumin/globulin ratio.

Table 5: Effect of Sodium butyrate, Sanguinarine Phytobiotic and Ampicillin on liver enzymes and kidney function in broiler chickens infected with *C. Perfringens*

Group	Liver enzymes (U/L)			Kidney function(mg/dl)	
	AST	ALT	ALP	Uric acid	Creatinine
G1	39.35±0.74^{c}	48.96±1.25^{c}	235.32±1.31^{c}	4.20±0.39^{c}	1.04±0.11^{c}
G2	55.59±0.66^{a}	55.29±0.93^{a}	350.10±1.42^{a}	7.58±0.48^{a}	2.21±0.18^{a}
G3	47.92±0.71^{b}	52.44±1.11^{b}	243.60±1.80^{b}	6.15±0.28^{b}	1.40±0.09^{b}
G4	46.66±0.48^{b}	52.53±1.22^{b}	245.02±1.81^{b}	6.25±0.27^{b}	1.35±0.04^{b}
G5	41.42±0.61^{c}	50.07±1.20^{c}	244.41±1.72^{b}	5.85±0.19^{b}	1.33±0.02^{b}

G1: Control; G2: Infected non treated, G3: Infected+Na But; G4: Infected +Phyto; G5: Infected + Amp. Values were means ± standard error. Means within the same column with different superscripts were significantly different (P<0.05). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase and ALP: Alkaline phosphatase.

Discussion

The current work was designed to investigate the efficacy of Sanguinarine phytobiotic, Sodium butyrate compared with ampicillin in treatment of *C. perfringens* infection in broilers with a special reference to their effects on growth performance, hemato-biochemical and immunological parameters. The clinical signs of NE in G1 may be due to toxin excreted by *C. perfringens* which caused high mortality rate (34). Also, Olkowski et al. (35) stated that, *C. perfringens* infections in poultry induced clinical signs as loss of appetite, depression, diarrhea, emaciation and high mortality rate. Broiler chickens infected with *C. perfringens* showed typical clinical signs, mortality rate and reduction in growth performance of chickens (36,37).

Medication of broilers infected with *C. perfringens* using sodium butyrate, sanguinarine phytobiotic or ampicillin was effective and resulted in disappearance of clin-ical symptoms and improved growth performa-nce and health status of infected birds. Lee et al. (13) reported that, infected chicks received phytobiotic showed improved body weight, weight gain and feed conversion due to phytobiotic had good effect on *C. perfringens* colonization in intestines of broiler chickens (38). Cross et al. (16) stated that, phytobiotic in ration probably prevent necrotic clinical signs and improved body performance by their direct antimicrobial effect on *C. perfringens***.** Good efficacy of a phytobiotic for improving clinical signs, lesion scores and body performance in experimental necrotic enteritis may be due could be attributed to the presence of essential oils in phytobiotic that could improve nutrient digestibility due to enhance the activities of trypsin and amylase (39,40).

Lesson et al. (41) recorded that, butyric acid play a role in controlling the proliferation of *C. perfringens* in broiler chicken**s** leading to improve body performance by increasing villus height lead to increase absorption of nutrients**.** Sodium butyrate has beneficial effects in controling NE in chickens by disappearing clinical signs and improving body weight (42). Furthermore, sodium butyrate have dose-dependent activity against necrotic enteritis (43,44).

Ampicillin was very effective in treatment of necrotic enteritis in broilers as it improved body weight, weight gain, feed consumption and FCR. These findings were attributed to that, *C. perfringens* was highly sensitive to ampicillin and antimicrobial compounds in poultry industry as improved poultry health and the health status of the infected chickens as evidenced by disappearance of clinical signs of the disease and improved body performance (45). While, broilers chickens infected with *C. perfringens* showed significant reduction in weight gain coupled with elevation in feed conversion rate (46). Moreover, another beta lactam (amoxicillin) have good potency in treatment of NE in broiler chickens and induced disappearance of clinical signs and improved body performance (37).

The current work revealed that, broiler chickens infected with *C. perfringens* showed significant decrease in total erythrocytic count, hemoglobin content and PCV, associated with significant increase in leukocyte, heterophil, lymphocyte eosinophil's basophils and monocyte. The changes in haematological parameters in broiler suffering from NE might be due to bacterial toxin (47)**.** The decrease in erythrogram might be due to intravascular hemolysis induced by *C. perfringens* (48). Clostridial toxins caused breakdown of phospholipids of erythrocytes membrane and cause hemolysis by damaging circulating erythrocytes (49). Similar findings were previously reported (50-52).

Our results revealed that, oral administration of sodium butyrate, sanguinarine phytobiotic or ampicillin for treatment of clostridial infection in broiler chickens induced significant improvement in both erythrogram and leukogram.

The essential oils which present in phytobiotics reduced *C. perfringens* count in broiler and stimulating the absorption of essential nutrients as minerals and protein induced increase in erythrocyte and hemoglobin content when compared with infec-ted non-treated group (53). Also, phytobiotics suppress intestinal bacteria due to its

antibacterial effect leading to enhancement of both erythrogram and leukogram (54,55).

Sodium butyrate had bacteriostatic or bactericidal effect by penetrating the bacterial cell wall and dissociate to H^+ and anions inside the cell, lowering pH and resulting in energy deficiency and osmotic problems in the organism. This led to improvement of biological function of host as blood picture (56).

Hematological parameters in chickens suffering from NE were improved towards the normal level after treatment with ampicillin, this improvement might be due to antimicrobials effects which suppress the invasion of *C. perfringens* so improved absorption of essential substance for erythropoiesis (57).

Our study revealed that broiler chickens infected with *C. perfringens* revealed significant reduction in phagocytosis, Phagocytic index, Nitric oxide and HI titers of ND compared with control group. According to Coles (58), Grilli et al. (59), Yitbarek et al. (60) and Chake et al. (61), *C. perfringens* infection in broiler revealed reduction in phagocytosis, phagocytic index, Nitric oxide and HI titers of ND.

Infected broiler chickens with *C. perfringens* and treated with sodium butyrate, sanguiranine phytobiotic or ampicillin showed improvement in phagocytosis and Phagocytic index, Nitric oxide and HI titers of ND..This in accordance with Wati et al. (62) that broiler chickens received ration contain phytobiotic prevent necrotic enteritis and improved phagocytosis and Phagocytic index and improved immunological parameters as Nitric oxide and HI titers of ND.

Sodium butyrate could stimulate host defense in chicken, by increase the activity of chicken phagocytosis, phagocytic index and nitric oxide production (63-65). Butyric regulates the macrophage activities in the intestine and the macrophage regulates the function of T cells and dendritic cells in the gut which have role in host immunity (66).

Elevation in liver enzymes (AST, ALT, and ALP), uric acid and creatinine in broiler chickens infected with *C. perfringens* might be due to liver and kidney damage by closterdial toxins (47). Reduction in proteinogram in infected chicks might be attributed to a state of anorexia and mal absorption of nutrients from inflamed intestine leading to inability of liver to synthesis proteins (67). Similarly, Mabrouk, (68) stated that broiler chicken infected with *C. perfringens* showed significant decrease in serum total protein, albumin and globulin associated with significant increase in liver enzymes (AST, ALT and ALP), Uric acid and creatinine. Medication of *C. perfringens* infected broiler chickens using sodium butyrate, phytobiotic or ampicillin showed improvement in serum total protein, albumin, globulin, liver enzymes (AST, ALT and ALP), uric acid and creatinine when compared with G2.

Phytobiotics suppressed *C. perfringens* infection in broiler chickens and improved protein picture. They act on the intestinal walls, promoting the absorption of more nutrients and secretion of digestive enzymes which enhance the nutrient digestibility leading to improved protein profile (69,70). The same results were reported by Lesson et al. (41) who stated that, sodium butyrate improved liver enzymes and protein picture in broiler suffering from NE. Ampicillin is very important in treatment of NE in chickens and improved both liver and kidney function and showed improvement in protein picture (44,50).

Conclusion

It could be concluded that experimental oral infection with *C. perfringens* in broiler chickens induced adverse effects on health state, growth performance, hemato-biochemical and immumological profile of birds which could be reversed or ameliorated by using Sanguinarine phytobiotic, Sodium butyrate or ampicillin. So, the study recommended the use of such tratments hand in hand with antibiotics for controlling of necrotic enteritis in broilers, but ampicillin had superior action aginst *Clostridium perfringens* infection.

Conflict of interest

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

References

1. Kamboh A, Zhu W. Effect of increasing levels of bioflavonoids in broiler feed on plasma anti-oxidative potential, lipid metabolites, and fatty acid composition of meat. Poult Sci.2013; 92: 454–61.

2. Hussain J, Rabbani I, Aslam S, Ahmed H. An overview of poultry industry in Pakistan. World Poultry Science Journal. 2015; 71: 689–700.

3. Florence L, Hakim S, Thong K. Determination of toxinotypes of inviromental *C. perfringens* by polymerase chain reaction. Trop. Biom. 2011; 28: 171–4.

4. Llanco L, Viviane J, Nakano J, Ferreira A, Avila-Campos M.J. Toxinotyping and antimicrobial susceptibility of *Clostridium perfringens*isolated from broiler chickens with necrotic enteritis. Inter. J. of Microbiol.2012; 4: 290–4.

5. Kaldhusdal M, Lovland A. The economical impact of *Clostridium perfringens* is greater than anticipated. World Poultry. 2000; 16: 50–1.

6. Caly D, L, D'Inca R, Auclair E, Drider D. Alternatives to Antibiotics to Prevent Necrotic Enteritis in Broiler Chickens: A Microbiologist's Perspective. Front Microbiol. 2015; 6, 1336. doi: 10.3389/fmicb2.015.01336

7. Cooper K, Songer J. Virulence of *Clostridium perfringens* in an experimental model of poultry necrotic enteritis. Vet Microbiol, 2010; 142: 323–8.

8. Gunal M, Yayli G, Kaya O, Karahan N, Sulak O. The effects of antibiotic growth promotors, probiotic or organic acid supplementation performance, intestinal microflora and tissue of broilers. International Journal of Poultry Science. 2006; 5: 149–55.

9. Dibner J, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res.,2002; 11: 453–63.

10. (10)Sultan A, Bilal M, Khan S, Hassan Z. Effect of chlorine dioxide (dutrion (R) on growth performance, gut histomorphology and pathogenic microbial count of meat type birds. Pak. Vet. J.,2015; 35: 183–7.

11. Khan S.H, Iqbal J. Recent advances in the role of organic acids in poultry nutrition. J. Appl. Anim. Res., 2016; 44: 359–69.

12. Awaad M., Atta A., Shalaby Basma H., Gharieb M., Elmenawey K., Farag Eman F. and Awad Amal, N. The Efficacy of Na-Butyrate Encapsulated in Palm Fat on Experimentally Induced Necrotic Enteritis and Enumeration of intestinal resident *Clostridium perfringens* in Broiler Chickens . J of Agriculture and Vet Sci.2014; 7: 40–4.

13. Lee K, Everts H, Frehner M, Losa R, Beynen A. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poult. Sci.2003; 44: 450–7 .

14. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. J Anim Sci, 2008; 86: 140–8.

15. Burt S. Essential oils: their antibacterial properties and potential applications in foods -a review. Int J Food Microb, 2004; 94: 223–53.

16. Cross D, McDevitt R, Hillman, K, Acamovic T. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. British Poultry Science, 2007; 48: 496–506.

17. Gharaibeh S, AlRifai R, Al-Majali A. Molecular typing and antimicrobial susceptibility of *Clostridium Perfringens* from broiler chickens. Anaerobe: 2010; 16: 586–9.

18. Goren E, De Jong W, Solkema A. some pharmacokinetical aspects of ampicillin trihydrate and its therapeutic efficacy in experimental *Escherichia coli* infection in poultry . Avian Pathology,1981; 10: 43–55.

19. El-Ghousein S, Al-Beitawi N. The Effect of Feeding of Crushed Thyme (Thymus valgaris L) on Growth, Blood Con-stituents, Gastrointestinal Tract and Carcass Characteristics of Broiler Chickens. Journal of Poultry Science. 2009; 46: 100–4.

20. Wafaa A, Abd El-Ghany. Comparative Evaluation on the Effect of Coccidostate and Synbiotic Preparation on prevention of *Clostridium perfringens* in broiler chickens. Global Veterinaria.2010; 5: 324–33.

21. Natt M, Herrick A. A new blood diluent for counting the erythrocytes and leukocytes of chickens. Poultry. Sci. 1952; 31: 735–8.

22. Cohen R. Anticoagulation, centrifugation time and sample replicate number in micro haematocrite method for avian blood. Poultry Sci., 1967; 46: 216.

23. Wintrobe M. Clinical Haematology. 6th ed. Lea and Febiger, 1967; Philadelphia: 1287.

24. Lucy F, Larry D. Ontageny and line difference in mitogenic responses of chicken lymphocyte, Poultry sci.1982; 62: 579–84.

25. Reitman S, and Frankel S. Acalorimetric method for determination of serum glutamic

oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol.1957; 28: 56–63.

26. Kind P, King E. Estimation of plasma phosphatase by determination of hydrolyzed phenol with aminoantipyrine. J. Clin. Path. 1954; 7: 322–6.

27. Doumas B, Carter R, Peers T, Schaffier R. A candidate reference method for determination of total protein in serum. Clin.Chem. 1981; 27: 1642.

28. Davis B. Disc electrophoresis. II. Method and application to human serum proteins. Ann. NY Acad. Sci., 1964; 121: 404–27.

29. Henry R, Cannon D, Winkelman J. Clinical Chemistry: Principals and Techniques. Harper and Row, Hagrstown. 1974; 437–40.

30. Husdan H, Rapoport, A. Estimation of creatinine. Clin. Chem.1968; 14: 222–38.

31. Green L, C, Wanger D, A, Glogowski J, Skipper P, L, Wishnok J, S, Tanninbaum S, R. Analysis of nitrites and nitrates in biological fluids. Analytical Biochemistry, 1982; 126: 131–8.

32. Villagas P. Newcastle Disease Virus Titration, Avian Virology (AM-508), Lab. Manual. 1991; 6–18.

33. Tamhane F, Dunlop N. Statics and data analysis from elementary to intermediate. Upper Saddle River, USA.Prentice Hall. 2000; 722.

34. Maddy F, Eman R, Hassan A, Radwan Nagwa S, Rady M. Effect of probiotic on necrotic enteritis in chickens with presences of immunosuppressive factors. Global Vet. 2012; 9: 44–50.

35. Olkowski A, Wojnarowicz M, Chirino T, Drewa M. Responses of broiler chickens orally challenged with *Clostridium perfringens* isolated from field cases of necrotic enteritis. Res. in Vet. Sci. 2006; 81: 99–108.

36. Gamal A. Concurrent use of diclazuril and lincomycin for controlling of severe necrotic enteritis in broiler chicks. MVSc., Thesis (pharmacology) Faculty of Vet Med, Zag University 2016.

37. Mohamed E, Mohamed A. Efficacy of Flagymox® (Amoxicillin and Metronidazole Combination) in Controlling *Clostridium perfringens* infection in Broiler Chickens. World J of Pharmacy and Pharmaceutical Sci.2017; 6: 80–95.

38. Mitsch P, Zitterl-Eglseer B, Kohler C, Gabler R, Losa K, Zimpernik I. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens*in the intestines of broiler chickens. Poult. Sci. 2004; 83: 669–75.

39. McReynolds J, Waneck J, Byrd K, Genovese S, Duke C, Nisbet D. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. Poult. Sci.2009; 88: 2075 – 2080.

40. Lee K, Kim J, Oh S, An B. Effects of dietary sanguinarine on growth performance, organ weight, cecal microflora, serum cholesterol level and meat quality in chickens. J Poult Sci. 2015; 52: 15–22.

41. Leeson S, Namkung H, Antongiovanni M, Lee E. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poultry Science, 2005; 84, 1418–22.

42. Timbermont L. A contribution to the pathogenesis and treatment of *Clostridium perfringens*-associated necrotic enteritis in broilers. PhD Diss. Ghent University, Faculty of Veterinary Medicine, Ghent, Belgium 2009.

43. Timbermont L, Lanckriet J, Dewulf N, Nollet K, Schwarzer F, Haesebrouck R, Ducatelle A, Van Immerseel F. Control of *C. perfringens*-induced necrotic enteritis in broilers by target-released butyric acid, fatty acids, and essential oils. Avian Pathol. 2010; 39: 117–21.

44. Jerzsele A, Szeker R, Csizinszky E, Gere C, Jakab J, Mallo K, Galfi P. Efficacy of protected sodium butyrate, a protected blend of essential oils, their combination, and Bacillus amyloliquefaciens spore suspension against artificially induced necrotic enteritis in broilers. Poultry Sci Assoc Inc.2011; 837–43.

45. Das B, Gupta G, Phukan A. Experimental production and treatment of necrotic enteritis in fowel. Indian Journal of Poultry Science. 1997; 32: 59-66.

46. Liu D, Guo Y, Wang Z, Yuan J. Exogenous lysozyme influences *Clostridium perfringens* colonization and intestinal barrier function in broiler chickens. Avian Pathol. 2010; 39: 17–24.

47. Doxy D. Clinical pathology and diagnostic procedure 2nd Ed. Baillier London.1983; 56–60.

48. Topley Y, Wilsons T. Microbiological and Microbial Infections 9 th Ed. Vol 3 Systemic bacteriology Oxford Univ. Press, USA. 1999; 52: 237–8.

49. Allam H, Dina M, Abdullaha S, Nahad G. Immuno-biochemical and pathological studies on necrotic enteritis in pekin duckling with trail of treatment. Mansoura Vet. Med. J. 2013; 12: 45–53.

50. El-Gharbawy E. Concurrent use of amoxicillin and metronidazol for controling closterdial problems in broiler chickens. M.Sc.

Thesis Fac. of Vet Med. (Pharmacology) Monefia University 2014.

51. Salah H, El Sayed M, Reham R, Abd El Hamid Eman S. Study on the effect of humic acid on growth performance, immunological, some blood parameters and control intestinal *Clostridium* in broiler chickens Zag. Vet. J. 2015; 43 (1)102–9

52. Sayed A, Sabry M, Osama E, Sarhan M. Concurrent use of ciprofloxacin and metronidazole for controlling of some bacterial infections in broiler chickens. Benha Vet Med J, 2016; 31, 2: 83–92.

53. Jamroz D, Orda I, Kamel C, Wiliczkiewicz A, Wertelecki T, Skorupinska I. The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. J. Anim. Feed Sci.,2003; 12: 583–96.

54. Vidanarachchi J, Mikkelsen L, Sims I, Choct M. Phytobiotics: alternatives to antibiotic growth promoters in monogastric animal feeds. Recent Adv. Anim. Nutr. Aust. 2005; 15, 131–44.

55. Ganguly S. Promising Pharmaceutical Effect of Various Biological and Inorganic Agents as Feed Supplements for Livestock and Poultry with Discussion on Research proven Facts and Establishment of Concept.*Research in Pharmacy and life Sci*, IJRPLS, 2013; 1: 115–20.

56. Dahiya J, Wilkie D, Van Kessel A, Drew M. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim. Fed. Sci. Tech.2006; 129: 60–88.

57. Kamel M. Interaction between danofloxacin and fofluperdone acetate in chickens MVSc, Thesis (pharmacology), Faculty of Vet Med, Zag. Univ. 2004.

58. Coles E. Veterinary clinical pathology. 4th Ed. W. B. Saunders Company Philadelphia U. S. A 1986; 17-19.

59. Grilli E, Catelli E, Piva A. Pediocin A improves growth performance of broilers challenged with *Clostridium perfringens*. Poult Sci.2009; 88: 2152–8.

60. Yitbarek A, Echeverry H, Rodriguez C. Innate immune response to yeast-derived carbohydrates in broiler chickens fed organic diets and challenged with *Clostridium perfringens*. Poult Sci. 2012; 91: 1105–12.

61. Chake K, Birger S, Robert S, Natalie M, Mingan C. An early feeding regime and a high-density amino acid diet on growth performance of broilers under subclinical necrotic enteritis challenge Anim Nutr. Mar; 2017; 3: 25–32.

62. Wati T, Ghosh T, Syed B, Haldar S. Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. Animal Nutrition, 2015; 1: 213–9.

63. Sunkara L, Achanta M, Schreiber N, Bommineni Y, Dai G, Jiang W, Lamont S, Lillehoj H, Beker A, Teeter R. Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. PLoS One. 2011; 6: e27225.

64. Van Deun K, Pasmans F, Van Immerseel F, Ducatelle R, Haesebrouck F. Butyrate protects Caco-2 cells from *Campylobacter jejuni* invasion and translocation. Br J Nutr. 2008; 100: 480–4.

65. Jahanian R. Effect of varing levels of butyric acid glycerides on performance, immune response and jejuna epithelium morphology of broiler chicks. In 18th European Symposium on poultry nutrition Izmir. Turkey. 2011; 213–5.

66. Belih S, EL-Hadad Seham F, Amen Ghada E, Basiony Maha R. Influence of sodium butyrate on *Salmonella* infection in broiler chicks. Benha. Vet. Med. J, 2016; 31: 21–32.

67. Kaneko J. Clinical biochemistry of domestic animals.4th ed. Academic Press, Inc., New York, London, 1989; 339 -365.

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

69. Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella Typhimurium*, *Closterdium perferenges* and *Listeria monocytogenes*. Food Cont., 2007; 18: 414–20.

70. Abudabos A, Alyemni A, Dafallah Y, Khan R. The effect of phytogenic feed additives to substitute in feed antibiotics on growth traits and blood biochemical parameters in broiler chicks challenged with *Salmonella typhiymurium.* Environ Sci. Poll. Res. 2016; 23: 24151–7.