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Effect of using synbiotics and essential oils on performance parameters and immune response of necrotic enteritis challenged broiler chicks.

Amal, H. T. Abdelnaser; Magda, M. A. Moustafa and Marwa, I. Abd El-Haleem
Department of Avian and Rabbit Diseases, Faculty of Veterinary Medicine, Benha University

A B S T R A C T

One hundred and eighty healthy one-day-old broiler chicks were used to evaluate the effect of synbiotics and/ or essential oils (EO) as alternatives to antibiotics on performance in induced necrotic enteritis (NE) and immunity. EO treatment improved the total feed conversion ratio (FCR) in comparison with challenged non-treated group and antibiotic treated group. While using synbiotics did not ameliorate the negative impact of necrotic enteritis challenge on feed intake and FCR. The treatments had positive impact on immune response to vaccination against Newcastle disease (ND), infectious bronchitis (IB), avian influenza (AI) and infectious bursal disease (IBD) as well as they increased the spleen relative weight. The synbiotic treatment alleviate the histopathological changes from vaccination in bursa of Fabricius (BF) as well as antibiotic treated group. The results suggest that EO and synbiotics, as replacements to antibiotics, may be an effective tool to augment performance and immune response of NE challenged birds.

Keywords: *Necrotic enteritis, performance, Synbiotics, immunity, Essential oils, broilers.*

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1. INTRODUCTION

Antibiotics have been common feed additive in poultry rations as they were frequently used for prophylaxis against diseases in birds (Sun *et al*., 2005). The continued feeding of antibiotics at subtherapeutic levels caused imbalance of gut microflora, antibiotic residue and developed drug-resistant bacteria (Jensen, 1998 and Andremont, 2000)]. Consequently, the Europe Union Commission banned antibiotics in animal feeds since 2006 (Europe Union Commission, 2005). Nevertheless, several countries implied to banning suffered from rearise of necrotic enteritis outbreaks in broilers (Van Immerseel *et al*., 2004).

Necrotic enteritis is an enterotoxemic multi-factorial disease that destroys the intestinal lining of the digestive tract of both wild and domestic birds especially chickens used for meat production all over the world. Some toxins secreted from *C. perfringens* types A and C are incriminated in the necrotic lesions in the intestinal wall and mortalities which in turn leads to high economic losses estimated to be more than $3 billion/ year (Jayaraman *et al.*, 2013).

The use of dietary additives as synbiotics and phytobiotics is gaining momentum and paid an attention to be used as alternatives to antibiotics because of their beneficial effects on performance and gut microflora (Mitsch *et al*., 2004 and Dahiya *et al*., 2006).

Synbiotics are mixture of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and / or activating the metabolism of one or a limited number of health promoting bacteria, and hence improving host welfare (Fooks and Gibson, 2002).

The phytobiotics are natural new class of additives that based on herbs, spices and fruits. Essential oils, as a category of phytobiotics, are volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation (Khan, 2014). They enhance the productivity through improvement of digestibility, nutrient absorption and elimination of pathogens in the gut as well as, they have a high acceptability among consumers (Kubkomawa *et al*., 2013).

The goal of this work is to evaluate the potential protective effects of synbiotic, essential oils and their combination as alternatives to antibiotic feed additives on performance and immunity in the NE challenged broiler chicken.

2. MATERILAS AND METHODS

2.1. Birds and management:

The present experiment conducted on 180, healthy one-day-old broiler chicks (Arbor Acres). Birds fed on well-balanced diet (Table 1) formulated according to (NRC, 1994). They were housed in a clean disinfected well-ventilated room (5m×7m). The room divided into 5 equal partitions for the first five groups (G1 – G5). The control group (G6) housed in a separate place. The rooms provided with suitable number of heaters, feeders and drinkers.

2.2. Experimental design:

The experimental birds were randomly divided into 6 groups (30 birds/3 replicates / group). 1, Antibiotic and anti coccidia (ABAC) group: challenged with *Eimeria spp. + C. perfringens* and fed basal diet with chlortetracycline Hcl at rate 1 kg/ ton (Atco pharma) and diclazuril at rate 200gm/ton (Atco pharma); 2, Synbiotic (SY) group: challenged with *Eimeria spp. + C. perfringens* and fed basal diet with synbiotic based on *Bacillus subtilis, B. licheniformis, Saccharomyces cerevisiae,* beta glucan and mannanoligosccharides at rate 250 gm/ton (organic chemical solutions, L.L.C. – USA); 3, Essential oils (EO) group: challenged with *Eimeria spp. + C. perfringens* and fed basal diet with essential oils of oregano, anise, and citrus peel at rate of 125 gm/ton (BIOMIN Singapore Pte Ltd); 4, Synbiotic and essential oils (SYEO) group: challenged with *Eimeria spp. + C. perfringens* and fed basal diet with combination of synbiotics and essential oils at same doses mentioned before; 5, Necrotic enteritis (NE) group: fed basal diet and challenged with *Eimeria spp. + C. perfringens* and 6, CN group (control negative): fed basal diet only. The SY and SYEO groups were sprayed at hatchery by probiotic based on 5×10^{12} CFU of *Enterococcus spp., Bifidobacterium spp., Pedicoccus spp. and Lactobacillus spp*. (BIOMIN Singapore Pte Ltd) at rate of 20 gm / 1000 bird. Experimental birds fed diet with additives from zero to 35 days of age and vaccinated against ND, IB, AI and IBD as shown in table (2).

2.2. Experimental induction of NE:

The induction of necrotic enteritis was done according to Gholamiandehkordi *et al*., (2007). At 12 d, 10-fold dose of commercial live attenuated coccidia vaccine (Coccivac B^{TM}-Schering plaugh animal health) based on *E. acervulina, E. maxima, E. Necatrix and E. tenella* given orally to birds of groups 1 to 5 to mimic the detrimental effect of coccidiosis. At 14 days ; the same groups were given 10^5 of intermediate IBD virus (Cevac^{TM} Gumbol - Ceva). At 17 days the birds of groups 1-5 were orally challenged once daily for three successive days by 1ml of 4×10^8 CFU/ml of type A α-toxigenic field strain of *C. perfringens* that obtained from the department of anaerobes; Veterinary Serum and Vaccines Research Institute in Abbasia.

2.3. Performance parameters:

2.3.1.Feed intake (FI):

The FI calculated weekly by dividing the amount of feed consumed in grams (by a certain group) by the number of chicks of this group during the same week.

2.3.2. Feed conversion ratio (FCR):

Feed conversion ratio calculated weekly according to Lambert *et al.,* (1936) by the following equation:

$$FCR = \frac{\text{Average feed intake (g) bird/week}}{\text{Average body weight gain (g) bird/week}}$$

2.4. Humeral immune response to viral vaccines:

Antibody titers against ND and AI vaccination were measured twice at 3rd and 5th weeks of

experiment by haem agglutination inhibition (HI) test according to OIE, (2012).
Antibody titer against IB and IBD vaccination were measured twice at 3rd and 5th weeks of experiment by using specific ELISA kits in accordance with the protocols specified by the OIE, (2012).

2.5. The relative weight of immune organs:

Bursa of Fabricius (BF), thymus and spleen were harvested from the euthanized birds at 23 day of age. These organs were weighed and their relative weights were calculated as organ weight/live BW×100 (Verma *et al.,* 2004).

2.6. Histopathological examination:

Bursa of Fabricius and thymus were collected at 23 d of age and preserved in 10% formalin solution and examined according to Bancroft *et al*., (1996).

2.7. *Statistical analysis*:

Differences between groups were analyzed by using One-Way ANOVA and Duncan's multiple comparison Post Hoc tests (Duncan, 1955).

Table 1: Composition of starter, grower and finisher diets.

Ingredients	%		
	Starter	Grower	Finisher
Yellow corn	53.85	58.40	61.24
Soyabean meal 47	34.6	28.70	28.70
Corn gluten meal	4.15	5.00	1.65
Vegetable oil	2.50	3.40	4.40
Sodium chloride	0.30	0.30	0.30
DL-Methionine	0.27	0.25	0.23
L-Lysine	0.22	0.35	0.12
Limestone	1.90	3.40	1.55
mono-calcium phosphate	1.43	1.20	1.10
Sodium bicarbonate	0.18	0.17	0.17
Vitamins and mineral premix	0.30	0.30	0.30

Table 2: Vaccination programme.

Age	Type of vaccine	Type and dose	Route	Company
7d	Cevac™ BIL (Hitchner+ IB)	Live - 10^9 / bird	Eye drop	Ceva
10d	Avian influenza (Subtype H_5N_2- N28 strain)	Inavtivated – 10^7 / bird	S/C	QYH- Biotech limited
14d	Cevac™ Gumbol (IBD-intermediate)	Live – 10^5 / bird	Eye drop	Ceva

3. RESULTS

3.1. Performance parameters:

During the third week all treated groups showed significant decrease in FI in comparison with the CN group, while there was non significant difference in FCR of challenged groups either treated or not when compared with the CN group (Table 3). In addition, At the end of the experiment, all challenged groups either treated or not recorded non significant difference when

compared to the CN group in TFI except the ABAC group (the highest value as 3.27 Kg) and in TFCR except the SY group (the worst value; 2.59).

3.2. Humeral immune response to vaccination:

The HI antibody titer against ND and AI vaccines shown as log 10 in **table (4)**. A significant higher titer (P<0.05) was recorded in EO and SYEO groups at 3rd week (1.11 and 1.10 respectively) in comparison with NE and CN groups (0). The antibody titer disappeared in serum of experimental chicks at 4th and 5th week of experiment except for the ABAC group, which showed 0.70 titer in 5th week. The HI antibody titer against AI vaccine in SYEO and ABAC group was 0.30 at 3rd week and this was lower than NE group (0.67). A significant high titer (P<0.05) was recorded in EO group (1.42) in comparison with NE group (1.10) at 5th week of experiment. The recorded improvement was insignificantly higher than titer recorded in ABAC group (1.20) (P>0.05).

At 3rd and 5th week of the experiment ELISA antibody titer against IB vaccine was significantly higher (P<0.05) in SY group (4385and 13772 respectively) than NE group (117 and 9101.3 respectively) and ABAC group (0 and 4344 respectively). At 3rd week, the titer in EO group was higher (2543.3) than NE group (117) and ABAC group (0) (P>0.05). The used biological treatments showed insignificant low antibody titer against IBD (634.67, 808.33 and 644.33 respectively) in comparison with NE group (922.33) at 3rd week (P>0.05). In addition, the ABAC group showed significantly lower titer (233) in comparison with NE group (P<0.05). The antibody titer of EO group was significantly increased at 5th weeks (293.33) in comparison with NE and ABAC groups (0) (P<0.05). Also, a significant increase in titer (P<0.05) was recorded in SYEO group (220) in comparison with NE group.

3.3. Relative weight of immune organs:

As shown in table (3), there was mild numerical fluctuation in relative bursal weight between treated birds and +ve challenge group. The ABAC group showed the highest bursal weight (0.14) while, the SY group showed the lowest weight ratio (0.08). The SY, EO and SYEO groups did not improve the relative thymus weight (0.42, 0.33 and 0.38) in comparison with CN and ABAC groups (0.58 and 0.46 respectively). The relative weight of spleen in The NE group showed lower relative spleen weight (0.10) in comparison with CN group (0.14) (P>0.05). The relative weight of spleen was significantly increased in SYEO group (0.15) (P<0.05) while, SY and EO groups showed improvement in its relative weight (0.13 and 0.12) in comparison with NE and ABAC groups (0.09) (P>0.05).

3.4. Histopathological findings:

The BF of SY group showed mild histo-pathological alterations same as ABAC group in comparison with NE group and other treatments that showed moderate histo-pathological changes **(**Plate 1). The thymus of SY and EO groups showed mild histopatho-logical alterations as hemorrhages and depletion of medullary thymocytes (Plate 2), the same degree of lesions shown in NE group, while the ABAC group showed the least degree of histopathological alteration.

Table 3: Effect of antibiotic, synbiotic and essential oils on performance parameters and relative weight of immune organs (means ± SE).

	ABAC	SY	EO	SYEO	NE	CN
FI 3rd w	0.44±0.02^{b}	0.44±0.01^{b}	0.41±0.01^{b}	0.42±0.02^{b}	0.42±0.02^{b}	0.54±0.06^{a}
Total FI	3.27±0.25^{a}	2.69±0.02^{b}	2.78±0.19^{ab}	2.53±0.16^{b}	2.61±0.05^{b}	2.56±0.23^{b}
FCR 3rd week	1.42±0.06^{a}	1.65±0.02^{a}	1.51±0.04^{a}	1.65±0.11^{a}	1.46±0.19^{a}	1.50±0.03^{a}
Total FCR	2.43±0.26^{ab}	2.59±0.31^{a}	2.15±0.07^{ab}	2.19±0.28^{ab}	2.22±0.17^{ab}	1.88±0.18^{b}
BF%	0.14±0.04^{a}	0.08±0.02^{a}	0.11±0.02^{a}	0.09±0.02^{a}	0.11±0.02^{a}	0.10±0.02^{a}
Thymus%	0.46±0.02^{a}	0.42±0.08^{a}	0.33±0.07^{a}	0.38±0.06^{a}	0.42±0.04^{a}	0.58±0.14^{a}
Spleen%	0.09±0.00^{c}	0.13±0.01^{abc}	0.12±0.00^{abc}	0.15±0.03^{a}	0.10±0.01^{bc}	0.14±0.02^{ab}

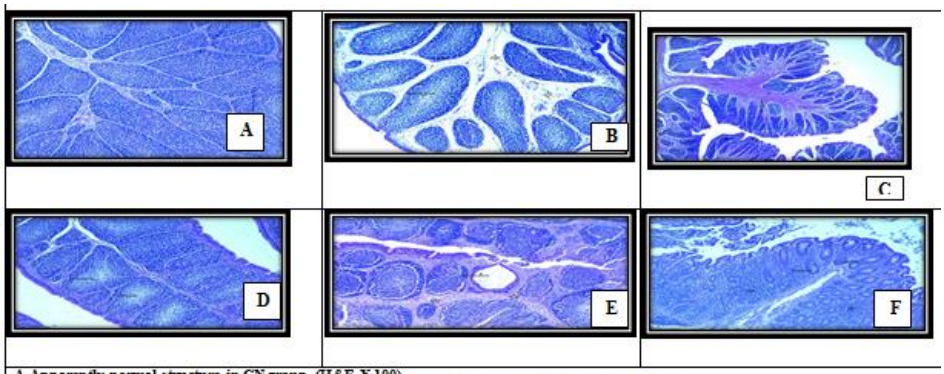
Duncan represents least significant differences between different groups at probability P< 0.05. Means with different superscripts (a, b, c, d) within a row are significantly different at P< 0.05.

Table 4: Effect of antibiotic, synbiotic and essential oils on humeral immune response to viral vaccines (means ±SE).

		ABAC	SY	EO	SYEO	NE	CN
ND	3 W	0.30±0.30 [ab]	0.00±0.00 [b]	1.11±0.56 [a]	1.10±0.20 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	5 W	0.70±0.70 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
AI	3W	0.30±0.30 bc	0.00±0.00 c	0.00±0.00 c	0.30±0.30 abc	0.67±0.12 ab	0.20±0.20 bc
	5 W	1.20±0.00 a	0.40±0.40 b	1.42±0.27 a	0.30±0.30 b	1.10±0.20 a	0.00±0.00 b
IB	3 W	0.00±0.00 b	4385.0±453.38 a	2543.3±21.18 b	0.00±0.00 b	117.00±8.88 b	142.00±8.00 b
	5 W	4344.0±446.77 c	13772±720.57 a	3594.0±162.26 c	3642.7±1102.29 c	9101.3±593.94 b	0.00±0.00 d
IBD	3 W	233.00±33.50 c	634.67±43.59 bc	808.33±95.13 ab	644.33±13.86 bc	922.33±14.19 ab	244.33±244.33 c
	5 W	0.00±0.00 [c]	0.00±0.00 [c]	293.33±23.33 [a]	220.00±11.54 [b]	0.00±0.00 [c]	0.00±0.00 [c]

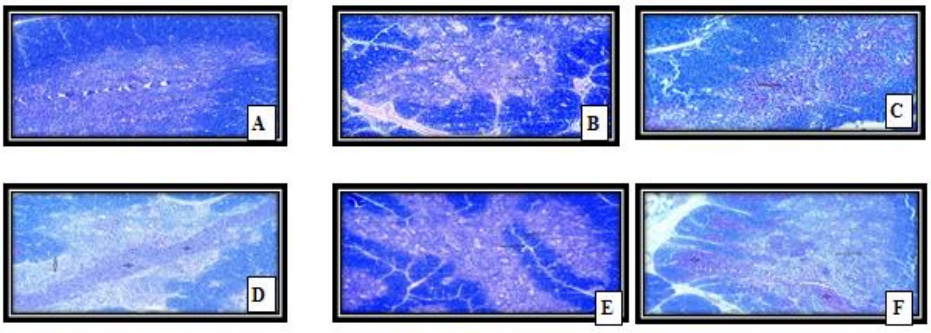
Duncan represents least significant differences between different groups at probability P< 0.05. Means with different superscripts (a, b, c, d) within a row are significantly different at P< 0.05.
*N.B.) Antibody titers against ND and AI vaccines were measured by HI test. Antibody titers against IB and IBD vaccines were measured by ELISA test.

Plate 1: Histopathological changes in bursa of Fabricius of euthanized chicks



A-Apparently normal structure in CN group. (H&E X 100).
B- Depletion of lymphocytes (arrow) with interfollicular edema (star) in ABAC group (H&E X 100).
C- Proliferation of connective tissue (arrow) and hyperplasia of lining epithelium (star) in SY group (H&E X 50).
D- Mid depletion of lymphocytes (arrow) in EO group (H&E X 100).
E- Depletion of lymphocytes, cysts (arrow) and interfollicular connective tissue (star) in SYEO group (H&E X 100).
F- Disappearance of lymphoid follicles with epithelization (arrow) and lymphocytes infiltration (star) in NE group (H&E X 100).

Plate 2: Hishological changes in thymus of euthanized chicks



A-Apparently normal thymus in CN group (H&E X 100).
B- Depletion of medullary thymocytes(arrow) in ABAC group (H&E X 50).
C- Focal hemorrhage (arrow) in SY group (H&E X 200).
D- Depletion of medullary thymocytes (arrow) and hemorrhage (star) in EO group (H&E X 50).
E- Severe depletion of medullary thymocytes (arrow) in SYEO group (H&E X 50).
F- Depletion of thymocytes (arrow) and hemorrhage (star) in NE group (H&E X 50).

4. Discussion:

The use of dietary additives as probiotics and phytobiotics are gaining momentum and paid an attention to be used as an alternative to antibiotic feed additives because of their beneficial effects on health of poultry as well as to avoid the hazards of continued using of antibiotics on human health.

Adding the EO alone or in combination with synbiotic has improved the total FCR in comparison with the NE and antibiotic treated groups while it did not have significant effect on feed intake. On the other hand, using the synbiotic did not enhance the aforementioned parameters in comparison with the positive control and ABAC groups. These results come along with those of Jerzsele *et al*., (2012) who found that using essential oils in diets of *C. perfringens* challenged broiler chicks has improved weight gain, while using probiotics (*B. amyloliquefaciens* spores) did not alleviate the drop in body weight gain associated with the NE challenge. The lake of efficiency on performance parameters when using biotic products with *C. perfringens* challenged birds was reported also by Shanmugasundaram and Selvarai (2013) and M·Sadeq *et al.,* (2015). Contaray, Geier *et al*., (2010) reported positive impact of the probiotic based products on BW and mortality % of challenged broilers. The EO ,as type of phytobiotics, are able to improve the taste and feed palatability, stimulate the secretion of bile, mucus and saliva and improve the digestive enzymes activities as well as, they have antimicrobial activity against *C. Perfringens* and other intestinal pathogens (Alloui *et al*., 2014).

Using synbiotics in diets of vaccinated broilers improved the antibody titer to IB vaccine while it did not affect the immune response to ND, AI and IBD vaccines. The additive did not improve relative weight of BF and thymus but increased spleen weight. Silva *et al*., (2009) and Seidavi *et al*., (2016) agreed with our findings as they reported absence of obvious significance in the antibody titer against AI, ND and IBD vaccines as well as absence of effect on weight of BF and thymus in broiler fed probiotics, prebiotic or their combination. Moreover, Tolba *et al*., (2007) reported improvement of spleen weight in Fayoumi hens fed diets containing commercial probiotic. On the other hand, the essential oils helped in hindering the antibody titer decline against ND and IB at third week of experiment and improved the immune response to AI and IBD vaccines at 5th week. EO did not affect weight of BF and thymus but increased spleen weight ($P>0.05$). The combination between synbiotics and EO showed to be effective in improving immune response to ND and IBD vaccination as well as significantly increasing the spleen weight. Sultan *et al*., (2017) and Chowdhury *et al*., (2018) agreed with our findings by reporting significant increase of antibody titer against ND and AI vaccination as well as absence of any significant effect on weight of the lymphoid organs (BF) when broiler chicks given herbal compound in feed or water ($P<0.05$). Contrary, Özek *et al*., (2011) found insignificant improvement in ELISA antibody titer ($P>0.05$) against IB and IBD vaccines when laying hens fed diets with essential oil. We attribute our results to presence of certain components in essential oils, which may bind to immunoglobulin G recceptors and stimulate the immune response as recorded by Nimmerjahn and Ravetch, (2010) and Ahmed *et al*., (2013). In addition, some plant bioactive compounds regulate expressions of various genes involved in immune response (Liu *et al*., 2014). The used biological treatments did not alleviate the histopathological changes in thymus and BF except for the synbiotic treatment, which showed milder lesions in BF, same as antibiotic treated birds, in comparison with NE group. These results disagreed with Madian and Abd El-Ghany (2006) as they reported that using antibiotic with vaccinated broilers showed severe histopathological changes in thymus and BF, while using biotic preparations showed lesser damage in them.

5. Conclusion

The current study suggests that essential oils may be used as effective alternative to antibiotics in broiler rations to augment drawbacks of NE challenge on performance and enhance immune response of broiler chicks. As well as, offering the consumers with healthy poultry products, free from antibiotic residues.

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