

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Immune Profile of Medication Vaccinated Broiler Chickens.

¹Kh. M. Elbayoumi*, ²M.M. Amer,¹ Zeinab M.S. Amin Girh, ¹A. EL-shemy, and ³Aziza M. Amer.

¹Dept of Poult. Dis., Vet. Res. Division, NRC, P.O. Code 12311 Dokki, Giza, Egypt

²Dept of Poult. Dis., Faculty Vete. Med., Cairo University, P.O. Code 12211 Giza, Egypt

³Dept. of Parasitology, Vet. Res. Division, NRC, P.O. Code 12311 Dokki, Giza, Egypt

⁴Dept of Pharmacology, Faculty Vet. Med., Cairo University, P.O. Code 12211 Giza, Egypt

ABSTRACT

This study was carried out to study effect of antibiotics and/ or prebiotics on total feed conversion rate (FCR) immune profile to Newcastle disease (ND), Avian Influenza (AI), Infectious Bronchitis (IB) and Infectious Bursal disease (IBD) vaccines as well as *Mycoplasma gallisepticum* (MG) infection were measured by ELISA- test as well as air sac lesion score at end of the 5th week of age in broiler chickens. A total number of 200, 1 day old broiler chickens were used. Chicks were divided into 8 equal groups; 25 chicks each; and treated as follows: group 1, 2,3,4,5 and 6 were medicated with tylosin + colistin, tylosin, tylosin and prebiotic, colistin, colistin + prebiotic and prebiotic ; respectively. While groups 7 and 8 were kept as non medicated vaccine and negative control; respectively. Chicken groups 1-7 were received the used vaccines. while group 8 was kept as control negative. Antibiotics were used at the first 3 days and 14-16 as well as 26- 28 days of life. Prebiotic (Multienzymes) was given in feed in a dose of 500 gm/ton from the 4-28 days of life. Live vaccines Hitchner B₁ and La Sota against ND at 5 and 18 days of age, H 120 strain against IB disease at 1- day old and 228E against IBD at 6 and 14 days of age all were given by eye drops instillation. Inactivated vaccine against AI and ND was given subcutaneously at 8 and 10 days old; respectively. At end of the 5th week of age: 20 blood samples/group for monitoring ELISA antibody titers and 10 birds were subjected to PM examination. Feed conversion rate (FCR) was calculated. FCR in medicated groups (1.55- 1.67) was higher than control non medicated 1.67 and vaccinated non treated 1.77 at the end of 5th weeks of age. The medicated groups showed the best in prebiotic group 7 (1.55) followed 1.57, 1.59, 1.60 and 1.65 in tylosin gr 3 , colistin gr 5, tylosin-colistin gr 2 , tylosin-prebiotic gr 4 and colistin - prebiotic gr 6; respectively. The recorded gross air sac lesions were varied from apparent normal to slight turbidity without marked difference between medicated groups, while negative control and vaccinated non medicated showed thickened air sac wall with fibrinous exudates. The means air sac lesion score was the highest 2.80 in vaccinated non treated group and 1.8 in control negative, while medicated groups varied from 1.55 to 0.68. These results indicated that the used drugs played a role in controlling infection and limitation of air sac gross lesions. ELISA titers against ND , AI , IB and IBD viruses at 1st day of life, as compared with that of 5th week of age indicated the normal decaying of maternal antibody titer without field challenge, while it was increased with MG that indicates stimulation of possible infection. Concerning means ELISA titer by 5th week of age days of age against: 1) MG ELISA : The vaccinated non treated and control negative groups showed higher titres 1891.8±121.5 and 1685.0±332.5 than vaccinated medicated groups where the titre range was 200.4±142.5 (Tylosin) to 1073.3±761.8 (Colistin). Combination of the used drugs showed moderate results. The result indicated that live vaccines activated Mycolasma of infection and used drugs suppressed this activation. 2) ND ELISA the lowest was 4492.0±1374.7 in gr. 2 , followed by 4496.4±1427.1 in gr. 5, 4736.9±1116.2 in vaccinated non treated gr. 8, 4853.7±1547.9 in gr. 7, 5378.2±1872.6 in gr. 6, 5496.5±1186. in gr. 3 and the highest was 5874.8±1171.3 in tylosin-prebiotic gr. 4. 3) AI ELISA tires the lowest was 2547.3±908.5 in gr. 2 followed by 3259.1±1475.7 in gr. 8, 3366.4±1198.2 gr. 4, 3682.7±1297.5 in gr. 7 3982.7±970.2 in gr. 5, 4006.0±1260.2 in gr. 3 and the highest was 4121.7±1329.6 in gr. 6. 4) IB mean titres lowest was 5887.1±826.8 followed by 7259.7±1410.6, 7935.7±2321.9, 8657.1±1050.5 , 8857.1±1326.7, 10700.0±998.1 and the highest.1titre was 10850.0±920 in gr2,gr 8, g r5 , gr 7,gr. 3,tylosin-prebiotic gr 4 and colistin-prebiotic gr. 6; respectively. 5) ELISA titer means against IBD the lowest was 1470.3±360, followed by 1660.7±423.9.3, 1726.4±360.2, 1760.8±563.7, 1930.6±525.3, 2105.1±535.5 and 2900.3±434.9 in gr 3, 4 , 5 , 2 , 8 , 7 and 6 ; respectively. The recorded CV% values of vaccines response in medicated groups were lower than vaccinated non medicated group and varied from good to excellent. Generally the multienzyme prebiotic with antibiotic induced higher titres and the combined antibiotic result in mordent levels than antibiotic alone. It could be concluded that the use of antibiotics and/ or prebiotic in broilers improved immune response against used vaccines , performance and reduced air sac lesion score. Therefore, we can recommended the usage of antibiotics and/ or prebiotic in broiler from MG suspected infected breeders and reared in uncontrolled hygienic condition to reduce spread of MG infection, limitation of air sac gross lesions and controlling its adverse effect on immune response and performance.

Keywords: broiler, ELISA, Antibiotic, prebiotic, FCR, immune profile, air sac lesion score. multienzyme.

*Corresponding author

INTRODUCTION

Antibiotic and antibacterial medications still used in poultry industry in several indications including therapeutic treatment, prevention or as traditional growth promoters [1] and [2]. However using of such antibiotics at time of vaccination is not well established yet and few data available in such indication.

In broiler and turkey production flocks, an effective monitoring program can be the regular sampling and testing of blood as they are slaughtered at the processing plant. This serologic monitoring will establish a baseline of antibody titers that are the result of both vaccination and field challenge [3]. Changes in the usually observed antibody titers may indicate a decrease or increase in vaccine efficiency [4] or an increased field challenge by a particular pathogen [5]. A regular serologic monitoring program is also helpful to determine whether a flock has been exposed to a new pathogen, not previously present in the region [6]. Evolution and diagnostic advantages of the graphic presentation of ELISA based flock profiling data in combination with gross and microscopic pathology data was described by Mallinson et al. [7]. The establishment of such profiles for different poultry diseases is facilitated by ELISA testing [8].

Prebiotics are non digestibility and selected ferments capability by some bacterial groups [9]. Most prebiotics are carbohydrates. Prebiotic ingredients are often made of several compounds. These molecules not only differ for the polymerization degree but also for the production technology (as, fractions can be obtained either by enzymatic hydrolysis or by extraction); these the two manufacturing processes lead up to different mixtures of final products. Intestinal bacteria metabolize these compounds in a different ways [10, 11,12].

Respiratory disease of poultry cause severe economic losses specially Avian influenza (AI) [13] , Newcastle disease (ND), Infectious bronchitis (IB) and Mycoplasma gallisepticum (MG) [14] these affections could be prevented by vaccination by triggering or boosting the bird's immune system to produce antibodies that in turn fight the invading causal organisms using live and inactivated vaccine against [15,16,17] as well as usage of antibiotics [18] . Infectious bursal disease (IBD) causes a variable degree of immunosuppression in the affected birds. Infection of chicks in the early age, displays a severe and prolonged immunosuppression [19]. **Vaccination** plays an important part in the health management of the poultry flock.

Talebi and Ghasemi-lak [20] compared ELISA titres of MG and MS infected broiler breeders at 35 weeks old before and after treatment with tylosin for 5 days and concluded that the antibiotics affect the outcome of the Mg and Ms infections in broiler breeders and reduce serological titres of Mg and Ms infected birds but do not completely cure the birds from the infections. Amer, et al. [21] reported that Tilmicosin titres in 1- day old commercial broiler chicks for the 1st 2 days of life and repetition at the 19 days of age for another 2 days was completely eliminate the serum positive titers for MG and partially eliminate it for MS as measured by ELISA and the prevalence of marked air sac gross lesions in non treated control group indicated the development of CRD, the lesions increased in severity with age in non treated. The treated groups showed milder lesions varied from normal to slight turbidity without marked difference between medicated flocks. The prevalence of gross lesions of the air sac in all the medicated groups was less than those of the infected non medicated [22,23]. Mohnl, et al. [24] found that the symbiotic had a comparable potential to improve broiler performance.

The CV is a measure of variation of antibodies within a group of serum samples. The lower the CV, the more uniform the antibody response. A low CV is typically associated with good vaccination procedures or with a recent antibody response after field exposure to a given pathogen. Because an ELISA titer or an ELISA titer range reflects simply a quantitative response, such titers should be used as follows: 1) as a reference for possible trends in seroconversion in a poultry company upon field challenges; 2) for identification of rapid seroconversion in paired acute and convalescent samples in a diagnostic situation; 3) for evaluations of vaccines and vaccine application procedures; or 4) to document the absence of antibodies against pathogens such as AIV, MG, or MS (IDEXX manual)

Both polymexins (colistin) and macroloids (tylosin) antibiotics used in this study has positive impacts in controlling of MG and enhanced immune response of broiler chickens to IB and IBD vaccines.

Prebiotic (betaine) to produce humoral immune response. A combination between antibiotic and prebiotic can be used to minimize the possible adverse effects of excessive use of antibiotic on vital organs [25,26].

The objective of present study was to evaluate immune performance of commercial broiler chickens to used vaccines when these chickens were raised with antibiotic and/ or prebiotic medicated. The parameters measured included: Feed conversion rate, humeral immune response to ND, AI, IB and IBD vaccines as well as MG infection were measured by ELISA- test as well as air sac lesion score at end of the 5th week of age .

MATERIAL AND METHODS

Experimental Chicks:

A total number of 200 commercial broilers chicks obtained from breeder farm not vaccinated against Mycoplasma as hatched were divided into 8 equal groups; 25 chicks in each.

Ration:

Commercial starter and grower broiler chicken ration were given till 21 and 32 days of age, respectively. The used commercial balanced ration based on yellow corn or soyabean that met the [27] broiler chicken requirements.

Vaccine Strains:

- Hitchiner B₁ vaccine : contains lentogenic Newcastle strains – Hipra lab. – Spain - Batch NO. 388V-3.
- La Sota vaccine: lentogenic Newcastle strains – IZO S.P.A. – Italy Batch no. 0722 F.
- Infectious bursal disease (IBD) intermediate stain vaccine Nobis Gumbro 228 E, Intervet.
- Inactivated ND Clone 30 virus " Newcavac vaccine" - Intervet UK Ltd- Batch no. S257A01.
- Inactivated oil VOLVAC[®] AI H5N2 Inactivated oil emulsion vaccine - Boehringer Ingelheim vetmedica S.A.De.C.V., Mexico. recommended dose according company instructions was 0.5 ml, used subcutaneously in neck region.

Natuzyme[®] Prebiotic:

It is a multienzyme poultry feed supplements commercial product, Novartis Limited- India, contains standardized components : Cellulase, xylanase, beta-lucanase, alpha-amylase and pectinases. It also contains phytase, protease, hemicellulase, amyloglycosidase, pentosanase and phyton activities. Dosage: 500 gm/ton of feed.

Antibiotics:

Colistin sulphate 6 MIU: each gm contains 6000.000 IU colistin sulphate. Lot No. 150415 . Jordan Vet. and Agr. Med. Ind. Co – Amman – Jordan.

Tylox[®] : tylosin water soluble powder 100gm - Lot. No. 150118. Jordan Vet. and Agr. Med. Ind. Co – Amman – Jordan.

Vaccination time and application methods:

Live vaccines applied by eye drops instillation against ND using live Hitchner B₁ and La Sota at 5 and 18 days of age by eye drops instillation methods while against IB disease using live H 120 strain at one day old and against IBD at 6 and 14 days of age. While inactivated vaccine against AI and ND was given through subcutaneous route at the back of the neck at 8 and 10 days old; respectively. Live vaccinal virus was inoculated in specific pathogen free (SPF) embrionated chicken egg (ECE) and EID₅₀ titer was calculated by method of [28].

Calculation of FCR:

Total weight (g) of food consumption by the birds of a group during a given period /total weight gain (g) of the birds of the same group during a given period (including weight gain of birds which died during the given period) according to *Sainsbury* [29].

Samples:

Blood samples for serum were collected for ELISA test at the end of the 5th week of life.

Serological ELISA test:

The sera obtained were tested to evaluate the antibodies titer against MG, ND, AI, IB and IBD antibodies procedure was performed using commercial ELISA kits: The ELISA test was performed according to the manufacturer's recommendations. The results were expressed in titer as recommended by the diagnostic kit producer.

- Indirect ELISA methods, including ProFLOCK Plus AIV Ab test kit (Synbiotics, USA), The indirect ELISA methods were performed.
- **ND:** Chicken serum samples were examined for NDV antibodies by indirect ELISA, using a commercial ELISA test kit ProFLOCK® NDV Plus (Synbiotics, San Diego, CA), run in 96-well microtiter plates containing NDV antigen.
- **IBD:** The sera obtained from blood of experimental chicks at various time points were tested for IBD antibodies using the PROFLOK® plus IBD Ab test kit (Synbiotics, San Diego, CA). The antigen used by this kit is purified extract from IBDV infected bursa tissue.
- **IB:** The PROFLOK® IBV ELISA Kit (Synbiotics, USA), which is a rapid serologic test for the detection of IBV Antibody in chicken serum samples.
- **MG:** The procedure used in this test was performed using commercial ELISA kits for the presence of anti-MG antibodies ProFLOCK® *Mycoplasma gallisepticum* Antibody Test Kit, Synbiotics Corp. - USA].

Air Sac Lesions scour:

The air sacs of in dead and sacrificed chickens were examined according to Guarini,et al. [30].

Experimental design:

A total number of 200 broilers Hubbard chicks were divided into 8 equal groups; 25 chicks in each. Chicks group 1, 2,3,4,5 and 6 were medicated with tylosin + colistin, tylosin, tylosin and prebiotic, colistin, colistin + prebiotic and prebiotic ; respectively. While groups 7 and 8 were kept as non medicated vaccine and negative control; respectively. Chicken groups 1-7 were received the used vaccines. while group 8 was kept as control negative. Antibiotics were used at the first 3 days and 14-16 as well as 26- 28 days of life. Prebiotic was given in feed in a dose of 500 gm/ton from the 4-28 days of life. At end of the 5th week of age: twenty blood samples for serum were collected for monitoring antibody titers using ELISA -test. Ten birds were subjected to postmortem examination.

Coefficient of variation (CV%) values:

The CV% is the standard deviation divided by the mean, multiplied by 100, whether we are relating to antibody titers. Interpretation of CV values in vaccinated birds can be done as: > 30% : Excellent; 30-50%: Good; 51-80%: Fair and >80%: poor.

RESULTS AND DISCUSSION

Respiratory disease of poultry cause severe economic losses specially AI [31], ND , IB and MG [14] these affections could be prevented by vaccination. The general health condition of birds is a factor in the

choice of vaccine, especially in flocks that are under heavy challenge from virulent respiratory viruses or severely immunosuppressed, sometimes a safer vaccine would be preferable to a more efficacious vaccine with some reactivity. Chronic respiratory disease was adversely affecting broiler performance and the impact of MG infection was exacerbated by an respiratory viral vaccination program [6,32,33]. Mycoplasmas may affect the cell-mediated immune system by inducing either suppression or stimulation of B and T lymphocytes, and inducing cytokines [34,35 and 36]. Some vaccines can also prevent infection with and reduce transmission of a field strain [37, 38]. In broiler production, an effective monitoring program can be the regular sampling and testing of blood as they are slaughtered at the processing plant. This serologic monitoring will establish a baseline of antibody titers that are the result of both vaccination and field challenge [3]. Changes in the usually observed antibody titers may indicate a decrease or increase in vaccine efficiency [4].

FCR (Table 1 and Fig 1) at the end of 5th weeks of age the best was 1.55 in prebiotic group 7, followed by group 3 (tylosin) which was 1.57, followed by group 5 (colistin) which was 1.59, followed by group 2 (tylosin-colistin) which was 1.60, followed by group 4 (tylosin-prebiotic) which was 1.65, followed by group 6 (colistin - prebiotic) which was 1.67, followed by group 1 (control negative) which was 1.75, followed by group 8 (vaccinated non treated group) which was 1.77, the latest group 8 was the mostly affected this maybe due to that vaccination itself considered stress on birds resulting in affecting feed conversion rate negatively this results was matched with *Xiaofei Wang, et al.* [39] who reported that immunization against ND virus at different vaccinal doses affect body weight gain and FCR, on the other hand *Nunes, et al.* [40] stated that use of enzymes in poultry feed improves feed conversion rate this maybe explain that group 7 which received enzyme mixture was the highest feed conversion rate compared to control group, all other treated groups were better than control non treated group this maybe due to that antibiotics used control pathogenic microorganisms which affect feed conversion rate negatively, this results was parallel with *Adel Feizi et al.* [41] who reported that use of tylosin not only control MG mortalities and lesions but also improves FCR together with body weight gain, also *Kuldeep Dhama et al.* [42] stated that use of antibiotics such as colistin improves poultry performance including feed conversion rate. Effect of antibiotics in improving performance due to its antimicrobial activities rather than having any direct effects on birds physiology [43,44].

The recorded gross air sac lesions were varied from apparent normal to slight turbidity without marked difference between medicated groups, while negative control and vaccinated nonmedicated showed thickened air sac wall with fibrinous exudates. Results of means air sac lesion score (Table 2 and Fig 2) revealed that the highest was group 8 (vaccinated non treated group) which was 2.80, followed by group 7 (prebiotic only) which was 1.80, followed by group 1 (control group) which was 1.55, followed by group 6 (colistin-prebiotic) which was 1.37, followed by group 4 (tylosin-prebiotic) which was 1.26, followed by group 2 (tylosin-colistin) which was 1.10, followed by group 3 (tylosin) which was 0.75, and the lowest mean air sac lesion score was group 5 (colistin) which was 0.68. Presence of air sac lesions maybe due to *Mycoplasma* spp. infection either vertically or horizontally transmitted [45,46]. This opportunistic microorganism under field stress factors including live vaccination will results in air sac lesions and disease conditions [47,48], under our experimental conditions the lowest lesion score was in antibiotic medicated groups rather than others group, this may be due to sensitivity of *Mycoplasma* spp. to tylosin [41,49] or effect of colistin on invaders complicating agent such as *avian pathogenic E.coli* resulting in decrease lesion and severity of such condition [50,51]. Also under our experimental conditions it was found that prebiotic enzymes has no role on lesion score caused by *Mycoplasma* infection, researchers reported that enzymes decrease lesion score only caused by *C. perfrungens* infection [52] together with improving gut flora in cocci-vaccinated broilers [53]. The result indicated that the used drugs played a role in controlling infection and limitation of air sac gross lesions [6,21,54].

ELISA maternal titers at 1st day of life in negative control group 1 against ND, AI, IB and IBD was 7038.5± 2372.4, 3217.6± 571.3, 1450.8± 687.6 and 1450.8± 687.6 as decreased to at the 5th week of age where it was 179.5 ± 89.4, 113.8±72.1, 65.5 ±47.8 and 80.5± 43.7; respectively. This result indicating normal decaying of maternal antibody titer without any field challenge.

ND virus ELISA titres at the 5th weeks in chicken groups the lowest was 4492.0±1374.7 in group 2 (tylosine – colistin), followed by 4496.4±1427.1 in group 5 (colistin), 4736.9±1116.2 in group 8 (vaccinated

non treated), 4853.7±1547.9 in group 7 (prebiotic) , 5378.2±1872.6 in group 6 (colistin-prebiotic), 5496.5±1186. in group 3 (tylosin) and the highest was 5874.8±1171.3 in group 4 (tylosin-prebiotic).

Mean ELISA titer against AI virus at 5th week of life was the lowest 2547.3±908.5 in group 2 followed by 3259.1±1475.7 in group 8, 3366.4±1198.2 group 4, 3682.7±1297.5 in group 7 3982.7±970.2 in group 5, 4006.0±1260.2 in group 3 and the highest was 4121.7±1329.6 in group 6.

IB mean titres was virus the lowest by group 2 (tylosin-colistin) which was 5887.1±826.8, followed by group 8 (vaccinated non treated) which was 7259.7±1410.6, followed by group 5 (colistin) which was 7935.7±2321.9, followed by group 7 (prebiotic) which was 8657.1±1050.5, followed by group 3 (tylosin) which was 8857.1±1326.7, followed by group 4 (tylosin-prebiotic) which was 10700.0±998.1, followed by group 6 (colistin-prebiotic) 10850.0±920.1.

ELISA titer means against IBD virus at revealed that the lowest was in group 3 (tylosin) which was 1470.3±360.3, followed by group 4 (tylosin-prebiotic) which was 1660.7±423.9, followed by group 5 (colistin) which was 1726.4±360.2, followed by group 2 (tylosin-colistin) which was 1760.8±563.7, followed by group 8 (vaccinated non treated group) which was 1930.6±525.3, followed by group 7 (prebiotic) which was 2105.1±535.5, followed by the highest titre in group 6 (colistin – prebiotic) which was 2900.3±434.9. Generally the multienzyme prebiotic with antibiotic induced higher titres and the combined antibiotic result in mordent levels than antibiotic alone. Under our experimental conditions it was noticed that prebiotics enzymes improves humoral immune response in broiler chickens, this results was parallel with *Zangiabadi and Torki* [55] who reported that enzymes prebiotics improves performance together with humoral immune response in broiler chickens, also improves of means ELISA titer in respiratory virus vaccines groups treated with colistin antibiotic when compared with vaccinated non treated groups revealed that colistin has role in improvement of humoral immune response, this results was matched with results found by *Der-Nan Lee* [56] and *Naoto Yoshino* [57]. On the other hand tylosin did not improves humoral immune response against IBD vaccination when compared to vaccinated control group, this due to that macroloids is indeed capable of altering the proliferative capacity of immune cell [58], promote production of pro-inflammatory cytokines such as interleukin 1(IL-1), interleukin 2 (IL-2), interferons (IFNs), and tumor necrosis factor alpha [59] this maybe due to positive effect of macroloids on macrophage. On the other hand, other authors reported that macroloids found to improves spleenocytes proliferations in chickens but in the same time antibody ELISA titers against IB was lower when compared with colistin [56] Interpretation of the CV value of the obtained ELISA results proved that non treated controls showed high homogenous titers as a result of active MG infection while it was lower in medicated groups. The recorded CV% values of vaccines response in medicated groups were lower than vaccinated non medicated group and varied from good to excellent (Table 3 and fig 3).

Table (1): Average feed intake (AFI), Average body weight (ABW) and Feed conversion rate (FCR) of vaccinated, medicated and control broiler chicken groups at end of the 5th week of age.

Gr. No	Group treatment	AFI	ABW	FCR
1	Negative control	3496	1990	1.75
2	Tylosin - colistin	3570	2227.5	1.6
3	Tylosin	3457	2194	1.57
4	Tylosin - prebiotic	3660	2221	1.65
5	colistin	3579	2250	1.59
6	Colistin - prebiotic	3625	2173	1.67
7	prebiotic	3582	2305	1.55
8	vaccinated non treated	3515	1987	1.77

Table (2): Mean ELISA titres \pm SD against MS,ND,AI, IB and IBD (n=20) as well as Mean Air sac lesion scour in vaccinated , medicated and control broiler chicken groups at end of the 5th week of age (n= 10).

Gr No	Treatment	Age/ days	n= 20												Mean Air sac lesion scour			
			MG		ND		AI		IB		IBD							
			Mean \pm SD	CV %	Mean \pm SD	CV %	Mean \pm SD	CV %	Mean \pm SD	CV %	Mean \pm SD	CV %						
1	Negative control	0	543.6	24.5	5	7038.5	2372.4	34	3217.6	571.3	18	1450.8	687.6	47	1450.8	687.6	47	1.55
		34	1685.0	332.5	20	179.5	89.4	45	113.8	72.1	63	65.5	47.8	54	80.5	43.7	54	
2	Tylosin - Colistin	0	924.6	156.5	17	4492.0	1374.7	31	2547.3	908.5	36	5887.1	826.8	14	1760.8	563.7	32	1.10
3	Tylosin	0	200.4	142.5	71	5496.5	1186.7	22	4006.0	1260.2	31	8857.1	1326.7	15	1470.3	360.3	25	0.75
4	Tylosin - Prebiotic	0	924.6	134.5	4	5874.8	1171.3	20	3366.4	1198.2	36	10700.0	998.1	9	1660.7	423.9	26	1.26
5	Colistin	0	1073.3	761.8	71	4496.4	1427.1	32	3982.7	970.2	24	7935.7	2321.9	29	1726.4	360.2	21	0.68
6	Colistin - Prebiotic	0	999.0	476.5	48	5378.2	1872.6	35	4121.7	1329.6	32	10850.0	920.1	8	2900.3	434.9	15	1.37
7	Prebiotic	0	625.7	89.5	14	4853.7	1547.9	32	3682.7	1297.5	35	8657.1	1050.5	12	2105.1	535.5	25	1.80
8	Vaccinated non treated	0	1891.8	121.5	6	4736.9	1116.2	24	3259.1	1475.7	45	7259.7	1410.6	19	1930.6	525.3	27	2.80

Table (3): Interpretation of CV values in ELISA results against MS,ND,AI, IB and IBD in vaccinated , medicated and control broiler chicken groups at end of the 5th week of age.

	MG		ND		AI		IB		IBD	
	CV%	Interpretation	CV%	Interpretation	CV%	Interpretation	CV%	Interpretation	CV%	Interpretation
Negative control	20	Excellent	45	Good	63	Fair	54	Fair	54	Fair
Tylosin + colistin	17		31		36		14		32	
Tylosin	71	Fair	22	Excellent	31	Good	15	Excellent	25	Excellent
Tylosin -prebiotic	4	Excellent	20		36		9		26	
colistin	71	Fair	32	Good	24	Good	29	Excellent	21	Excellent
Colistin -prebiotic	48	Good	35		32		8		15	
prebiotic	14	Excellent	32	Excellent	35	Excellent	12	Excellent	25	Excellent
Vaccinated non treated	6		24		45		19		27	

Fig (1): Levels of Feed conversion rate (FCR) of vaccinated , medicated and control broiler chicken groups at end of the 5th week of age.

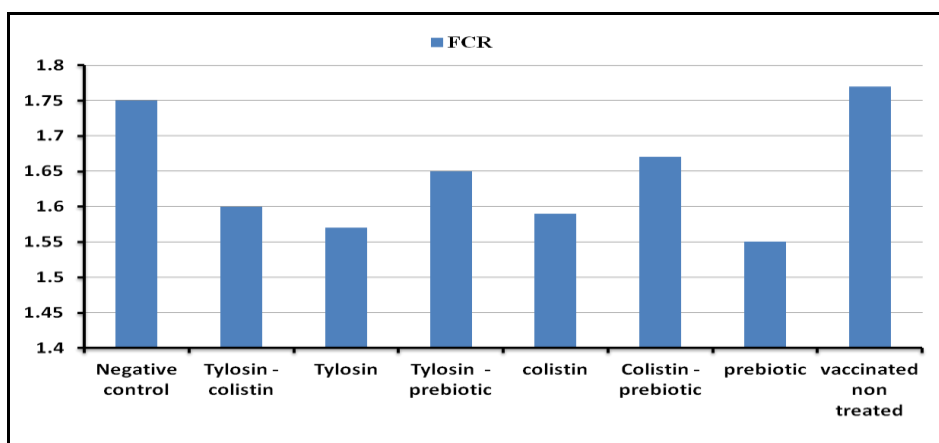


Fig (2): Levels of mean air sac lesion scour of vaccinated , medicated and control broiler chicken groups at end of the 5th week of age.

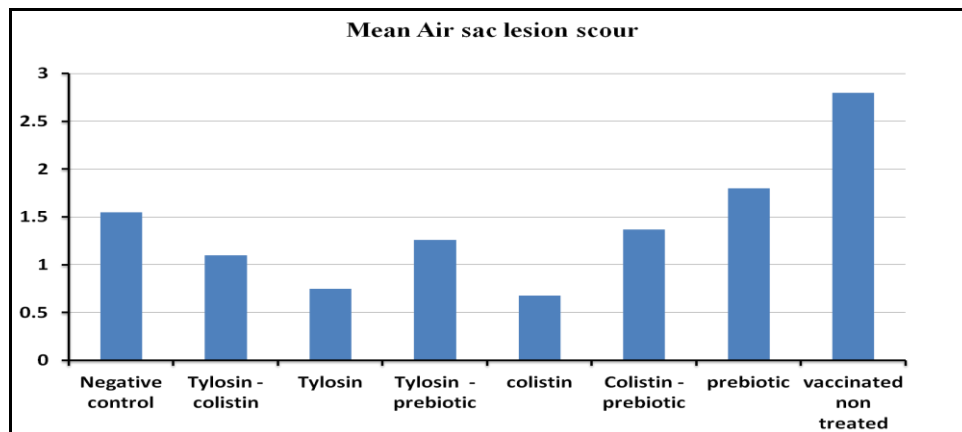
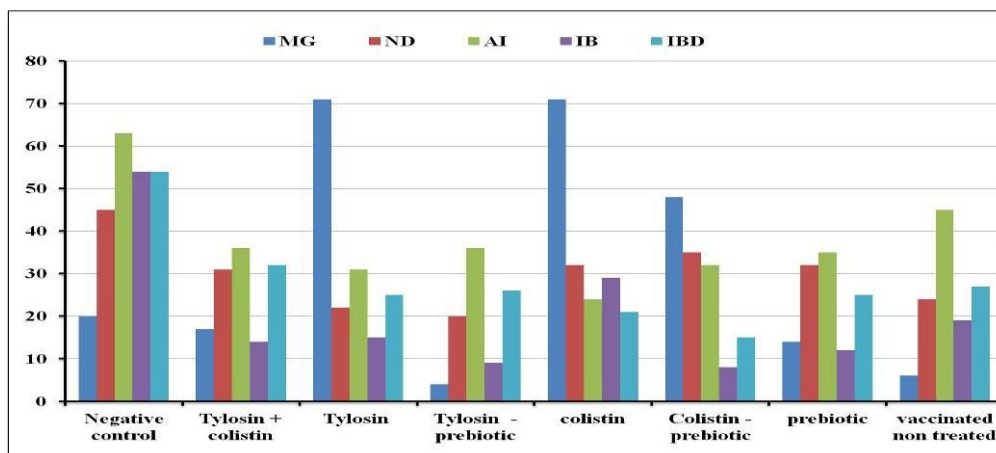


Fig (3): Levels of CV% values in ELISA against MS, ND, AI, IB and IBD in vaccinated , medicated and control broiler chicken groups at end of the 5th week of age.



It could be concluded that the use of antibiotics and/ or prebiotic in broilers improved immune response against used vaccines , performance and reduced air sac lesion score. Therefore, we can recommended the usage of antibiotics and/ or prebiotic in broiler from MG suspected infected breeders and reared in uncontrolled hygienic condition to reduce spread of MG infection, limitation of air sac gross lesions and controlling its adverse effect on immune response and performance.

REFERENCES

[1] Miles, RD; Butcher, GD ; Henry, PR; Littell RC (2006): Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poult Sci* ;85(3):476-85.

[2] Moussa S. Diarra and Francois Malouin (2014): Antibiotics in Canadian poultry productions and anticipated alternatives. *Front Microbiol*;5:282 doi:10.3389/fmicb.2014.00282.

[3] Qingsong Han, Xiaolong Gao, Pengpeng Wu, Sa Xiao, Xinglong Wang, Peng Liu, Lina Tong, Huafang Hao, Shuxia Zhang, Ruyi Dang and Zengqi Yang (2016): Re-evaluation the immune efficacy of Newcastle disease virus vaccine in commercial laying chickens. *Res. in Vet. Sci.*,111: 63–66.

[4] Kai Zhao, Gang Chen, Xing-ming Shi, Ting-ting Gao, Wei Li, Yan Zhao, Feng-qiang Zhang, Jin Wu, Xianlan Cui, and Yun-Feng Wang (2012): Preparation and Efficacy of a Live Newcastle Disease Virus Vaccine Encapsulated in Chitosan Nanoparticles. *PLOS One*, 7 (12): e53314. doi: 10.1371/journal.pone.0053314.

- [5] Swayne DE, Suarez DL, Spackman E, Jadhao S, Dauphin G, Kim-Torchetti M, McGrane J, Weaver J, Daniels P, Wong F, Selleck P, Wiyono A, Indriani R, Yupiana Y, Sawitri Siregar E, Prajitno T, Smith D and Fouchier R (2015): Antibody titer has positive predictive value for vaccine protection against challenge with natural antigenic-drift variants of H5N1 high-pathogenicity avian influenza viruses from Indonesia. *J. Virol.*; 89 (7): 3746-62. doi: 10.1128/JVI.00025-15.
- [6] Saif, Y. M., Barnes, H. J., Fadly, A. M., Glisson J. R. and Swayne, D. E. (2003): *Poultry Diseases*, 11th Edition., Iowa State Press, Iowa, 719 –774.
- [7] Mallinson, E. T., D. B. Snyder, W. W. Marquardt, and S. 1. Gorham. (1988): In *Principles of Disease Prevention: Diagnosis and Control* by Alex J. Bermudez and Bruce Stewart-Brown in *poultry diseases* Saif, Y. M.; Barnes, H. J.; Fadly, A. M.; Glisson, J. R. and Swayne, D. E. (2003), 11th Ed., Iowa State Press, Iowa. A Blackwell Publishing Co. page.3-45.
- [8] Gharaibeh S and Mahmoud K (2013): Decay of maternal antibodies in broiler chickens. *Poult Sci.*;92(9):2333-6. doi: 10.3382/ps.2013-03249.
- [9] Joanne Slavin (2013): Fiber and Prebiotics: Mechanisms and Health Benefits. *Nutrients* ; 5(4): 1417–1435.
- [10] Roberfroid, M. B., Van Loo, J. A., and Gibson, G. R. (1998): The bifidogenic nature of chicory inulin and its hydrolysis products. *J Nutr.*, 128, 11-19.
- [11] Perrin, S., Fournies, C., Grill, J., Jacobs, H., and Schneider, F. (2002): Fermentation of chicory fructooligosaccharides in mixtures of different degrees of polymerization by three strains of bifidobacteria. *Can. J. Microbiol.*, 48, 759- 763
- [12] Kaplan, H., and Hutkins, R. (2003): Metabolism of fructooligosaccharides by *Lactobacillus paracasei* 1195. *Appl. Environ. Microbiol.*, 69, 2217-2222.
- [13] Ilaria Capua and Stefano Marangon (2006): Control of Avian Influenza in Poultry. *Emerging Infectious Diseases* • Vol. 12, No. 9.
- [14] Proceedings of the sixty-fifth western poultry disease conference (2016). Vancouver, BC, Canada.
- [15] Abdelwhab E.M and Hafez M. Hafez (2012): Insight into Alternative Approaches for Control of Avian Influenza in Poultry, with Emphasis on Highly Pathogenic H5N1. *Viruses*. 4(11): 3179–3208.
- [16] Patti J. Miller , Claudio L. Afonso, John El Attrache, Kristi M. Dorsey, Sean C. Courtney , Zijing Guo and Darrell R. Kapczynski (2013): Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. *Dev. and Comp. Immunol.*, 41(4) 505–513.
- [17] Kuldeep Dhama, Shambhu Dayal Singh, Rajamani Barathidasan, P.A. Desingu, Sandip Chakraborty, Ruchi Tiwari and M. Asok Kumar (2014): Emergence of Avian Infectious Bronchitis Virus and its Variants Need Better Diagnosis, Prevention and Control Strategies: A Global Perspective. *Pakistan J. of Biological Sci.* 17 (6) .751-767.
- [18] Somaye Bagheri Farsani , Maryam Eslami Farsani , Fakhroldin Asadi Farsani , Shahram Aroufzad and Sabri Ban (2013): Relationship between organizational learning and organizational performance among employees in physical education organizations. *Euro. J. Exp. Bio.*, 3(1): 540-544.
- [19] Lukert, P.D. and Saif, Y.M. (1991): *Infectious bursal disease*. *Diseases of poultry*, 9th Ed. Iowa State. University press. Ames, Iowa USA,. 1060-1085.
- [20] Talebi, A. and Ghasemi-lak, M. (2004): Investigation of antibiotic effects on serological titers of infected Ross broiler breeders with Mg and Ms. *J. of the Facult. of Vet. Med., University of Tehran*. 59 (3) : 271-275.
- [21] Amer, M.M. ; Zohair, G. A.; EL-Bayomi, K. M., and Zenab, M. S. Gera (2012): Effect of tilmicosin in control of mycoplasmosis in broiler chickens from infected breeders using ELISA test for evaluation. *J. of Amer. Sci* ;8(3) 696-700.
- [22] Jordan, F.T.W. and Horrocks, B. K. (1996): The minimum inhibitory concentration of tilmicosin and tylosin for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and a comparison of their efficacy in the control of *Mycoplasma gallisepticum* infection in broiler chicks. *Avi. Dis.* 40 (2) : 326-334 .
- [23] Stipkovits, L T .(2000): Current questions of the control of *Mycoplasma synoviae* infection. *Magyar Allatorvosok Lapja*. 122 (3) :165-167.
- [24] Mohnl, M., Y. Acosta Aragon, A. Acosta Ojeda, Fujun Rodriguez Sanchez and B.S. Pasteiner (2007): Effect Nitric oxide in both symbiotic feed additive in comparison to antibiotic growth promoter on performance of broilers. *Poult. Sci.*, 86 (suppl. 1): 217.
- [25] Amer, M.M.; Elbayoumi, Kh. M.; Zeinab M.S. Amin Girh; Eman R. Hassan and M. A. Bosila (2016): Studies on effect of prebiotic on immune response of broiler chicken to ND -AI combined

- inactivated vaccine. *International J. of Chem. Tech. Res.*, 9 (12) 182- 190. CODEN (USA) : IJCRGG, ISSN: 0974-4290, IS SN(Online): 2455-9555
- [26] Elbayoumi Kh.M., Zeinab M.S. Amin Girh, Eman R. Hassan, Aziza M. Amer, Ghazi .A. M. Zohair, and Amer, M.M. (2016): Enhancement of immune response against IBD and IB in antibiotic treated *Mycoplasma gallisepticum* serologically positive broiler chickens. *International J. of Chem. Tech. Res.*, 9 (12) 934-942. CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN (Online): 2455- 9555.
- [27] NRC (National Research Council) (1984): National requirement for poultry. 9th Ed., Washington DC, National Academy Press.
- [28] Reed, L. J. and Muench, H. (1938): Simple method of estimating 50 per cent end point", *Am. J. Hyg.*, 27: 493-499.
- [29] Sainsbury, D., (1984): System of management in "Poultry health and management". 2nd Edition. Granda Publishing (TD), 8 Grafton street, London. WIX 3LA.
- [30] Guarini, C.P. B.; Massi., P. and Tosi, G. (1999): Evaluation of clinical efficacy of a new generation macrolide, Pulmotil AC (tilmicosin), in the treatment of *Mycoplasma*-associated respiratory disease. *Selezione Veterinaria.* (8/9): 603-610.
- [31] Ilaria Capua and Stefano Marangon (2006): Control of Avian Influenza in Poultry. *Emerg. Infect. Dis.* 12(9): 1319–1324. doi: 10.3201/eid1209.060430
- [32] Fabricant, J. and P. P. Levine. (1962): Experimental production of complicated chronic respiratory disease infection ("air sac" disease). *Avian Dis.* 6:13–23.
- [33] Gross, W. B. (1990): Factors affecting the development of respiratory disease complex in chickens. *Avian Dis.* 34:607–610.
- [34] Amer, J. A. Newman, P. Singh, and A. Silim. (1998): Lymphoproliferative responses of specific-pathogenfree chickens to *Mycoplasma gallisepticum* strain PG31. *Avian Pathol.* 27:277–283.
- [35] Razin, S., D. Yogevev, and Y. Naot. (1998):Molecular biology and pathogenicity of mycoplasmas. *Microbiol. Mol. Biol. Rev.*; 62:1094–1156. 329.
- [36] Lam, K. M. (2004):*Mycoplasma gallisepticum*-induced alterations in cytokine genes in chicken cells and embryos. *Avian Dis.* 48:215–219.
- [37] Kleven, S.H., H.-H. Fan, and K.S. Turner. (1998): Pen trial studies on the use of live vaccines to displace virulent *Mycoplasma gallisepticum* in chickens. *Avian Dis.* 42:300-306.
- [38] Turner, K.S., and S.H. Kleven.(1998): Eradication of live F strain *Mycoplasma gallisepticum* vaccine using live ts-11 on a multiage commercial layer farm. *Avian Dis.* 42:404-407.
- [39] Xiaofei Wang, Qinqin Zhou, Jing Shen, Junhu Yao and Xiaojun Yang (2015): Effect of difference doses of Newcastle disease vaccine immunization on growth performance, plasma variables and immune response of broilers. *J. of Animal Sci. and Biotechnol.* 6:20.DOI 10.1186/s40104-015-0019-Y.
- [40] Nunes JO , Abreu RD, Brito JAG, Silva RF da, Oliveira LS and Jesus NA (2015): Enzyme Supplementation of Broiler Feeds with Reduced Mineral and Energy Levels. *Brazilian J. of Poult. Sci.*, 17 no.spe Campinas . ISSN 1516-635X.
- [41] Adel Feizi, Soroosh Babakhani and Hossein Nikpiran (2013): Comparative survey of tiamulin and tylosin in control of *Mycoplasma gallisepticum* in broiler chickens. *Europ. J. of Experimental Biol.*, 3(1):536-539.
- [42] Kuldeep Dhama, Ruchi Tiwari, Rifat Ullah Khan, Sandip Chakraborty, Marappan Gopi, Kumaragurubaran Karthik, Mani Saminathan, Perumal Arumugam Desingu and Lakshmi Tulasi Sunkara (2014): Growth Promoters and Novel Feed Additives Improving Poultry Production and Health, Bioactive Principles and Beneficial Applications: The Trends and Advances-A Review. *Int. J. Pharmacol.*, 10 (3): 129-159.
- [43] Stanley, V.G., C. Gray, M. Daley, W.F. Krueger and A.E. Sefton, (2004): An alternative to antibiotic-based drugs in feed for enhancing performance of broilers grown on *Eimeria* sp. infected litter. *Poult. Sci.*, 83: 39-44.
- [44] Dibner, J.J. and J.D. Richards, (2005): Antibiotic growth promoters in agriculture: History and mode of action. *Poult. Sci.*, 84: 634-643.
- [45] Elgnay F.S. and Azwai S.M. (2013): Seroprevalence of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* in one day old broiler chickens in Libya. *J. of Animal and Poult. Sci.*, 2(1): 11-18.
- [46] Khalda A. Khalifa, Egbal Sidahmed Abdelrahim, Magdi Badwi, and Amal M. Mohamed (2013): Isolation and Molecular Characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in Chickens in Sudan. *J. of Vet. Med.*, Article ID 208026, 4 pages

- [47] Nakamura K., Narita M., Imai K., Matsumura T., Maeda M. and Tanimura T. (1992): The effect of mixed live vaccines of Newcastle disease and infectious bronchitis on the chicken respiratory tract. *J. Comp. Pathol.*;106 (4):341-350.
- [48] Nakamura K. , Ueda H., Tanimura T. and Noguchi K. (1994) : Effect of mixed live vaccine (Newcastle disease and infectious bronchitis) and *Mycoplasma gallisepticum* on the chicken respiratory tract and on *Escherichia coli* infection. *J. Comp. Pathol.* ;111(1):33-42.
- [49] Kleven S.H. (2008) : Control of Avian *Mycoplasma* Infections in Commercial Poultry. *Avian Diseases* 52(3): 367-374.
- [50] Agnes Agunos, Dave Leger, and Carolee Carson (2012): Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada. *Can Vet J.*; 53(12): 1289–1300.
- [51] Chafik Redha Messai, Khatima Aït-Oudhia, Djamel Khelef1, Taha Mossadek Hamdi, Nadia Safia Chenouf and Mohamed Ramzi Messai (2015): Serogroups and antibiotics susceptibility pattern of avian pathogenic *Escherichia coli* strains responsible for Colibacillosis in broiler breeding farms in the east of Algeria. *Afr. J. Microbiol. Res.* 9(49): 2358-2363. DOI: 10.5897/AJMR2015.7600.
- [52] Jia W., Slominski B.A., Bruce H.L., Blank G., Crow G. and Jones O. (2009): Effects of diet type and enzyme addition on growth performance and gut health of broiler chickens during subclinical *Clostridium perfringens* challenge. *Poult. Sci.* , 88 (1): 132-140.
- [53] Parker J, Oviedo-Rondón EO, Clack BA, Clemente-Hernández S, Osborne J, Remus JC, Kettunen H., Mäkivuokko H. and Pierson E.M. (2007): Enzymes as feed additive to aid in responses against *Eimeria* species in coccidia-vaccinated broilers fed corn-soybean meal diets with different protein levels. *Poult Sci.*; 86(4):m643-653.
- [54] Saggiolato, M., P. Massi S., Pretolani and G. Tosi, (2000): Use of tilmicosin in drinking water (*Pulmotil* ACREg.) to control *Mycoplasma synoviae* infection in broilers. *Selezione Veterinaria*, 8/9: 701-704.
- [55] Zangiabadi H. and Torki M. (2010): The effect of a beta-mannanase-based enzyme on growth performance and humoral immune response of broiler chickens fed diets containing graded levels of whole dates. *Trop. Anim. Health Prod.* 42.(6):1209-1217. doi: 10.1007/s11250-010-9550-1.
- [56] Der-Nan Lee, Ching-Feng Weng, Shiau-Ru Lyu, Ruo-Chi Wang, Hen-Wei Wei and Bao-Jichen (2008): Growth performance, immune response, and gastrointestinal health of Taiwan red-feathered native chickens fed diets supplemented with growth-promoting antibiotics. *J. Chin. Soc. Anim. Sci.*37(4):233-247.
- [57] Naoto Yoshino, Masahiro Endo, Hiroyuki Kanno, Naomi Matsukawa, Reiko Tsutsumi, Ryosuke Takeshita, Shigehiro Sato (2013): Polymyxins as Novel and Safe Mucosal Adjuvants to Induce Humoral Immune Responses in Mice. *PLOS ONE.* 8(4) e61643.
- [58] Baba, T.; Yamashita, N.; Kodama, H.; Mukamoto, M.; Asada, M.; Nakamoto, K.; Nose, Y.; McGruder, E. D. (1998): Effect of tylosin tartrate (Tylan Soluble) on cellular immune responses in chickens. *Poult. Sci.*, Savoy, 77(9) 1306-1311.
- [59] Szymańska-Czerwińska, M.; Bednarek, D.; Zdzisińska, B; Kandefer-Szerszeń, M. (2009): Effect of tylosin and prebiotics on the level of cytokines and lymphocyte immunophenotyping parameters in calves. *Central Europ. J. Immunol.*, Warsaw, 34(1) 1-6.