



# Effect of Antibiotic and Prebiotic On Immunity and Reproductivity of Broiler Breeder Flock

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## ABSTRACT

This study was carried out to explore effects of antibiotics (Tylosin, Colestin) and prebiotic (enzymes) on immune status and reproduction of commercial Ross broilers breeder flock of 6000 females and 700 males to use viral vaccines (ND, AI, IB, IBD and CIA) as well as MG and MS infection by ELISA- test. Results revealed that mean ELISA titers against MS was  $685 \pm 120.7$ ,  $1200.4 \pm 322.7$ ,  $924.6 \pm 99.9$ ,  $1373 \pm 168.5$  and  $925.7 \pm 133.4$  at 6, 27, 35, 44 and 61 weeks, respectively; while CV% still is below 30%. Mean ELISA titer against MG was  $2891.8 \pm 146.64$ ,  $608.5 \pm 208.5$ ,  $2961 \pm 1319.5$ ,  $3199.2 \pm 1331.2$  at 6th, 27th, 35th and 44th week, respectively. While CV% was 5.1 at 6th week and increased gradually to be 48.4%. The fluctuation in MS and MS ELISA titers might indicate active infection and/or low challenge frequency that was suppressed by the use of drugs.

ELISA for viral vaccines including ND was  $7594 \pm 4810$ ,  $18906 \pm 3562$ ,  $17543 \pm 5478$  and  $16232 \pm 2718$  at 6th, 15th, 35th and 61st week, respectively. CV% was higher with 63.3 at 6th week (week) and after that was good and excellent. ELISA titer for AI was  $6214.6 \pm 1571.3$  at 6th week and gradually increased to  $14805.8 \pm 2260.7$  at 35th week with CV% less than 30%. Titers against IBD virus was  $3716 \pm 1188$  at 6th week, increased to  $9476 \pm 4578$  by 35th weeks, then decreased to  $3232 \pm 1780$  at 61st weeks. ELISA titer against IB virus started with  $3642 \pm 1048$  and increased to  $11192 \pm 3458$  at 53rd week. CIA titers started with  $1188 \pm 410$ ,  $7848 \pm 1923$  and  $6056 \pm 1385$  at 6th, 27th and 53rd week.

Interpretation of the recorded ELISA titers with CV% indicating uneven immunity at 6th week followed by varied increase from good to excellent. This indicates good uniform flock immune by repeated vaccination, medication and good management; while the decreased titers in last weeks were due to normal decaying of antibody.

Cumulative mortality rate in the flock was 2.5% lower than farm stander. Regarding flock productivity as compared with flock stander resulted in high egg production percent in the first 10 weeks. Decreased egg production and hatched eggs at the last weeks (16, 64) can be attributed to big sized eggs. An increase was seen in both average fertility with 1.35% and average hatchability 0.66% as well as cumulative produced chicks/hen by 5 chicks/hen than farm stander.

Conclusion: Based on serological mortality and reproduction results, this study pointed out that the applied vaccination program and preventive medication (Tylosin, Colestin and enzyme prebiotic) in ration or water to broiler breeders resulted in a higher immunity and reproductive performance.

**Key Words:** Broiler breeder chicken, ELISA test, Antibiotic, prebiotic, reproduction, immune response.

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## INTRODUCTION

Vaccination of breeder flocks providing 1 day old chicks with MDA, protect chicks from infection in the 1<sup>st</sup> – 3<sup>rd</sup> week [1;5]. Vaccine strategies are based on the prevention

of vertical and horizontal transmission of virus to very young chicks by immunization of breeder flocks and have been successful in reducing the incidence of chicken infectious anemia

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(CIA) in young chicks [6]. Antibodies reduced virus shedding from infected chickens [1,7,8] and keep birds in high productivity [9]. The success of passive immunity depends upon antibody titer of the breeder hen, the higher titer (measure of circulating antibodies) in the hen, the greater transfer to the chick. For Newcastle disease (ND), Avian influenza (AI) and Infectious bursal disease (IBD), there is about 50% transfer of titer from the breeder hen to the chick [1, 10, 11]. High uniform titers in breeders are usually obtained with vaccination programs that involve initial administration of a live virus (primer) followed by a killed virus at some older age.

The broiler breeder hen usually starts egg production at the age of 23-24 weeks and produces around 183 hatchable eggs out of 199 total hens housed eggs produced in 65 weeks of its laying cycle [12]. Farooq et al. [13] reported smaller per hen-housed egg production ( $188 \pm 0.56$  eggs) representing  $88 \pm 0.23\%$  hatchable and  $14 \pm 0.18\%$  unhatchable eggs than that reported by North [12]. Probable reason for that could be shorter egg laying cycle and poor rearing environment in former than in later case. Higher mortality rate adversely affect production performance of broiler breeders. Mortality and its negative association with net profit was reported [13,14]. Moreover, North [12] and Ali [15] reported poor economic performance of breeders at mortality level of above 10% and maximum level of mortality 13.11%, respectively.

Serologic monitoring will establish a baseline of antibody titers that are the result of both vaccination and field challenge. Changes in the regular observed antibody titers may indicate a decrease in the efficacy of vaccine administration or an increased field challenge by a particular pathogen. A regular serologic monitoring program is also helpful to determine whether a flock is exposed to a new pathogen [16]. Evolution and diagnostic advantages of the graphic presentation of ELISA are based on flock-profiling data in combination with gross and microscopic pathology data [17].

Prebiotics are non-digestibility and selected ferments capability by some bacterial groups. Most prebiotics are carbohydrates. Moreover, ingredients are often made of several compounds. These molecules not only differ in the polymerization degree but also in the production technology (as, fractions can be obtained either by enzymatic hydrolysis or by extraction); these two manufacturing processes lead up to different mixtures of final products. Intestinal bacteria metabolize these compounds in different ways [18, 19, and 20].

Respiratory disease of poultry cause severe economic losses specially AI, ND, Infectious bronchitis (IB) and Mycoplasma gallisepticum (MG). These affections could be prevented by vaccination that plays an important role in the health management of the poultry flock 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 usage of antibiotics. IBD and CIA cause to a variable degree of immunosuppression in the affected birds [16]. Infection of chicks in the early age displays a severe and prolonged immunosuppression [21].

Talebi and Ghasemi-lak [22] compared ELISA titers 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 in infected birds but do not completely cure the birds from the infections. Antibiotic antibacterial medications are still used in poultry industry in several indications including therapeutic treatment, prevention or as traditional growth promoters [23, 24]. However, using such antibiotics during vaccination has not been established well yet and few data are available in such indication. Both MG and MS are reported as spread chicken pathogens among Egyptian chicken flocks by either serological and /or isolation and identification methods [25, 26]. The eradication of mycoplasma infection can be achieved through improvements in hygiene and management practices, therapeutic treatment of breeder layers and/or hatching eggs and better monitoring procedures. Antimycoplasma drugs are used intensively to improve productivity and reproduction of laying [16, 27, 28]. Stipkovits and Kempf [29] stated that antibiotics could be used for therapeutic treatment or prophylactic medication. Fewer MG and fewer positive reactors was recovered from infected medicated chicks [30]. Administration of very low levels of tylosin in feeding MG infected layers in multiple-age complexes was found to lessen egg production losses [31].

The CV is a measure of variation of antibodies within a group of serum samples. Lower CV indicates the more uniform antibody response and 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 colistin (polymexins), Tylosin (macroloids) antibiotics and prebiotic used in this study showed positive impacts in controlling MG and E.coli and bacterial complication with such bacteria together with enhancement immune response of broiler chickens to use viral vaccines [32,33,34,35].

The objective of present study was to evaluate immune status of commercial broiler breeder chicken flock raised with antibiotic and/ or prebiotic medicated to some used viral vaccines by ELISA- test against ND,AI, IB, IBD and CIA as well as antibody against MG and MS infection in relation to flock reproductively.

## MATERIAL AND METHODS

### 1- Experimental Chicken flock:

A total number of 6700 Ross broilers breeder flock of 6000 females and 700 males were housed in semiautomatic deep litter house.

**2. Ration:**

Commercial breeder chicken ration were given according to Ross breeding manual and [36]. The used commercial

balanced ration was based on yellow corn or soya bean that met with [37] broiler breeder chicken requirements.

**3- Vaccine Strains and vaccination:**

The used viral vaccines, time and route of vaccination are shown in table (1).

**Table (1):** Vaccination Program for breeder chickens

no	Age	Vaccine	Method	Manufacturer
1	0 D	THV	sc	merial
2	4 D	coccivac	feed spray	shering plough
3	7 D	ND CLONE	D.W	INTERVET
4	8 D	AI(H5)+ND and REO LIVE	im/sc	merial/intervet
5	15D	Gumboro D78	D.W	INTERVET
6	20D	Clone HB	D.W	IZO
7	25 D	Gumboro D78 intermediate	D.W	INTERVET
8	6 ws	ILT	E.D	INTERVET
9	7 ws	REO,IB,IBD,ND	S,C	INTERVET
10	8 ws	Vectormune (pox)	W.W	Biomune
11	9 ws	Colone HB	D.W	IZO
12	10 ws	AI(H5) + ND+IB	sc	Lohhman/Merial
13	12 ws	ILT	E.D	INTERVET
14	13 ws	CAV	D.W	Lohhman
		TRT Live	D.W	INTERVET
15	14 ws	Clone ND	D.W	INTERVET
16	15 ws	EDS and AI(H5)+ND	im/sc	Biomune/Lohhman
17	17 ws	IBH120	D.W	INTERVET
18	19 ws	ND La Sota	D.W	INTERVET
19	20 ws	TRT inac+Reo+IB+ND+IBD	im/sc	INTERVET/Biomune
20	21 ws	AI(H5) +ND La Sota	s.c/ D.W	INTERVET
34 week & Every 5 ws		ND La Sota	D.W	INTERVET

**4- Natuzyme® Prebiotic:**

It is a multienzyme poultry feed supplements commercial product, Novartis Limited, India contains: 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. Prebiotic was given in ration in the first 28 days, then 3 days before vaccination.

**5- Antibiotics:**

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

b- Tylox® : tylosin water soluble powder 100gm - Lot. No. 150118. Jordan Vet. and Agr. Med. Ind. Co – Amman – Jordan. Both Tylosin and colestin were at first 3 days and 28-30 days followed by every 45 days dose of tylosin + colistin 2 days in water.

**6- Samples:**

Blood samples for serum were collected for ELISA test at the end of the week 6, 15, 24, 35, 44, 53 and 61 of life to detect ELISA antibody titers against ND, AI, IB, IBD, CIA , MG and MS. Results are shown in tables (1 - 3).

**7- Serological ELISA test:**

The collected sera were tested to evaluate the antibodies titer against ND, AI, IB, IBD MG, CIA and MS

antibodies procedure performed using commercial ELISA kits according to the manufacturer's recommendations:

a. AI: Indirect ELISA methods, including ProfLOCK plus AIV Ab test kit (Synbiotics, USA), indirect ELISA methods were performed.

b. 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 micro-titer plates containing NDV antigen.

c. IBD: The sera obtained from blood of experimental chicks at various time points were tested for IBD antibodies using the PROFLOCK® plus IBD Ab test kit (Symbiotics, San Diego, CA).

d. IB: The PROFLOCK® IBV ELISA Kit (Synbiotics, USA), which is a rapid serologic test used for the detection of IBV antibody in chicken serum samples.

e. CIA: All samples were analyzed by Elisa kit (Synbiotic Corp., USA) in the same condition. According to the kit's instruction, the serum samples with the S/P ratio equal or less than 0.349 were considered zero titer and S/P ratio equal or greater than 0.350 ( $\geq 1472$ ) were considered positive serum.

f. MG: The procedure used in this test was performed using commercial ELISA kits for the presence of anti-MG antibodies ProfLOCK® MG Antibody Test Kit, Synbiotics Corp. - USA].



g. MS: commercial ELISA kit (Synbiotics Corp., ProFlock, USA) to detect specific antibodies against MS were used according to the manufacturer's instructions.

**8- 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.

**9. Reproduction:**

Reproduction of the flock under study was calculated at 27<sup>th</sup>, 35<sup>th</sup>, 44<sup>th</sup>, 53<sup>rd</sup> and 61<sup>st</sup> week of life according to North [12] as compared with breed stander as well as the farm stander [36], which is the average of reproduction of last 3 successive flocks from starting of egg laying at 24<sup>th</sup>

– 64<sup>th</sup> week (40 production weeks). The obtained results are seen in table (4-6).

**RESULTS AND DISCUSSION**

Good management, vaccination program and preventive medication are required to obtain high performance and production as well as hatchery parameters in poultry farms. Results of table (2) revealed that mean ELISA titers against MS was 685±120.7 and increased to 1200.4±322.7 till 27<sup>th</sup> week, then slightly decreased to 924.6±99.9 at 35<sup>th</sup> week, then increased again at 44<sup>th</sup> week to 1373±168.5 and decreased at 61<sup>st</sup> week to 925.67±133.4, ELISA titers reduction at 35<sup>th</sup> week might be due to using antibiotic at this time and susceptibility of infected strain to such antibiotic [39,40] resulting in decreased bacterial population while such titer rose at

**Table ( 2 ):** Main ELISA titers against MS and MG in vaccinated and medicated breeder flock.

Age/ weeks	No of samples	MS			MG		
		mean	SD	CV%	mean	SD	CV%
6	31	685	120.7	17.6	2891.8	146.64	5.1
15	45	1924.6	50.4	2.6	1446.2	176.8	12.2
27	30	1200.4	322.7	26.9	608.5	208.5	34.3
35	30	924.6	99.9	10.8	2961	1319.5	44.6
44	35	1373.3	168.5	12.3	3199.2	1331.2	41.6
53	20	1099	313.5	28.5	668.3	281.5	42.1
61	48	925.67	133.4	14.4	364.9	176.6	48.4

44<sup>th</sup> week indicating activation of latent infection again, which gradually decreased at 53<sup>rd</sup> and 61<sup>st</sup> week, which indicate normal decaying of antibody [41], while CV% still is below 30% all observation time indicate persistent infection in spite of antibiotic treatment, which indicates resistance to used antibiotic or persistence on material in poultry environment [42]. Moreover, other researchers stated that antibiotic treatment against MS could decrease symptoms of the disease but did not eliminate infection [43]. While mean ELISA titer against MG was

2891.8±146.64 at 6<sup>th</sup> week of life, gradually decreased till 27<sup>th</sup> week of life and become 608.5±208.5, then sharply increase again at 35<sup>th</sup> and 44<sup>th</sup> week of life to 2961±1319.5 and 3199.2±1331.2, respectively, which indicates active infection [44]. Then, again decreased gradually in next 53 and 61 weeks of life which indicates possible normal decaying of antibody [41], while CV% was 5.1 at week 6 and increased gradually to 48.4 % indicate uneven immunity due to challenge frequency.

**Table ( 3 ):** Main ELISA titers against ND and AI in vaccinated and medicated breeder flock

Age/ week	No of samples	ND			AI		
		Mean	SD	CV%	Mean	SD	CV%
6	31	7594	4810	63.3	6214.6	1571.3	25
15	45	18906	3562	18.8	10113.8	1372.1	14
27	30	12083	3239	26.8	12547.5	1908.5	15
35	30	17543	5478	31.2	14805.8	2260.7	15
44	35	14762	3217	21.8	6366.4	2260.2	36
53	20	15682	3458	22.1	4986.5	2310.5	46
61	48	16232	2718	16.7	4121.7	2260.2	55

Results of mean ELISA for ND at 6 weeks of age was 7594±4810, which increased by 15 weeks of age to 18906±3562 indicating good vaccination immune response [45] by 27 weeks of age. This titer become 12083±3239, which indicate field challenge [46], then increased by 35 weeks of age to 17543±5478 good immune response due to vaccination, then decreased by 44 weeks of age to 14762±3217 indicating field

challenge due to indemicity of such disease and increase again and still was high at 53 and 61 weeks of age to be 15682±3458 and 16232±2718, respectively, which maybe indicate good immune response due to vaccination and absence of field challenge, while CV% was higher with 63.3 at 6 weeks of age and decreased very good flock immune uniformity [47]. ELISA titer for AI (Table 3) was 6214.6±1571.3 at 6 week-old, which gradually



increased weekly to 14805.8±2260.7 at 35 week-old with CV% less than 30, which indicate good vaccination immune response with no challenge [48], then decreased by week 44 to 6366.4±2260.2, which indicate maybe field challenge [49].

Mean ELISA titers against IBD virus at 6 week-old was 3716±1188, started to increase 9476±4578 by 35<sup>th</sup> week. This indicates good immune response due to vaccination without any field challenge [50, 51], then decreased gradually to 3232±1780 at 61<sup>st</sup> weeks indicating normal decaying of antibody [41]. While mean antibody titer

against IB virus starts with 3642±1048 and increases gradually to 21170±13329 at 27<sup>th</sup> weeks due to vaccination with no evidence of field challenge then decrease gradually to 11192±3458 at 53<sup>rd</sup> week [52]. Concerning CIA mean antibody titers start with 1188±410 and increase gradually to 7848±1923 at 27<sup>th</sup> week indicating good antibody response due to vaccination [53] and decreased by 35 and 44 weeks of age then increase again to 6056±1385 at 53<sup>rd</sup> week.

**Table (4):** Main ELISA titers against IBD, IB and CIA in vaccinated and medicated breeder flock

Age/ week	No of samples	IBD			IB			CIA		
		Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
6	31	3716	1188	32.0	3642	1048	28.8	1188	410	34.5
15	45	2088	1356	64.9	15501	3256	21.0	7078	1536	21.7
27	30	4083	929	22.8	21170	13329	63.0	7848	1923	24.5
35	30	9476	4578	48.3	18006	14325	79.6	4008	2847	71.0
44	35	7119	2137	30.0	16377	17321	105.8	2780	732	26.3
53	20	5574	2158	38.7	11192	3458	30.9	6056	1385	22.9
61	48	3232	1780	55.1	15176	12718	83.8	5394	1278	23.7

Interpretation of the recorded ELISA titres to CV%, indicates uneven immunity at 6 weeks and followed by increased titres and to be varied from good to excellent. This indicates good flock immune uniformity by repeated vaccination field challenge; while the decreased titres in last weeks were due to normal decaying of antibody.

The recorded cumulative mortality (Table 5) rate (12.5%) in the flock was 1.5 % lower than farm stander (14%). This result indicates improvement and higher production performance and immunity of broiler breeders [12,13,14,15].

**Table (5):** Average weekly Egg production % , egg produced and hatching eggs / hen housed / week of farm stander and treated flocks.

Age/weeks	Egg production %		Egg/Housed hen/week		Hatch Egg/Housed hen/week	
	Farm Standar	Treated	Farm Standard	Treated	Farm Standard	Treated
27	55.0	56.5	3.85	3.66	3.96	3.81
35	84.0	86.9	5.88	5.57	6.08	5.40
44	75.0	79.5	5.15	4.82	5.57	4.90
53	66.5	67.5	4.66	4.40	4.73	4.43
61	56.0	45.5	3.92	3.75	3.18	3.10
64	48	46.3	3.36	3.23	3.24	3.08
Average egg production %	63.01	65.13				
Cumulative egg			178.56	164.73	180.32	173.50
Cumulative Mortality%	14	12.5				

\* Hen-week (%) is based on the assumption that mortality in lay is 8% with 0.2% mortality per week.

\*\* A hatching egg is considered to be an egg which is 50- 70 g.

Regarding effect of Treatment of flock productivity as compared with flock stander (Table 5), resulted in increased egg production percent in the first 10 weeks. This was matched with Torki et al. [54] who found that enzymes improves productive performance and egg quality of laying hens, moreover use of both tylosin and colistin could improve egg production percent due to controlling infectious agents such as Mycoplasma spp. and /or complicating agents such as avian pathogenic E.coli and Mycoplasma [32,38,34] affected both quantity

and quality of produced egg [55,56]. Decreased egg production and hatched eggs at the last weeks (61 and 64) can be attributed to big sized eggs.

Results of reproduction (Table 6) increased average fertility with 1.35% and average hatchability 0.66% with improvement of shell characters. This may be due to that controlling infectious agents such as MG, MS, E.coli and Salmonella by antibiotic used resulting in improving fertility and hatchability [22,32,57,58].

**Table (6):** Weekly Hatchability and hick Production, average Fertility and Hatchability as well as cumulative chick/hen and total culls percentage in treated and farm stander of breeder chickens.

Age/weeks	Farm Standard		Treated	
	Hatchability (%)	Chicks/Week Hen-Housed	Hatchability (%)	Chicks/week Hen-Housed
27	80.6	2.35	81.8	2.47
35	90.5	5.60	92.3	5.63
44	86.5	4.62	86.8	4.78
53	79.9	3.62	77.6	3.68
61	73.6	2.35	75.6	3.18
64	70.2	2.45	71.8	2.62
Average Fertility %	93.60		94.95	
Average hatchability	82.0		82.66	
Cumulative		168.5		173.5
Culled chick%	1.71		1.55	

NOTES: \* Hatchability is based on an average egg age of 3 days. Hatchability will drop by 0.5% per day of storage between 7 and 11 days.

Cumulative produced chicks per hen was increased due to treatment by 5 chicks/hen than farm stander. This may be due to controlling poultry pathogens leading to embryonic death in hatcheries, such as avian pathogenic mycoplasma [16], E.coli [59], and Salmonella spp. [60] as both pathogens affect fertility and hatchability.

From both serological results and productivity, we can notice that flock under test had good immune response and production parameters.

In conclusion, our field study pointed out that the applied vaccination program and preventive medication (Tylosin, Colestin and enzyme prebiotic) in ration or water to broiler breeders resulted in a higher immunity and reproductive performance as compared with farm stander as control.

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