

# Evaluation of Antibiotics to Control *Mycoplasma gallisepticum* in Broiler Breeder Chickens

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## Abstract

This study aims at the evaluation of the efficacy of Pulmotil and Denagard in comparison to the generic tylosin against *Mycoplasma gallisepticum* (MG) infection in broiler breeders. A total of 600 twenty four-week old broiler breeder pullets along with their 60 roosters of the Ross 308 strain, were equally subdivided into four treatments of 150 birds each with 3 replicates per treatment (50 pullets and 5 roosters/pen). The MG-free birds of Group 1, the Control, were kept in a separate house and left with no MG-challenge or drug administration while those of groups 2, 3, and 4 were raised in another house and previously challenged with MG at 2 and 24 weeks of age respectively. Every 4 weeks and for 3 consecutive days, birds of group 3 were administered Pulmotil (3–21 Weeks) and Denagard (25–44 weeks), whereas those of Group 4 were treated throughout the trial with generic Tylosin. Production performance parameters were not significantly different among the differently treated birds. At 30 weeks of age, Pulmotil-Denagard treatment significantly reduced the tracheal MG counts to a similar level to that of the controls and restored the fertility of the MG challenged birds at 39 weeks of age. At 35 weeks of age, Pulmotil-Denagard treatment significantly reduced the frequency of day-old progeny with positive airsac lesions. It also reduced MG colonization in the airsacs of the day-old offsprings of the 35 and 39 week old breeders. As the Pulmotil-Denagard treatment significantly reduced the MG colonization of bird tissues, it reduced MG sera titers at 12, 20 and 30 weeks of age, and increased titers against IB, IBD and ND at later stages. In conclusion, the use of Pulmotil/Denagard program is highly recommended in MG-infected breeder farms, and protects against any potential MG endemic infection.

**Keywords:** Pulmotil; Denagard; Tylosin; Broiler breeder; *Mycoplasma gallisepticum*; Performance; Pathology; Immunity

## Introduction

*Mycoplasma gallisepticum* (MG) is a main causative agent of complicated chronic respiratory disease (CCRD). MG affects also the egg production in chickens and results in reduced feed conversion efficiency, condemnation and downgrading of broilers carcasses at slaughter because of airsacculitis. Consequently, MG infection increases medication costs and decreases hatchability and quality of one day old chicks [1,2]. Different types of vaccines are used to prevent MG infections in poultry. Most of these vaccines are either killed (bacterins) or mutant live ones, namely ts-11 that showed significant impact in protecting breeders and layer flocks. In spite of the availability of MG vaccines, the level of protection conferred by these vaccines to poultry flocks is not consistent [3,4].

Control of MG infection by chemotherapy is another practical way to control and minimize the economic losses due to MG infection [5]. Alun and Ching-Ching [6] and Pakpinyo and Sasipreeyajan [7] reported strong resistance to erythromycin, while Ellakany et al. [8] found that MG field isolates from Egypt were highly sensitive to tiamulin, tylosin, but moderately sensitive to enrofloxacin. However, it was reported in literature that resistance to tylosin increased in the last decades [9,10].

Abd El Hamid et al. [11] mentioned that tiamulin was found to be the most effective drug against 10 MG field isolates with minimal inhibitory concentration (MIC) of 0.0125-0.4 µg/ml, followed by doxycycline (MIC: 0.003-0.4 µg/ml), then tylosin (MIC of 0.025-0.4 µg/ml), Enrofloxacin (MIC of 0.0125-0.1 µg/ml), Ciprofloxacin (MIC of 0.2-1.6 µg/ml) and finally Erythromycin (MIC of 3.2-6.4 µg/ml). Accordingly, periodical antibiotic sensitivity testing of new drugs to the emerging field isolates of MG is needed as a guide to mass treatment [12]. Tiamulin and Tilmicosin were equally efficient in reducing MG-

caused respiratory illness in broilers and layers as demonstrated in the works of Zakeri and Kashefi [13]; however, Tiamulin efficacy in treating MG infected broiler breeders is not yet reported in literature.

This study aimed at the evaluation of the anti-mycoplasmal efficacy of two commercial drugs, namely Pulmotil (Tylosin) and Denagard (Tiamulin) against *Mycoplasma gallisepticum* (MG) infection in broiler breeders. Performance, pathological and immunological parameters were assessed in this study following the administration of these two commercial drugs to the control and MG-challenged birds.

## Materials and Methods

### Birds and housing

This experiment was conducted at the research facilities of the American University of Beirut in the Beqaa region where equipped poultry houses are available. A total of 720 two-week old broiler breeder pullets of the Ross 308 strain, were received and equally subdivided into four groups of 180 birds each (three floor pens/group with 60 birds/pen). Also 150 two-week old males of the same strain were provided and grown in 3 floor pens with 50 birds per pen. A poultry house with 9 floor pens was used to grow the males (3 pens) separately from the females (3 pens). Another poultry house was also

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used to grow the rest of the females in 9 individual floor pens at the rate indicated above. Birds in both houses were offered water *ad libitum* and daily feed quantities as per the ROSS 308 Parent Stock Performance recommendations provided by the company. At arrival, tracheas of 20 birds were collected to confirm that the birds are MG free using Frey's culturing method [14]. Males and females were raised separately according to ROSS 308 Manual. At 22 weeks of age, the females were selected in homogeneous groups (birds with extreme body weights were removed) and only 50 pullets were retained per pen. Males were introduced to each pen in a ratio of 1 male to ten females.

### Preparation of MG challenge strain

The MG strain used for challenge was previously isolated from a suspectedly infected laying hen and confirmed as *Mycoplasma gallisepticum* following its culture on Frey's medium and slide agglutination test at the American University of Beirut, and biochemical characterization tests at the Avian Medicine Laboratory at "Istituto Zooprofilattico Sperimentale delle Venezie", Italy. The field isolate was serially subcultured over 50, then 500 ml then one liter of Frey's broth, and the count is adjusted finally to  $8.3 \times 10^6$  cfu/ml. It is worth noting that the Avian Medicine Laboratory confirmed the in-vitro susceptibility of this isolate to tilmicosin, tiamulin, tylosin, enrofloxacin, erythromycin, oxytetracyclin and doxycycline, and resistance to Lincomycin.

### Treatments

Female birds were assigned to 4 treatments so that unchallenged birds (one treatment and all the males) were kept in one house with three replicates per treatment, whereas all challenged female birds (3 treatments) were kept in the other house. The four treatments are indicated in Table 1.

In order to obtain accurate data, unchallenged control birds of treatment 1 were raised in a separate building and served by another animal attendant to eliminate the possibility of cross contamination among the groups.

### Blood collection for seroconversion studies

A volume of around 2 ml of blood was collected from the wing vein of 5 birds/pen (15 birds/treatment) into non-heparinized tubes at 12, 23, 30, 35, 39 and 44 weeks of age. Blood was centrifuged for 10 minutes at 2000 rpm to collect sera that were analyzed for ELISA titers to MG, Infectious Bursal Disease (IBD), Infectious Bronchitis (IBV), and NewCastle Diseases (NDV).

### Observations

The parameters observed in this study were:

#### Performance parameters

- Hen weekly body weight during both rearing and egg production periods

- Egg production
- Egg weights and 1-day old baby chick weight among different groups
- Fertility and hatchability percentages at 30, 35 and 39 weeks of age. For this purpose, a total of 19 eggs/group/setting were incubated for 21 days.

#### The pathological studies included

- Determination of the mortality percentage among breeders
- Determination of MG load in the breeders' trachea at 30, 35, 39 and 44 weeks of age: Five tracheal swab/pen were pooled in 5 ml of Frey's broth suspension. The quantitation of MG in these suspensions was determined using real time qRT-PCR according to Grodio et al. [15].
- Determination of the livability of the progeny: a total of 10 chicks/week/pen (30 chicks per treatment) were transferred to a brooder cage for a period of one week. Livability percentage was recorded at the end of the seventh day.
- Recording the air sac lesion score and tracheitis of three-day-old chicks/pen (9 chicks per treatment) at 35 and 39 weeks of breeders age.
- Determination of MG vertical transmission from 35 and 39 weeks old breeders to the progeny: airsacs and tracheas were collected from three-day-old chicks/pen and pooled separately. The quantitation of MG in trachea and airsac pools was determined using real time qRT-PCR according to Grodio et al. (2010).
- ELISA seroconversion to MG at 12, 23, 30, 35, 39 and 44 weeks of age. Five serum samples were collected from each pen (15 samples from each group). ELISA seroconversion to IBV, IBDV and NDV was also determined at 30, 35 and 39 weeks of age (IDEXX kits, One IDEX X Drive Westbrook, Maine 04092 USA).

### Experimental period

This research trial lasted for 44 weeks and the work was granted the approval of the Institutional Animal Care and Use Committee (IACUC) of the American University of Beirut.

### Statistical design and analysis

The design of the trial is a complete randomized design with four treatments replicated 3 times with 50 females and 5 roosters per replicate. Data were analyzed using the GLM procedure and means were compared following the Tukey's method of SAS [16].

Group	Infection with MG suspension <sup>a</sup>	Medication
1	-	-
2	Weeks 3 (intrathoracic), 4 (ocular), 23 (intrathoracic) and 24 (ocular)	-
3	Weeks 3 (intrathoracic), 4 (ocular), 23 (intrathoracic) and 24 (ocular)	Pulmotil/Denagard <sup>b</sup>
4	Weeks 3 (intrathoracic), 4 (ocular), 23 (intrathoracic) and 24 (ocular)	Tylosin <sup>c</sup>

<sup>a</sup>MG field isolate is grown in Frey's Broth, the MG count is adjusted to  $8.3 \times 10^6$  cfu/ml. Birds were challenged intrathoracically with 0.5 mL and ocularly with 50  $\mu$ L of the prepared suspension.

<sup>b</sup>Pulmotil AC at a rate of 15mg/kg body weight was administered in drinking water at three weeks of age, for a period of 3 days and then every 4 weeks in drinking water for 3 successive days, and Denagard at 25 weeks of age for 3 successive days at a rate of 12.5 mg/kg body weight and repeated every 4 weeks.

<sup>c</sup>Tylosin was administered in drinking water at three weeks of age, for a period of 3 days and then every 4 weeks in drinking water for 3 successive days at a rate of 15 mg/kg body weight.

**Table 1:** Allocation of treatments to experimental broiler breeder groups.

## Results and Discussion

### Performance of birds

Mortality during the rearing period in males and pullets was very low and did not exceed 3% throughout the 24 week-period. During this period, birds in both houses had body weight and daily feed intake figures comparable to those of ROSS 308 (Aviagen, 2014). The performance of birds from 24 to 44 weeks of age in terms of body weight change, mortality rate, percent egg production, along with feed conversion values were not significantly different among the different treatments ( $P>0.05$ ) and averaged 546 g, 5.33%, 72.6% and 2.724 kg feed per dozen of eggs, respectively.

### MG load in tracheal swab suspensions

Table 2 shows the MG load in tracheal swab suspensions collected from the broiler breeders at 30, 35, 39 and 44 weeks of age. At 30 weeks of age, Pulmotil/Denagard treatment (Group 3) significantly reduced the tracheal Log<sub>10</sub> MG counts to 1.23, non-significantly different from that of the control Group 1. Birds of the tylosin-treated Group (4) showed significantly lower tracheal MG count in comparison to those challenged and deprived of any medication (Group 2,  $P<0.05$ ) but higher values than those of the Pulmotil/Denagard treated group (3,  $P > 0.05$ ). At later stages, namely 35, 39 and 44 weeks of age, birds of the MG challenged group 2 showed significantly higher tracheal MG counts in comparison to the rest, indicating the success of the MG challenge model used in this study [4,17].

### Egg and day-old chick weights

Table 3 shows the average weight of eggs and day-old chicks of experimental breeders at 39 and 44 weeks old. Egg weight in group 1 (control) was significantly higher than the eggs of group 2 and numerically higher than the eggs of the rest probably because the birds of group 1 were larger in size than those of the other groups [18]. Regarding the groups with MG challenge, the average egg weight was numerically the lowest in group 2 in comparison with all other groups. It is worth mentioning that treatment with Pulmotil/Denagard (group 3) and tylosin (group 4), improved the weight of eggs at 39 and 44 weeks of age. The same pattern was further reflected by the day-

old chick weight, indicating that both the Pulmotil/Denagard and the generic tylosin redressed the egg weight and consequently the day-old chick weight following an early MG infection.

### Fertility and hatchability of eggs

Table 4 shows the fertility and hatchability percentages of eggs collected from the experimental broiler breeders at 30, 35, 39 and 44 weeks of age. The MG challenged group 2, that was deprived of medication showed a consistent lower fertility and hatchability percentage in comparison to those of other groups as of 30 weeks of age. These results indicate the success of the MG challenge model that was adopted in this study, drastically affecting the reproductive performance [19]. Both Pulmotil-Denagard program and generic tylosin treatments succeeded in restoring the fertility and hatchability levels, with non-significantly higher values recorded for Pulmotil-Denagard program treated group (3) in comparison to Tylosin-treated group (4).

### Lesions scores and MG colonization of the tracheas and airsacs of the progeny

Table 5 presents the frequency of day old chicks with positive tissue lesion score from 35 and 39 weeks old breeders. Positive tissue lesions were not observed in air sacs and tracheas of day old chicks hatched from eggs obtained at 35 and 39 weeks from the control group. At 35 weeks of age, Pulmotil - Denagard treatment program (Group 3) significantly reduced the frequency of day-old chicks with positive air sac lesions. Consequently, there was no significant difference between Group 3 and the controls (3/9 vs. 0/9, respectively,  $P>0.05$ ). However, birds of groups 2 (MG challenged) and 4 (MG challenged and treated with tylosin) showed the highest frequency of day-old chicks with positive air sac lesion score (6/9 and 4/9, respectively). The same pattern was observed when the breeders were 39 weeks-old.

As for tracheitis, the frequency of day old chicks showing this lesion was null to very mild, with no significant differences among the progeny of breeders at 35 and 39 weeks of age (Table 5). These results might indicate that the MG isolate that was used in this experiment has the tendency to infect mostly the tissues of the lower respiratory system of the chicks [20].

Treatment <sup>b</sup>	Log <sub>10</sub> CFU count of MG/mL of tracheal swab suspension <sup>a</sup> at			
	30 weeks	35 weeks	39 weeks	44 weeks
1	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>
2	6.37 <sup>C</sup>	8.69 <sup>B</sup>	5.31 <sup>B</sup>	6.4 <sup>B</sup>
3	1.23 <sup>A,B</sup>	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>
4	2.29 <sup>B</sup>	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>
SEM <sup>c</sup>	0.37	0.47	0.3	0.34

<sup>a</sup>Day-old breeders were free from any MG infection as revealed by culture of tracheas (collected from 20 birds) in Frey's medium  
<sup>b</sup>1) Control; 2) MG challenged; 3) MG challenged and treated with Pulmotil and Denagard; 4) MG challenged and treated with Tylosin.

<sup>c</sup>Pooled Standard error of mean

<sup>A-C</sup>Means in the same column with different superscripts are significantly different ( $p<0.05$ ).

**Table 2:** *Mycoplasma gallisepticum* colonies count (Log<sub>10</sub> CFU count of MG/mL of tracheal swab suspension<sup>a</sup>) collected from the experimental breeders at different ages.

Treatment <sup>a</sup>	Average Egg weight (g), 39 weeks	Average chick weight (g), 39 weeks	Average Egg weight (g), 44 weeks
1	67.6 <sup>B</sup>	48.7 <sup>B</sup>	71.1 <sup>B</sup>
2	63.9 <sup>A</sup>	44.8 <sup>A</sup>	66.8 <sup>A</sup>
3	65.8 <sup>A,B</sup>	47.9 <sup>A,B</sup>	68.0 <sup>A,B</sup>
4	64.7 <sup>A,B</sup>	47.6 <sup>A,B</sup>	67.5 <sup>A,B</sup>
SEM <sup>b</sup>	0.36	0.4	0.47

<sup>a</sup> 1) Control; 2) MG challenged; 3) MG challenged and treated with Pulmotil and Denagard; 4) MG challenged and treated with Tylosin.

<sup>b</sup> Pooled Standard error of mean

<sup>A,B</sup> Means in the same column with different superscripts are significantly different ( $p<0.05$ ).

**Table 3:** Average weight of eggs and day-old chicks hatched from MG breeders.

Treatment <sup>a</sup>	1	2	3	4	SEM <sup>b</sup>
Fertility 30 wk	94.7 <sup>A</sup>	84.2 <sup>B</sup>	94.7 <sup>A</sup>	93.0 <sup>A</sup>	1.39
Hatchability 30 wk	91.2 <sup>A</sup>	66.7 <sup>B</sup>	86.0 <sup>A</sup>	84.2 <sup>A</sup>	2.82
Fertility 35 wk	96.3 <sup>A</sup>	70.2 <sup>C</sup>	94.7 <sup>AB</sup>	89.5 <sup>B</sup>	1.98
Hatchability 35 wk	91.0 <sup>A</sup>	56.1 <sup>C</sup>	86.0 <sup>AB</sup>	73.7 <sup>B</sup>	2.62
Fertility 39 wk	98.0 <sup>A</sup>	87.7 <sup>C</sup>	96.5 <sup>A</sup>	94.7 <sup>B</sup>	0.92
Hatchability 39 wk	94.5 <sup>A</sup>	59.6 <sup>B</sup>	93.0 <sup>A</sup>	87.7 <sup>A</sup>	2.75
Initial number of birds 35 wk	11	9.7	11.3	14	NA
Livability 35 wk (%)	100	100	96.7	100	1.26
Initial number of birds 39 wk	10	5.3	10.7	9.3	NA
Livability 39 wk	96.3	100	100	100	1.75

<sup>a</sup> 1) Control; 2) MG challenged early; 3) MG challenged early and treated with Pulmotil and Denagard; 4) MG challenged early and treated with Tylosin.

<sup>b</sup> Pooled standard error of means

<sup>A-C</sup> Means in the same row with different superscripts are significantly different (P<0.05)

**Table 4:** Percent fertility and hatchability of eggs produced by MG treated Ross 308 breeders along with livability of hatched chicks during the first 10 days of life.

Treatment <sup>a</sup>	positive airsac lesion score, 35 weeks	Tracheitis, 35 weeks	positive airsac lesion score, 39 weeks	Tracheitis, 39 weeks
1	0/9 <sup>A</sup>	0/9	0/9 <sup>A</sup>	0/9
2	6/9 <sup>B</sup>	01-Sep	6/8 <sup>B</sup>	01-Aug
3	3/9 <sup>A,B</sup>	0/9	3/9 <sup>A,B</sup>	01-Sep
4	4/9 <sup>B</sup>	0/9	4/8 <sup>B</sup>	01-Aug

<sup>a</sup> 1) Control; 2) MG challenged early; 3) MG challenged early and treated with Pulmotil and Denagard; 4) MG challenged early and treated with Tylosin.

<sup>A-B</sup> Frequencies in the same column with different superscripts are significantly different (P<0.05)

**Table 5:** Frequency of day-old chicks, with positive tissue lesion score, from the progeny of the MG breeder trail.

Treatment <sup>a</sup>	Trachea, 35 weeks	Airsacs, 35 weeks	Trachea, 39 weeks	Airsacs, 39 weeks
1	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>	<0.9	<0.9 <sup>A</sup>
2	1.34 <sup>B</sup>	3.41 <sup>B</sup>	1.8	4.79 <sup>B</sup>
3	<0.9 <sup>A</sup>	<0.9 <sup>A</sup>	<0.9	<0.9 <sup>A</sup>
4	<0.9 <sup>A</sup>	0.93 <sup>A</sup>	<0.9	0.93 <sup>A</sup>
SEM <sup>b</sup>	0.34	0.4	0.37	0.38

<sup>a</sup> 1) Control; 2) MG challenged; 3) MG challenged and treated with Pulmotil and Denagard; 4) MG challenged and treated with Tylosin.

<sup>b</sup> Pooled standard error of means

<sup>A-B</sup> Means in the same column with different superscripts are significantly different (P<0.05)

**Table 6:** *Mycoplasma gallisepticum* colonies counts as Log<sub>10</sub> CFU count of MG/g of tissue homogenate collected from the progeny of the MG breeder trail.

Table 6 shows the *Mycoplasma gallisepticum* colonies count in homogenates of tracheas and air sacs collected from the progeny of experimental breeders at 35 and 39 weeks of age. Regarding the progeny of 35 weeks-old breeders, the highest MG count in tracheas and air sacs was recorded in Group 2, which had an MG challenge with no antibiotic treatment. This proves, again, the success of the MG challenge model that is adopted in this study, reflected this time by a vertical transmission of the bacterium to the progeny [21]. All other groups showed very low MG count in the trachea, below the detectable levels of the adopted q-PCR method. The same pattern applies for the progeny of 39 weeks-old breeders. This is another indication that the MG isolate used in this study doesn't colonize heavily the tissues of the upper respiratory tract [20].

As for MG colonization of the air sacs of the progeny of 35 weeks-old breeders, Pulmotil - Denagard program significantly reduced the Log<sub>10</sub> MG count to less than 0.9/g of tissue (Group 3) as compared to 3.41 for the challenged non-treated group 2. Tylosin also reduced significantly the Log<sub>10</sub> MG count in the air sacs but to a level of 0.93/g of tissue. The same pattern was observed for the progeny of the breeders at 39 weeks of age.

### Humoral response to MG and vaccination

Sera titers of different experimental breeder's groups to MG,

at different ages, are presented in Table 7. The highest titers were recorded for group 2, which had an early MG challenge but deprived of any treatment. This reflects the high MG colonization rate in tissues of these birds, as demonstrated in Table 2. The Pulmotil-Denagard treatment (Group 3) significantly reduced the MG colonization of bird tissues, resulting in lower titers to MG specifically at 12, 20 and 30 weeks of age (287.21, 402.4 and 986.92 respectively). Tylosin-treated birds (Group 4) showed also lower titers to MG, however the decrement was not as prominent as that observed for the Pulmotil-Denagard treated Group 3. These results confirm the superiority of Pulmotil-Denagard treatment program over tylosin in reducing MG infection in challenge birds. It is worth noting that birds of Group 1, the control, showed significantly lower titers to MG, indicating the absence of any cross contamination with MG, and the success of the biosecurity measures that were taken during the whole study [3,22].

Tables 8-10 show the breeders sera titers to IBV, NDV and IBD at 30, 35 and 44 weeks of age, respectively. The pattern was almost similar for the birds that had an MG challenge (groups 2, 3 and 4) and the control (Group 1). Titers to IBD, IBV and NDV were lower in Pulmotil-Denagard treated group (3) at earlier stages (30 weeks of age), in comparison to those of the controls (Group 1), Tylosin-treated (Group 4) and non-treated (Group 2) birds. Afterwards, the sera titers observed for Group 3 recovered progressively and recorded the highest values at 44 weeks of age [23]. These results confirm the positive impact



Treatment <sup>a</sup>	MG 12 weeks	MG 20 weeks	MG 23 weeks	MG 30 weeks	MG 35 weeks	MG 44 weeks
1	328 <sup>A</sup>	171 <sup>A</sup>	287 <sup>A</sup>	160 <sup>A</sup>	131 <sup>A</sup>	166 <sup>A</sup>
2	3201 <sup>C</sup>	5011 <sup>B</sup>	4037 <sup>C</sup>	1837 <sup>A,B,C</sup>	3429 <sup>B,C</sup>	2024 <sup>C</sup>
3	287 <sup>A</sup>	402 <sup>A</sup>	987 <sup>A,B</sup>	828 <sup>A,B</sup>	1773 <sup>B</sup>	1792 <sup>B,C</sup>
4	1839 <sup>B</sup>	990 <sup>A</sup>	1316 <sup>A,B</sup>	2458 <sup>B,C</sup>	3025 <sup>B,C</sup>	1808 <sup>B,C</sup>
SEM <sup>b</sup>	153.9	252.8	245.2	198.4	350.6	186

<sup>a</sup> 1) Control; 2) MG challenged; 3) MG challenged and treated with Pulmotil and Denagard; 4) MG challenged and treated with Tylosin.  
<sup>b</sup> Pooled standard error of means  
<sup>A-C</sup> Means in the same column with different superscripts are significantly different (P<0.05)

**Table 7:** Average sera titers to *Mycoplasma gallisepticum* (MG) of experimental breeders at different ages.

Treatment <sup>a</sup>	IBV 30 weeks	IBV 35 weeks	IBV 44 weeks
1	5770	4372	3803 <sup>A,B</sup>
2	5809	3110	1881 <sup>A</sup>
3	3533	4088	4096 <sup>B</sup>
4	5987	5560	2604 <sup>A,B</sup>
SEM <sup>b</sup>	237.2	252.8	209.9

<sup>a</sup> 1) Control; 2) MG challenged; 3) MG challenged and treated with Pulmotil and Denagard; 4) MG challenged and treated with Tylosin.  
<sup>b</sup> Pooled standard error of means  
<sup>A-B</sup> Means in the same row with different superscripts are significantly different (P<0.05)

**Table 8:** Average sera titers to Infectious Bronchitis (IB) vaccine of breeders of MG trail.

Treatment <sup>a</sup>	NDV 30 weeks	NDV 35 weeks	NDV 44 weeks
1	6396 <sup>B</sup>	5511 <sup>B</sup>	5016
2	5071 <sup>B</sup>	3676 <sup>A,B</sup>	3783
3	2593 <sup>A</sup>	2592 <sup>A</sup>	5590
4	5877 <sup>B</sup>	5060 <sup>B</sup>	4983
SEM <sup>b</sup>	183.2	195.1	214

<sup>a</sup> 1) Control; 2) MG challenged; 3) MG challenged and treated with Pulmotil and Denagard; 4) MG challenged and treated with Tylosin.  
<sup>b</sup> Pooled standard error of means  
<sup>A-C</sup> Means in the same row with different superscripts are significantly different (P<0.05)

**Table 9:** Average sera titers to New Castle Disease (ND) vaccine of breeders of MG trail.

Treatment <sup>a</sup>	IBD 30 weeks	IBD 35 weeks	IBD 44 weeks
1	5117 <sup>A</sup>	3972 <sup>A,B</sup>	4599 <sup>A</sup>
2	8242 <sup>B</sup>	6501 <sup>B,C</sup>	8491 <sup>B,C</sup>
3	5450 <sup>A</sup>	6626 <sup>B,C</sup>	9764 <sup>C</sup>
4	8390 <sup>B</sup>	8655 <sup>C</sup>	7824 <sup>B,C</sup>
SEM <sup>b</sup>	212.6	285.3	298.4

<sup>a</sup> 1) Control; 2) MG challenged; 3) MG challenged and treated with Pulmotil and Denagard; 4) MG challenged and treated with Tylosin.  
<sup>b</sup> Pooled standard error of means  
<sup>A-C</sup> Means in the same row with different superscripts are significantly different (P<0.05)

**Table 10:** Average sera titers to Infectious Bursal Disease (IBD) vaccine of breeders of MG trail.

of Pulmotil-Denagard treatment program on the immune system, as it reduces the MG colonization burden, leaving enough space for the humoral immunity to peak against IBV, IBD, and NDV, specifically at later stages.

In conclusion, the performance criteria of all challenged birds irrespective of the treatment offered were not affected among treatments tested in this trial [24]. The Pulmotil/Denagard treatment significantly rectified the impact of MG challenge in broiler breeders, cleared the MG tracheal colonization and reduced the MG vertical transmission to the progeny. This was reflected by higher egg weight and chick weight, higher fertility and hatchability percentages. Pulmotil/Denagard treatment had also a positive impact on the immune response of MG-challenged breeders. It reduced the MG colonization from the breeders' respiratory tissues resulting in a reduced antibody titer to MG and left the space for the immune system to increase its response against IBV, IBD and NDV vaccines [25]. In addition, the Pulmotil/Denagard treatment was more efficient in reducing the vertical transmission of MG to the progeny than tylosin.

The use of Pulmotil/Denagard program is highly recommended in breeder farms suffering from MG infection and offers a strong protection against any potential MG endemic infection.

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#### Disclosure Statement

No potential conflict of interest was reported by the authors.

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