



## The Influence of Tilmicosine and Enrofloxacin in Mycoplasmas Control, Hatchery Performance and Quality of Eggs and Day Old Chicks

### KEYWORDS

Mycoplasma, Tilmicosine, Enrofloxacin, Breeder, hatchability, broiler

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### ABSTRACT

The aim of this study is to evidence the role of antibiotics Tilmicosine and Enrofloxacin in mycoplasmas (MG) control, their impact in hatchery performance and quality of eggs and day old chicks in breeder broiler chickens.

The experiment took place for 63 weeks in a breeder broiler farm in Ioannina's area in Greece. From this area Albanian farmers import the meager number of day old chicks.

The 3 groups, were positive in *M.gallisepticum* (Eliza test, Check flock Mg -IDEEX and cultural control).

The best results were in the Tilmicosine group, not only in *M.gallisepticum* control, but also in the hatchability which was 85.6 %, 84.15% and 83.2% respectively for the Tilmicosine, Enrofloxacin and control group.

The average of eggs and day old chicks weight in different days of laying curve (day 210, 280, 350) were better in the Tilmicosine and Enrofloxacin groups, than the control group.

### INTRODUCTION

The hatchability in the incubator and quality of eggs and day old chicks in breeder broiler chickens, is a very important indicator for farmers of the parental flocks. Many factors play an important role in the percentage of the hatching of eggs like the genetic strain (Abudabos, 2010) quality of eggs (Yoho et al, 2008), age of broiler breeders (Tona et al, 2004., Dikmen et al, 2007), egg shell and incubation condition (Christensen et al, 2005), nutrition (Ayidin et al, 2004., King'ori, 2010), Environmental factors (King'ori, 2011), egg storage position (Tiwari et al, 2005), males and females rapports ( Sapp, 2004), eggs size (Abiola, 2008) etc.

Different microorganisms, especially mycoplasmas have an affect on the fertility and the embryonic deaths and as a result on the percentage of hatching of the eggs in the incubator. (Levisohn et al, 1985., Lockaby et al, 1999., King'ori, 2011)

The chick's vitality, besides others, has also to do with the chick's weight which is interdependent from the egg's weight.

Lots of authors mentioned that the one day broiler chicks weight, can affect its weight in slaughter house at the age of 38-40 days (Leeson et al, 2000).

Besides other factors, the egg's and hatched chick's weight, can be influenced by several factors with microbe origin such are Mycoplasmas.

In order to see concretely the influence of *Mycoplasma gallisepticum* in the quality of eggs and hatched chicks, we organized the following experiment:

### MATERIAL AND METHOD

In a breeder broiler farm that produces eggs for broiler incubation, there have been created three groups with 3500 female chicks on each. In the two of the three groups we used the antibiotic Tilmicosine and Enrofloxacin in the first 3 days that the chicks just entered in the stable with dose 30ml Tilmicosine 25% in 100L/drinking water (Pulmotil AC, Elanco) and 50ml of Enrofloxacin 10% in 100L/drinking water (Baytril, Bayer). Then, Tilmicosine was used for 5 other times for one

day with dose 20mg/kg.b.w with four-week intervals till the beginning of the eggs' lay. The same scheme but for 2 days was using the Enrofloxacin 10mg/kg body way. Upon the beginning of the eggs' lay. No more antibiotics were used till the removal of the breeder stock from the stable which lasted for 63 weeks. In the control group no antibiotic was used. Before the antibiotics usage, 15 chicks for every group were made the blood infusion in specified days and the control for the presence of Mycoplasma antibodies was made, through the ELIZA's test, Flock check MG-IDEEX. There were also made cultural controls in determined days in respective cultures. The cultural tests have taken place in microbiology laboratory of the Faculty of Medicine in Ioannina-Greece. The terrains PLO agar and bujon medium of the company Difco were used , adding the supplement Mycoplasmas: yeast extract, horse serum, penicillin and thallium acetate according to the assigned methodology. From the laboratory, clinical and anatomy-pathological check, we had no other microbe factors to affect the experiment result. But however, the conditions in the three experimental groups were equal.

We collected the eggs for each group and put in the incubator separately under strict control on every detail.

Then we scaled with electronic scale by 100 eggs taken occasionally from each group, and we got out the average for one egg, placing them separately for incubation.

The chicks hatched by these eggs were scaled with the electronic scale, separately for every group getting out by this way the average for one chick.

For this purpose, the eggs have been taken in assigned days that coincided with the peak of the eggs' production (lay). On the 210<sup>th</sup> day on which the eggs' production was 88%, later on the 280<sup>th</sup> day on which the eggs' production was in the middle of the production curve over 70%, and at the end of the production period on the 350<sup>th</sup> day on which the percentage of eggs' lay was about 50%.

### RESULTS AND DISCUSSION

In table no.1 are presented the results of serological analysis.

In the second day we don't have the presence of the antibodies of *Mycoplasma gallisepticum* in the blood serum infused to the newly arrived chicks in stable. This demonstrates that grandparents flock is free of *Mycoplasma gallisepticum*, under the conditions of a rigorous bio-security.

**Table No. 1. Serological analysis, Elisa test**

Groups	ELISA 2 <sup>nd</sup> day	ELISA 30 <sup>th</sup> day	ELISA 120 <sup>th</sup> day	ELISA 210 <sup>th</sup> day	ELISA 280 <sup>th</sup> day
Control group	-	+	+	+	+
Tilmicosine	-	-	+	+	+
Enrofloxacin	-	+	+	+	+

**Table No. 2. Cultural test: The presence of MG in different days**

Groups	Cultural test on the 30 <sup>th</sup> day	Cultural test on the 120 <sup>th</sup> day	Cultural test on the 210 <sup>th</sup> day	Cultural test on the 280 <sup>th</sup> day
Control group	1/15	4/15	6/15	7/15
Tilmicosine	0/15	1/15	2/15	3/15
Enrofloxacin	0/15	2/15	3/15	5/15

The serological analysis of the 30<sup>th</sup> day showed the presence of mycoplasma's antibodies in the blood serum in the control group and Enrofloxacin group, such a fact shows the horizontal infection of chicks because of the lack of a non rigorous bio-security.

In the serological analysis after the 120<sup>th</sup> day, we have the presence of mycoplasma's antibodies in all groups. The serological analysis was assisted from the cultural tests too for the increase of MG presence.

In table no.2 are presented the results taken from the cultural tests. The presence of MG on the 30<sup>th</sup> day was verified only in the control group and not in the group where Tilmicosine and Enrofloxacin were used. In the controls of the 120<sup>th</sup> day, we have the mycoplasmas presence in all groups, in the serological control and in the cultural tests.

The MG presence is higher in the samples of the control group (4 positive samples in 15), compared to the group where the antibiotics were used (Tilmicosine 1 positive sample and Enrofloxacin 2 positive samples in 15). This shows that the antibiotic usage with one-month intervals, keeps in a control the growing chain of mycoplasmas which are held in a relatively lower level. On the other side, this shows that the antibiotic usage in long intervals is not capable of controlling perfectly the increase and multiplying of mycoplasmas, especially when we also have other stresses such as the post-vaccine ones or the lack of a non rigorous bio-security (Ley and al, 1997., Jerome et al, 2006).

In the laboratory control of the 210<sup>th</sup> and 280<sup>th</sup> day, when chickens are just in the middle and in the peak of their eggs production, we have an increase in the number of mycoplasmas samples. This shows that the eggs produced and incubated in this period are in considerable percentage (relatively high), infected by *Mycoplasma gallisepticum* and carry the possibility of broilers' vertical infection (Barbour et al, 1998). But it is important to emphasize that the infection is lower in Tilmicosine group compared to the Enrofloxacin and especially to the control group.

This increase of mycoplasmas in control group, in our opinion has to do with the non-usage of antibiotics for a long time, and consequently the MG has the possibility to continue its multiplying.

Our results are in contradiction to the conclusions of some authors that have applied the antibiotics usage only in pullets impeding the transmission of mycoplasmas through eggs in the newly hatched chicks (Elanco Italy, 2003). Our results showed that under the conditions of a non-perfect bio-security, the antibiotic usage only in pullets till the beginning of the eggs' lay, is not able to control the mycoplasmas' transmission through eggs in the newly hatched chicks.

These results show that the frequent monitoring of breeder stock is necessary, in order to take precaution for the mycoplasmas control, through the increase of bio-security and through the frequent usage of antibiotics in chickens during the laying period (Barbour et al, 1998., Nascimento et al, 2005).

**Table Nr.3 The production, the incubation and the percentage of the hatching of the eggs in the three groups.**

Groups	Total egg production	Incubation eggs	Hatching eggs	Hatching Percentage
Control group	503720	476015	396044	83.20%
Tilmicosine	574030	548770	469800	85.60%
Enrofloxacin	543060	516995	435050	84.15%

In table nr. 3 it can be seen that in the total amount of eggs produced, in absolute value, there is a difference of 30 to 70 thousand in the Tilmicosine's group versus the group that uses Enrofloxacin and the control group.

In total the percentage of the hatching in the incubator is obviously different. Which is 73 756, more hatched birds in Tilmicosine's group in comparison to control group and 34 750 in comparison to Enrofloxacin.

Tilmicosine's group has 2,6 % higher percentage than control group and 1,45% higher than Enrofloxacin's group. The percentage of the hatchability were 85.6 %, 84.15% and 83.2% respectively for the Tilmicosine, Enrofloxacin and control group.

These results are of a big interest for an economy that deals with egg production and the hatching of birds in incubators. So the economical effect of Tilmicosine should be taken under consideration.

**Table Nr.4 The weight of eggs and chicks in different days of production curve**

Groups	Weight on the 210 <sup>th</sup> day (30 weeks)		Weight on the 280 <sup>th</sup> day (40 weeks)		Weight on the 350 <sup>th</sup> day (50 weeks)	
	Egg/gr	Chick/gr	Egg/gr	Chick/gr	Egg/gr	Chick/gr
Control group	53,8 gr	39,35gr	60,8 gr	44,6 gr	62,9 gr	46,2 gr
Tilmicosine	55,7gr	40,78gr	63,9gr	46,92gr	65,7gr	49,3gr
Enrofloxacin	54.6gr	39.95gr	62.2gr	45.65gr	64.3 gr	47.2 gr

From the careful observation of tables' no.4, it results clearly that the eggs produced from chickens on the 210<sup>th</sup> day, which coincides with the peak of egg's production, are smaller, so with average weight that is smaller compared to the weightings on the 280<sup>th</sup> and 350<sup>th</sup> day. Not only the average weight of their eggs, but also that of the chicks hatched from them, is smaller.

This average weight of both eggs and chicks, increases on the 280<sup>th</sup> day and reaches the biggest weight on the 350<sup>th</sup> day.

A certain progress of egg's weight, and consequently of the chicks' weight that are hatched from them, is physiological (Leeson et al, 2000., Yanacopoulos et al,1987). The egg weight is in the right correlation with chickens' body weight, which increases continuously till the end of the laying period (Leeson et al, 2000).

The weight of chicks hatched from eggs in the peak of their production, so from small eggs, must be taken into consideration from the importers of our country that debate and require from the farmers of the parent stock of Greece and Italy to receive chicks that come from as big eggs as possible and that chicks have the most possible weight.

However, in every phase of weighting we have differences between the three groups concerning the average weight of the eggs and of the chicks hatched from them. In the group where we have used the antibiotic macrolid Tilmicosine, the weight is bigger compared to the Enrofloxacin group and both eggs from groups treated with antibiotics were bigger than control group. Certainly, in absolute values, this difference seems inconsiderable, but by the biological point of view it has noticeably influenced in the hatch of a more vital chick which can pass easier the initial stresses, the receipt and assimilation of nutrition etc. Furthermore, a vital chick is very resistant towards the different infections, including also the respiratory infections (Dufour-Zavala,1999) such are mycoplasmas which are frequent in poultry industry.

The differences between groups and ages of weighting, is verified also by the results' statistical processing.

Other authors have studies the correlation between the chicks' weight in hatch and those in slaughter house. As big as the weight of chicks is in the hatch phase, as big is it during the growth and also in slaughter house (Halevy et al, 2004).

The difference in the percentage of the incubated eggs' hatch and of the quality of the chick's weight is dedicated to the antibiotics concentration by lots of authors, especially

to pharmacokinetics of macrolide antibiotics such as Tilmicosine in the egg, in egg yolk and in the egg embryo controlling well and decreasing the microbe number of Mycoplasmas (Goetting, 2011).

The decrease of Mycoplasmas' number, in our case MG, decreased their negative impact in eggs and in eggs' embryo, increasing so not only the fertility and hatchability, but also the weight and vitality of the chicks hatched from them (Mohamet et al, 1987., Scorenaux et al, 1998., Nascimento et al,2005).

## CONCLUSION

- 1.- The usage of the macrolide antibiotic – Tilmicosine, gives better results in the control of *Mycoplasma gallisepticum* in the breeder broiler chicken, affecting positively in the decrease of MG microbe number, verified in laboratory through the serological analyses (Elisa test) and through cultural tests in the respective terrains.
- 2.- The usage of Tilmicosine and Enrofloxacin antibiotics influenced to increase the percentage of hatchery of the eggs and to increase the weight of eggs and chicks hatched from them giving us more vital chicks compared to the control group.
- 3.- Taking the measurements of bio-security, quarantine and disinfections, must be as complete as possible in the breeder broiler chicken, in order to control the horizontal transmission of *Mycoplasma gallisepticum*.
- 4.- According to literature, Tilmicosine is concentrated in eggs, so the non incubated eggs must not be given for public consumption.

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