



*Original Contribution*

**METAFILLACTIC EFFICIENCY OF FLORFENICOL, APPLIED TO THE FODDER OF THE PIGS FROM THE FATTENING INFECTED WITH *MYCOPLASMA HYOPNEUMONIAE***

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**ABSTRACT**

The field research has been conducted with 390 pigs, which have are the same weight and age and are affected by a subclinical form of enzootic pneumonia. The pigs have been divided into two groups. First group has been receiving concentrated forage, combined with the antibiotic medicine Florfenicol in dose 2.0 kg Feniveex-2% premix/1000 kg fodder, for 7 days. Second group has been receiving the same fodder, but with the supplement Tylosin in dose 1.0 kg Tylovet-10% premix/1000 kg, for 21 days (positive control). The pigs from both groups have been observed for a period of 30 days, after the beginning of the treatment with medical fodder. The field research has demonstrated, that the Florfenicol used in the beginning of the fattening has proved effective in the control of the enzootic pneumonia, in comparison with the group that has been receiving Tylosin. It is established improvement of the clinical status of the animals, decrease in death, lowering the damage of the lungs and an increase in the average daily growth.

**Key words:** *Mycoplasma hyopneumoniae*, pigs, Florfenicol, metaphylaxis.

**INTRODUCTION**

The enzootic pneumonia is a chronic, contagious pig disease caused by *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) (1). The disease cause the serious economic damages in pigs - breeding all over the world, although, it is difficult to determine the specific economic effect of the mycoplasmal pneumonia on a herd level. A review from different studies shows that the disease leads to decreasing of the average daily growth approximately with 17% and with 14% it decreases the conversion of forage. It is also proved that for every 10% of affected from pneumonia lungs the average daily growth decreases with 37 grams. A survey, made in USA, shows that the cost for a pig, that is suffering from a mycoplasmal pneumonia, is 4.08\$. The annual cost for the whole country counts approximately 367 billion dollars (2).

Effective antibiotics applied with the food and water of the pigs is an effective control of the

Respiratory diseases, including the enzootic pneumonia (EP). This leads to an improvement of the economic parameters - better conversion of the forage and increased average daily growth, also to a decreasing of the clinical symptoms and lung lesions of the disease and prevents it from secondary bacterial infection (1, 3). From the other hand, the inappropriate application of antibiotics increases the possibility of antimicrobial resistance and antimicrobial residue in the slaughtered pig corpses (4). And for that reason, after the end of the treatment new cases of the disease could be presented (5, 6).

The purpose of this field research was to evaluate the effect of applying Florfenicol in the forage to clinical and economic parameters of pigs, suffering from enzootic pneumonia.

**MATERIALS AND METHODS**

**Study animals**

The field research was conduct with 390 pigs, equal by age and weight, affected by a subclinical form of enzootic pneumonia. The organization of the technological process in the pig farm involved weaning the pigs at 35 days of age and with a period of growing old of 73 days. Immediately after transitioning the pigs

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into the fattening group we formed two study groups:

- First group - 205 pigs, 15 weeks old with average live weight of  $55 \pm 2.15$  kg. All of the animals have been given concentrated forage, combined with the antibiotic medicine Florfenicol (Feniveex-2% premix<sup>®</sup>, Reveex), with the following application: 2.0 kg Feniveex-2% premix<sup>®</sup>/1000 kg fodder for 7 days.
- Second group - 185 pigs (positive control), 15 weeks old with average live weight of  $55 \pm 3.07$  kg, fed with the special forage mix „grower”. At the early stages of the fattening of the pigs their forage was modified with the antibiotic medicine Tylosin (Tylovet-10% premix<sup>®</sup>, Biovet), with the following application: 1.0 kg Tylovet-10% premix<sup>®</sup>/1000 kg fodder for 21 days.

All of the rooms, used for the experiment, were thoroughly cleaned and disinfected. All of the medical forages were made at the pig farm and were given to the pigs *ad libitum*. The water was also given *ad libitum*, from autonomous nipple water troughs. The pigs from the two groups were bred by the same conditions. All of the parameters of the ventilation, lightning and temperature in the rooms were exactly the same as the requirements of the GMP for a contemporary intensive pig farm.

### Clinical studies

We made the observation over the pigs for 30 days after starting the medical forages. We monitored the pigs for the following indicators every day: rectal temperature, general condition, dyspnea, cough and nasal discharge. The different medical indicators were ranked by a specific scale, described by Lang et al. (7), and we described the clinical condition of the pigs as very good, good, satisfactory and poor.

### Necropsy

Pathological anatomical exams were performed to all of the dead and killed by emergency pigs. The severity and extent of pulmonary lesions typical for EP in percentage from the whole pulmonary surface were evaluated according to the Kristensen et al. (8).

### Serological test

From all of the two groups of pigs were taken 20 blood samples in total before the treatment (D0) and at the end of the experiment (D30). The serums were tested for proving the presence of specific antibodies against *M. hyopneumoniae*. The test were performed by blocking ELISA with the help of the testing kit

Ingezim M. hyo compact<sup>®</sup> (Ingenaza-Spain), according to the instructions of the manufacturer.

### Polymerase chain reaction (PCR)

We have achieved the confirmation of *M. hyopneumoniae* in 20 nasal swabs of pigs via PCR with one primer couple from the „Jena Bioscience” company: MHP950-1L (5'-AGG AAC ACC ATC GCG ATT TTT A-3') and MHP950-1R (5'-ATA AAA ATG GCA TTC CTTTT A-3'). They are responsible for the synthesis of a DNA amplification product of 910 bp in size that is specific for *M. hyopneumoniae*. We have isolated the bacterial DNA with Buccal Swab DNA Kit (Geneshun Biotech, China). For the reaction we have used 3.5 µl of DNA, 10 pmol of each primer, 12.5 µl 2<sup>x</sup>PCR Master Mix, and distilled water up to 25 µl. We have performed the amplification in thermocycler with the following temperature sequence: initial denaturation – 95 °C/5 min; 35 cycles of amplification including denaturation – 95 °C/30 sec; annealing – 53.5 °C/40 sec; elongation – 72 °C/1 min; final elongation – 72 °C/7 min, followed by cooling to 4 °C. We controlled the obtained DNA and the PCR products by gel electrophoresis at 120 V for 40 minutes.

### Nested polymerase chain reaction (nPCR)

All samples of nasal swabs in PCR, were further analyzed using nPCR. The sequences of the primers (Jena Bioscience<sup>®</sup>) used for nPCR are present below: MHP950-2L (5'-CCC TTT GTC TTA ATT TTT GCA A-3') and MHP950-2R (5'-GCC GAT TCT AGT ACC CTA ATC C-3'). They are responsible for the synthesis of DNA amplified specific for *M. hyopneumoniae* product with a size 807 bp. For the reaction: 1.0 µl PCR product, 2.0 µl mixed primers (2L-2R), 12.5 µl MasterMix and 9.5 µl distilled water, were used. The amplification cycle consisted of an initial 5 min denaturation at 95 °C, followed by 35 cycles of 30 sec at 95 °C; annealing – 56.5 °C/40 sec; elongation – 72 °C/1 min; final elongation – 72 °C/7 min, followed by cooling to 4 °C. Obtained PCR products were visualized in gel electrophoresis (120 V for 40 min).

### Statistical analysis

Statistical analysis was performed using the StatMost software (StatMost 3.6, Dataxiom Software, 2003). Data represent mean values with standard error of the means (Mean $\pm$ SE) determine by one-way ANOVA. Results were considered statistically significantly different when  $P < 0.05$ .

## RESULTS AND DISCUSSION

The economic and epidemiological results from the clinical study of applying Florfenicol to the forage of pigs for fattening and to control the EP are shown on **Table 1**. From the results it became clear that the symptoms of a respiratory disease are presented at both groups. From the other hand morbidity rate is with 20.1% lower in the group where the pigs were treated methafilactic with Florfenicol compared to the positive control group, where the pigs were treated with Tylosin-premix. In mortality rate calculated as a total number of dead animals from all of the receptive animals in the group, is lower in the group where the animals were medicated with Florfenicol

(0.5%), compared to the other one where the animals were treated with Tylosin. Best economic results we received from the group, treated with Florfenicol-premix. They refers to obtaining a higher average daily gain of 50 g more than those treated with Tylosin and to higher percent of clinically recovery pigs, respectively 97.5% for the ones treated with Florfenicol and 94.6% for the other ones-treated with Tylosin. Our results confirm the information from Ciprián et al. (9) concerning significantly higher average daily weight gain of the Florfenicol-treated pigs than that of the non-treated ones, affected of chronic infection of *M. hyopneumoniae*.

**Table 1.** Economic and epidemiological results of the clinical testing of Florfenicol-premix in fattening pigs, affected by EP

Parameters	Units	I group (experimental)	II group (positive control)
1. Treated pigs	n	205	185
2. Medicament	-	Feniveex 2%- premix <sup>®</sup>	Tylovet 10 % - premix <sup>®</sup>
3. Active substance	-	Florfenicol - 2.0 g/100 g	Tylosine phosphas - 100.0 mg/1g
4. Dose	kg/t	2.0	1.0
5. Course of treatment	days	7	21
6. Start of treatment	weeks	15	15
7. Finish of treatment	weeks	16	18
8. Duration of the experiment	days	30	30
9. Average live weight at beginning of experiment	kg	55±2.15*	55±3.07*
10. Average live weight at the end of the experiment	kg	79±3.29**	77.5±2.83
11. Derived growth	kg	24	22.5
12. Average daily gain	kg	0.800	0.750
13. Morbidity rate	n	12	48
	%	5.8	25.9
14. Mortality rate	n	1	2
	%	0.5	1.1
15. Emergency slaughtered	n	4	8
	%	2.0	4.3
16. Clinically healed	n	200	175
	%	97.5	94.6

\*  $p < 0.05$  \*\*  $p < 0.01$  \*\*\*  $p < 0.001$

The clinical efficiency of Florfenicol-premix against mycoplasmal pneumonia is presented at **Table 2**. All of the collected data shows that during the first 7 days of the experiment, all of the pigs that have been given Florfenicol are in better medical condition (69.3%) compared to the other group (64.9%). During this period at both groups there are no dead or slaughtered animals. During the period from 8<sup>th</sup> to 14<sup>th</sup> day

the difference between the pigs in very good medical condition at both groups significantly starts to increase, as in the first group the percent is 64.9% and in the second one 32.4%. Clinical symptoms of a respiratory disease (e.g. cough, nose secretion, dyspnea, anorexia, depression) were presented as follow 1.5% in the first group and 11.4% in the second group of pigs. In both groups the percentage of

deaths reached 0.5%, while the percentage of slaughtered pigs was 1.1% more in Tylosin treated. During the third week (from 15<sup>th</sup> to 21<sup>st</sup> day) the tendency for a higher morbidity rate and mortality rate at the second group (treated with Tylosin) were retained. During the end of the methafilactic course (from 22<sup>nd</sup> to 30<sup>th</sup> day) the medical condition of the pigs from the first group remain as “very good” in 57.2% in the cases and only in 1.0% symptoms of a respiratory disease were presented. Among the

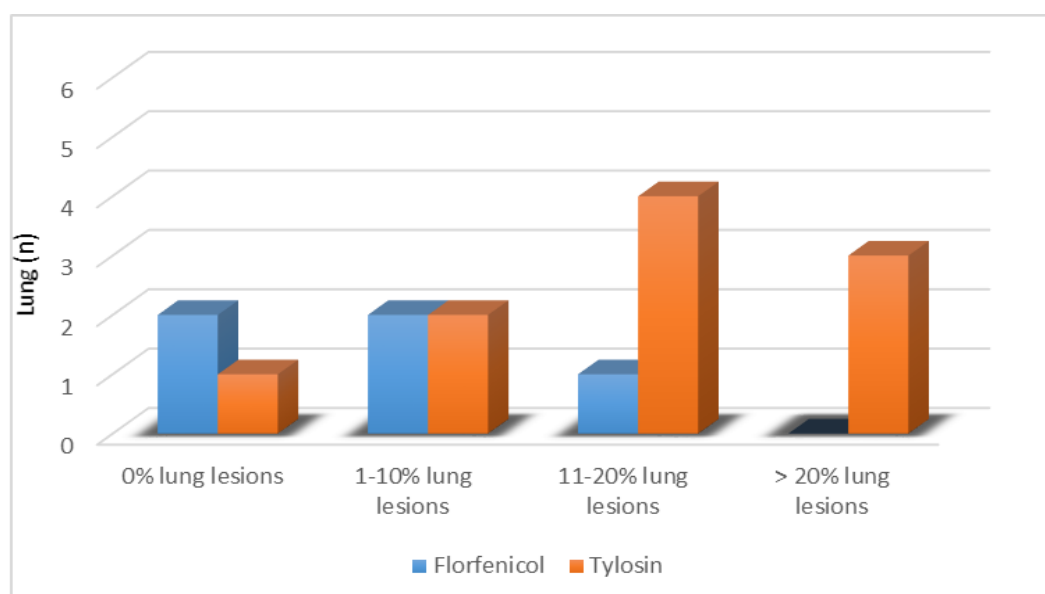
pigs in the second group only 20.4% were in “very good” condition and 3.4% of them have shown symptoms of a respiratory disease. During this final stage of methafilactic in both groups, we did not record any differences in the percentage of deaths and slaughtered pigs. Similar results are obtained from Ciprián et al. (9) in Florfenicol treated pigs which have lower body temperature, lighter cough and dyspnea, compared with untreated pigs affected by *M. hyopneumoniae* infection.

**Table 2.** Clinical status of fattening pigs treated with Florfenicol-premix and Tylosin-premix

Treated group	Day of experience	Clinical status					
		very good (%)	good (%)	satisfactory (%)	poor (%)	died (%)	slaughtered (%)
Florfenicol (n=205)	7	69.3	19.0	9.8	1.9	0	0
	14	64.9	23.9	8.7	1.5	0.5	0.5
	21	60.1	30.0	7.4	1.5	0	1.0
	30	57.2	31.8	9.5	1.0	0	0.5
Tylosin (n=185)	7	64.9	27.0	5.4	2.7	0	0
	14	32.4	37.8	16.3	11.4	0.5	1.6
	21	22.1	48.6	17.7	8.8	0.6	2.2
	30	20.5	59.7	15.9	3.4	0	0.5

The results of the pathological exams of the dead and dropped out pigs during the research are presented at **Figure 1**. The table shows that among the pigs treated with Florfenicol predominate lungs without any lesions which are common for EP. At both groups there were no differences among the lungs affected slightly (with lesions to 10% of the lungs surface). In the second group (treated with Tylosin) the percentage of moderately affected lungs was higher (lesions from 11% to 20% of

the lungs surface). Among the pigs, treated with Florfenicol there were no severe cases of lungs lesion (lesion with more than 20% affected area of the lungs) due to *M. hyopneumoniae*, which were very common among the pigs treated with Tylosin. Our results are similar with the results received from Ciprián et al. (9) concerning lower average percentage of pneumonic gross lesions in treated pigs with Florfenicol, compared to *M. hyopneumoniae* infected non-medicated.



**Figure 1.** Assessment of lung lesions caused by *M. hyopneumoniae* in both treatment groups

The results of the serological experiments show that before the treatment of the animals with Florfenicol the percentage of seropositive pigs is 50% (5/10 pigs) and in the end of the experiment it increases up to 70% (7/10 pigs). In the control group treated with Tylosin there weren't registered differences in the percentage of the seropositive pigs before the treatment - 80% (8/10 pigs) in the end of the experiment - 80% (8/10 pigs). The short course of medicating the pigs with Florfenicol (7 days) led to enough natural exposition with the pathogen, which can stimulate effectively the active immunity against *M. hyopneumoniae*. The long treatment of the pigs with Tylosin (21 days) in the control group may be the cause some animals to remain immunologically defenseless. This can cause risk of repeating infections with *M. hyopneumoniae* at stopping medication. Our results are similar with the

results received from Walter et al. (10) and Del Pozo Sacristan et al. (11). The analysis of the results from the molecular-biological exams from a nose secretion didn't show any significant differences between the two groups. In the group treated with Florfenicol the percentage of nasal secretions positive for *M. hyopneumoniae* before the treatment is 70% (7/10 pigs) and in the end of the experiment - 80% (8/10 pigs). In the control group treated with Tylosin the percentage of positive for *M. hyopneumoniae* nasal secretions before the treatment is 90% (9/10 pigs) and in the end of the experiment - 100% (10/10 pigs). This leads to conclusion that the clinical recovery after the treatment is not accompanied with full disappearance of the pathogen which is confirmed by the existing lung lesions in both groups, reported also by Thacker (1) (**Table 3**).

**Table 3.** Results of serological and molecular-biological studies of fattening pigs affected by EP

Treated group	Number of seropositive and nPCR-positive pigs/total number of pigs sampled			
	ELISA		nPCR	
	Before treatment (D0)	After treatment (D30)	Before treatment (D0)	After treatment (D30)
<b>Florfenicol</b>	5/10	7/10	7/10	8/10
<b>Tylosin</b>	8/10	8/10	9/10	10/10

The results from our research show that the methafilactic treatment of pigs with Florfenicol (2.0 kg/t) added to the forage leads to reducing the clinical symptoms and lungs lesions and all of this leads to a better economic results. Similar to our results are ones from Del Pozo Sacristan et al. (12) research. Their research was to determine the efficiency of a single treatment of pigs with Florfenicol in dosage 30 mg/kg at experimental infection with *M. hyopneumoniae*. The good results of applying Florfenicol to control the mycoplasmal pneumonia and unsatisfactory effect of applying Tylosin are in unison with Vicca et al. (13) and Vicca (3) research which describes first cases of acquired resistance of field strains of *M. hyopneumoniae* against macrolides, lincosamides and fluoroquinolones and the absence of one against Florfenicol.

At the stationary farms for EP as a prophylactic precaution is suited medicine to be given along with the forage. This makes easier the applying of the medicine and also it reduces the stress from the animals. And this is the reason why the medical forages are preferred in the industrial pig-breeding where the pigs must be treated as a group. Important

moment in the enzootic pneumonia's treatment the proper choice of antibiotic which is in connection with the increasing multi resistance to antimicrobials. This must pay attention to the veterinary doctors that they have to be applied whenever they are needed and they have to be applied properly.

## CONCLUSION

It is established a good methafilactic efficiency of Florfenicol applied in dose 2.0 kg Feniveex-2% premix/1000 kg forage for 7 days to control the enzootic pneumonia in pigs. This is presented by reducing the clinical symptoms and lung lesions and by increasing the average daily gain.

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