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Interactive Effect between Florfenicol and Tylosin Tartrate for Use in the Therapy and Prevention of Diseases in Veterinary Medicine

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ABSTRACT

An in vitro test on the pharmacological interaction of the antibiotic combination between florfenicol and tylosin tartrate was performed. An in vitro challenge method was used for this purpose. The results were used to calculate their interaction using the graphical representation known as ISOBOLOGRAM. The pure salts of florfenicol and tylosin tartrate were used. The agar bioassay method was used. The following criteria were used to quantify the results: A synergistic effect was defined when the actual/theoretical effect (SMEF) was greater than 1. The additive effect was defined when the SMEF was equal to 1. The area between antagonism and additive effect was defined as the zone of indifference. When studying the interaction between florfenicol and tylosin tartrate it was observed that the best SMF of this antibiotic combination was 1.45 when the concentration ratio between the antibiotics was 1 : 4 (florfenicol tylosin tartrate respectively). Consequently, it can be postulated that the combination of florfenicol with tylosin tartrate showed a synergistic effect and can be recommended in poultry and swine for the treatment of mycoplasma infections, most gram + and gram- germs and other infectious agents sensitive to the formulation.

KEYWORDS: Florfenicol tylosin combination premix, poultry, swine.

INTRODUCTION

Since the beginning of chemotherapy when sulphonamide was first used and penicillin was discovered, veterinarians have sought ways to combine them to increase the effectiveness against diseases caused by infectious germs susceptible to each other. The use of antibiotic combinations is useful when mixed infections in veterinary patients are detected, and the combination to be used is synergistic especially with resistant germs reducing the risk of promoting antimicrobial resistance to antibiotics. In this particular case it is necessary to mention florfenicol and thylosin tartrate, which have been very important in the therapy of infections in productive animals in intensive farms.

Nowadays, there is both laboratory (Bennet et al. 1966) and graphical (King, 1981; Hamilton, 1985; Rahal, 1978) methodology that allows to establish the antimicrobial trends of antibiotics, alone or in combination, pharmacological information (mini-review) of each of the antibiotics used in this study is obligatory.

FLORFENICOL

Analogue of chloramphenicol. The presence of fluorine makes florfenicol less susceptible to bacterial resistance mechanisms. Its potency is higher than that of chloramphenicol and thiamphenicol. It is distributed in all tissues. Almost completely excreted in the urine in its active form. No toxicity problems have been reported. Its excretion form makes it recommended for the treatment of urinary tract infections. It is used in aquaculture and in the veterinary field to target clinically susceptible infections and is very useful in the treatment of respiratory infections in calves and young pigs.

In susceptible bacteria it inhibits protein synthesis by combining simultaneously with the 50S and 70S ribosomal units to affect peptidyl transferase activity, preventing amino acid transfer to the growing peptide chains and inhibiting protein formation,

In bovine respiratory diseases it is reported to be bactericidal when the problem is caused by Mannheimia (Pasteurella)

haemolytica and Pasteurella multocida when dosed correctly to reach the minimum inhibitory concentration (MBCs) concentration which is very close to the MICs.

For Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus, MICs were observed to be 3.1 µg/ml. For 42 Actinobacillus pleuropneumoniae isolates, MICs were ~0.2 to ~0.39 µg/ml in seven thiamphenicol-resistant strains. The average MIC was 0.25 µg/ml reported for 108 A. pleuropneumoniae strains isolated from porcine lungs.

ABSORPTION: Bioavailability. Intramuscular

In calves aged 3-6 months a dose of 20 mg/kg has a bioavailability of 78.5% (range 59.3 to 106%). The duration of concentration <1mcg/L is 22 hr and after iv administration is 11 hr.

In **lactating cows** a dose of 20 mg/kg bioavailability is 3814%.

In horses a dose of 22 mg/kg the bioavailability is 81%. Not recommended for use in horses because it affects the intestinal flora.

Oral administration

In calves from 2 to 6 weeks of age at a dose of 11 to 22 mg/kg the bioavailability is 89% but is very variable, it decreases when administered with milk replacer.

In horses at a dose of 22 mg/kg the bioavailability is 83.3%.

PHARMACOKINETICS

As a fat-soluble neutral compound, Florfenicol readily crosses cellular barriers. In addition, it has a relatively low protein binding rate (30-45%), which allows it to diffuse rapidly throughout the body, reaching tissues and fluids such as brain, cerebrospinal fluid or internal structures of the eye that are inaccessible to other antibacterial drugs. Its apparent volume of distribution is high, more than 1 Lt/kg in most species, allowing it to reach poorly irrigated tissues.En animales monogástricos y terneros jóvenes, el Florfenicol se absorbe bien a través del tracto gastrointestinal. En los rumiantes adultos, por el contrario, el antibiótico es inactivado en el rumen, probablemente, debido a la actividad bacteriana. En perros y gatos se puede administrar por vía oral, intramuscular o por inyección intravenosa lenta.

It is generally well distributed in the body, including the CNS and eye. In cerebrospinal fluid it reaches very high concentrations (30-50% of plasma concentrations in the absence of meningitis) and brain concentrations are maintained longer than plasma concentrations.

Cattle: In 2- to 5-week-old animals, after oral administration of 11 mg/kg every twelve hours (seven doses), florfenicol was well distributed in many tissues, reaching concentrations of 4 to 8 mcg/gram in lungs, heart, pancreas, skeletal muscle, spleen and synovium. These concentrations were at least as high as serum concentrations. Concentrations in brain (1 to 2 mcg/gram), cerebrospinal fluid (2 to 3 mcg/ml) and aqueous humour (2 to 3 mcg/ml) have been found at one-quarter to one-half the serum concentration in healthy calves.

Atlantic salmon: Florfenicol is distributed to all organs and tissues at a dose of 10 mg/kg when water temperature is 8.5 to 11.5 °C. Concentrations in muscle and blood are similar to serum concentrations, while fat and the central nervous system (CNS) have lower concentrations. Only 25% of serum concentrations are present in the brain.

BIOTRANSFORMATION: Cattle: Approximately 64% of a 20 mg/kg intramuscular/48h dose, administered twice, is excreted as pure drug in the urine. Urinary metabolites include florfenicol amine, florfenicol alcohol, florfenicol oxamic acid and monochloroflorflorfenicol. Florfenicol and its metabolites, such as onochlorofloroflorfenicol and florfenicol oxamic acid, are also excreted in the faeces. Florfenicol amine is retained as a metabolite mainly in the liver, and is therefore used as a marker residue for stone removal**.**

Atlantic salmon: Florfenicol is rapidly metabolised at temperatures of 8.5 to 11.5 °C and its main metabolite is florfenicol amine**.**

The elimination of Florfenicol in calves, less than 8 weeks of age:

approximately 50% of an intravenous dose of 22 mg/kg is eliminated unchanged in the urine within 30 hours. In pigs, at a dose of 20 mg/kg orally for 5 days 76% was excreted via urine and 24% via faecal excretion within 3 to 19 days after the last dose**.**

In poultry, elimination after a dose of 20 mg/kg for 3 days orally was excreted in ranges from 93.7 to 98.2% within 7 days after the last dose.

Indications:

Atlantic salmon: In the treatment and control of furunculosis caused by susceptible pathogens.

Pigs and chickens: Treatment of diseases caused by germs sensitive to florfenicol.

Dosage:

Salmon: 10 mg/kg per day for 10 days.

Pigs: 20 mg/kg per day for 5 days in drinking water.

Chickens: 30 mg/kg per day for 3 days in drinking water.

Tolerance: Florfenicol is well tolerated at therapeutic doses. However, there are cases of mild diarrhoea, decreased feed consumption, decreased water consumption, all of which are usually transient.

Compatibility: The bacteriostatic action of florfenicol may inhibit the bactericidal action of b-lactam antibiotics, so they cannot be used together. On the other hand, the presence of florfenicol prolongs the pharmacological effects of other drugs with intense hepatic metabolism, as it is a potent

inhibitor of cytochrome P-450. It cannot be co-administered with ionophores such as monensin or lasalocid, as it may cause muscle degeneration in some species**.**

Florfenicol, associated with oxytetracycline, is useful in respiratory processes caused by E.coli, Pasteurella and Mycoplasma. It also acts in gastrointestinal processes, and is especially effective against Salmonella spp. However, its indiscriminate use in meat poultry farming favours the appearance of salmonella strains resistant to phenicols, and the proliferation of Campylobacter by competitive substitution.

Contraindications: Hypersensitivity to Florfenicol.

Withdrawal time: When administered orally in pigs after 5 days of medication the withdrawal time is 14 days. In cattle the withdrawal time is 1 to 2 days when administered orally and 7 days when parenterally injected intramuscularly. In LA injectable presentations the withdrawal time is 30 days.

TYLOSINE

Antibiotic of the macrolide group, produced by a strain of Streptomyces fradiae, different from that which produces neomycin. Aqueous solutions of tylosin are stable at pH 5.5 to 7.5 at temperatures of 25°C for up to three months. At acidic pH (4.5 or less) it degrades, first to desmicosin and then to inert products. Tylosin base is poorly water soluble (5 mg/ml at 25oC), but dissolves readily in organic solvents. It combines with minerals and organic acids to form highly soluble tylosin salts. It is approved for use in domestic animals; mainly dogs, cats, cattle and pigs. It has a pKa of 7.1.

ANTIBACTERIAL SPECTRUM: Attacks gram-positive microorganisms, especially Mycoplasma gallisepticum S6. In vitro attacks PPLO of chickens, turkeys, pigs, cows and goats. Also various strains of spirochetes and leptospires. It is useful against the causative agent of swine erysipelas; Haemophilus pertussis, Moraxella bovis, Vibrio and some gram-negative bacteria.

BACTERIAL RESISTANCE TO TYLOSINE: Microbes develop little resistance to this antibiotic. When present, especially in Staphylococcus aureus, partial cross-resistance with erythromycin has been observed. However, it has been the author's experience that tylosin loses all effectiveness as resistance develops to the compound through indiscriminate use as a growth promoter. It is found in many blends of feed concentrates for various species of domestic animals. MECHANISM OF ACTION: It is bacteriostatic and interferes with bacterial protein production by inhibiting the function of the 50S subribosomal unit. Inhibits protein synthesis by interfering with mRNA**.**

ABSORPTION, METABOLISM AND EXCRETION: Tartrate salt is readily absorbed by the digestive tract of chickens, turkeys and pigs. In hens, this salt can be applied subcutaneously. Phosphate salt is sometimes mixed with pig feed, but seems to be more difficult to absorb than tartrate salt. It is administered parenterally, but intramuscular administration is preferred. To the preparation for intramuscular application, 4% V/V benzyl alcohol is added as a bactericide to the base tylosin, dissolved in propylene glycol and water. After oral or parenteral administration, it is efficiently distributed in organ tissues. It does not cross the brain barrier. But it passes into the lungs and milk in concentrations higher than plasma. It is excreted by the liver and kidneys. The LD50 (Lethal Dose 50%) in pigs is 5 g/kg orally and 1 g/kg intramuscularly.

USES OF TYLOSINE: Tartrate salt is effective in the treatment or prevention of chronic respiratory disease (CRD or ECR). Tylosin is useful after vaccinations or any other stress. In turkeys it is useful as a support in infectious sinusitis and in prevention of respiratory forms of the same disease. In pigs tylosin can be administered with tartrate in water or as phosphate salt in feed, when treating or preventing Vibrio enteritis. It is recommended to continue treatment with tylosin phosphate, even if the acute symptoms of the disease have disappeared. For cows and calves, tylosin base injected intramuscularly is useful in pneumonia, scabies and metritis. In pigs it is applied intramuscularly against erysipelas, pneumonia and dysentery. In cats and dogs, tylosin base is used intramuscularly for upper respiratory infections, otitis externa, cellulitis, metritis, leptospirosis and secondary infections caused in the normal course of viral diseases. It is also used in postoperative treatment**.**

CONTRAINDICATIONS IN THE USE OF TYLOSIN: Tylosin is not administered to hens in production, because the egg may carry high concentrations of the antibiotic. For human consumption, hens shall not be slaughtered for at least three days after the last parenteral application of tylosin, or for 24 hours before if they received the drug orally. In turkeys, after administration of the antibiotic, five days must be allowed before slaughter for human consumption. Lactating cows should be removed from the milking line for 96 hours so that their milk is not consumed. Pigs should also not be slaughtered for 21 days following tylosin administration. It is used in intestinal bacterial overpopulation in dogs, administered with feed three times a day. Orally administered to cows it can produce serious diarrhoea, in horses it also produces diarrhoea which can lead to death, when administered by any route.

DOSAGE: BIRDS, hens and turkeys: 0.5 g/l water for as long as necessary. Hens: subcutaneously, 1 ml/kg bodyweight of 50 mg/ml or 200 mg/ml solution, depending on the severity of the infection. In this case, the total dose should not exceed 2.5 ml. If inflammation persists, a second treatment can be given after 10 days. DOG, CAT: 2 to 10 mg/kg/day intramuscularly for three consecutive days. If there is no response, continue the medication; in addition, laboratory tests should be carried out. SWINE: 9 mg/kg twice daily, treatment should not exceed three days. CATTLE: 4 to 10 mg/kg/day intramuscularly for three consecutive days, in parallel; antibiograms will be carried out.

CONTRAINDICATED IN HORSES.

SHEEP: 10 mg/kg per day, treatment should not exceed 5 days. In case of Vibrio abortion, tylosin tartrate salt can be administered. It is applied intramuscularly in a total dose of 400 mg per day. Only two applications will be given to control the outbreak in about 10 days. In Mexico, indiscriminate use allows the development of resistance in most bacteria. At present, its clinical medication needs re-evaluation.

INTERACTIONS: Tylosin may increase the toxicity of cardiac glycosides. Combined with florfenicol it has a synergistic effect. As a growth promoter it has been used in pigs, accelerating the maturation of the intestinal microbiota with similar effect in piglets combined with colistin and/or florfenicol.

In poultry, tylosin has also been reported to be an effective growth promoter (Kim et all, 2004).

TYLOSIN RESIDUES AND WITHDRAWAL TIME

Residues of tylosin depend on the route of administration.

In poultry, the withdrawal time of tylosin is considered to be at least 6 days.

In dairy cows it is recommended not to use milk from 6 milkings, tylosin residues in dairy cows were no longer detectable at 108-144 hours.

In pigs tylosin 100g/ton in feed no amounts were observed within lps MRLs and not detectable at the recommended 21 days withdrawal.

MATERIAL AND METHODS

Medicinal products: Florfenicol and Tylosin tatrate in their pure salts were obtained from LAPISA SA de CV. Mexico.

In vitro tests were performed according to the National Committee for Clinical Laboratory Standards of the United States of America. And adapted and modified according to the methodology suggested by Bennet et al. (1966). Suspensions of B. subtilis were made by adding the contents of two ampoules of Bacillus subtilis spore suspension (DIFCO) to 100 ml of sterile normal saline and 2.5 ml of a solution containing 0.3825 dibasic potassium phosphate and 0.0833 g of monobasic potassium phosphate to bring the pH of the B. subtilis solution to a value of 7.0.

The antibiotic standards tested were vacuum dried for a minimum of 48 hours and then carefully weighed and added to a solution to achieve a concentration of 1000µg/ml. This concentration serves as a stock solution from which dilutions are made for in vitro testing.

The effect of the combination of florfenicol and tylosin tartrate was tested by making serial dilutions of the two antimicrobial agents, which were mixed in such a way that each row and column consisted of a fixed amount of one agent and increasing amounts of the other antimicrobial. The concentration ranges used were based on the MICs obtained for each of the anti-infective agents used and the bacteria used as test bacteria. Dilutions covered 4 x MIC (antagonistic action) and 0.25 x MIC (synergistic action): Aliquots of 75 µL of bacteria (c. 1 x 106 cfu/mL) and 75 µL of each antibiotic were added to each microtitre plate. As controls, the MIC of each antibiotic alone and their combinations were determined on each plate

Plates were incubated overnight at 37°C and bacterial growth was visually inspected and then confirmed by photometer (Bausch & Lomb) at an optimum density of 540 nm.

The results were collated and where synergistic trends were observed, the changes in MIC were plotted and their trend observed with the resulting isobolograms.

RESULTS AND DISCUSSION

Figure 1 below shows the experimental setup to study the antibiotic properties of florfenicol combined with tylosin tartrate; it represents an agar plate with 96 wells in which the reference germs and the different concentrations of the antibiotics are deposited and from which six solutions were prepared. The uncoloured or clear wells functioned as controls (no antibiotic and no bacteria), the dark wells functioned as controls for bacteria (no antibiotic 0% growth inhibition) and the wells labelled 1-6 functioned as medicated wells for six combinations of antibiotic dilutions, in triplicate, while the wells in row H received the highest concentration of antibiotic combination. Two 96-well plates were made with row H2 representing solutions 4 to 6 of the second 96-well agar plate.

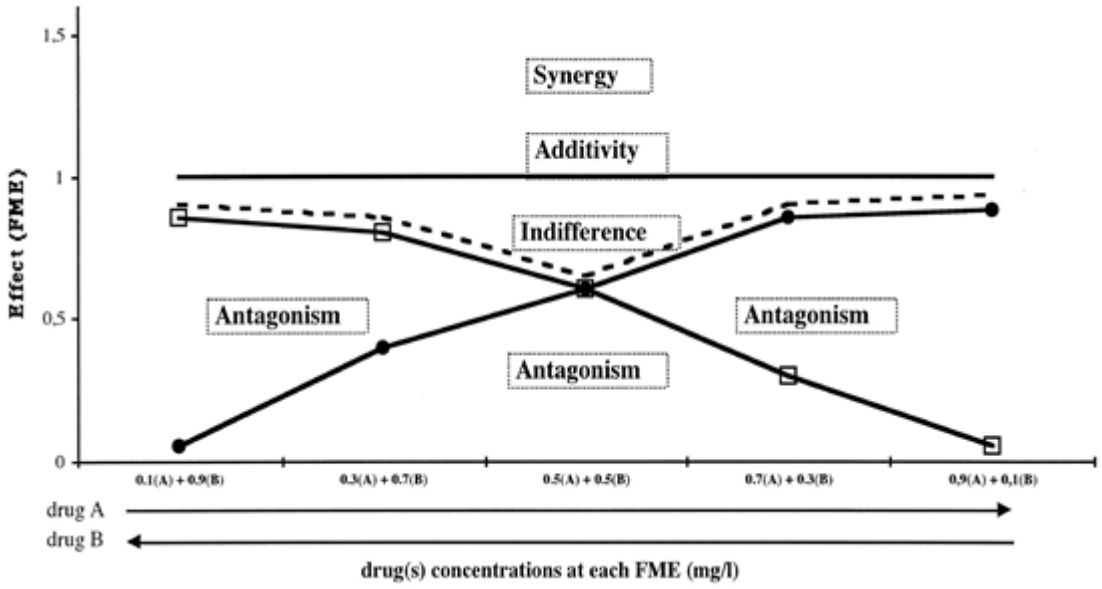
In the antibiotic combinations the proportions used A corresponds to florfenicol while B corresponds to tylosin tartrate.

NOTE: This test shows results that are similar or equal to those performed with other commercial products using the same antibiotic compounds for use in premixes for poultry and pigs.

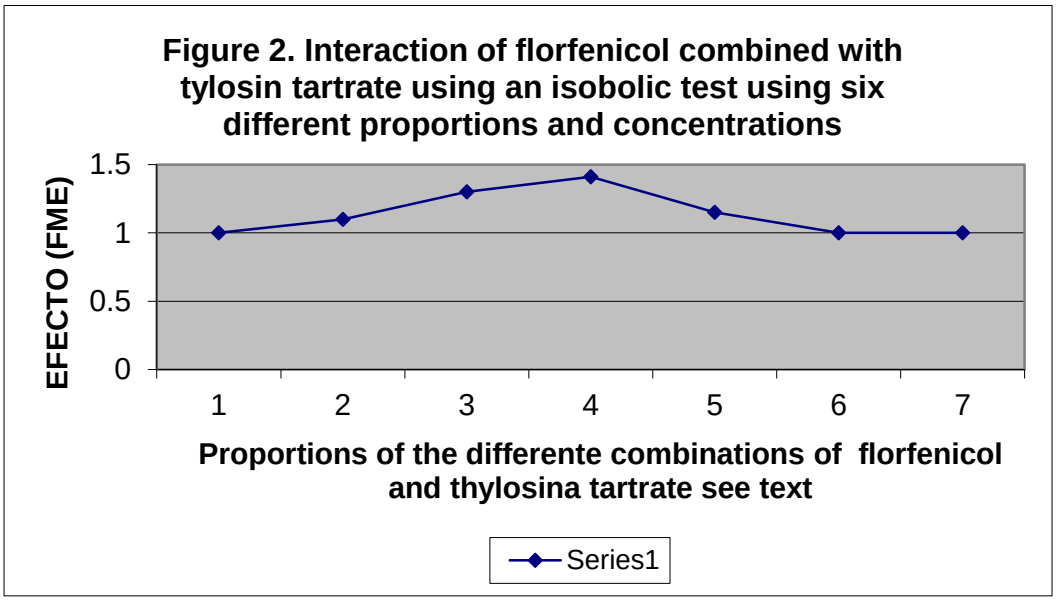
Table 1. The values of the graphical representation of the changes in MIC and observe their trend with the resulting isobolograms in a combination of florfenicol with tylosin tartrate in the solutions with the mentioned ratios.

Solución	proporción de A con B		
	Antibiotico A	antibiotico B	Valor de MIC
1	5	0	1.0
2	4	1	1.1
3	3	2	1.2
4	2	3	1.45
5	1	4	1.15
6	0	5	1.0

The graph of our results was interpreted the following scheme (:Desbiolles and Cols.2000)



When the corresponding results observed in table 1 are applied, the resulting graph is as shown in figure 2, and in which the trends of the combinations can be observed in terms of the proportions of the same used in the interaction tests.



It can be argued that the graph does not agree with what is expressed in the introduction, for this interpretation the effect of the antibiotic combinations used should be taken into account according to the scheme presented as a reference. (Desbiolles et al. 2000).

In this study it can be observed that there is a tendency towards synergy in combinations 2 to 5, but it becomes apparent that combinations 3 and 4 are the most representative of a desirable antibiotic synergy. In this combination, a 1:4 ratio is used for florfenicol and tylosin tartrate, respectively, results which are in agreement with similar studies with different chemical compounds and antibiotics (Ulvatne, 2001) against *E. coli*.

CONCLUSION

Based on the results obtained with this study, it can be postulated that the antibiotic combination used between ratios 3 and 4 is suitable for use in pig and poultry diseases produced by germs susceptible to the combination studied. To finally conclude that the antibiotic combination of florfenicol tylosin tartrate ratio of 3 : 2 and 2 : 3 respectively, which have the best synergistic effect, can be used for the treatment of diseases caused by germs susceptible to the combination studied.

REFERENCES

1. Bennet, J. V., Brodie J. L.; Benner, E. J.,Kirby, W. M.M. 1966 Simplified, accurate method for antibiotic assay of clinical specimens. App. Microbiol. 14: 170 -177
2. Desbiolles N, Piroth P, Lequeu C, Neuwirth C, Portier H, Chavanet P. 2001Fractional Maximal Effect Method for In Vitro Synergy between Amoxicillin and Ceftriaxone and between Vancomycin and Ceftriaxone against Enterococcus faecalisand Penicillin-Resistant Streptococcus pneumonia. Antimicrobial Agents and Chemotherapy 45: 12, 1: 3328-3333.
3. Fuentes Hernandez, V.O.Antibioticos 2020. Farmacología Veterinaria. ISBN 970-27-0165-1 Comision Editorial de la Universidad de Guadalajara Mexico p
4. Gaynor M. and Mankin A.S**.** (2005). Macrolide antibiotics: binding site, mechanism of action, resistance. Front. Med. Chem. 2, 21-35.
5. Hamilton-Miller, J. M. T**.** 1985. Rationalization of terminology and methodology in the study of antibiotic interaction. J. Antimicrob. Chemother. **15:**655-658
6. Kim, J., Guevarra R B., NguyenS G.,Lee J H.,Jeong D K.., Tatsuya Unno T. 2016. Effects of the Antibiotics Growth Promoter Tylosin on Swine Gut Microbiota. J. Microbiol. Biotechnol. 2016; 26(5): 876-882
7. King, T. C., D. Schlessinger, and D. J. Krogstad**.** 1981. The assesment of antimicrobial combinations. Rev. Infect. Dis. **3:**627-633
8. Lewicki K. 2006 TYLOSIN A review of pharmacokinetics, residues in food animals and analytical methods. ftp://ftp.fao.org/ag/agn/food/tylosin_2006.pdf
9. LI J., SHUANGYANG D, SUXIA ZHANG, CUN LI, XIAOWEI LI, ZHONGWEI LIU, JINFENG LIU, AND JIANZHONG SHEN 2006 Residue Depletion of Florfenicol and Its Metabolite Florfenicol Amine in Swine Tissues after Intramuscular Administration J. Agric. Food Chem. 54, 9614-9619
10. National Committee for Clinical Laboratory Standards**.** 1994. Performance for antimicrobial susceptibility testing. Standard M100-S5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. Norbert Desbiolles, Lionel Piroth, Catherine Lequeu, Catherine Neuwirth, Henri Portier, and Pascal Chavanet. 2001. Fractional Maximal Effect Method for In Vitro Synergy between Amoxicillin and Ceftriaxone and between Vancomycin and Ceftriaxone against *Enterococcus faecalis* and Penicillin-Resistant *Streptococcus pneumoniae*.Antimicrobial Agents and Chemotherapy, December 2001, p. 3328-3333, Vol. 45, No. 12
12. Rahal, J. J**.** 1978. Antibiotic combinations: the clinical relevance of synergy and antagonism. Medicine **57:**179-195
13. Ulvatne, H., Karoliussen, S., Stiberg, T., Rekdal, O., Svendsen, J., 2001**.** Short antibacterial peptides and erythromycin act synergically against *Escherichia coli.* Journal of Antimicrobial Chemotherapy (2001) **48**, 203-208
14. Voorspoels, J., D'Haese, E., de craene, B.A., Vervaet, C., De Riemaecker, D., Deprez, P., Nelis., H., and J. P. Remon **1999.** Pharmacokinetics of florfenicol after treatment of pigs with single oral or intramuscular doses or with medicated feed for three days. The Veterinary Record,145, (14), 397-399
15. Zhanel G.G., Dueck M., Hoban D.J., Vercaigne L.M., Embil J.M., Gin A.S. and Karlowsky J.A**.** (2001). Review of macrolides and ketolides. Focus on respiratory tract infections. Drugs 61, 443-498.