C.3./S.II.

Sulfonamides & Diaminoyrimidines (DAP)
Substances which have in their structure the sulphonamide group: $\text{SO}_2\text{-NH}_2$, structure similar to that of paraaminobenzoic acid (PABA), essential precursor to vitamin B.

**Classification:**

*antimicrobial, diuretic, hypoglycemic and antithyroid currently are known, three types of derivatives outgoing from para aminobenzene sulphonamide:*

- substituted at position N1
- substituted at position N4 and
- double substituted at positions N1 and N4.
- the most important are those of the first type.
- There are other compounds considered sulphonamides:
  - *homosulfonamide* which has interposed between the amine function and the benzene ring a methylene function;
  - *sulfones*, which acts on *Hansen bacillus* (germ of leprosy in humans)
Solubility
- influence the effectiveness = tissue concentration is governed by rate of absorption at the injection site and their concentrations in the body compartments.

the rate of absorption is proportional with their hydrophilicity

Chemical properties
- derivatives of para-aminobenzene sulfonamide are white crystalline powders, odorless, bitter taste, insoluble in water, mineral acids

Solubilization is made at extremes pH.
Identification:

**Qualitative methods**
- Osadchenko method in the presence of HCl and lignin (cellulose)

Commercial presentation:
- powders, tablets, injectable solutions, buvable solutions, external solution (eye drops), ointments, intrauterine suppositories and tablets.
- There are also: **oral paste and bowls for horses.**
Absorption and diffusion

Old sulphonamides:
**only p.o. (absorption rate is proportional with solubility), or topically**

Absorption:
**variable with the species, dependent on the degree of intestinal fullness:**
- **dogs and cats** - rapidly absorbed, almost completely,
- **in cows**, absorption rate is much longer (is recommended to administer in warm milk to close the oesophageal tray and encourage direct passage to abomasum.

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Monosodium salts: strictly i.v.

Exception: sodium sulfadimidine and sodium sulfadimethoxin, which may be administered deeply i.m.

Diffusion of sulfamide:
- in tissues and organs is very good (those that absorb easily have general action).

diffuses into:
tissues and organs, including barriers, in fetal circulation are reaching up to 50% of maternal therapeutic concentration.
Diffusion coefficient in lungs very important, as well as the one of diffusion in organs, in which infection is mainly localized.

**Penetrate into:**

figurative elements, serosae, synovial fluids, transudates, exudates, with beneficial effects in therapy **(condition: that there is no pus)**.

**Diffuses well in:** cerebrospinal fluid crossing the blood-brain barrier.

**High concentrations in:** kidney and liver

In these organs is achieved the concentration of sulphonamides, exceeding the blood concentrations of the substance.
Administered sulfonamides

By oral route:  
are well absorbed.

By parenteral route:  
uptake is based on the used path, the i.v. one being rapid and achieving maximum concentrations immediately.

By serous route:  
good absorption, high blood levels.

By rectal route:  
gives uneven absorption and is less useful in veterinary medicine

By i.m. route:  
just some oily solutions.
in blood, sulfonamides couples to plasma proteins. From this point of view are divided into:

classical sulfonamides:
- small percentage of coupling and a shorter duration of action in the body

retard sulfonamides:
- couples massively to plasma proteins and acts for a long time in the body, being released under a free from in blood.

Concentrations in blood should provide a minimum inhibiting level on bacteria or germs on during the entire treatment.

**M.I.C:** 3 mg sulfonamide/100 ml blood.

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Metabolization

It is achieved in the liver, by biotransformation, or conjugation.

An important mechanism is sulfonamide acetylation at the amine function (found in classic sulfonamides).

Acetylation at the amine function of sulfonamides has the disadvantage that the metabolites precipitates in urine; in acid urine may even form microcrystals (that can block the renal tubules).
Acetylation is an important route especially for old sulfonamides. An advantage of pirimidinic sulfonamides (sulphadiazine, sulphamerazine, sulphadimidine) is that the acetyl derivative is more soluble, as the other sulfonamides.

An important route of metabolization in retard sulfonamides is glucuroconjugation which allows the formation of some metabolites with easy elimination.
Elimination

Via kidneys:
The uncoupled sulfonamide is excreted by the glomerulus, ionized molecules being excreted actively by proximal tubules.

Reabsorption:
occurs in the uriniferous tubules through processes of passive diffusion of unionized liposoluble compounds.
Sulfonamide reabsorption occurs in the tubules through passive diffusion of unionized liposoluble compounds.
The proportion of unionized drug will be dependent on $pK_a$ and on the pH of the tubular fluid.
From the point of view of the rate of renal elimination, sulphonamides are divided into 6 categories:

1. sulphonamides with ultrafast elimination (Sulfathiourea / Badional) - useful therapeutic time: 2-3 hours;

2. sulphonamides with fast elimination (Sulfathiazole) - useful therapeutic time 4-6 hours;

3. sulphonamides with average elimination (Sulfafenazole, Oris, Sulfamethoxazole, Gantanole, Sulfisomidine or Elkosin) - useful therapeutic time 12 hours;
4. sulfonamides with slow elimination
(Sulphamerazine, Sulfodimerazine, Sulfametine, Bayren, Sulfanetoxipirimidazine or Retamid)
- which are eliminated from the body after 24 hours;

5. sulfonamides with very slow elimination
(Sulfamethoxipirazine, Longun or Sulfalen)
- duration of action in the body of 48 hours;

6. sulfonamides with ultraslow elimination
(Sulfadimetoxipirimidine, Sulfadoxine or Sulformetoxin)
- useful therapeutic time of 72 hours (in oral administration to birds can get up to 5 days).
• Besides the renal elimination after oral administration there is also a smaller or greater amounts of sulfonamides in feces, depending on the degree of absorption, and some times in milk.

Sometimes increased concentrations in milk can produce problems in the cheese industry stopping fermentation.

Can be eliminated through eggs, elimination is done both in the egg white and in the yolk.

• Theoretical risk of sulfamidotherapy is crystalluria as a result of precipitation of poorly soluble sulfonamides in tubules and ureters. This will result in reduced production of urine, pain and the the presence of albumin in urine.
The drawbacks can be minimized by rules:

- watering animals with water ad libitum, because dehydration leads to
- urine concentration,
- making treatments max. 7 days,
- use of associations of sulfamidics (ex. sulfapiridin + sulfamerazina + sulfadiazine),

Prolonged dosage in ruminants determines:
- symbiotic flora suppression,

Dosage for very long period causes:
- diarrhea and problems related to the destruction of the production of vitamin K by the ruminal flora.

The toxicity of sulphonamides in poultry is recognized by decreasing production and egg quality. In the case of sulfaquinoxaline haemorrhagic diathesis can be found

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Spectrum

**Includes:** Gram-negative and Gram-positive cocci (especially streptococci, staphylococci, pneumococci, Gram negative bacteria) and Gram positive (B. anthracis, Pasteurela), Clostridium, B. mallei, in actinobacillosis in actinomycosis and on Corynebacterium spp.

**Give good results in:** avian coccidiosis,

**They are not active in:** ricketsiosis, on small viruses an in case of acid-alcoollo - resistant bacillus.
Mode of action

**Bacteriostatic**

**Causes** inhibition of bacterial multiplication, easily damages the bacterial cells.

This will subsequently allow **phagocytosis of bacterial cells.**

- In the presence of sulfonamides phagocytosis is activated, cells are more vulnerable.

**The process of phagocytosis is so intense, that does not form immunity.**

- associating sulphonamides with sulfonamides amplifiers (potentiated sulphonamides) gives bactericidal effect.
Bacteriostatic action is maximum in high bacterial multiplication phase and especially on germs after the invasion multiplication.

The optimal one is the one after the logarithmic phase of bacterial multiplication by depletion of para-aminobenzoic acid and use by substitution of sulphonamides.
Mechanism of action

interfering metabolic synthesis of folic acid, but there is also bacteria which does not require the action of PABA in the formation of nucleic acids but they can get already synthesized metabolic compounds (folic acid).

sulfonamide molecule is similar to that of para-aminobenzoic acid, such as size, spatial structure, interatomic distances and distribution of electrical charges.
The size of the PABA molecule is 6,7 Å, compared to 6,9 Å length and 2,3 Å width compared to 2,4 Å in sulfonamides.

Spatial distribution:
similar when there is a substitution at the amide position, atomic distribution of electrical charges and interatomic distances having an important role in the three-dimensional distribution.

Carboxyl group -COOH
has a more powerful electronegative character than the sulfanyl one.

• Sulfonamides that have at the amidic nitrogen a radical have higher spatial similarity to PABA and this substituent influence much the bacteriostatic action
Sulphonamides antagonists

• The competitive activity of sulphonamides has been demonstrated by the addition of some metabolic compounds which the body is unable to synthesize in the presence of sulfonamides.

• They have eliminated the antibacterial activity of sulfonamides, any of the following metabolites being considered antagonists:
  - paraaminobenzoic acid and the related structures, such as local anesthetics with $p$ nucleus–aminobenzoic (ex. procaine, butacaine, benzocaine), some antibiotics (ex. procaine penicillin);
other members of the B complex
nicotinamide, folic acid, choline) and their precursors including
many amino acids (ex. glutamic acid, methionine)
some proteins
can combine partially with sulfonamides and this way, can
temporarly block their antibacterian activity (gelatin, albumin,
peptone and serum protein),
sulfonamides are antagonized by the tissue fluids and by blood
through which is circuiting
The resulting products consecutive of cell and tissue death,
especially pus, which acts as a mechanical barrier (non-
vascular).
Toxicity and side effects

- The main risk is crystalluria, in the tubules and ureters, urmarea precipitării cazul sulfamidelor slab solubile.
- Effects on blood picture in the first hours are: leukocytosis, lymphocytosis, leukopenia up to agranulocytosis.
- High doses and prolonged treatment, lead to methemoglobinemia. In such cases treatment is made using methylene blue for the reduction of hemoglobin in oxyhemoglobin.
- Sulfonamide dosage in ruminants may be followed by suppression of development of specific rumen flora.
• in ruminats long term administration may be followed by diarrhea, depression and haematological problems (bleeding) due to suppression of the production of vitamin K by specific rumen flora.

• In poultry toxicity is reported by decreasing egg production and egg shell thinning. Haemorrhagic diathesis (in particular Sulfaquinoxaline) is frequently met.

• Thrombocytopenia is more pronounced in poultry in the case of Sulfoquinoxaline, responsible for specific hemorrhagic syndrome
• In poultry, cause destruction of symbiotic flora from the intestine, occurrence of avitaminoses or hypovitaminosis K, which is why sulfonamides treatments are often accompanied by the administration of vitamin K in drinking water.

• In carnivores, administration of large amounts of sulfonamides may give rise to nervous phenomena, especially in youth. I.v. administration of sulfathiazole leads to immediate onset of central nature vomiting.

• In sulfonamides treatments in carnivores, large quantities of liquid and the use of sodium bicarbonate for alkalinization of urine are recommended.
Indications and contraindications

• Sulfamidotherapy **is applied only in the case of naturally sensitive germs to sulfonamides** **sulfamidogram is recomanded prior to treatment.**
  • The effectiveness of treatment is better **during the multiplication of the bacteria**, when they heavily synthesized folic acid, necessary to nucleic acid synthesis.
  • It will start with a loading dose, which is larger than the other doses of that day.
  • Treatment is done using decreasing doses, the highest dose being the first day of treatment.
• **General recommendations in sulfamidotherapy**
• When treatment is discontinued does not resumed with sulfonamides.
• Associations are recomanded under the form of di or tri-sulfonamides, based on each ones kinetics.
• Association with sulphonamides amplifiers are recomanded, with stronger activity and the resistance installs more difficultly.
• Combination with other chemotherapeutic agents or bacteriostatic antibiotics is recomanded.
• Sulfonamides association with vitamin H1, B9, brewery yeast, local anesthetics of para-aminobenzoic acid structure, sulfur-containing amino acids and feeding meat is contraindicated.
In the case of prophylactic treatments (avian cholera), prophylactic therapy should be supported, using sulfonamides with slow or ultraslow elimination. Therapy for supporting the organism including nonspecific therapy, is recomanded, to complete the effect of sulphonamides by bacterial phagocytosis.

- As a method of administration they are administered orally or parenterally.
- The oral route used in mass treatment, or in a mixture with feed, or with drinking water (in poultry and swines). Administration to other species is usually done individually.
• **Parenteral administration** is most often intravenous.
• **Administration of sulfathiazole, 20% injectable solution** is prohibited s.c or i.m. route (phlegmons or abscesses).
• Locally sulfonamides may be administered orally in the case of enteritis, using hardly absorbable sulfonamides.
• At the surface of the skin and mucous membranes, sulfonamides can be applied as powders on wounds, being a good surface cicatrizing or under the form of sulfamidate hemobandage.
Sulfamidoresistance

May be:
• natural or
• gained/acquired resistance.

Natural resistance:
of bacteria and microorganisms which may take
preformed folic acid from the environment, that can not
synthesize their own.

Acquired resistance:
occurs more rapidly and it is longer. It is based on
enzymatic adaptation of the bacterial metabolism or of
a species of bacteria or mediation of factor R.
• Sulfonamides used in veterinary therapeutics
Systemic sulfonamides

**Sulphanilamide (mother sulfonamide)**
under the form of powder on wounds, alone or in combination, local applications as ointments or intrauterine application.

**Sulfadimidine (sulfamethazine)**
semiretard action, of 12-24 hours (t½: 8–12 h). It absorbs quickly, coupling approx. 75% to plasma proteins and achieved high levels in the blood, diffuses well in lungs.

**Is administered in:** infectious diseases, infections, local lesions, sol. 33% or 20%, administered s.c. or i.v. to the livestock

**Orally:** 8-10 cg/kgbw. (products are formulated as a bolus, for bovines) and in coccidiosis in poultry and avian cholera.

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Sulfadiazine

- first pyrimidine sulfonamide.
- is well absorbed in the digestive tract but not as sulphanilamide.
- quickly and evenly distributed in the tissues
- It is absorbed in the CSF and has
- $T_{1/2}$ for about 3 hours.

The therapeutic importance lies in particular from association with trimethoprim.
Sulfadimethoxin (sulfadoxine)
structurally similar to sulfadiazine, important for the high concentrations that it produces in the livestock categories, where $T_{1/2}$ is de 11–15 h.

Sulfadoxine has:
ultraslow elimination, is maintained in the body at therapeutic value for 72 h.
Metabolized by glucuronidation and eliminated without renal side effects.
used in potentiated preparations the the form of injectable solutions 40%: Borgal, Trivetrin.
The therapeutic dosages are 50–55 mg/kg bw, initial dose, orally, followed by 25–30 mg/kg bw parenterally (s.c. or i.v.)

**sulfadimethoxin**
Sulfathiazole

on most bacteria, coupled with plasma proteins 40-45%
Is prepared at strongly alkaline pH, solubilization medium being made in NaOH at a pH=10-12.
Compared with sulfadimidine and sulfadimethoxin, sulfathiazole = more toxic and in therapeutics the derivative Phthalylsulfathiazole it is preferred, orally. In pigs, where intravenous administration is challenging i.p. rouye is used.
it can be administered: p.o. in the form of tablets or powders, parenterally sol. 20% and externally in ointments 10%. 

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Sulfamethoxypyridazin
used as such or in sulfamidic combinations for livestock. plasma peak is reached after 6-8 hours after single administration.
There are conditioned oral type controlled slow release bolus, which can maintain therapeutic plasma levels for 2-3 days after a single dose (usual dose: 55 mg/kg bw).

Sulphaphenazol
semiretard sulfonamide with very good absorption and diffusion, realise a coupling with plasma proteins in a proportion of 80%. Is maintained in the body for 12 ore.
Sulfametin is coupled massively with plasma proteins (90%). It is rapidly absorbed orally, with slow elimination, maintaining a therapeutic concentration in the body for 24-36 h. It is realised under: Injectable, powder, tablet formulations.

Sulfathiourea (Badional) is included in other chemotherapeutic preparations, its time of action is short. Associated with Marfanil, form Marbadal preparation, used in different local administration and especially in the intrauterine ones.
Sulfacetamide

heavily acetylated sulfonamide at the amine function, is readily absorbed but is rapidly cleared.

Used as the sodium salt and administered most often in topical and ophthalmic combinations, external (collyrium with sulfacetamide 10%) in conjunctivitis and keratitis, being non-irritating to eyes, the drug diffuses well into the eye.

Sulfacetamide can also be used in rhinopharyngeal applications, in external otitis or enteric sulfonamide (phthalyl form).
Sulphathiourea (Badional)

included in other chemotherapeutic preparations, its time of action is short. Associated with Marfanil, form preparation Marbadal, used in different local administration especially in the intrauterine ones.

Neoxazole

sulfonamide with *local urinary action*, which focuses very much in the kidney and is very active in urinary infections with *E.coli, Proteus, Pseudomonas*, etc. supplied as tablets or solutions 40%.

Sulphanilamide (mother sulfonamide)

under the form of powder on wounds, alone or in combination, local applications as ointments or intrauterine application.
Enteric sulfonamides

The most important representatives of this group are:
- sulfaguanidine,
- Phthalylsulfathiazole,
- phthalylsulfacetamide
- succinylsulfathiazole,

sulfonamides which are of very low solubility and are less absorbent, except sulfaguanidine, which is absorbed in a proportion of 50%, after single administrations, and will no longer have, strictly intestinal activity.
Presentation of enteric sulphonamides is under the form of white insoluble powders.

- **Sulphamerazine** - has time of action 8 hours.

- **Neoxazole** - sulfonamide with *local urinary action*, which focuses very much in the kidney and is very active in urinary infections with *E. coli, Proteus, Pseudomonas*, etc. supplied as tablets or solutions 40%.

- **Sulfametin** - coupled massively with plasma proteins (90%).
  
is rapidly absorbed orally, 
  **It is realised under:** injectable, powder, tablet **formulations.**
Associations of sulfonamides
(disulfamide and trisulfamide)

• **Suzodil powder** - disulfamide formed from Sulfamethazine and Sulfacetamide in proportion of 1:1. Is administered orally.
• **Suzotril powder** - trisulfamide formed from Sulfathiazole, Sulfacetamide and Sulfadimidine sodium, in proportion of 1:1. Is administered orally.
• **Suzotril injectable** - 30% solution, which contains Sulfacetamide sodium 10p, Sulfamethazine 10p, Sulfathiazole 10p, NaOH and distilled water ad. 100. It is given iv injections.
• **Suzodril** – injectable sol. which contains Sulfathiazole sodium 16,3%, Sulfacetamide sodium 15% distilled water ad 100.

• **Ametosulfin** - injectable sol. 30% which contains Sulfathiazole, Sulfacetamide sodium and Sulfametetine each 10 p.

• **Sulfotin** - preparation based on sulfachlorpiridanise sodium associated with Trimethoprim and an antihistamine. It is used in colibacillosis and enteritis in calves.

• **Sumetrolim** - based on Sulfamethoxazole and Trimethoprim. Is a n injectable solution 24% and is administered once daily, deep intramuscular 1 ml/10 kg bw.
• **Tetramidan** - French product, as a powder mix, to be taken orally and a mixture of three sulfonamides, and in the form of an injectable solution 25% includes three other sulfonamides.

• **Neodiar** - suspension containing 3 sulfonamides, a chemotherapeutic - Furazolidone - and an antihistamine - Neomycin. It is used in the treatment of enteritis in pigs orally with a syringe.

• **It is used once a day for 7 kg piglets and two doses per day for 7-12 kg, piglets and the treatment lasts three days.**
Homosulfonamides and sulfones

Typically used in human medicine, but also in the veterinary therapeutics (homosulfonamides).

**Marfanil** - makes more difficult resistance and used in the form of injectable preparations like *Supronal*.

**Diazona** is a sulfone is used in the treatment of leprosy in humans. It is also encountered in some preparations for the treatment of coccidiosis in poultry.
Antiprotozoal sulfonamides

The best known mode of action of these sulfonamides is mainly on immature, asexual stages, but also on schizonts where is interfering folic acid / PABA route. 

*Sulfaquinoxaline* (4-amino-2N-quinoxalinyl-benzen-sulfonamide) active on coccidia and is used in the curative therapy of avian cholera. It is well absorbed in the intestine.

In high concentrations has negative effects: producing ovarian follicular degeneration in poultry with compromising the reproductive function, adrenal degenerative phenomena.
**Sulfaquinoxaline**

is administered in the drinking water or feed pre-mix in coccidiosis in chickens, turkeys, rabbits, often in association with amprolium.

**Dosage**

- turkeys, chickens and other poultry: **125 ppm/day** feed or water for **8 days**, preventive and **500 ppm** in water for **7 days**, curative.
- rabbits, preventive, **250 ppm** daily, in feed
- **1000 ppm** in water, curative.

- for calves, the preventive dosage is **12 mg/kg bw.**
Waiting period for poultry meat is 5 days.

Side effects - laying hens, medication should be stopped one month before laying there is the danger of damaging the ovarian follicles.

Taking into account the side effects of Sulfacoccidin Sulfaveridine was prepared, which is a combination consisting of 2.5% sulfaquinoxaline and 2.2% Ethoxydiaveridine. It is used in the drinking water at a concentration of 2 ‰.

Dimerasol

prepared in 33% solutions, active against Gram negative and positive germs but also on coccidia. It is administered orally or parenterally (i.v, i.m or s.c).

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anthelmintic sulfonamides

- Studies have demonstrated clorsulon efficacy against trematodes, especially on *Fasciola*, adult stages, and on immature forms, young forms of 6 weeks in sheep and of 8 weeks in calf, have been eliminated with only a single. The drug is rapidly absorbed, in proportion of 75% and couples to plasma (25%) and red blood cells, here the activity on trematodes.
Clorsulon
4-amino-6-(tricloroethenyl)-1,3-benzen-disulfonamide.
Clorsulon is not mutagenic or teratogenic, having safety index of more than 25 times the usual dose, however it will not be administered to dairy cows.

Dosage
Bovines and sheeps: 7 mg/kgbw., p. o. and 4 mg/kgbw., s.c.
Waiting period for meat is 8 weeks.
• Diaminopyrimidines and sulphonamide associations
Combination sulfonamide + potentiating drug = bactericidal effect and maintain sulfonamides in the modern antibacterial therapeutic arsenal.

- Efficacy may be enhanced by combination with inhibitors of dihydrofolate reductases, i.e. 2,4-diaminopirimidines, or similar structures

Trimethoprim

first discovered diaminopyrimidine, antibacterial activity without the drawbacks sulphonamides, without side effects and which has the ability to potentiate each other with the sulfonamides.
• Result = increasing the effect and transformation from bacteriostatic to the bactericidal, expressed by several times increase of the therapeutic index.

• note that, combination is antagonized by the presence of thymidine (involved in diversion of DNA formation).

Action sites of the sulphonamids and of trimethoprim
Trimethoprim

Main compound used as chemotherapeutic and potentiating drug.

- Close range of bacteriostatic action to sulfonamides
- Major diseases of the dog, cat and farm animals
- Tract diseases: respiratory, urinary, gastro-intestinal, genital.

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Aminosalicylates: Sulfadoxine, sulfasalazine and sulfadiazine

- The most common combination with trimethoprim are sulfadiazine and sulfadoxine.
- Combination with Trimethoprim is done by 1:5 proportion.
- The most important mixtures are made with: sulfamethoxazole, sulfadiazine, sulfadoxine, sulfachlor-pirimidine.
- Trimethoprim excretion is made via urine.
The combination of **trimethoprim + sulfadiazine** is present in several formulations:

- **injectable** (80mg trimethoprim + 400mg sulfadiazine/ml)
- **bolus** (0.2 g trimethoprim + 1 g sulfadiazine).
- **oral powders** (0.2 trimethoprim + 1g sulfadiazine/10g pulv.)
- **oral susp.** (80mg trimethoprim + 400mg sulfadiazine/ml)
- **tablets** (80 mg and 400 mg)

In: **L.A., dogs**, 15 mg/kg x 2 times/day, **cats**, 120 mg/day
Other sulfonamide potentiating drugs

Diaveridine
• potentiating drug with **own chemotherapeutic actions**, active in **coccidiosis** in poultry. It is used in combination especially for the treatment of coccidiosis in poultry.

Ethoxydiaveridine
• compound where the **methoxy** function (of Diaveridine) is replaced by an **ethoxy** function.
• crystalline powder, slightly yellow, odorless, bitter taste, insoluble in water, soluble in acids. **acting on coccidia** no side effects and can also be used in laying hens.
Conclusions
• Thank You For Your Atention!