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FOOD POISONING BY ANTIBIOTICS RESIDUES IN RABBITS

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INTRODUCTION

The toxicity of various antibiotics in rabbits hab been reported for a long time by different authors (Milhaud 1976 - Morisse 1978 - Camguilhem 1980).
In rabbits and in rodents the administration of such antibiotics leads to a disrupture of the intestinal flora balance and enhanced dramatically the growth of pathogenic populations of E. coli (Renault 1973, Prohaszka 1980, Peeters 1984) and Clostridia (Carman 1982 - Rolfe 1984).
Those knowledges are henceforth classical so that the accidents resulting from a direct use by veterinarians, feed manufacturers or rabbit keepers are quite unusual.
Recently (Morisse, 1986) has reported the toxic effect in rabbits of an antibiotic used as a coccidiostat in broilers : "Narasin". Narasin like Monensin (previously considered as toxic in rabbits) (Morisse 1979) belong to the termed ionophores family in reason of their ability to transport ions (mainly Na ions) across biological membranes.
It is likely that their ionic ability is the basis of both the eimericid activity and toxicity in addition of their intestinal dysbacteriosis property.

Circumstances of the narasin poisoning were reported as follows :

- The toxic was detected in the feed whereas the accidental adding of medicated premix was impossible.

- Heavy losses have been experimentaly reproduced by both suspicious feed and control feed added narasin 30 ppm.

- Among different samples (corresponding to different feed bags of a single manufacturing) some of them were free of toxic while in others the toxic level was as high as 35 ppm.

- Quite obviously, the first bags of rabbit feed had been contaminated by a previous broiler feed manufacturing added narasin.

As all french manufacturers produce various types of feed, they try to keep clear of contaminations by a very strict manufacturing programme and by the use of ground cereals sent as a cleaner agent into the lines before rabbit feed processing.
The aim of the present paper is to report the techniques and the results of antibiotics investigations in suspicious feed as well as to evaluate the efficiency of cleaning procedures after manufacturing medicated feed.

MATERIAL AND METHODS

Agar diffusion test "AD test"

This classic method is used as a simple screening test.
Extraction of active susbstances is obtained by mixing during 15 minutes, 10 g of feed with 25 ml of both solvants : Methanol - HCl and Methanol-water; 2 ml of each extract is added with 2 ml of Chloroform, giving four different samples.
100 µ l of each extract is deposed into 10 mm diameter pits of agar seeded with Bacillus subtilis and Micrococcus luteus .
The spots diameters are measured after 18-24 hours incubation. Respective activity of the four extracts allows to adjust the choice of solvants for following methods.

Thin layer chromatography (with bioautography) "T.L.C."

T.L.C. technique has been adapted to assay animal feed by Freres et Valdebouze (1973).
That technique is specially adapted to identify and to semiquantitate polyethers antibiotics (ionophores) used in broilers feed as coccidiostats.
The migration distances of extracts on a silica gel plate in ethyl acetate are compared with those of the reference standards of the four polyethers commonly used as feed additives in broilers : Monensin and Narasin (Eli Lilly) Salinomycine (Roussel) and Lasalocid (Hoffman La Roche).
After migration, the bioautography is obtained by overlaying directly the plate with molten 50° C medium containing B. subtilis.
After 24 hours at 30° C the migrations distances revealed by inhibitory spots are compared in feed extracts and in reference standards.

High voltage electophoresis (with bioautography) "H.V.E."

As the previously used techniques do not allow to separate different mixed antibiotics, HVE described by Smither and Vaughan (1978) has been used ; its characteristic is that the migration of different antibiotics molecules in both agar and agarose gels is obtained between electrodes connected to a power unit of 1200 volts an 200 mA.
The electrolytes are composed by succinic acid and Tris buffer pH 6 and 8.
After migration during 1 h 45 minutes, visualization is obtained by bioautography.
Identification and titration of antibiotics is based upon the migration distances, and the form of spots compared with characteristics of reference standards spots.
The minimum level of detection varies with the nature of antibiotic ; it can be as low as 2 mg/kg for Ampicillin.

Controls have been performed in 2 kinds of materials.

- Samples of feed suspected of being in relation with sudden outbreaks of losses. In each circumstance, the plausibility of food poisoning has been evaluated by a previous reliable inquiry

- The cleaning efficiency of manufacturing process has been checked on samples of ground wheat used as a cleaner agent after manufacturing of a broiler feed added Salinomycin 60g/T (Salinomycin not toxic for rabbits is used as a contamination tracer).
  2 consecutive cleanings using 100 kg ground wheat each, are performed and samples are taken in 4 different sites of the process : mixer, molasses blending system, end of line and pelleting press.

R E S U L T S

Results of investigations performed on suspect feed and on process cleaning efficiency are summarized in table 1 and table 2.

D I S C U S S I O N

Antibacterial activity in suspect feed.

In 35 different feed samples considered as responsible of heavy losses after a reliable inquiry, the abnormal presence of an antibacterial activity (regardless of scheduled drugs) is detected in 65 p.cent of samples.
Contamination by ionophores is the most frequent one (50 p.cent of samples).
When Ampicillin , Narasin and Monensin are concerned, the relationship between feed and pathology is unquestionable (in 35 p. cent of studied cases)
The frequency of Salinomycin contamination (23 p. cent of samples) is the reflect of its widespread use by french manufacturers. Salinomycin is considered as harmless but its presence confirm the frequency of rabbit feed contamination by small amounts of broiler feed remaining in manufacturing processes.
It is obvious that antibiotic residues are not found in 100 p. cent of suspect feed ; used techniques are based upon antibacterial activity of extracts and they cannot detect mycotoxins, chemical pollutants (as pesticides) or toxic chemical additives suspected to be used as pelleting agents in some feedstuffs (bran or straw).

Control of cleaning procedures.

The cleaning of manufacturing processes after medicated feed processing tends to become a routine procedure but controls give evidence that even when a mixer is tidily emptied out by opening of the lower part, large amounts of material remain stuck on the walls.
Other parts of the processing lines (pipes, molasses blending system, pelleting press etc...) are heavily contaminated in the same way.
After a first 100 kg ground cereals passage, the absence of residues

could be expected; in fact, a second ground cereales passage, simulating a feed manufacturing ; show that residues can be still present.
The release of residues seems to be quite hazardous and contamination of only a part of a manufacturing is possible ; so is explained that among rabbit units supplied on the same day from the same manufacturing, only a few of them (sometimes a single one) can be affected.

C O N C L U S I O N S

Some of the medicated premixes used in mills are highly toxic for rabbits (and other species like horses) and no doubt that residual 20-25 ppm of active substances (as Monensin, Narasin or Ampicillin) are sufficient to induce heavy losses in rabbits units.
As 20-25 ppm of such drugs have been experimentally proved to induce heavy losses in rabbits, it is doubtful that lower doses (5-10 ppm) are quite harmless on growth and sanitary conditions.
Feed manufacturers are quite aware of risks and they do their best to provide the safest feed ; unfortunately the problem is so complex and the new drugs so active, that even thorough and sophisticated cleaning procedures, cannot totaly protect susceptible species against poisoning risks.
The authors opinion is that nothing but specific lines for rabbit feed processing, can give a complete guarantee.

TABLE 1 Determination of antimicrobial activity on 35 suspect feed samples by AD ; TLC and HPE methods.

Total antimicrobial activity (AD)	Ionophores (TLC)	Antibiotics (sensu stricto)(HVE)
	17/35	6/35
22/35	Narasin 7; 10-35 ppm Monensin 1; 5-10 " Lasolocide 1; 5-10 " Salinomycin 8; 3-5 "	Ampicilline 5;20-40 ppm Furans 1; 30-40 ppm

TABLE 2 Salinomycin titration (ppm) in ground cereals used as a cleaner agent after manufacturing of a medicated broiler feed (Salinomycin 50 ppm)

	1	2	3	4
1st passage	20 10-20*	25	20-25	
2nd passage	2,5-5	20		50 15* 10-15**

1 = mixer 2 = molasses blending system, 3 = end of line
4 = pelleting press

* about 50th kilo

** last kilos

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SUMMARY

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35 feed considered as being at the origin of heavy losses in rabbit units have been assayed for antibiotics detection by 3 techniques: Agar Diffusion (AD), Thin Layer Chromatography (TLC) and High Voltage Electrophoresis (HVE).
In 22 feed (63 p.cent) an abnormal antibacterial activity is detected by AD. Polyethers (ionophores) are found in 17 feed (49 p.cent) by TLC. Ampicillin and Furans are identified by HVE in 6 cases.
According to the high susceptibility of rabbits to monensin, narasin and ampicillin, the food origine of intoxications is considered as unquestionable in at least 13 cases (37 p.cent).
The contamination of processing lines by previous medicated feed is at the origin of rabbit feed pollution.
The control of cleaning measures performed by feed manufacturers gives evidence that even after a thorough emptying of processing lines and cleaning them by a passage of ground cereals, the total absence of drugs residues cannot be obtained.
The authors suggest that the only way to provide a total safety would be to use specific lines for rabbit feed processing.

RESUMEN

INTOXICACIONES ALIMENTARIAS POR RESIDUOS DE ANTIBIOTICOS EN EL CONEJO

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Tres técnicas distintas : difusion en agar, cromatografia en capa delgada y electroforesis de alta tension, fueron empleadas para investigar antibioticos en 35 alimentos considerados, a partir de una encuesta, como causantes de severas mortandades en criaderos de conejos.
Se detecto una actividad antibiotica anormal en 22 muestras (63 p.cent). Se encontraron poliéteres (ionoforos) en 17 muestras (49 p.cent) y 6 de ellas (17 p.cent) contenian o ampicilina o furanos.
Debido a la sensibilidad del conejo al monensin, al narasin y a la ampicilina, no caben dudas sobre el origen alimentario de las intoxicaciones en 13 casos (37 p.cent).
El origen de estos problemas es la contaminacion de los circuitos de fabricacion por alimentos medicados procesados anteriormente.
El control de la eficacia de la limpieza que efectuan los fabricantes, muestra que aun vaciando cuidadosamente los circuitos y limpiandolos por pasaje de cereales molidos, no es posible garantizar totalmente la ausencia de residuos.
Los autores sugieren que el unico medio para lograr una seguridad total es utilizar una cadena de fabricacion especial para el alimento destinado a los conejos.