ANTIBIOTICS IN FOODSTUFFS

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When we discuss different uses of antibiotics in agriculture as a hygienic problem, we have in mind above all the problem of assessing their residues in foods, or we are concerned with their effect on foods and the human organism resp. At present antibiotics are used in agriculture when they are added to fodder as protective substances in plant cultivation, and also in the processing of meats where they are used as a preservative. We must not underrate either the problem of residues in milk following the treatment of mastitis in cows.

Since 1950 successful trials were made where antibiotics were used for stimulating the growth of farm animals, particularly poultry and pigg. The trials, where along with antibiotic vitamin B12 was administered, were most successful. The optimum concentration was 5—10 mg. antibiotic per kg. fodder. This concentration has been raised since to 20—100 mg. per kg. According to data reported in the literature the literature the weight increment of farm animals increases on an average by 10—20% due to the antibiotics.

The mechanism of action of the antibiotic administered with fodder has not been elucidated completely so far. It is not clear whether a direct growth effect is involved or a nutritional effect via the influence exerted on the intestinal microflora. Most authors maintain, however, that the effect of antibiotics is due to the specific action on the intestinal microflora and that the activity of some toxin-producing microorganisms is reduced or eliminated and the nutrients are thus utilised more readily. It was for instance revealed that when simultaneously with penicillin penicillinase was administered, which inactivated the penicillin, the growth effect was lacking.

Some authors maintain, however, that by raising the sanitary standard in animal husbandry to an optimum level an equal effect can be obtained.

The most remarkable stimulatory effect was obtained with antibiotics of the tetracycline series which are still used in most countries, incl. the CSSR. During the long-term administration of certain antibiotics a decrease of the growth effect of these substances was observed and therefore recently new antibiotics were introduced for feeding farm animals. Therefore at present in this country trials with bacitracin, oleandomycin and erythromycin for poultry feeding are conducted.

As regards sensitization caused by antibiotics and also the possible induction of bacterial resistance, the problem of antibiotic residues in tissues of animals fed antibiotics is important. Broquist, Kohler, Anderson et al. found in sera of chicks fed antibiotics 0.014—0.061 gamma chlortetracycline per ml., when 50—100 mg. chlortetracycline per kg. of fodder was used. These authors also found residues of the antibiotic in the liver and meat of these chickens. Recently in the CSSR the problem of retention of chlortetracycline by individual organs and tissues resp. after large therapeutic doses was investigated. It was revealed that 24—48 hours after the last dose of chlortetracycline or tetracycline no residues of the above antibiotics are left in tissues except bones where residues of tetracycline
were found even after seven days. The position is different under various pathological conditions where after seven days residues of the antibiotics are still found in the damaged organs.

Most data in the literature on residues of antibiotics particularly tetracyclines used for feeding farm animals suggest that the addition of 20 mg./kg. of food does not cause the presence of detectable antibiotic residues in meat. When amounts of 100–200 mg./kg. are used, residues in tissues can be, however, detected. It is obvious that the detection of antibiotics in meat depends on the sensitivity of the method used.

In experiments on chicks we investigated the deposition and metabolism of chlortetracycline administered with the diet throughout the period of development, i.e. till the animals reached the weight of 1 kg. (10 weeks). The preparation Aureovit 12—20 mg. of the effective substance per kg. of fodder—was used.

The results of the experiment suggest that the antibiotic penetrates while chlortetracycline is being administered into all organs and throughout this period higher concentrations are detectable in the liver, kidneys and bones. In the bones they persist longest, as there antibiotics residues were found more than three weeks after the elimination of chlortetracycline from the diet.

Chlortetracycline retention in bone is known and is explained by the fact that chlortetracycline being a strongly chelating agent forms a complex with several elements among which the complex of chlortetracycline with calcium is most stable and can be detected by an intense yellow fluorescence. The bones of chickens fed chlortetracycline have an intense yellow fluorescence in ultraviolet light.

Málek and Bewelander and Alexandrov resp. provided evidence that tetracyclines influence the development of foetal bones (limbs are short and deformed, impaired mineralisation), while even high doses of chlortetracycline are without effect on developed bone. Vargová and Kovalčík found that even small amounts of antibiotic are transmitted from the uterus to the foetal tissue. The transmission through the mammary gland is even greater.

Under the condition that the period without antibiotic administration and the zero tolerance of the antibiotic in meat are strictly respected, in 1957 permission was given in the CSSR to use chlortetracycline for animal feeding. The maximum amount permitted is 20 mg. per kg. of food. For this purpose a special preparation Aureovit 12 is produced which contains 10,000 I. U. per 1 g. The animals must not be slaughtered sooner than 72 hours after the last dose. Special regulations were issued for the handling of antibiotics.

The mechanism of action of tetracycline antibiotics has not been elucidated so far. It is, however, known that the biological activity of some substances depends on their chemical structure and spatial arrangement of different functional groups. This finding was applied when investigating the degradation products of chlortetracycline which are formed on exposure to heat. In previous experiments it was revealed that after heating chlortetracycline solutions of foods containing chlortetracycline their biological activity declines considerably.

Today we know already about the breakdown of chlortetracycline by alkaline or acid hydrolysis, data on its breakdown by heat are, however, lacking.

For the separation of the products formed we used a chromatographic method and we assessed their absorption spectra in the ultraviolet and infrared spectrum.

From our estimations it appears that heating of neutral solutions of chlortetracycline at 100° for 30—90 mins. no substantial changes take place, i.e. the tetracycline nucleus remains intact. The exposed specimens have, contrary to standards, a different ratio of stereoisomers. Only some of the stereoisomers of chlortetracycline, tetracycline and oxytetracycline which are present, are
biologically active. The change of biological activity need not necessarily be associated with a change
in the structure of the substance, as apparent from our experiments with changing the structure
of the substance, and a change in the spatial arrangement of the molecule, is sufficient.

Based on our results which revealed that after acronisation antibiotic residues remain in meat
which are not completely inactivated by boiling, in CSSR chlortetracycline must not be used for the
acronisation of meat.

Since 1956 in the CSSR experimentally an antibiotic preparation Plantasol, containing strepto-
mycin sulphate and chlortetracycline, is used for spraying vegetables. This preparation serves to
improve the health of plants and to stimulate their growth. The persistence of streptomycin in the
fruit of plants thus treated depends on the season and type of vegetable.

In the above trials streptomycin was used which has a specific action in medicine. Residues of
this preparation persist according to our results 10 days after administration. The presence of resi-
dues would imply in case of zero tolerance a slower distribution of vegetables. Therefore the use of
this preparation for this purpose was not authorised except for cultivation of seeds.

In the CSSR experiments were also conducted with feeding Mycelia of penicillin which is a by-
product in the industrial production of penicillin. According to data of the manufacturer it contains
protein, vitamins and enzymes. Because the fresh mycelium still contains 200—1,000 I. U. penicillin,
it is subjected before use to thermal degradation.

In order to render the control of thermal degradation of penicillin in the mycelium possible, we
elaborated a procedure for estimating the penicillin content of the mycelium for the purpose of
hygienic control. As basis the well known plate diffusion methods (with Bac. subtilis) and the test
tube method taken over from dairy practice, was used.

In conjunction with this we must remind also of the problem of residual amounts of penicillin
in milk after therapeutic measures in cows. Vickers et al. and Erstin describe cases of allergy
after ingestion of milk containing penicillin. According to the above authors allergic reactions in
man can be produced already by milk containing 3 millionth I. U. of penicillin.

In the CSSR milk from penicillin treated animals must not be consumed sooner than 72 hours
after the last dose of penicillin.

As from the hygienic aspect the problem of bacterial resistance produced by residual amounts
of antibiotics in meat from animals fed antibiotics is important, we paid attention to this problem.
Experiments revealed that as a result of acronisation with chlortetracycline adapted of perma-
nently resistant forms of bacteria develop from bacteria which originally were sensitive to chlorte-
tracycline.

The importance of the hygienic problem of antibiotics as preservatives and supplements to fodder
is apparent also from the fact that the World Health Organization was concerned with this problem
and the findings are summarized in a monograph which in Japan is no doubt well known. At the
same time it was mentioned that the problem has not been resolved yet and that research will have
to be focussed also in future on the problem of antibiotic residues in foods. Czechoslovak depart-
ments are working intensely on these problems.
食糧品中の抗生物質について

農薬において抗生物質の様々な使用を衛生的な問題として取上げる場合、まず第一に食糧品の中にある抗生物質の跡の問題、または抗生物質が人体に及ぼす影響の問題と考えられる。現在抗生物質を飼料に入れたり或は草木にスプレーしたり、肉の保存薬として肉に加えたり、牛の乳腺炎の治療に用いられたりするが、乳牛の中に残る抗生物質の問題を軽視してはならない。

1950年以降農村の家畜特にニワトリと豚の成長を促進するため、抗生物質の使用が試みられ、大成功であった。抗生物質は飼料1kgにつき5〜10mgから20〜100mgに引上げられて実験され、家畜の成長率は10〜20%以上上げられた。抗生物質の家畜の成長に影響するかについて、直接成長に影響がある。或は間接的に腸内の微生物を通じて栄養的な結果がもたらされるのか不明であるが、大部分の研究者は後者を見解をとっている。次に用いる抗生物質をみるに、テトラサイクリン列に最も強い促進効果がみられた。ただ、或は抗生物質を長期間にわたると、成長効果がだんだん減少するので、新しい抗生物質を用いることになる。

今日、養鶏において、ベチトラチン、オレアンドマイシン、エリトロマイシンを用いて実験している。

抗生物質によって起された銅感作と細菌の抗生物質に対する抵抗の問題は、抗生物質を食べさせられた動物の内に残る。抗生物質の跡の問題である。飼料1kg当りクロロメトトリクリネ50〜100mgを食べさせた鶏の血清1ml中0.014〜0.061gamma、肝臓や肉の中にも抗生物質の跡が発見された。治療の為に与えられたクロロメトトリクリネが器官と組織の中にどのように残るか研究したところ、24〜48時間後には組織の中に残らないが、ただ骨の中では7日後でも跡は発見され、ある病気の場合には7日後病気の器官に抗生物質が残ることが判っている。またテトラサイクリンが胎児の骨の発育に影響する報告がある。（四肢が短かく変形、やわらかすぎる骨。骨が完全に発育する場合は大体完発残されない。また、抗生物質が数日でも子宮から胎児に移行することも重要な問題である。テトラサイクリンの影響のメカニズムはまだよく理解されていないが、或ものの生物学的活動、その化学的な構造と機能的なグループの場所の配列によることは知られている。我々は熱を加える時に生ずる腐敗生成物を研究しているが、紫外外のスペクトルを利用して、クロロメトトリクリネを30〜90分、100℃で熱すると本質的変化は見られず、テトラサイクリン核はそのまま残る。ステロイドメタンの発生比率をもつ。そして研究の結果、わが国では現在のアクリニューソンのため、クロロメトトリクリンを使っていなければならない。また、ブラントノルを草木にスプレーし、草木の健康を良くし成長を促進させる効果があるが10日間の残存するので、野菜のスプレーは禁止されている。また牛の病気治療のあと、ベニリンが牛乳中残り、アレルギーを起こすので、治療後72時間たって飲むことが許されている。等々食糧品の中に残されている抗生物質の問題は将来大いに研究されなければならない課題であるのでチェコスロバキアに於ては様々な研究機関で研究している。

（内田）