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| Autor: | Rutczyska-Skonieczna, Eugenia Maria / Nikonorow, Maksym |
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Effect of dose and period of administration of oxytetracycline (OTC) to hens upon the level of that antibiotic in eggs, meat and giblets. Hygienic evaluation of these products

Eugenia Maria Rutczyńska-Skonieczna and Maksym Nikonorow

Department of Food Research, State Institute of Hygiene, Warsaw

Most publications deal with the positive effect of antibiotics on the breeding of cattle and fowl. Fewer concern with the accumulation of antibiotics in tissues of animals receiving the said compounds in forage mixtures. There is particular divergence of opinions on the transmission of antibiotic to meat and eggs of hens (1—8). Present work has been intended mainly to elicit, if feeding hens with OTC in prophylactic doses and for therapeutic or breeding purposes brings about the passage of the said antibiotic to eggs, meat and giblets, as well as learning the dynamics of disappearance of OTC from hen organism.

Need for informations concerning the influence of the culinary thermal treatment on possible OTC residue in eggs, giblets and meat of hens, and determination of conditions protecting consumer's health pointed to the next purposes.

Experimental material consisted of eggs, meat and giblets from laying hens of Rhode Island Red stock. They were 16 months old at the beginning of the experiments (these from groups fed with prophylactic and therapeutic doses), and 8—9 months old (these put on forage dose).

Daily doses of 5 mg were performed for 60 days; of 22 mg for 30 days; and of 88 mg per hen for 6 days. OTC hydrochloride containing 900 µg of active substance in 1 mg of the preparation produced by Tarchomin Pharmaceutical Works «Polfa», Poland, was administrated in the sterile distilled water (doses of 5 mg) and in 15 % saccharose solution (dose of 22 and 88 mg). The antibiotic solution (3 ml) containing half of the daily dose of OTC was administrated to hens per os by means of the syringe with metal canule twice a day with intervals of about 10 hours.

The basal feed given to experimental and control animals was industrial DK mixture (without the antibiotic), grain and greens. The vitaminic food «Paszovit» and mineral mixture «Formosan» containing microelements made up the additional forage.

Hens were within enclosed space without any yard. Their weight ranged from 1,51 kg to 2,95 kg. Forty seven samples of raw meat (chest muscles, with a skin) and 141 giblets' samples (muscular stomach, heart, liver) were examined extracting the antibiotic within 3 hours following slaughter of hens.

There were 230 raw eggs examined.

OTC content was determined separately in yolks and glairs not later than within 24 hours from laying. Cylinder-plate method with *Bac. cereus* 8145 as a test organism was performed (9).

Eggs, meat and giblets of hens fed with 88 mg of OTC per hen daily for 6 days, as well as eggs from control hens having OTC injected in dose of 12 mg per hen were subjected to culinary thermal treatment. Fifty four eggs, 10 meat samples, 10 giblets samples and 5 bouillon samples were tested.

OTC content in thermally treated products was given translated into terms of the dry substance.

Results

Results of the raw eggs study are presented in Table 1. Observing the dynamics of the antibiotic disappearance from hen body, OTC-free eggs were found on 5-th day following the dose of 22 mg given for 30 days and on 8-th day following the dose of 88 mg for 6 days. The comparison of results obtained from raw meat and giblets studies is presented in Table 2. The statistically significant

Table 1
Relationship between the period of OTC administration (dose of 5,22 and 88 mg) and the antibiotic content in eggs

| Doses mg per hen daily | Period of ad- minis- tration (days) | Yolk $\mu\text{g/g}$ | | in whole yolk-ball μg | | White $\mu\text{g/g}$ | | in whole white μg | | Whole egg μg | |
|---------------------------------|---|-------------------------|--------|--|--------|--------------------------|------|---------------------------------|------|----------------------------|-------|
| | | from | to | from | to | from | to | from | to | from | to |
| 5 | 14 | | | not found | | | | not found | | — | — |
| | 28 | | | not found | | | | not found | | — | — |
| | 42 | | | not found | | | | not found | | — | — |
| | 56 | | | not found | | | | not found | | — | — |
| 22 | 5 | 0 | traces | 0 | traces | | | not found | | — | — |
| | 10 | 0 | traces | 0 | traces | | | not found | | — | — |
| | 20 | traces | | traces | | | | not found | | 2,12 | 3,17 |
| | 30 | 0,12 | 0,17 | 2,12 | 3,17 | | | not found | | 1,25 | 3,68 |
| | | traces | | traces | | | | | | | |
| | | 0,08 | 0,21 | 1,25 | 3,68 | 0,00 | 0,08 | 0,00 | 2,06 | | |
| 88 | 1 | | | not found | | 0,00 | 0,19 | 0,00 | 5,46 | 0,00 | 5,46 |
| | 2 | 0,00 | 0,18 | 0,00 | 3,31 | 0,11 | 0,26 | 3,50 | 7,37 | 3,50 | 10,54 |
| | 3 | 0,00 | 0,29 | 0,00 | 5,50 | 0,13 | 0,24 | 3,87 | 7,39 | 3,87 | 11,47 |
| | 4 | 0,00 | 0,41 | 0,00 | 7,13 | traces | | traces | | | |
| | 5 | 0,25 | 0,48 | 4,89 | 9,21 | 0,13 | 0,26 | 3,99 | 9,93 | 3,50 | 17,05 |
| | 6 | 0,17 | 0,48 | 2,91 | 9,02 | traces | 0,18 | 0,32 | 5,40 | 9,70 | 4,89 |
| | | | | | | (0,00) | | (0,00) | | | |
| | | | | | | 0,12 | 0,26 | 4,49 | 8,20 | 2,91 | 17,22 |

increases (means compared in pairs according to Student's t test at the coefficient of confidence of 0,95) were ascertained merely for livers after 6-day administration of 88 mg dose; the decreases were observed after 24- and 72-hours waiting periods.

Table 2 OTC content in meat and giblets following doses of 5,22 and 88 mg

| Doses mg per hen daily | Period of adminis- tration (days) | Time from the last dosage to the hen slaughter (hours) | Meat μg/g | | Giblets μg/g | | | | | |
|---------------------------------|--|---|------------------------|------|------------------------|------|------------------------|------|---------------------|--------|
| | | | | | Stomach | | Heart | | Liver | |
| | | | from | to | from | to | from | to | from | to |
| 5 | 30 60 | 6 24 | not found not found | | not found not found | | not found not found | | traces not found | traces |
| 22 | 15 | 6 | 0,00 | 0,30 | 0,00 | 0,47 | 0,00 | 0,41 | 0,00 | 0,83 |
| | 30 | 6 | 0,68 | 1,05 | 0,83 | 2,20 | 1,00 | 1,15 | 1,05 | 2,50 |
| | | 24 | 0,00 | 0,45 | 0,00 | 0,70 | 0,00 | 0,39 | 0,00 | 0,57 |
| 88 | 2 | 24 | 0,00 | 1,00 | 0,00 | 0,58 | 0,00 | 0,60 | 0,20 | 0,59 |
| | 6 | 24 | traces | | traces | | traces | | | |
| | | | 0,21 | 3,90 | 0,16 | 3,15 | 0,16 | 3,50 | 1,30 | 4,80 |
| | 6 | 72 | 0,00 | 0,35 | 0,00 | 0,65 | 0,00 | 0,33 | 0,00 | 1,65 |

Table 3

Ammount of OTC regained from eggs after thermal treatment (evaluated respectively for OTC content found in raw egg mass)

| | Eggs examined | OTC content in eggs (μg/g) | | per cent of OTC regained | |
|--|--|-------------------------------|----------------------|-----------------------------|--------------------------|
| | | from | to | from | to |
| OTC injected | after ½ min. frying after 8 min. boiling after 3½ min. boiling | 0,32 0,47 0,50 | 0,44 0,57 0,64 | 47,06 73,44 78,13 | 69,84 89,06 100,00 |
| Eggs from hens receiving OTC per os. | after ½ min. frying | 0,43 | 1,19 | 72,88 | 91,54 |

In Table 3 are collected the results of studies on boiled and fried eggs.

No antibiotic was found in meat nor in giblets after one-hour thermal treatment. Bouillon was OTC-free, too. On the other hand in liver after 3 minute-long frying (inner temperature circa 65 ° C) about 50 % of OTC content in raw organ was recovered.

The following conclusions for the hygienic control purposes resulted from the above discussed study:

1. Eggs from hens receiving prophylactic doses of OTC not exceeding 22 mg daily per hen for one month may be allowed to consumption after the lapse of 5 days. Eggs from hens receiving therapeutic doses of OTC not exceeding 88 mg per hen daily for 6 days may be allowed for consumption only after 8 days since this antibiotic has been withdrawn.

2. Slaughter of butcher fowl (chicken) fed with forage doses not exceeding 5 mg per head daily may be executed only 24 hours after the antibiotic withdrawal.

3. The time between hen slaughter and the antibiotic withdrawal must not be shorter than 72 hours for fowl fed with prophylactic doses and 6 days for that receiving the therapeutical doses. Completing that time one may expect the zero tolerance for OTC in meat and giblets of hens.

4. Tests for OTC in liver should be recognized for hygienic evaluation index of carcasses of fowl fed with the said antibiotic per os.

5. When slaughter must be done ahead of the higher mentioned time, liver of hens fed with prophylactic or therapeutic doses of OTC has not be allowed for consumption. Meat and others giblets (heart, muscular stomach) may but only after thermal treatment of at least one hour duration.

Summary

Examination of the effect of dose and period of administration of oxytetracycline to hens upon the level of that antibiotic their eggs, meat and giblets. Determination of the time necessary, after the end of administration, to get antibiotic free eggs, meat and giblets.

Résumé

On a constaté que l'antibiotique oxytétracycline, administré à la poule, passe dans la chair, les œufs et certains organes. On a examiné combien de temps il faut attendre, après la fin de l'administration, pour que la chair, les œufs et les organes soient exempts d'oxytétracycline. Ce temps varie suivant la dose d'antibiotique et la durée du traitement.

Zusammenfassung

Es wurde festgestellt, daß mit dem Futter Hühnern verabreichtes Oxytetracyklin ins Fleisch, in die Eier und in gewisse Organe (Herz, Magen, Leber) übergeht. An Hand von Versuchen wurde die Zeitspanne bestimmt, welche nach Abschluß der Verfütterung dieses Antibiotikums nötig ist, um oxytetracyklin-freies Fleisch, Eier und Organe zu erhalten. Diese Zeitspanne schwankt je nach der verfütterten Dosis und der Dauer der Behandlung.

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Dosage colorimétrique du p-nitro-benzaldéhyde (PNB)

Par R. Saba, D. Monnier et F. E. Khalil

Laboratoire de chimie analytique de l'Université de Genève

Il existe peu de méthodes de dosage colorimétrique du PNB. La plus utilisée est celle de Puga (1), qui consiste à faire réagir la solution hydroalcoolique de faible quantité de PNB avec une solution d'hydrosulfite de sodium et une solution de 2—4-dinitrophénylhydrazine à 100°. La coloration rouge-orange se développe après addition d'une solution de pyridine et de NaOH.

La méthode que nous avons mise au point a été inspirée par les considérations suivantes: on peut identifier et doser l'indol par le p-diméthylbenzaldéhyde (2, 3). C'est une réaction de condensation de ces deux molécules avec élimination d'eau. Si on opère en milieu HCl concentré, on obtient une coloration rouge intense stable en présence d'un grand excès de réactif. J. M. Turner (4) le remplace par le p-diméthylaminocinamaldéhyde et augmente ainsi la sensibilité de 2,2.

Nous avons donc examiné le comportement du PNB en présence d'indol en excès (Fig. 1). En milieu sulfurique on obtient une coloration rouge très sensible, proportionnelle à la concentration de PNB, qui permet donc le dosage de ce dernier.

Etude analytique

Réactifs. 1. solution d'indol dans l'alcool absolu à 0,5 g/100 ml (peu stable, doit être renouvelée chaque semaine). 2. solutions concentrée et diluée à 50 % de H₂SO₄. 3. solutions aqueuses de PNB de 1 à 10 µg/ml.