Transfer of Dietary Chlortetracycline into Eggs and its Disappearance from Eggs and from the Liver

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In the series of studies on the transfer and disappearance of dietary antibiotics in eggs, it was found that the behaviour of two macrolide type antibiotics with lactone rings in their chemical structure, i. e. spiramycin¹⁾ and tylosin²⁾, was different, the former being easier to be absorbed and transferred into eggs and staying longer than the latter. The behaviour of oxytetracycline reported in the previous paper³⁾ resembles rather that of tylosin than spiramycin.

In this paper, the behaviour of chlortetracycline, another tetracycline antibiotic widely used as feed ingredient, was reported.

Experimental

A premix of chlortetracycline, abbreviated as CTC hereafter, containing 88,800 ppm of CTC, was mixed in the basal diet at the level of 8,000 ppm. The composition of basal diet was the same as used in the previous experiments¹⁻³⁾. The supplementation of the CTC was made by replacing wheat bran in the basal diet.

Total 35 white Leghorn hens of about 8 months old were divided into a group of 10 hens and 5 groups of 5 hens each. The hens were reared in individual cages and fed the diet containing 8,000 ppm of CTC for 7 days and thereafter fed the basal diet free from antibiotics for another 7 days.

All of the eggs laid by 10 hens of the first group during the experimental period of 14 days were kept individually in a refrigerator of -20°C . Among the 10 hens of the group, 5 hens, who laid more eggs continuously during 14 days of experimental period than the rest of the hens, were selected and all of the eggs laid by them during 14 days of experimental period were analyzed for their CTC content.

At 0, 1, 2, 3 and 5 days after the withdrawal of dietary CTC, i. e. at 7, 8, 9, 10 and 12 days after the start of the experiment, one group each of 5 hens was sacrificed to get the sample of the liver.

Individual egg white, egg yolk and the liver were homogenized and diluted by 5 times with 0.1 M phosphate buffer solution of pH 4.5. Content of CTC in the diluted sample was determined by cup-plate method using *Bacillus cereus* var. *mycoides* ATCC

11778⁴⁾. The threshold sensitivity of the assay was 0.05 μg equivalent per g of the sample. The content of CTC in the whole egg was calculated from the contents in the egg white and yolk as described previously²⁾.

Content of CTC in the whole egg during the feeding period of CTC and after the withdrawal of dietary CTC was analyzed statistically as randomized block design, using individual hen as block. Data of 5th day after the start of the experiment were discarded since only 2 hens out of 5 laid on that day. Content of CTC in the whole egg of other days, when no egg was laid, was estimated statistically by the procedure to estimate missing value in factorial experiment design⁵⁾. Then, three regression analyses were carried out, i. e. the regression between CTC content in the eggs and days after the start of feeding 8,000 ppm of CTC, the regression between logarithm of CTC content in the egg and days after the withdrawal of dietary CTC, and the regression between CTC content in the liver and days after the withdrawal of the dietary antibiotic.

Results

Content of CTC in egg white and egg yolk of the eggs laid during feeding the diet containing 8,000 ppm of CTC and after the withdrawal of dietary CTC is shown in Table 1. After estimating the missing value statistically and discarding the data of 5th day, average of 5 estimates in egg yolk, egg white and whole egg are plotted in Fig. 1, to show the daily change in CTC content in the egg.

The increase in CTC content in egg white was very rapid and reached plateau

Table 1. Chlortetracycline content in egg white and yolk of hens fed the diet containing 8,000 ppm of chlortetracycline¹⁾ (μg equivalent/g)

Hen No.	Days on CTC ²⁾ feeding							Days after withdrawal						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Egg white:														
1970	0.13	0.27	0.28	0.43	— ³⁾	0.32	0.20	0.14	0 ⁴⁾	0	0	0	0	0
1971	0.10	—	0.13	0.15	0.19	0.21	0.18	0.10	0	0	—	0	0	0
1972	0.26	0.29	0.44	0.36	—	0.39	0.31	0.16	0.05	0	0	0	0	0
1981	0.14	0.24	0.31	0.24	—	0.17	0.20	0.11	0	0	0	0	0	0
1983	0.15	0.42	0.31	—	0.21	0.31	0.28	0.11	0.06	—	0	0	0	0
Egg yolk:														
1970	0	0.06	0.11	0.18	—	0.27	0.28	0.24	0.21	0.19	0.12	0.09	0	0
1971	0	—	0.06	0.06	0.09	0.11	0.14	0.17	0.18	0.14	—	0.06	0	0
1972	0	0.08	0.14	0.30	—	0.39	0.39	0.39	0.47	0.32	0.19	0.08	0	0
1981	0	0.09	0.13	0.28	—	0.38	0.28	0.28	0.28	0.23	0.18	0.08	0	0
1983	0	0.08	0.13	—	0.17	0.27	0.28	0.30	0.25	—	0.18	0.09	0	0

1) Threshold sensitivity was 0.05 μg equivalent/g.

2) Chlortetracycline

3) No egg was laid.

4) Zero indicates the content below the sensitivity level.

Fig. 1. Chlortetracycline content in the eggs laid by hens fed the diet containing 8,000 ppm of chlortetracycline

already on the 2nd day of CTC feeding. After the withdrawal of dietary CTC, content of CTC in egg white decreased rapidly and only small amount of CTC, i. e. 0.05 and 0.06 μg equivalent/g, which is equal to and only 0.01 μg over the threshold sensitivity level, respectively, was found in 2 eggs out of 5 on the 2nd day after the withdrawal of CTC. On the other hand, CTC content in egg yolk increased almost linearly after feeding CTC, reached plateau on the 5th~6th day on CTC feeding, and decreased almost linearly after the 3rd day of the withdrawal of dietary CTC.

Combining the contents in egg white and egg yolk, content of CTC in whole egg was found to increase rapidly and reached plateau on the 3rd day of CTC feeding. Thereafter CTC content during feeding period was almost constant with average value of 0.271 μg equivalent/g. The following Equation 1 was found fit to describe the linearly increasing tendency of CTC in the eggs after feeding CTC,

$$y=0.015+0.0782\ T_1 \dots\dots\dots(1)$$

where, T_1 is days on CTC feeding and y is average CTC content (μg equivalent/g) in whole egg laid at T_1 day. The correlation coefficient was 0.970, which is significant at 5% level. However, the constant in Equation 1, i. e. 0.015, was not significant statistically, so the relationship between T_1 and y could be simply described by Equation 2.

$$y=0.0782\ T_1 \dots\dots\dots(2)$$

The intersection between Equation 2 and $y=0.271$, i. e. average CTC content at

plateau, was 3.5 days.

The decrease in CTC content in the whole egg after the withdrawal of dietary CTC could be well described by the following Equation 3,

$$\log y = -0.5672 - 0.1860 T_2 \dots\dots\dots (3)$$

where, T_2 is days after the withdrawal of dietary CTC and y is average CTC content (μg equivalent/g) in whole eggs laid at T_2 day. The correlation coefficient was -0.993 , which was highly significant statistically at 1% level. Equation 3 can be transformed into Equation 4, which is shown in Fig. 1.

$$y = 0.271 \cdot e^{-0.4282 T_2} \dots\dots\dots (4)$$

Although CTC in egg white was detected for only 2 days after the withdrawal of diethdrawal of dietary CTC, the following Equation 5 was induced from the averages of CTC in egg white on 0, 1 and 2 days after the withdrawal of dietary CTC,

$$\log y = -0.4846 - 0.5536 T_2 \dots\dots\dots (5)$$

where, T_2 is days after the withdrawal of dietary CTC and y is average CTC content (μg equivalent/g) in egg white laid on T_2 day. Average CTC content of egg white at plateau, i. e., white of the eggs laid on the 2nd 3rd, 4th, 6th and 7th day on CTC feeding was used as the value for 0 day to get more reliable value than the actual average of data at 0 day. The correlation coefficient was fairly large, being -0.979 , but not significant statistically mainly due to the smallness of sample size, i. e. 3.

Table 2. Chlortetracycline content in the liver of hens fed diet containing 8,000 ppm of chlortetracycline for 7 days (μg equivalent/g)

Days after withdrawal of dietary chlortetracycline				
0	1	2	3	5
6.00	0.26	0.29	0 ¹⁾	0
3.41	0.28	0.31	0	0
3.10	0.38	0.30	0	0
3.30	0.26	0.25	0	0
3.38	0.49	0.34	0	0

1) Zero indicates the content below the sensitivity level of 0.05 μg equivalent/g.

Content of CTC in the liver of the hens after the withdrawal of dietary CTC of 8,000 ppm is shown in Table 2. Linear relationship between logarithm of average content of CTC in the liver of 5 hens and days after the withdrawal of dietary CTC was observed. The relationship could be well described by the following Equation 6, which can be transformed into Equation 7.

$$\log y = 0.2196 - 0.6290 T_2 \dots\dots\dots (6)$$

$$y = 1.66 \cdot e^{-1.4488 T_2} \dots\dots\dots (7)$$

where, T_2 is days after the withdrawal of dietary CTC and y is average CTC content (μg equivalent/g) in the liver at T_2 day. Correlation coefficient between T_2 and $\log y$ was -0.992 , which was highly significant statistically at 1% level.

Discussion

In the previous experiments with spiramycin¹⁾, tylosin²⁾ and oxytetracycline³⁾, content of antibiotics in whole egg laid by the hens fed the diet of extremely high level of the antibiotics increased linearly within 7 days of feeding period. These findings do not mean that the content of the antibiotics in the egg increases without limitation. Instead, they rather mean that content of the antibiotics in the egg should reach plateau when the speed of absorption of dietary antibiotics into hens' body and that of disappearance from hens' body are in balance. The latter speed is a function of body deposition of the antibiotics. Therefore, the speed will be accelerated by the increase of body deposition by longer feeding of antibiotics. It is certain that 7 days of feeding spiramycin at dietary level of 1,000 ppm, tylosin at 8,000 ppm and oxytetracycline at 4,000 ppm were not long enough to reach plateau of content of the antibiotics in the egg. However, 3.5 days of feeding CTC at 8,000 ppm was enough to reach plateau.

The findings reveal that the speed of absorption of CTC is slower, or the speed of disappearance of CTC from the hens' body is more rapid than those of the other 3 antibiotics tested already, resulting in earlier balance between absorption and disappearance of CTC than that of the other antibiotics.

It should be mentioned that maximum content of CTC in whole egg at plateau was 0.271 μg equivalent/g with dietary CTC level of 8,000 ppm, while content of oxytetracycline in whole egg on the 7th day on feeding 8,000 ppm of oxytetracycline was estimated to be 1.98 μg equivalent/g from Equation 2 or 8 in the previous paper³⁾, although the value may be lower than the maximum value at plateau. Considering the values together with the difference in the behaviour of CTC and the other 3 antibiotics it is easily supposed that no CTC will be detected in both egg white and yolk of the eggs laid by hens fed the diet containing 20 ppm of CTC, which is the dose allowed to use in feed for growth promoting purpose. Though only 8,000 ppm of dietary level of CTC was tested in this experiment, it is also suspected that little CTC will be detected from the eggs laid by hens fed the diet containing 500 ppm of CTC, which will be close to the upper level of CTC for therapeutic use. The findings on the residue of CTC in broiler⁴⁾ support the above supposition.

Combining Equations 2 and 4, following Equation 8 is induced to describe the behaviour of CTC in the eggs during and after feeding CTC at dietary level of 8,000 ppm,

$$y = 0.0782 T_1 \cdot e^{-0.4282 T_2} \dots\dots\dots (8)$$

where, T_1 is days on CTC feeding, T_2 is days after the termination of CTC feeding, and y is average CTC content (μg equivalent/g) in the whole eggs laid at $T_1 + T_2$ days after the start of CTC feeding. In Equation 8, T_1 should be less than or equal to 3.47 days regardless of the length of CTC feeding over 3.47 days. In Equation 8, T_2 should be zero during CTC feeding and T_1 should be a fixed value after the termination of CTC feeding.

Equation 8 for CTC, though it does not contain the term of x , i. e. dietary concentration of CTC, corresponds to Equation 1 for spiramycin, Equation 9 for oxytetracycline

in the previous paper³⁾ and Equation 9 for tylosin in the previous paper²⁾. From these 4 equations, average content of antibiotics in the whole eggs laid by the hens fed the antibiotics at a certain dietary level for certain days can be calculated. For example, if 8,000 ppm of antibiotics was fed for 3 days for therapeutic purpose, though the level is unusually high from the practical point of view, average content of spiramycin, tylosin, oxytetracycline and chlortetracycline in the whole eggs laid on the 3rd day of antibiotic feeding will be estimated to be 8.3, 2.8, 0.8 and 0.2 μg equivalent/g, respectively.

Since no term for x , i. e. dietary concentration of CTC, is included in Equation 8, the easiness of dietary CTC to be transferred into egg cannot be compared with those of the other antibiotics, as we compared those of oxytetracycline and spiramycin in the previous paper³⁾.

As discussed repeatedly in the previous papers¹⁻⁴⁾, the coefficient for T_2 in Equations 3 and 6 indicates the rapidness of disappearance of CTC from whole egg and from the liver, respectively, after the withdrawal of dietary CTC. To understand the rapidness more easily, biological half lives and their 95% confidence intervals were calculated and are shown in Table 3, with biological half lives of the other antibiotics for the convenience to compare. Biological half life of CTC in egg white could also be estimated from Equation 5, but its confidence interval was not calculated, since correlation coefficient was not significant statistically as mentioned above.

Table 3. Biological half lives of various antibiotics.

Antibiotics	calculated from	Biological half life	
		mean	95% confidence interval
chlortetracycline	whole egg	day 1.6	day 1.7~1.5
	egg white	0.5	— ¹⁾
	liver (layer)	0.5	0.6~0.4
Oxytetracycline ²⁾	whole egg	1.1	1.6~0.8
	egg white	0.6	—
Tylosin ³⁾	whole egg	1.3	1.8~1.0
	egg white	0.6	—
	liver (layer)	0.6	—
	liver (broiler) ⁴⁾	0.6	0.9~0.4
Spiramycin ⁵⁾	whole egg	2.4	4.7~1.6
	egg white	2.4	3.6~1.8
	liver (layer)	2.4	4.6~1.6
	liver (broiler) ⁶⁾	2.0	—

1) No interval was estimated since the exponential relationship was not confirmed statistically.

2) Literature 3.

3) Literature 2.

4) Literature 6.

5) Literature 1.

6) Literature 4.

As shown in Table 3, biological half lives of CTC in the liver and egg white are almost identical, being 0.5 days in average, while that in whole egg is longer, being 1.6 days. The findings that biological half lives of CTC in whole egg, egg white and in the liver resemble those of tylosin suggest that CTC disappears from the hens' body as fast as tylosin dose, but much faster than spiramycin does. As discussed previously²⁾, antibiotics content in whole egg is important from the viewpoint of pollution of table egg. The biological half life of CTC in whole egg was 1.6 days in average, which is almost comparable to those of oxytetracycline and tylosin, since their confidence intervals overlapped each other.

Summary

A diet containing extremely high level of 8,000 ppm of chlortetracycline (abbreviated CTC) was fed to 35 laying hens for 7 days, and thereafter, the hens were fed the basal diet free from CTC for another 7 days. Five hens each were sacrificed on 0, 1, 2, 3 and 5 days after the termination of CTC feeding to analyze CTC content in the liver micro-biologically. Among the rest of the hens, 5 hens, who laid more eggs continuously for 14 days than the others, were selected and CTC content of all of the eggs laid by them were analyzed.

Content of CTC in egg white and whole egg increased rapidly after feeding CTC and reached plateau on the 2nd and 4th day, respectively. After the withdrawal of dietary CTC, the content of CTC in whole egg, egg white and the liver decreased exponentially.

Combining the data, an equation was induced to describe the behaviour of dietary CTC at 8,000 ppm, which resembles that of tylosin.

Biological half life of CTC in whole egg was calculated to be 1.6 days with 95% confidence interval from 1.7 to 1.5 days, and that in the liver to be 0.5 days from 0.6 to 0.4 days.

Literature

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飼料中のクロルテトラサイクリンの鶏卵中への移行ならびに 鶏卵と肝臓よりの消失について

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クロルテトラサイクリン (以下 CTC) の極端な高濃度 (8,000 ppm) を含む飼料を, 産卵鶏 35 羽に 7 日間給与し, その後, CTC 無添加飼料をさらに 7 日間与えた。CTC 給与中止後 0, 1, 2, 3 および 5 日に, それぞれ 5 羽ずつ殺して肝臓中の CTC 含量を微生物定量法により測定した。残りのうち, 14 日間の産卵数の多い 5 羽を選び, その 14 日間の卵をすべて分析に供した。

卵白および全卵中の CTC 含量は, CTC 給与開始後急速に増加し, それぞれ 2 および 4 日目にほぼ一定値となった。CTC の給与を中止すると, 全卵, 卵白および

肝臓中の CTC 含量は指数曲線的に減少した。

これらのデータを総合することにより, 8,000 ppm の CTC を与える場合の飼料中の CTC の動きを示す式を導くことができた。これは, タイロシンの動きを示す式と似ている。

全卵中の CTC の生物学的半減期を計算すると, 1.6 日で, その 95% 信頼区間は 1.7~1.5 日であった。肝臓中の CTC の半減期は 0.5 日で, 0.6~0.4 日の区間であった。

(家禽会誌, 10, 261~268, 1973)