Decoquinate: Estimation of Residues in Chicken Tissues before and after Cooking

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A method of estimation of decoquinate (ethyl 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate) based on thin-layer chromatography and the measurement of fluorescence of the drug on the plate has been used to study the absorption and elimination of decoquinate in the chicken. The drug is found mainly in the fat, the residues in the flesh being minute after day 0. The product is rapidly excreted and, after 3 days, the residues are less than 0.05 ppm in chickens which have received 40 g per ton of drug in the diet. The distribution of the product is not greatly altered by cooking of the chicken wrapped in aluminum foil at 220° C. Most of the residual decoquinate is found in the juices.

Decoquinate (ethyl 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate) is used as a broiler chicken coccidiostat (Ball *et al.*, 1968; Yvore, 1968). It is necessary to know the amount and distribution of residues in the tissues of chickens after medication with the drug.

Some studies on this subject have already been made using fluorescence measurements in solution (Button *et al.*, 1969) and by means of radiochemically labeled drug (Filer *et al.*, 1969). We now give the results obtained by the direct measurement of fluorescence on a thin-layer plate (Laurent *et al.*, 1971).

The study was carried out for three purposes: to know the rate of decline of decoquinate in different tissues of chicken fed with feed containing various drug levels; to measure the residue levels of decoquinate in the organs of chickens treated with the normal dose of drug; and to discover the influence of cooking on the decoquinate residue levels.

EXPERIMENTAL

Feeding Studies. The chickens were fed at the "Station de Pathologie Aviaire de l'I.N.R.A." at Nouzilly, France. Two factors were varied: the concentration of decoquinate in the feed and the time between withdrawal of medication and slaughter. Groups of 11-day old chicks were fed with feed containing 38, 82, or 385 ppm of drug for 10 weeks. The drug was withdrawn for 0, 1, 2, or 3 days and the birds were fed unmedicated control food before slaughter.

The drug content of the feed used in these experiments was checked by a method (Laurent *et al.*, 1968) similar to that employed for the determination of residues in chicken tissues (Laurent *et al.*, 1970).

The feed analyses are shown in Table I.

Tissue Collection, Cooking. The chickens were stored frozen. The tissues were cut off from the thawed chicken in the following manner. The whole liver and the two kidneys were taken and stripped of most of the fat and weighed. The flesh was taken from the breast and legs, mixed, sampled, and weighed (10 g). The fat was taken from the yellow fatty bulks.

To study the influence of cooking, samples were taken, before cooking, from the breast and leg muscles and the fat of half of a bird. Half of the liver was cut off. The other half of the same bird (including half the liver) was cooked, and samples were then taken from the same muscles, the juice (taken in two separate layers), and liver.

The cooking of the half-bird (with heart, lungs, and stomach) wrapped in aluminum foil was carried out at 215–220° C for 90 min. The wrapping was sealed and no mechanical losses occurred.

The study was carried out on one control bird and eight birds which were medicated with decoquinate at the usual levels.

One must emphasize that this experiment was particularly severe, as the tissues were continually in contact with all of the cooking juices.

Method of Analysis. The method of analysis has been described in detail in another paper (Laurent *et al.*, 1971). It consists of four main stages: maceration and extraction with ethanolic hydrochloric acid; purification by partition between light petroleum and acetonitrile; column chromatography; and tlc. The amount of drug is then evaluated by direct measurement on the tlc plate by comparison of the fluorescence of the decoquinate-magnesium salt complex with that of a range of samples of drug treated in the same manner on the same plate. The nature of the complex is not known, but we refer to it as the decoquinate-magnesium salt complex. Its fluorescence is much greater than that of decoquinate itself (Laurent *et al.*, 1971).

RESULTS AND DISCUSSION

Recovery of the Drug. The percentage recovery has been previously studied by analysis of decoquinate added to tissues of unmediated chickens. The results are given in another paper (Laurent *et al.*, 1970). The mean percentage recovery for each tissue was 89% for muscle, 87% for liver, 78% for kidney, and 80% for fat.

To confirm that the analytical method was satisfactory, decoquinate was added to the two main tissues, flesh and liver, of a number of birds which had received decoquinate medication. It did not seem useful to again check the validity of the method for the fat. The weight of the kidneys was too small to perform two analyses. The results are given in Table II.

Sampling of Muscle. Some measurements were carried out to study the influence of the origin of the muscle (breast or leg). Twelve 10-g samples were taken from a treated chicken—six from breast muscle and six from leg muscle. The results are shown in Table III. There is no significant difference between breast and leg muscle. At the level of 0.2 ppm the range of these results is less than ± 0.03 ppm. The maximum relative error of the analytical method is therefore ±15%.

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Table I. Decoquinate in Feeds

Decoquinate in ppm	
Level added	Level found
38	41
82	82
385	344

Table II. Analyses of Muscle and Liver Fortified with 1 ppm

Initial concentration	Concentration found after fortifying	Difference	% Recovery
	Muscle		
<0.05	0.74	0.74	74
<0.05	1.09	1.09	109
<0.05	0.76	0.76	76
<0.05	0.70	0.70	70
0.07	0.94	0.87	87
0.33	1.17	0.84	84
	Liver		
<0.05	0.96	0.96	96
<0.05	0.87	0.87	87
<0.05	0.95	0.95	95
0.08	0.92	0.84	84
0.09	0.89	0.80	80
0.82	1.96	1.14	114

Mean Recovery: 83% (muscle), 93% (liver); Range: 70–114%.

Table III. Influence of the Origin of Flesh (Results in ppm)

Experiment No.	Breast Muscle	Leg Muscle
1	0.19	0.15
2	0.22	0.15
3	0.21	0.18
4	0.17	0.20
5	0.18	0.18
6	0.19	0.17
Range	0.17–0.22	0.15–0.20
Mean	0.19	0.17

Residue Studies. The results found for the different tissues of chicken fed with feed containing different levels of medication are summarized in Table IV. The influence of the length of time between the withdrawal of the medication and slaughter (0 to 3 days) is also summarized.

The analyses of muscle and liver of birds fed with feed medicated at the 38 ppm level were repeated four times.

At the start (day 0), the decoquinate is found mainly in the fat and liver. In all the tissues the drug disappears rapidly from the birds which received 38 or 82 ppm drug in feed, and within 3 days the residues fall below 0.1 ppm (except in the fat and in the kidneys of the birds which have received feed containing about 10 times the normal level of drug).

Table IV. Distribution and Decline of Decoquinate in Tissues

Level in feed	Withdrawal period (in days)	Residues of decoquinate (ppm)									
		Muscle				Liver				Kidney	Fat
0 control		<0.05				<0.05				<0.05	<0.05
38	0	0.09	0.05	0.10	0.09	0.26	0.10	0.38	0.14	0.19	1.05
	1	<0.05	<0.05	<0.05	<0.05	0.16	<0.05	0.05	<0.05	0.15	0.19
	2	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.06	<0.05	<0.05	<0.05
	3	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.09	<0.05
82	0	0.05				1.3				0.64	2.2
	1	0.07				0.05				0.39	0.5
	2	<0.05				0.08				<0.05	0.11
	3	<0.05				0.08				0.06	0.07
385	0	<0.05				1.4				0.6	2.4
	1	0.05				0.81				0.6	1.3
	2	0.26				0.47				2.1	[a]
	3	<0.05				<0.05				0.6	2.4

[a] No fat could be removed from this bird. The absence of fat perhaps explains the rather high results found in the muscle and kidney.

Table V. Effect of Cooking

Withdrawal period (days)	Weight of the chicken (g)	Residues of decoquinate (ppm)									
		Muscle				Liver		Raw fat	Juices from cooking		
		Breast		Leg					Weight in g	Upper layer (fat)	Lower layer (aqueous)
		Raw	Cooked	Raw	Cooked	Raw	Cooked				
Control	1000	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	71	<0.05	<0.05
0	1100	0.12	0.10	0.09	0.09	0.21	0.31	0.94	123	1.7	0.09
1	1030	<0.05	0.22	<0.05	0.23	<0.05	0.05	1.10	77	1.09	0.21
2	1190	<0.05	≤0.05	<0.05	≤0.05	0.05	0.18	0.95	30	0.76	0.24
3	880	0.05	0.17	<0.05	0.18	0.17	0.19	0.83	34	0.97	0.36
0	975	0.23	0.08	0.11	0.08	0.36	0.24	0.54	70	0.87	0.07
1	1100	<0.05	<0.05	<0.05	<0.05	0.12	0.23	0.36	83	0.46	0.10
2	1150	≤0.05	0.22	0.06	0.26	0.29	0.11	0.40	75	0.24	0.10
3	1000	0.07	0.07	0.07	0.07	≤0.05	≤0.05	0.24	19	0.36	0.11

In muscle, the residues are negligible even at day 0 at the highest medication level. The differences between the residue levels at 38 and at 385 mg per kg doses are small. It seems as though there is a threshold value for the accumulation of residues in the tissues.

The reproducibility on muscle and liver at 38 ppm level may be considered as good. Some variations are observed in liver at day 0; it seems that these may be explained by differences of a few hours in the withdrawal period and slightly different rates of elimination of decoquinate from one bird to another.

It is therefore sufficient to withdraw medication of decoquinate 2 days before slaughter. To conform with the usual practice and to allow a safety factor, a period of 3 days might perhaps be suggested.

The results showing the influence of cooking on residues are summarized in Table V, together with the weights of the birds and the juices.

A comparison of the levels found on the same tissue before and after cooking enables one to draw the following conclusions, taking into account the fact that a transfer of fat and loss of water has occurred in the organs and tissues.

There is no significant difference between breast and leg muscle. In both cases the changes due to cooking are small, and neither consistently increases nor decreases the residues. Liver behaves in a similar manner to muscle. The residue in the upper layer of the cooking juices (the fatty part) is approximately that of the initial value for the fat, and none of the drug has been destroyed. The different weights of the fatty and aqueous layers were not determined because of the difficulty of separating them completely.

It appears that cooking (under the conditions we employed) has little effect on the residues. The drug which is mainly present initially in the fat ends up in the juices. The small variations produced by cooking the flesh and the liver may be attributed to the decoquinate which is carried in the fat.

To sum up, at the normal use level the residues of decoquinate are very low, even if medication is continued until slaughter. The product is eliminated rapidly and after 2 or 3 days it cannot be detected in the several tissues examined. Under these conditions, the use of decoquinate as a coccidiostat does not present any hazard to the consumer if the drug is withdrawn 3 days before slaughter.

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