Effect of Cooking on Veterinary Drug Residues in Food. 6. Lasalocid

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The effect of heat on lasalocid in model solutions and as residues in food was investigated. The drug was found to be relatively stable to heat in neutral and acid conditions but unstable in basic conditions as was demonstrated by stability experiments at 100 °C in simple aqueous buffers. Lasalocid was found stable at pH 5.5 and 7.0 but unstable at pH 10.0 with a half-life of 30 min. In sunflower cooking oil at 180 °C lasalocid was not stable with a half-life of 15 min. In food, lasalocid residues in tissue from treated chickens were stable to cooking in chicken muscle but unstable when egg was cooked. A loss of 59% was observed for the preparation of an omelet, and a 27% loss was observed for the preparation of scrambled egg. Only a small amount (<5%) of lasalocid came out of tissue with exuded juices or into the liquid used for cooking. Residues in raw tissue from treated chickens were found to be evenly distributed in edible muscle.

Keywords: *Lasalocid; coccidiostat; cooking; stability; veterinary drug residues*

INTRODUCTION

Most information about drug residues in food (meat and animal products) is related to concentrations in raw tissue. Since most of this type of food is cooked before consumption, more information about the effect of cooking on residues is required to give a more accurate estimate of consumer exposure to these chemicals and any breakdown products.

Lasalocid A, 6-[(7*R*)-[(5*S*)-ethyl-5-(5*R*)-ethyltetrahydro-5-hydroxy-(6*S*)-methyl-2*H*-pyran-(2*R*)-yl)tetrahydro-(3*S*)-methyl-(2*S*)-furanyl]-(4*S*)-hydroxy-(3*R*),(5*S*)-dimethyl-6-oxononyl]-2-hydroxy-3-methylbenzoic acid (Figure 1), is produced by *Streptomyces lasaliensis* and was among the first three polyether antibiotics isolated by Berger *et al.* in 1951 (Berger *et al.*, 1951). Lasalocid is an ionophore and will form complexes with monovalent and divalent cations forming lipid soluble structures which aid membrane transport and passive diffusion of cations (Galitzer and Oehme, 1984). Although lasalocid is active against some Gram-positive bacteria, the major uses have been as a coccidiostat in poultry (Avatec, Roche) since 1977 and as a growth promoter for cattle (Bovatec, Roche) since 1982 (Novilla, 1982). Effective control of coccidiosis is achieved in broilers by the addition of 75−125 mg of lasalocid/kg of feed with a withdrawal period of 5 days before slaughter (Gu, 1983). It is not approved for laying hens, although recent surveillance has shown the incidence of residues of this compound in some chicken and egg samples (Kennedy *et al.*, 1996).

Polyether antibiotics were not used as veterinary medicines until 16 years after their discovery due to their high toxicity (Mallams, 1978). Toxic effects have been observed in some animals following use of these compounds. The acute oral toxicity of lasalocid is measured as an LD_{50} for poultry of 40 mg/kg of body weight and 100 mg/kg of body weight for cattle (Galitzer and Oehme, 1984). Ionophores are particularly toxic to horses with an LD_{50} estimated at 21.5 mg/kg of body weight (Hanson *et al.*).

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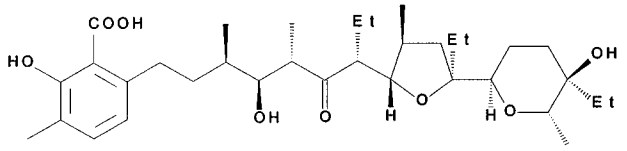

Figure 1. Structure of lasalocid.

Because of the incidence of residues of lasalocid in the food supply, the paucity of data about the stability of this compound to heat, and the concern about its toxicity, this compound was viewed as an important target for an investigation into the effects of cooking. Data generated could be used to give better consumer intake estimates and dietry exposure calculations.

MATERIALS AND METHODS

Materials. All solid chemicals were analytical grade, and HPLC grade solvents were used throughout. Water was obtained from an in-house Elga water purification system. Lasalocid standard was obtained from Sigma (Poole, U.K.).

Samples. Chicken and egg samples from a supply known to have been fed on a diet containing lasalocid were obtained from the Department of Agriculture, Northern Ireland (DANI).

Temperature Measurements for Cooking Experiments. The temperatures of each cooking procedure were monitored using a fiber thermometer inserted into the interior of the food sample. This consisted of an optical fiber with a GaAs crystal temperature sensor at one end. Light from the instrument illuminated the crystal, which re-emits light at a wavelength dependant on temperature. The model used was suitable for temperature measurement between 0 and 200 °C with an accuracy of 0.1 °C.

Procedure. A single piece of tissue was used for each cooking investigation. Replicate analysis of raw and cooked tissue from the same sample was carried out in a single analytical batch. Each batch contained one blank sample and one fortified at a known concentration to provide a measure of analytical recovery.

Distribution of Lasalocid within the Tissue. Several different birds were used for this study. They were all fed on the same diet, but it is not possible to assess data obtained from raw tissue samples originating from different chickens for homogeneity since these variations may arise due to metabolic or other differences between individuals. The homogeneity of lasalocid residues was assessed for one raw chicken sample where six replicates were analyzed.

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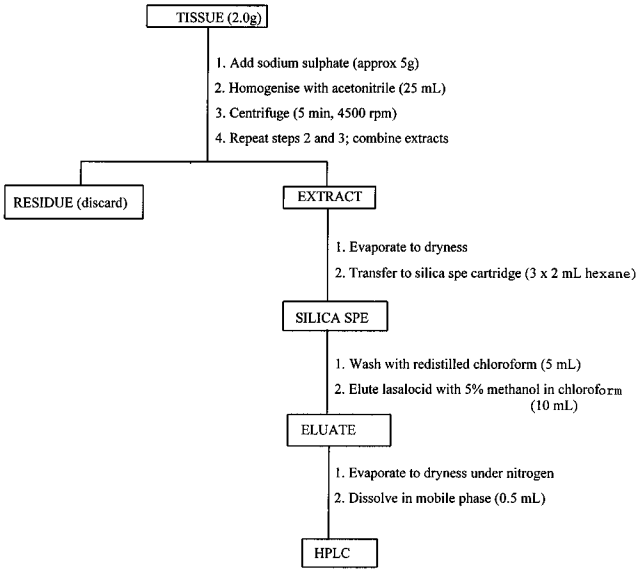

Figure 2. Schematic of the procedure for the determination of residues of lasalocid.

Table 1. Validation Data: Recovery in Blank Tissue Fortified at 40 μg/kg

tissue	*n*	mean (%)	CV (%)
raw chicken	6	88	17
raw egg	6	59	19
cooked chicken	5	49	20
cooked egg	6	49	6.1

Lasalocid Stability to Cooking. Chicken was cooked by microwaving, boiling, roasting, frying, and grilling methods. Egg was cooked by frying (omelet) and scrambling. Standard kitchen utensils and equipment were used in the cooking experiments. The recipes used were as follows.

Chicken Muscle by Microwaving. Chicken (266 g) was placed in a covered Pyrex dish and cooked for 7 min on medium power (700 W microwave). The product was allowed to stand for 2 min.

Chicken Muscle by Boiling. Meat was cut from the bone and cut into pieces. About 100 g was added to boiling water and cooked for 5 min.

Chicken Muscle by Roasting. A piece of chicken (283 g) was placed in a Pyrex dish and roasted for 30 min at 150 °C in a fan-assisted oven (equivalent to 190 °C in a conventional oven).

Chicken Muscle by Frying. The chicken (51 g) was removed from the bone and cut into small pieces which were formed into a pattie. This was fried in a little sunflower oil, with frequent turning.

Chicken Muscle by Grilling. A pattie (64 g) was formed as described above. This was grilled for 10 min, turning once halfway through the cooking process.

Egg by Frying (Omelet). Homogenized egg (51 g) was beaten with 15 mL of water. About 10 g of butter was heated in a small frying pan, and the mixture was poured in. The mixture was stirred, and the edges were lifted to ensure even cooking. Cooking time was 4 min at a moderate heat setting.

Egg by Scrambling. Homogenized egg (55 g) was beaten with 45 mL of milk. About 10 g of butter was heated in a saucepan, and the egg mixture was added. The mixture was stirred over medium heat for 4 min until the mixture had set into a creamy consistency.

There was normally a weight loss associated with evaporation during cooking, the only exception to this being the preparation of scrambled egg where additional ingredients resulted in a net gain. These changes were considered in calculating any overall change in the amount of lasalocid due to cooking, and data are included in Table 3.

Analytical Method. A solid phase extraction−high-performance liquid chromatography (HPLC) method of analysis was used to determine the lasalocid concentration of samples. This method has previously been reported by this laboratory (Tarbin and Shearer, 1992). A schematic of the procedure is shown in Figure 2. The tissue or juices from cooking were extracted with acetonitrile. The organic layer was evaporated, and the residue was applied to a silica gel solid phase extraction column. The lasalocid was then eluted with 5% methanol in chloroform solution which was subsequently evaporated under nitrogen. The residue was dissolved in mobile phase (0.01 M borax buffer, pH 10.0:acetonitrile, 40:60) and analyzed by reversed-phase (polymer column) HPLC with fluorescence detection, excitation wavelength 310 nm, emission 420 nm.

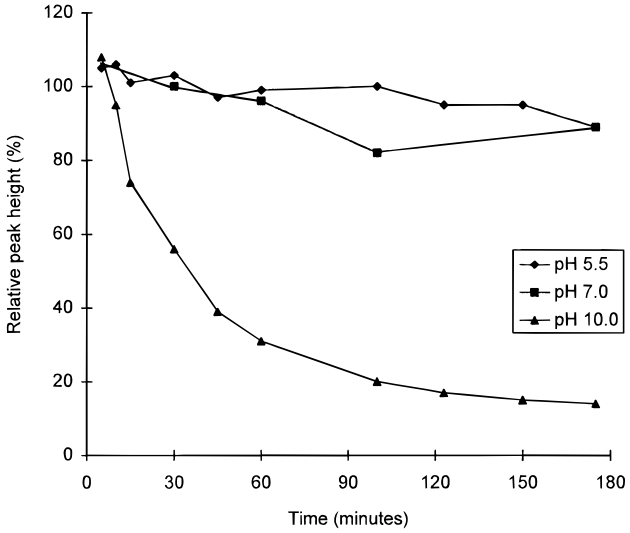

Figure 3. Stability of lasalocid against time at 100 °C in buffers of pH 5.5, 7, and 10.

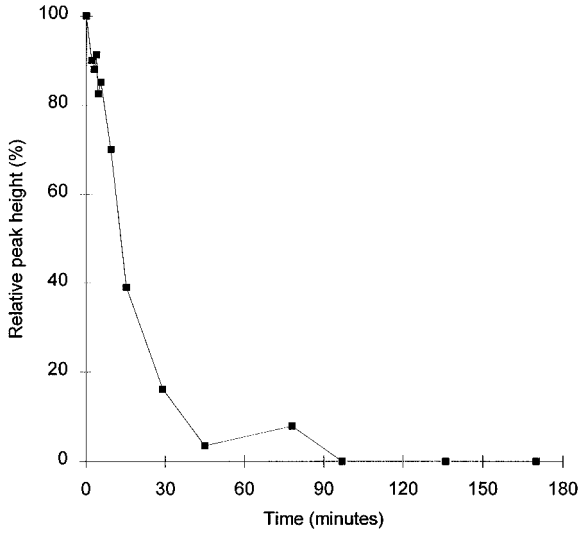

Figure 4. Stability of lasalocid in cooking oil at 180 °C.

Table 2. Homogeneity Data

tissue	recovery (%)	*n*	mean (μg/kg)	CV (%)
chicken	41	6	110	19
egg	59	5	18700	6.4

Lasalocid Stability. *In Hot Water.* A 1 mL aliquot of 20 μg/mL aqueous lasalocid standard solution was diluted to 50 mL with water. The solution was used to fill 20 sealed vials which were placed in a thermostatic oven. Single vials were removed at intervals over a period of 3 h. The experiment was repeated at temperatures of 60, 80, and 100 °C. The experiment also was conducted at 100 °C for phosphate buffers of pH 5.5 and 7.0 and borax buffer, pH 10.0. Temperature was monitored using a mercury in glass thermometer. HPLC analysis was performed directly on the aqueous samples.

Table 3. Results of the Effect of Cooking on Residues of Lasalocid in Food (μg/kg)

	chicken muscle					egg	
sample no.	microwaving	boiling	roasting	frying	grilling	omelet	scrambled
recovery (%)	41	51	71	71	50	59	59
raw							
1	94	77	60	39	88	16740	20140
2	136	77	53	52	69	18130	19650
mean (μg/kg)	115	77	57	45	79	17435	19895
cooked							
1	153	77	81	87	103	6672	11290
2	180	84	78	68	100	7761	12260
3	145	78	69	65	104	9822	15130
4	132	79	64	69	108	7310	13700
5	146	81	52	61	97	7271	12660
6	125	106		71			12940
mean (μg/kg)	147	84	69	70	103	7767	12997
CV (%)	11.9	12.0	15.4	11.7	3.6	14	9.2
fluid							
1	7.5	<LOD[a]	110				
mass (g)							
raw	266	68	283	51	64	51	55
cooked	173	49	210	31	39	49	62
fluid	51	340	6.1				
total lasalocid (μg)							
raw	30.5	5.2	16.0	2.3	5.0	883	1088
cooked	25.4	4.1	14.4	2.2	4.0	381	799
fluid	0.4	< 4.2	0.7				
net change (%)	−15	$-21 \le x \le +59$[b]	−5.6	−4.3	−20	−59	−27

[a] LOD: limit of determination. [b] The net change lies within the range shown depending if the concentration of lasalocid in the water is assumed to be 0 or at the LOD.

In Hot Cooking Oil. Lasalocid (2 mL, 200 μg/mL in MeOH) was added to a small portion of sunflower cooking oil which was diluted to 200 g with oil at 180 °C. Samples of cooking oil were removed at intervals over a period of 3 h and cooled before subsamples of 1.0 g were taken for analysis using the standard method described above.

RESULTS AND DISCUSSION

All results quoted were corrected for analytical method recovery. The method used was validated with raw and cooked tissue by analyzing batches containing both blank samples and samples fortified with a known concentration of lasalocid prior to extraction. This method has been widely applied in our laboratory, and further more extensive validation data were obtained previously. The validation data obtained associated specifically with this investigation are shown in Table 1.

There is no reference in the literature to the effect of cooking and heat stabilty of lasalocid or related drugs as residues in food. Lasalocid was found in this investigation to be stable in water at all temperatures measured up to 100 °C over a period of 3 h. A plot of lasalocid concentration in buffer solutions of pH 5.5, 7.0, and 10.0 at 100 °C against time is shown in Figure 3. Over a period of 3 h lasalocid was stable at pH 5.5 and 7.0. At pH 10.0 lasalocid was not stable with a half-life of about 30 min. In sunflower cooking oil at 180 °C the half-life was 15 min (Figure 4).

The concentration of lasalocid was determined in each of the chicken and egg samples used for experimental work. Since samples originated from different birds and different eggs, it is not appropriate to collate these data, but the homogeneity data collected for replicate analyses of the same raw samples of chicken and homogenized egg are collated in Table 2. These show that the residues in egg and chicken were distributed evenly, *i.e.*, the experimental data were within the analytical precision of the method. The precision observed for replicate analysis of cooked tissue with incurred residues of lasalocid supports this conclusion.

The results of the cooking experiments are shown in Table 3. The net change in concentration was calculated by comparing the total amount of lasalocid before and after cooking. Apparent increases in concentration of lasalocid in cooked tissue are accounted for by weight loss during the cooking process. Changes in the range of −20% to +20% were taken to reflect the analytical precision of the method. Cooking did not therefore affect the amount of lasalocid in chicken muscle. Less than 5% of the residue was found in juices which came from the meat when it was cooked by microwave and roasting. A small peak was seen in the water used for boiling, but the concentration was too small to accurately quantify due to the dilution effect in the relatively large amount of fluid present in this experiment. The net change in the amount of lasalocid was calculated assuming the concentration in water was 0 and at the limit of determination (LOD) yielding a range between a 21% loss and a 59% increase in lasalocid. Cooking decreased the amount of residue measured in egg by 27% for the preparation of omelet and 59% for scrambled egg. Neither of the cooking methods used for egg resulted in fluid available for analysis.

Chicken muscle is known to be about pH 5.7 and egg yolk about pH 6.0−6.8. Egg white can be as high as pH 9.0. The loss of lasalocid in egg may therefore be due to base-catalyzed hydrolysis of lasalocid, possibly a reverse of an aldol type condensation reaction at the 1,3-hydroxy−ketone function within the molecule. This supports the observation that the amount of lasalocid measured in egg samples containing residues can decrease between analyses if the egg is allowed to stand at temperatures above freezing for periods of about 1 day or more between replicate analyses. It also is consistent with the stability experiments carried out in simple buffers at 100 °C where lasalocid was found to

be stable at neutral and low pH but unstable at pH 10.0 with a half-life of 30 min.

A comparison with drugs studied previously showed that the amount of lasalocid which came from the tissue with juices which exuded during cooking was considerably less than observed for levamisole (Rose *et al.*, 1995a), sulphadimidine (Rose *et al.*, 1995b), oxytetracycline (Rose *et al.*, 1996), ivermectin (unpublished), and oxfendazole (Rose *et al.*, 1997) but more than for clenbuterol (Rose *et al.*, 1995c) where none was detected. This amounted to less than 5% of the total residue.

CONCLUSIONS

Lasalocid is stable to heat in simple aqueous solutions except at high pH. In cooking oil at 180 °C it is not stable with a half-life of 15 min. Residues of lasalocid in food are stable to heat in neutral and acidic conditions but unstable to heat in high-pH foods such as egg. A small amount of leaching from solid tissue into the surrounding fluid or with juices which exuded from the food as it was cooked was observed. Incurred residues of lasalocid were evenly distributed within the tissue.

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