



**Investigation of the causes for occurrence of residues of the anticoccidial feed additive nicarbazin in commercial poultry**

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Complete List of Authors:	O'Keeffe, Michael; Ashtown Food Research Centre, Teagasc, Food Safety Department Capurro, Emiliana; Ashtown Food Research Centre, Teagasc Danaher, Martin; Ashtown Food Research Centre, Teagasc Campbell, Katrina; Queens University Belfast Elliott, Chris; Queens University Belfast
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1 **Investigation of the causes for occurrence of residues of the anticoccidial feed additive nicarbazin in commercial poultry**

4 Michael O’Keeffe<sup>1\*</sup>, Emiliana Capurro<sup>1</sup>, Martin Danaher<sup>1</sup>, Katrina Campbell<sup>2</sup> and  
5 Christopher T. Elliott<sup>2</sup>

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7 <sup>1</sup> Food Safety Department, Ashtown Food Research Centre, Teagasc, Dunsinea, Ashtown,  
8 Dublin 15, Ireland

9 <sup>2</sup> Institute of Agri-Food & Land Use, Queen’s University Belfast, Stranmillis Road,  
10 Belfast BT9 5AG, Northern Ireland

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13 *Investigations were undertaken to identify causes for the occurrence of high levels of the*  
14 *zootechnical feed additive nicarbazin in broiler liver at slaughter. The first investigation*  
15 *on 32 commercial broiler flocks involved sampling and analysis for nicarbazin (as*  
16 *dinitrocarbanilide, DNC) in liver from birds during a 3-10 day period after withdrawal*  
17 *of nicarbazin from their feed and prior to commercial slaughter. DNC residues in liver*  
18 *samples of broilers scheduled as being withdrawn from nicarbazin for  $\geq 6$  days ranged*  
19 *from 20 to  $> 1600 \mu\text{g}/\text{kg}$  (the specified withdrawal period for nicarbazin is 5 days and*  
20 *the JECFA MRL is  $200 \mu\text{g}/\text{kg}$  liver). Further on-farm investigations on 12 of these flocks,*  
21 *selected on the basis of the feeding system in use and the levels of DNC residues*  
22 *determined in liver, identified issues in feed management contributing to elevated*  
23 *residues in broiler liver. A significant correlation ( $0.81, p < 0.01, n = 10$ ) between DNC*  
24 *residues in liver samples and in feed samples from the feeding pans was observed. The*  
25 *second investigation on 12 commercial broiler flocks involved sampling and analysis for*  
26 *DNC in liver samples and feed samples from feeding pans and from the feed mill at the*  
27 *three thinnings of birds for commercial slaughter. In the case of one flock, a clear*  
28 *relationship between nicarbazin in feed from the feed mill ( $10.5 \text{ mg}/\text{kg}$  DNC), in feed*  
29 *from the feeding pans ( $6.6 \text{ mg}/\text{kg}$  DNC) and in liver ( $583 \mu\text{g}/\text{kg}$  DNC) at first thinning (9*  
30 *days scheduled withdrawal from nicarbazin) was observed. Such a clear relationship was*  
31 *not observed in other cases, particularly at second and third thinnings, pointing to re-*  
32 *exposure of birds to nicarbazin late in the flock production cycle, probably from the*  
33 *litter. Guidelines outlining best farm practice to eliminate nicarbazin residues in poultry*  
34 *have been published in booklet and poster format for broiler producers and deal with*  
35 *feed system cleaning, feed bin management, feed deliveries, feed usage and records.*

36  
37 **Keywords:** nicarbazin, poultry, commercial production, residues, feed, liver

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<sup>\*</sup> To whom correspondence should be addressed. michael.okeeffe@teagasc.ie

## 41 Introduction

42  
43 The anticoccidial drug, nicarbazin, is widely used as a zootechnical feed additive in the  
44 earlier stages of poultry (broiler) production to prevent coccidiosis caused by species of  
45 *Eimeria*. The commercial product, Maxiban®, is a premix product marketed by the  
46 company Elanco and contains equal quantities of nicarbazin and the ionophore narasin  
47 which act synergistically to prevent coccidiosis. Typically, Maxiban® is incorporated  
48 into starter and grower poultry feeds and is provided to birds up to 3-4 weeks of age. The  
49 feed additive product is authorised for use at a dosage of 40-50 mg/kg each of nicarbazin  
50 and narasin, with a withdrawal period of 5 days (European Commission 2004).  
51 Thereafter, the nicarbazin-medicated feed is replaced with a feed containing an  
52 ionophore, such as monensin, and subsequently the birds are fed with finisher feed,  
53 containing no anticoccidial feed additives, for a period of some days prior to first thinning  
54 at approximately 5 weeks of age (O’Keeffe 2003). The process of “thinning” refers to the  
55 removal of a portion of the flock for slaughter; this practice provides birds of a particular  
56 weight category for the market and provides additional space for the remaining, larger  
57 birds. The commercial slaughtering of a flock may comprise of three thinnings, over a  
58 period of approximately 2 weeks; no anticoccidial treatment should occur during the  
59 thinnings process.

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61 Nicarbazin is an equimolar mixture of 4,6-dimethyl-2-hydroxypyrimidine (DHP) and  
62 4,4'-dinitrocarbanilide (DNC). The DHP component is excreted rapidly following  
63 withdrawal of the drug, but DNC is less rapidly eliminated (Porter and Gilfillan 1955).  
64 The FAO/WHO Joint Expert Committee on Food Additives (JECFA) has established  
65 DNC as the marker residue for nicarbazin and has set a maximum residue limit (MRL)  
66 for DNC of 200 µg/kg in edible tissues, including muscle and liver (Wells 1999). No  
67 MRL has been set for nicarbazin within the European Union, and the JECFA MRL is  
68 commonly used as a guideline by the UK and Irish authorities.

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70 The monitoring programmes in the UK and Ireland have identified the occurrence of  
71 DNC in poultry liver at levels in excess of the JECFA MRL at an incidence of 17% to 9%

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3 72 of samples tested (Veterinary Residues Committee 2001 to 2005) and of 19% to 7% of  
4 samples tested (O’Keeffe and Coen 2005, O’Keeffe *et al.* 2006), respectively. Similarity  
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7 74 in the incidence of MRL exceedances determined in the monitoring programmes in the  
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9 75 UK and in Ireland suggest similar usage patterns for nicarbazin by the poultry industries  
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11 76 in both jurisdictions. Although there have been decreases in the incidence of non-  
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13 77 compliant poultry liver samples in recent years, there is still a substantial problem. While  
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15 78 residues of nicarbazin in muscle (poultry meat), as distinct from liver, tend to be of the  
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17 79 order of 10-20 times lower, thereby reducing the food safety issue, the occurrence of  
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19 80 residues in tissues have given rise to consumer concerns (Young and Craig 2001) and a  
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21 81 negative image for the broiler industry. Previous research has identified contamination of  
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23 82 ostensibly nicarbazin-free feed during feed manufacturing as a potential source for  
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25 83 residues in eggs and in broiler liver (Dorn *et al.* 1988, Cannavan *et al.* 2000, Cannavan  
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27 84 and Kennedy 2000). In particular, McEvoy *et al.* (2003) identified practices in feed  
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29 85 manufacturing related to recycling of fines and of surplus material from the pelleting  
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31 86 process as factors contributing to nicarbazin contamination of withdrawal feed. Another  
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33 87 important source for nicarbazin residues in broiler tissues is faeces recycling from litter;  
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35 88 Castelli *et al.* (1989) determined the main source of nicarbazin contamination resulting in  
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37 89 residues in the livers of broilers, four weeks after withdrawal of medicated feed, to be the  
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39 90 litter on which they were housed. Penz *et al.* (1999) demonstrated that DNC was  
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41 91 detectable in the liver of birds at slaughter from the last of three crops of birds reared on  
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43 92 the same litter, where nicarbazin had only been given to the first crop of birds. Cannavan  
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45 93 and Kennedy (2000) found that where broilers were fed with nicarbazin according to the  
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47 94 product licence conditions, DNC residues in liver exceeding the JECFA MRL of 200  
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49 95 µg/kg were found in broilers housed on deep litter but not in broilers housed on wire  
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51 96 flooring, pointing to faecal recycling from litter as the probable cause.  
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55 98 Therefore, the practices applied in feed manufacturing, feed delivery, feed storage and  
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57 99 use and poultry housing are all potential contributors to the problem of nicarbazin  
60 100 residues in broilers in commercial poultry production. A comprehensive study was  
101 developed to address this problem on the island of Ireland. Much of the previous research  
102 in this area had been undertaken on research flocks in highly-designed and controlled

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3 103 experiments. In contrast, the research reported here was undertaken with commercial  
4 104 flocks, representing the conditions applying in normal commercial production of broilers.  
5 105 As part of this research, studies with commercial poultry flocks were designed to  
6 106 investigate poultry production practices, particularly feed management, that might be  
7 107 contributing to the high incidence of DNC-positive liver samples at slaughter. These  
8 108 studies encompassed (a) pre-commercial sampling of poultry from commercial flocks  
9 109 together with on-farm visits to some of these flocks to investigate feeding practices and  
10 110 (b) sampling of poultry from selected flocks at each of the commercial thinnings to track  
11 111 the occurrence of nicarbazin residues in poultry at the later stages of the flock production  
12 112 cycle. The aim of the research was to develop knowledge that would be used to produce  
13 113 practical guidelines for poultry producers to assist them in reducing or eliminating the  
14 114 incidence of nicarbazin residues at levels above the JECFA MRL in broiler livers.  
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## 28 117 **Experimental**

### 29 118 30 119 *Sampling at pre-commercial slaughter*

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32 120 A major poultry company was used for this study. In conjunction with routine testing for  
33 121 *Salmonella*, representative samples of poultry (livers of five birds) and of feed were  
34 122 obtained from 32 commercial flocks. Each feed sample consisted of a composite of  
35 123 individual feed samples taken from 10 feeding pans in the house at the same time as the  
36 124 poultry livers were sampled. The flocks were selected to be representative of different  
37 125 feeding systems (single bin, split bin, double bin) and to be at that stage of the production  
38 126 cycle where nicarbazin-medicated feed had been withdrawn. The samples of liver and  
39 127 feed were analysed for DNC content by high performance liquid chromatography  
40 128 (HPLC). Investigative visits to 12 selected flocks were made within one week of testing  
41 129 the liver and feed samples. The selection of flocks for further investigation was based on  
42 130 the results of the liver sample analyses (including both low and high levels of DNC) and  
43 131 the type of feeding system(s) in use on the farms. During these visits aspects of the  
44 132 poultry production system were studied and recorded, including, in particular, the feeding  
45 133 system used, feed delivery records and feed management.  
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**135 *Sampling at commercial thinning***

136 A second major poultry company was used for this study. 12 flocks, representing single  
137 bin (2 flocks), split bin (6 flocks) and double bin (4 flocks) feeding systems, were  
138 investigated. Samples of liver (livers from five birds) were obtained at commercial  
139 slaughter on three occasions, first thinning (approximately 35 days of age), second  
140 thinning (approximately 42 days of age) and third thinning (approximately 49 days of  
141 age). According to the coccidiosis treatment regime in use by the company, these  
142 slaughter times would represent 7, 14 and 21 days (approximately) following withdrawal  
143 of nicarbazin-containing feed from the birds. On each occasion that the birds were  
144 removed at the three thinnings, a sample of feed was collected from the feeding pans (a  
145 composite feed sample from 10 feed pans). In addition, the feed samples retained by the  
146 company's feed mill for all finisher and withdrawal feeds supplied by the company to the  
147 flocks under study were made available for analysis. All samples of liver and of feed  
148 from feeding pans and from the feed mill were analysed for DNC content by HPLC.

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**150 *Analysis of liver and feed samples*****151 *Reagents and chemicals***

152 DNC standard material was from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and  
153 methanol (both HiPerSolv grade), dimethylsulphoxide and n-hexane (Analar grade) were  
154 obtained from BDH (Merck, Poole, Dorset, UK). Bond Elut™ cartridges (C<sub>18</sub>, 500 mg, 3  
155 ml) were from Varian (Harbor City, CA, USA). The primary standard stock solution (1  
156 mg/ml) was prepared in dimethylsulphoxide. Secondary standard stock solutions (100  
157 and 10 µg/ml were prepared by dilution of the primary standard stock in methanol.  
158 Calibration standards (50-2000 ng/ml), used to quantify the DNC content in liver and  
159 feed samples, were prepared by diluting the 10 µg/ml solution in acetonitrile/water  
160 (80/20, v/v). The primary stock standard solution was prepared every 3 months and was  
161 stored in a glass test-tube covered in aluminium foil at room temperature in the dark;  
162 storage at room temperature prevented solidification of the stock solution. Secondary  
163 standard stock solutions (in methanol) and calibration standards for HPLC (in  
164 acetonitrile/water) were prepared monthly and stored at 4°C.

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5 166 *HPLC conditions*

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7 167 The HPLC system consisted of a model 600E HPLC pump with a model 717 autosampler  
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9 168 and model 484 UV detector (set at 350 nm), all from Waters (Milford, MA, USA). The  
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11 169 separation was carried out on a stainless-steel analytical column (250 mm × 4.6 mm i.d.)  
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13 170 equipped with a Securiguard™ pre-column, both packed with Hypersil BDS C<sub>18</sub> material  
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15 171 (Phenomenex, Cheshire, UK). The column temperature was maintained at 40°C. The  
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17 172 mobile phase, consisting of water/acetonitrile (55/45, v/v), was pumped at 1 ml/min.  
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19 173 Under these conditions, the retention time for DNC was approximately 13.5 min. A  
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21 174 Waters 746 data processing module was used for recording and processing  
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23 175 chromatograms. The injection volume for standards and sample extracts was 25 µl.

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25 17626 177 *Liver analyses*

27 178 Composite samples of liver (comprising the livers from 5 individual birds) were chopped  
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29 179 and mixed thoroughly and a representative subsample taken for analysis. Liver samples  
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31 180 were analysed for DNC content according to the method described by Capurro et al  
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33 181 (2005). This method involves extraction of a 2 g liver sample in a 30 ml polypropylene  
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35 182 tube by homogenising the sample with 10 ml acetonitrile, using a Polytron™  
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37 183 homogeniser, vortexing (2 min), sonicating (3 min) and shaking (15 min) before  
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39 184 centrifuging (2500 rpm, 10 min, 4°C) and transferring the supernatant to a clean  
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41 185 polypropylene tube. The sample was re-extracted with 5 ml acetonitrile plus 1 ml water  
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43 186 and the supernatants combined and defatted using n-hexane (2 × 10 ml). The acetonitrile  
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45 187 layer was evaporated to dryness and reconstituted in acetonitrile/water (70/30, v/v, 500  
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47 188 µl). Sample extracts were purified by passing through C<sub>18</sub> SPE cartridges and eluting with  
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49 189 2.5 ml acetonitrile/water (70/30, v/v). The purified extracts were evaporated to dryness  
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51 190 under nitrogen (60°C) and reconstituted in acetonitrile/water (80/20, v/v, 500 µl) for  
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53 191 HPLC analysis. Representative chromatograms for liver samples are shown in Figure 1.

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57 193 Performance of the method during sample testing was evaluated by fortifying control  
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59 194 liver samples, in duplicate, with DNC at 25 and 250 µg/kg. These quality control samples  
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195 were included with each batch of test samples; mean recovery of added DNC was

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3 196 typically 77 – 114% for both fortification levels. The limit of determination of the method  
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5 197 was 12.5 µg/kg, based on the lowest standard in the calibration curve.  
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9 199 *Feed analyses*

10 200 Feed samples were mixed thoroughly and representative 20 g subsamples were ground in  
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12 201 a swinging hammer mill (Glen Creston) through a 1 mm sieve. Following further manual  
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14 202 mixing of the ground feed sample, 2.5 g was weighed into a 50 ml screw-cap  
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16 203 polypropylene tube. Feed samples were analysed for DNC content according to the  
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18 204 method described by De Jong et al (2004). Water (5 ml) was added to the sample which  
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20 205 was mixed and allowed to stand for 10 min, ensuring that the total sample was wetted.  
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22 206 Methanol/acetonitrile (50/50, v/v, 35 ml) was added to the sample and the contents of the  
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24 207 tube were mixed thoroughly by hand. The tube was placed in a water bath (50°C) for 15  
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26 208 min, with the contents of the tube being mixed thoroughly by hand mid-way through the  
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28 209 incubation period. The tube was removed from the water bath and mixed on an end-to-  
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30 210 end shaker (15 min). Following extraction of the DNC, the tube contents were allowed to  
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32 211 settle and the supernatant was removed and filtered through paper filters (Whatman),  
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34 212 prior to final filtration through an HPLC sample filter (0.45 µm, Acrodisc® LC 13 mm  
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36 213 PVDF). An aliquot (3 ml) of the filtered feed sample extract was evaporated to dryness  
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38 214 under nitrogen (60°C) and reconstituted in acetonitrile/water (80/20, v/v, 300 µl) for  
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40 215 HPLC analysis. Representative chromatograms for feed samples are shown in Figure 2.  
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43 217 Performance of the method during sample testing was evaluated by fortifying control  
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45 218 feed samples, in duplicate, with DNC at 1.6 and 8.0 mg/kg. These quality control samples  
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47 219 were included with each batch of test samples; mean recovery ± standard deviation (n =  
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49 220 14) of added DNC was 95 ± 13.3 % and 83 ± 10.1 % for fortification at 1.6 and 8.0  
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51 221 mg/kg, respectively. The limit of determination of the method was approximately 0.1  
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53 222 mg/kg, based on the lowest standard in the calibration curve.  
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56 224  
57 225 **Results**

58 226 *Studies on poultry during the period prior to commercial slaughter*  
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4 227 Table 1 provides data on the samples of poultry liver taken from pre-slaughter flocks,  
5 228 representing 32 individual flocks on 23 different farms; the data comprise results for  
6 229 testing for nicarbazin residues in liver and details of the feeding system used for each  
7 230 flock. Measurable nicarbazin residues were determined in all samples of liver taken from  
8 231 the 32 flocks included in the initial study. Nicarbazin residue content of liver samples  
9 232 varied from approximately 20 to 5000 µg/kg DNC. 15 of the 32 liver samples contained  
10 233 nicarbazin residues at concentrations exceeding the JECFA MRL value of 200 µg/kg  
11 234 DNC.  
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20 236 Table 2 provides additional information on those flocks/farms selected for further  
21 237 investigation; selection of flocks/farms for further investigation was based on the results  
22 238 of the pre-slaughter sample analysis (including both low and high levels of nicarbazin)  
23 239 and the type of feeding system(s) in use on the farms. Data for the 12 poultry flocks, on 8  
24 240 farms, selected for further study, showed that 10 of these poultry flocks were scheduled  
25 241 as being on nicarbazin-free feed for between 6 and 10 days, i.e. 1 to 5 days longer than  
26 242 the specified withdrawal period of five days (Table 2). Liver samples from 4 of these 10  
27 243 flocks contained nicarbazin residues in excess of 200 µg/kg DNC (Figure 3), indicating  
28 244 that for these flocks, in particular, the withdrawal period was not effective and that on  
29 245 these farms there was continued exposure of birds to nicarbazin for extended periods after  
30 246 feed changeover. Samples of feed taken from the feeding pans on which the birds were  
31 247 feeding at pre-commercial slaughter sampling were analysed for nicarbazin (Table 2). For  
32 248 birds that were scheduled as being withdrawn from nicarbazin-containing feed for periods  
33 249 of 6 to 10 days, residue levels in liver were generally related (correlation coefficient 0.81,  
34 250  $p < 0.01$ ,  $n = 10$ ) to drug concentration in the feed from feeding pans; in the exceptional  
35 251 case of farm/flock O2, the relatively high nicarbazin content in the feeding pan was not  
36 252 reflected by a high liver content of nicarbazin. Elevated drug concentration in feed at 6 to  
37 253 10 days after planned feed changeover, resulting in elevated nicarbazin residue in liver,  
38 254 indicates significant feed contamination in these feeding systems. For farm/flock C1 and  
39 255 L2, where the scheduled times off nicarbazin-medicated feed were 4 and 3 days,  
40 256 respectively, only in the case of farm/flock L2 was a high liver nicarbazin residue content  
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3 257 associated with a high feed nicarbazine. For farm/flock C1, the elevated liver nicarbazine  
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5 258 residue may have been related to drug persistence.  
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9 260 The on-farm investigations did not show clear relationships between the feeding system  
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11 261 used (5 double bin, 5 single bin, 2 split bin), including the presence and type of hopper,  
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13 262 and the nicarbazine residue levels determined in flocks. There was a tendency for liver  
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15 263 samples from flocks with single bin systems to have a greater incidence of relatively high  
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17 264 concentrations of nicarbazine residue, but high levels of nicarbazine residue in pre-  
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19 265 slaughter liver samples were associated, also, with double bin and split bin systems. The  
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21 266 on-farm investigations strongly suggested that the feed management system used on the  
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23 267 particular farm may be the most important issue in determining whether birds remain  
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25 268 exposed to nicarbazine following feed changeover.  
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#### 28 270 *Studies on poultry at commercial slaughter*

29 271 Complete details for the farms/flocks included in the study are shown in Table 3,  
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31 272 including type of feeding system used and, for each of the three commercial thinnings,  
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33 273 scheduled number of days withdrawal from nicarbazine-containing feed and nicarbazine  
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35 274 content determined in samples of feed from the feed mill, feed from the feeding pans and  
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37 275 liver from the slaughtered birds. Of the 36 samples of feed (both feed mill and feeding  
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39 276 pan samples) and liver targeted for sampling, in total 32 samples of liver, 30 samples of  
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41 277 feed from feeding pans and 25 samples of feed from the feed mill were received for  
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43 278 analysis.  
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#### 46 280 *Results for first thinnings*

47 281 Nicarbazine residue was determined in all samples taken at the first thinning, ranging in  
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49 282 concentration from 25 to 600 µg/kg DNC. These samples were from birds that had been  
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51 283 scheduled as being withdrawn from nicarbazine for 6 days or more. No pattern of  
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53 284 nicarbazine residue in liver being related to feeding system was discernible; the three  
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55 285 samples containing nicarbazine residue in excess of 200 µg/kg DNC were each from  
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57 286 single, split and double bin systems. Where samples of feed from the feeding pans were  
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59 287 available, there was a relationship between nicarbazine residue in liver and nicarbazine in  
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4 288 feed. The two highest liver samples, 583  $\mu\text{g}/\text{kg}$  DNC (farm/flock EE2) and 292  $\mu\text{g}/\text{kg}$   
5 289 DNC (farm/flock HH1) were associated with feed nicarbazin levels of 6.6 mg/kg DNC  
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7 290 and 0.81 mg/kg DNC, respectively. All other liver samples (containing 25 - 128  $\mu\text{g}/\text{kg}$   
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9 291 DNC) were associated with feed nicarbazin levels of not detectable (ND) to 0.42 mg/kg  
10 292 DNC.

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14 294 Only in one case (farm/flock EE2) was a potential linkage from nicarbazin residue in  
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16 295 liver (583  $\mu\text{g}/\text{kg}$  DNC) through nicarbazin in feed in the feeding pans (6.6  $\mu\text{g}/\text{kg}$  DNC) to  
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18 296 nicarbazin in feed supplied by the feed mill (10.5  $\mu\text{g}/\text{kg}$  DNC) discernible. Interestingly,  
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20 297 the same batch of feed from the feed mill was supplied to another flock on this farm  
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22 298 (EE1), into a split bin system. In this case, the birds were slaughtered one day earlier and  
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24 299 nicarbazin residue in liver (124  $\mu\text{g}/\text{kg}$  DNC) and feed in the feeding pans (0.36 mg/kg  
25 300 DNC) were considerably lower. These differences in nicarbazin content between birds  
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27 301 (liver samples) and feeding pan feeds for the two flocks on the same farm may be due to  
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29 302 the different feeding systems used for the two flocks and/or may indicate that the feed  
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31 303 mill sample, containing 10.5 mg/kg DNC, may not be representative of the total feed  
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33 304 delivery, particularly the feed delivered to farm/flock EE1.

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### 36 306 *Results for second and third thinnings*

37 307 Nicarbazin residue levels in liver from birds taken at the second thinnings were lower,  
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39 308 generally, than levels in birds from the first thinnings, ranging in concentration from ND  
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41 309 to 208  $\mu\text{g}/\text{kg}$  DNC. These samples were from birds that had been scheduled as being  
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43 310 withdrawn from nicarbazin for 11-19 days. The associated feed from feeding pans  
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45 311 showed corresponding lower nicarbazin levels, also, ranging from ND to 0.25 mg/kg  
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47 312 DNC. Some relatively high levels of nicarbazin residue in liver from birds taken in the  
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49 313 second thinnings, for example farm/flock BB1 (208  $\mu\text{g}/\text{kg}$  DNC), CC1 (135  $\mu\text{g}/\text{kg}$  DNC)  
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51 314 and GG1 (129  $\mu\text{g}/\text{kg}$  DNC), could not be explained by the levels of nicarbazin  
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53 315 determined in the corresponding feed samples. These results suggest some alternative  
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55 316 source of nicarbazin to the birds, particularly since birds from these flocks showed lower  
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57 317 liver nicarbazin residue levels at first thinning.

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3 319 The results for samples from the third thinnings show no further decrease in liver  
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5 320 nicarbazin concentration overall, ranging from ND to 195 µg/kg DNC. Samples of the  
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7 321 feed supplied by the feed mill show generally similar nicarbazin contents as were found  
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9 322 in feed being fed to birds at the time of second thinnings, i.e. ND to 0.24 mg/kg DNC.  
10  
11 323 However, the nicarbazin content in feed samples from feeding pans was relatively high in  
12  
13 324 some cases, such as farm/flock AA1 (7.1 mg/kg DNC) and farm/flock BB1 (0.60 mg/kg  
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15 325 DNC), and, overall, was higher than the levels found in feeding pan samples at the  
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17 326 second thinnings. These elevated nicarbazin levels in feeding pan samples may be caused  
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19 327 by older, nicarbazin-medicated feed in the feeding system and at the bottom of feeding  
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21 328 pans becoming available when the cycle of poultry production is finishing. For a number  
22  
23 329 of farms/flocks, such as AA1, FF1, JJ1, there appears to be a re-contamination of poultry  
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25 330 with nicarbazin between the second and third thinnings, not always related to the  
26  
27 331 nicarbazin level determined in the feed samples from the feeding pans. As was observed  
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29 332 for the second thinnings, these latter findings suggest an alternative source of nicarbazin  
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31 333 contamination to the birds.

334

### 335 Discussion

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337 The pre-commercial slaughter sampling and the follow-on investigations on farms  
338 identified the following issues as important contributors to exposure of birds to  
339 nicarbazin following feed changeover:

- 340 (a) poor understanding by some farmers of the different feed types (nicarbazin-medicated  
341 feed, ionophore-medicated feed and non-medicated withdrawal feed) and the  
342 necessity not to mix them
- 343 (b) poor communication between feed supply and farms; in some cases it was found that  
344 feed deliveries were made before bins were empty, and identification of bins in  
345 double bin systems or of compartments in split bin systems appeared to be deficient
- 346 (c) poor management of feed bins on farms; in some cases rotation of bins and/or  
347 compartments for the different deliveries of feed did not appear to be based on the  
348 feed additive contents and the need to avoid cross-contamination

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4 349 (d) deficient cleaning and checking (that is, visual inspection of feed bins/hoppers for  
5 350 lodged feed) of feeding equipment between feed changeovers; in some cases even  
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7 351 where bins were emptied before feed changeover, hoppers and feeding lines might not  
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9 352 be emptied - the importance of this aspect was identified by the thorough cleaning  
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11 353 practice observed on one farm with a single bin system (farm/flock E1) for which the  
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13 354 pre-slaughter sampling, at 6 days off nicarbazin-medicated feed, showed a very low  
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15 355 level of nicarbazin residue in liver.  
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18 357 The second study, involving analysis for nicarbazin of poultry liver and feed samples  
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20 358 from the feeding pans and from the feed mill at the three commercial thinnings for 12  
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22 359 farms/flocks, gives some interesting insights into the potential causes for nicarbazin  
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24 360 residue in broilers at slaughter. In one case (farm/flock EE2, first thinning), there was a  
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26 361 clear "cause and effect" relationship between contaminated feed supplied from the feed  
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28 362 mill, contaminated feed on farm and relatively high nicarbazin residue levels in liver.  
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30 363 Relatively high nicarbazin residue levels were determined in poultry liver from some  
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32 364 farms/flocks at each of the three thinnings but there does not appear to be a consistent  
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34 365 pattern for individual farms/flocks across the three thinnings, nor any clear relationship  
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36 366 with feeding system used (single bin, split bin, double bin). The results obtained at the  
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38 367 second and, especially, at the third thinnings are particularly difficult to explain and  
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40 368 appear to point to issues such as (a) re-contamination of birds with nicarbazin and (b)  
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42 369 feed samples taken from feeding pans not being representative of what the birds were  
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44 370 consuming at time of third thinning. These latter results suggest that birds may be  
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46 371 exposed to alternative sources of nicarbazin close to slaughter, such as older nicarbazin-  
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48 372 medicated feed from the feeding system or from the litter (Cannavan and Kennedy,  
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50 373 2000).

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54 375 The aim of the investigations was to obtain comprehensive knowledge in commercial  
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56 376 poultry production systems on the causes for nicarbazin occurring in poultry at slaughter  
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58 377 and to use this knowledge to develop guidelines for poultry producers on prevention of  
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60 378 nicarbazin residues in broilers. Multiple potential causes for nicarbazin residues occurring  
379 in edible tissues of birds at slaughter were identified, including contamination of feed at

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3 380 the feed mill or during transport, supply of incorrect feed type, delivery of feed into  
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5 381 incorrect feed bin, inadequate cleaning of feeding system on farm prior to feed delivery  
6  
7 382 and feed changeover, inadequate withdrawal periods being observed, poor management  
8  
9 383 of feed on farm leading to re-exposure of birds to nicarbazin close to slaughter, and  
10  
11 384 recycling of nicarbazin by birds from litter. Guidelines have been developed in two  
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13 385 formats to address the observed deficiencies in management of feed on poultry farms.  
14 386 One format is a leaflet that provides poultry producers with information on how to  
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16 387 manage their enterprise to prevent, or at least reduce, the occurrence of nicarbazin  
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18 388 residues in poultry meat (O’Keeffe 2006). The other format is a poster giving an 11-  
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20 389 point plan for proper feed management on farm (Figure 4); the material is provided on a  
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22 390 laminated poster, 60 cm × 85 cm, for display in poultry production units.  
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## 393 **References**

- 26 393  
27 394  
28 395 Cannavan, A., Ball, G. and Kennedy, D.G., 2000, Nicarbazin contamination in feeds as a  
29 396 cause of residues in eggs. *Food Additives and Contaminants*, **17**, 829-836.  
30 397  
31 398 Cannavan, A. and Kennedy, D.G., 2000, Possible causes of nicarbazin residues in  
32 399 chicken tissues. *Food Additives and Contaminants*, **17**, 1001-1006.  
33 400  
34 401 Capurro, E., Danaher, M., Anastasio, A., Cortesi M. L. and O’Keeffe, M., 2005, Efficient  
35 402 HPLC method for the determination of nicarbazin, as dinitrocarbanilide in broiler liver.  
36 403 *Journal of Chromatography B*, **822**, 154 - 159.  
37 404  
38 405 Castelli, S., Brambilla, G.F., Riberzani, A. And Macri, A., 1989, Nicarbazina: deplezione  
39 406 della componente DNC dal tessuto muscolare ed epatico di broilers. *Industrie Alimentari*,  
40 407 **28**, 947-949.  
41 408  
42 409 De Jong, J., Tomassen, M., Driessen, J., Keukens, H., Putzka, H.-A. and Brambilla, G.,  
43 410 2004, Liquid chromatographic method for nicarbazin in broiler feeds and premixtures:  
44 411 development, validation, and interlaboratory study. *Journal of AOAC International*, **87**,  
45 412 1269-1277.  
46 413  
47 414 Dorn, P., Neudegger, J. and Knoppler, H.O., 1988, Zur problematic der  
48 415 wirkstoffverschleppung bei der mischfutterherstellung am beispiel nicarbazin.  
49 416 *Tierarztliche Umschau*, **43**, 524-529.  
50 417  
51 418 European Commission 2004/C 50/01, 25 February 2004, List of the authorised additives  
52 419 in feedingstuffs published in application of article 9t (b) of Council Directive  
53  
54  
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56  
57  
58  
59  
60

- 1  
2  
3 420 70/524/EEC concerning additives in feedingstuffs. *Official Journal of the European*  
4 421 *Union*, **C50**, 12.  
5 422  
6 423 McEvoy, J.D.G., Smyth, W.G. and Kennedy, D.G., 2003, Contamination of animal  
7 424 feedingstuffs with nicarbazin: investigations in a feed mill. *Food Additives and*  
8 425 *Contaminants*, **20**, 136-140.  
9 426  
10 427 O'Keeffe, M., 2003. *A Review of Coccidiostat Residues in Poultry*, safefood, Food Safety  
11 428 Promotion Board, Cork, 37 pp.  
12 429  
13 430 O'Keeffe, M. and Coen, K., 2005. *National Food Residue Database Report 2005*,  
14 431 Teagasc, Dublin, ISBN: 184170 397 4, 57 pp.  
15 432  
16 433 O'Keeffe, M., 2006. *Prevention of nicarbazin residues in broilers: guidelines for poultry*  
17 434 *producers*, safefood – the Food Safety Promotion Board, Cork, 6pp.  
18 435  
19 436 O'Keeffe, M., Rehmann, F.J. and Coen, K., 2006. *National Food Residue Database*  
20 437 *Report 2006*, Teagasc, Dublin, ISBN: 1 84170 462 3, 60 pp.  
21 438  
22 439 Penz, A.M.Jr., Vieira, S.L. and Ludke, J.V., 1999, Nicarbazin residues in broiler tissue  
23 440 and litter. *Journal of Applied Poultry Science*, **8**, 292-297.  
24 441  
25 442 Porter, C.C. and Gilfillan, J.L., 1955, The absorption and excretion of orally administered  
26 443 nicarbazin by chickens. *Poultry Science*, **34**, 995-1001.  
27 444  
28 445 Veterinary Residues Committee, 2001. *Annual Report on Surveillance for Veterinary*  
29 446 *Residues in Food in the UK 2001*, Veterinary Residues Committee, Surrey, UK, 36 pp.  
30 447  
31 448 Veterinary Residues Committee, 2002. *Annual Report on Surveillance for Veterinary*  
32 449 *Residues in Food in the UK 2002*, Veterinary Residues Committee, Surrey, UK, 44 pp.  
33 450  
34 451 Veterinary Residues Committee, 2003. *Annual Report on Surveillance for Veterinary*  
35 452 *Residues in Food in the UK 2003*, Veterinary Residues Committee, Surrey, UK, 48 pp.  
36 453  
37 454 Veterinary Residues Committee, 2004. *Annual Report on Surveillance for Veterinary*  
38 455 *Residues in Food in the UK 2004*, Veterinary Residues Committee, Surrey, UK, 56 pp.  
39 456  
40 457 Veterinary Residues Committee, 2005. *Annual Report on Surveillance for Veterinary*  
41 458 *Residues in Food in the UK 2005*, Veterinary Residues Committee, Surrey, UK, 48 pp.  
42 459  
43 460 Wells, R.J., 1999, Wells, Nicarbazin. In *FAO Food and Nutrition Paper 41/11*, Food  
44 461 and Agriculture Organisation of the UN, Rome, 83-93.  
45 462  
46 463 Young, R. and Craig, A., 2001. *Too Hard to Swallow – the truth about drugs and poultry*,  
47 464 Soil Association, Bristol, UK, 66 pp.  
48 465  
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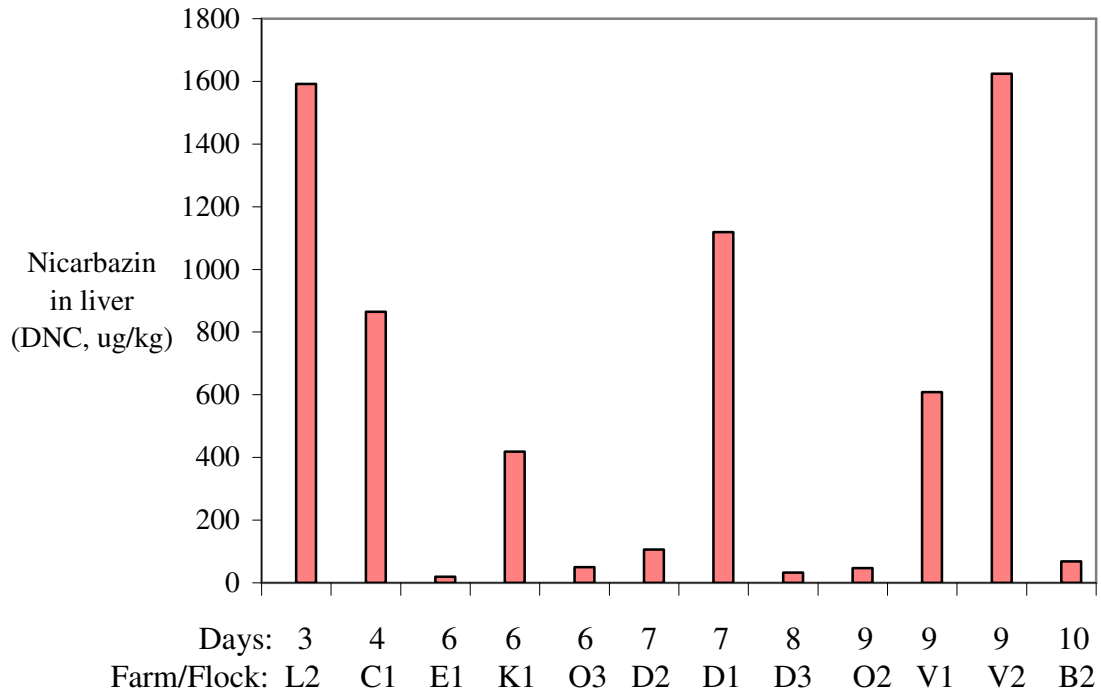
**Table 1.** Results of pre-commercial slaughter testing for nicarbazin in poultry flocks by HPLC

Farm/Flock	Feeding system	Nicarbazin (DNC, µg/kg)	
A1	double bin	86	
B2	double bin	68	*
C1	single bin	865	*
D1	single bin	1119	*
D2	split bin	106	*
D3	double bin	33	*
E1	single bin	19	*
F1	single bin	139	
F2	double bin	51	
G1	double bin	31	
G2	double bin	97	
H1	double bin	354	
I1	double bin	210	
J1	double bin	109	
K1	single bin	419	*
L1	double bin	48	
L2	single bin	1592	*
M1	single bin	241	
N1	double bin	85	
O2	double bin	47	*
O3	double bin	50	*
P2	double bin	243	
Q1	double bin	251	
R2	double bin	112	
S1	double bin	354	
T2	double bin	4897	
U1	double bin	44	
U2	double bin	359	
V1	double bin	609	*
V2	split bin	1624	*
W1	single bin	121	
W2	double bin	224	

\* Farms selected for further investigation.

**Table 2.** Details for farms/flocks subjected to further investigation

<b>Farm/ Flock</b>	<b>Nicarbazin residue in liver (DNC, µg/kg)</b>	<b>Feeding system details</b>	<b>Scheduled days withdrawal from medicated feed</b>	<b>Nicarbazin in feed (DNC, mg/kg)</b>
B2	68	double bin (separate hopper)	10	0.26
C1	865	single bin (no hopper)	4	1.2
D1	1119	single bin (separate hopper, old feeders)	7	4.9
D2	106	split bin (separate hopper)	7	0.67
D3	33	double bin (separate hopper)	8	0.14
E1	19	single bin (no hopper)	6	0.48
K1	419	single bin (hopper in/under bin)	6	9.2
L2	1592	single bin (hopper in/under bin)	3	22.6
O2	47	double bin (hopper in/under bin)	9	2.9
O3	50	double bin (hopper in/under bin)	6	0.70
V1	609	double bin (hopper in/under bin)	9	3.4
V2	1624	split bin (hopper in/under bin)	9	11.0



**Figure 3.** Nicarbazine residue levels in poultry liver samples as a function of scheduled time of withdrawal from medicated feed

**Table 3.** Details of samples taken and nicarbazin content in feed and liver samples

Farm/ Flock	Feeding system (bin type)	First thinning			Second thinning			Third thinning					
		Scheduled days off Nicarbazin	Nicarbazin (DNC)			Scheduled days off Nicarbazin	Nicarbazin (DNC)			Scheduled days off Nicarbazin	Nicarbazin (DNC)		
			Feed mill (mg/kg)	Feed pan (mg/kg)	Liver ( $\mu\text{g}/\text{kg}$ )		Feed mill (mg/kg)	Feed pan (mg/kg)	Liver ( $\mu\text{g}/\text{kg}$ )		Feed mill (mg/kg)	Feed pan (mg/kg)	Liver ( $\mu\text{g}/\text{kg}$ )
AA1	Split	9	ND	-	293	16	ND	0.12	44	21	-	7.1	97
BB1	Split	6	ND	-	101	11	0.70	0.25	208	21	ND	0.60	ND
CC1	Double	6	ND	ND	45	13	ND	ND	135	19	0.24	0.17	55
DD1	Single	7	-	ND	27	19	0.24	ND	ND	-	-	-	-
DD2	Double	7	-	0.17	25	15	-	ND	ND	-	-	-	-
EE1	Split	8	10.5	0.36	124	15	0.12	ND	37	21	0.24	0.24	31
EE2	Double	9	10.5	6.6	583	16	0.12	ND	ND	21	0.24	ND	16
FF1	Split	9	0.70	0.17	128	19	ND	ND	36	23	ND	0.31	106
GG1	Double	8	ND	ND	65	15	ND	ND	129	17	ND	0.29	15
HH1	Single	7	0.12	0.81	292	-	-	-	-	20	-	0.44	31
II1	Split	9	-	0.42	64	15	-	ND	ND	23	ND	ND	44
JJ1	Split	-	-	-	-	15	ND	ND	44	22	ND	ND	195

ND: No residue detected (limits of determination 0.1 mg/kg in feed, 25  $\mu\text{g}/\text{kg}$  in liver)

-: Sample not available for analysis



## 11 POINT PLAN TO PREVENT NICARBAZIN RESIDUES IN POULTRY



- |           |  |
|-----------|--|
| <b>1</b>  | Clearly number all feed bins, including each side of split bins.   |
| <b>2</b>  | Empty and clean the total feeding system — feed bins, hoppers, feed lines, feed pans — before delivery of Nicarbazine-free feed.                                     |
| <b>3</b>  | Check that there is no lodging of feed in bins, hoppers and feed pans.   |
| <b>4</b>  | Clearly specify the feed bin that is to receive the feed delivery and lock all other bins.   |
| <b>5</b>  | Take and store a sample of each feed delivered.  |
| <b>6</b>  | Follow a planned rotation of feed bins to prevent mixing of Nicarbazine-free feed with Nicarbazine-containing feed.  |
| <b>7</b>  | Prevent feed spillage into litter and clean up any Nicarbazine-containing feed that has spilled.   |
| <b>8</b>  | Ensure that all Nicarbazine-containing feed in feed pans is consumed by withdrawing feed from the birds for a period before the changeover to Nicarbazine-free feed. |
| <b>9</b>  | Ensure that birds do not receive Nicarbazine-containing feed again after they have been changed to Nicarbazine-free feed.  |
| <b>10</b> | Ensure that thinning of birds for slaughter is scheduled so that the full withdrawal period of 5 days off Nicarbazine-containing feed is observed.                   |
| <b>11</b> | Maintain accurate records on feed deliveries, feed usage and thinnings.  |



**Figure 4.** Poster with 11-point plan to prevent nicarbazine residues in poultry

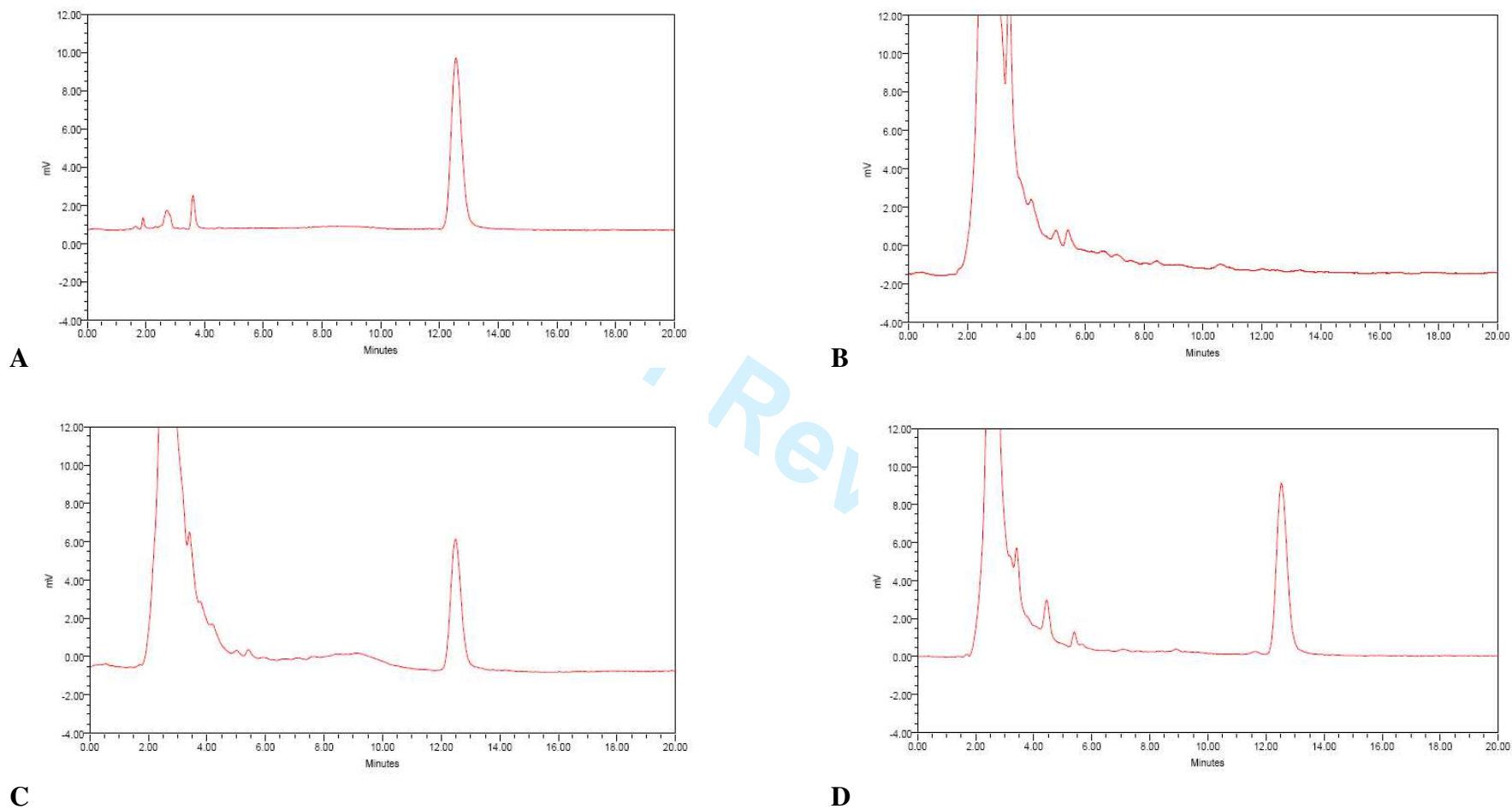
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## *safefood* 11 POINT PLAN

### TO PREVENT NICARBAZIN RESIDUES IN POULTRY

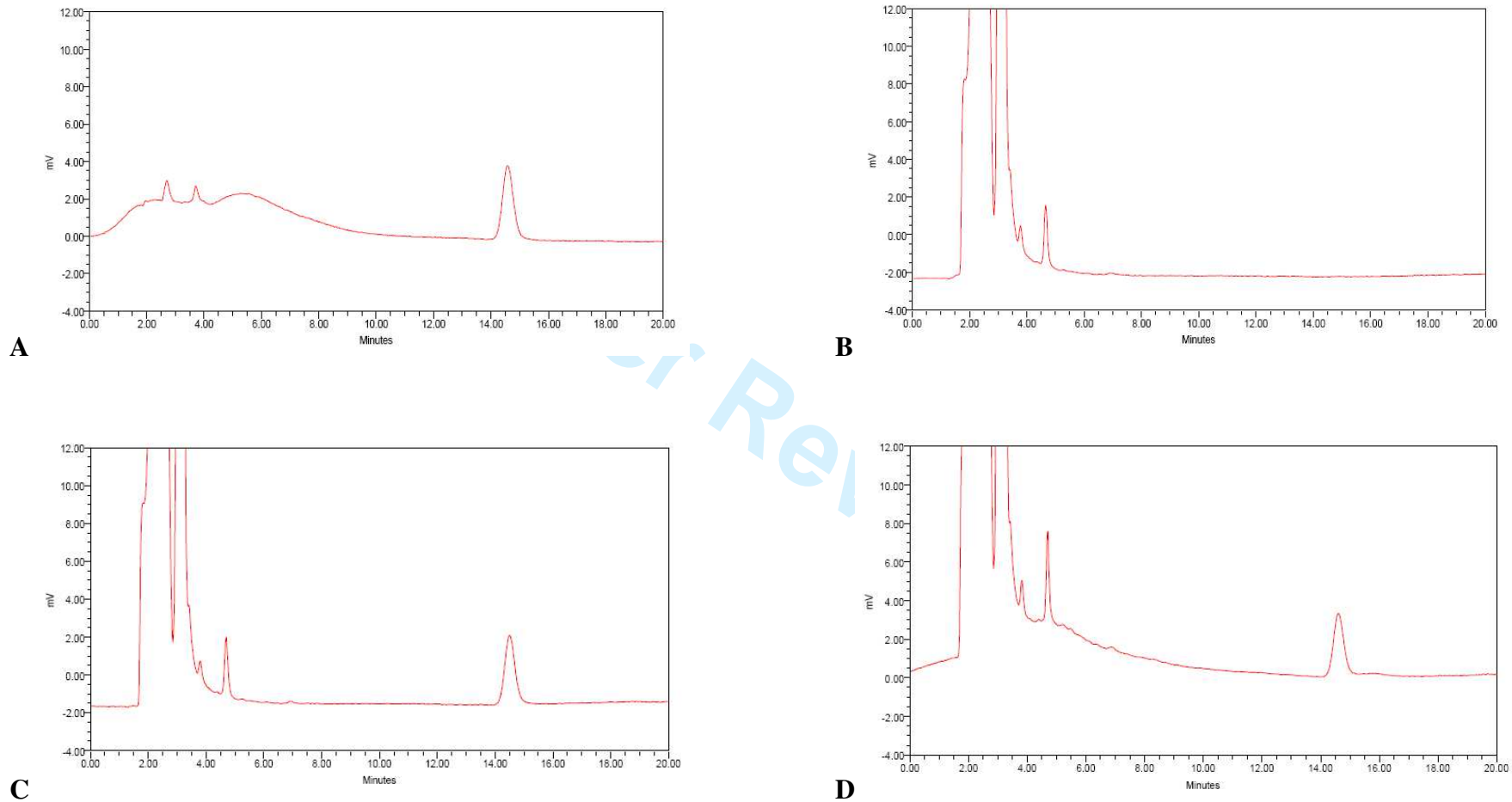
1. Clearly number all feed bins, including each side of split bins.
2. Empty and clean the total feeding system – feed bins, hoppers, feed lines, feed pans – before delivery of Nicarbazine-free feed.
3. Check that there is no lodging of feed in bins, hoppers and feed pans.
4. Clearly specify the feed bin that is to receive the feed delivery and lock all other bins.
5. Take and store a sample of each feed delivered.
6. Follow a planned rotation of feed bins to prevent mixing of Nicarbazine-free feed with Nicarbazine-containing feed.
7. Prevent feed spillage into litter and clean up any Nicarbazine-containing feed that has spilled.
8. Ensure that all Nicarbazine-containing feed in feed pans is consumed by withdrawing feed from the birds for a period before the changeover to Nicarbazine-free feed.
9. Ensure that birds do not receive Nicarbazine-containing feed again after they have been changed to Nicarbazine-free feed.
10. Ensure that thinning of birds for slaughter is scheduled so that the full withdrawal period of 5 days off nicarbazine-containing feed is observed.
11. Maintain accurate records on feed deliveries, feed usage and thinnings.

**Figure 4.** Safefood 11-point plan to prevent nicarbazine residues in poultry



**Figure 1.** Representative chromatograms for DNC in liver samples, retention time 12.5 min (A: DNC standard 1000 ng/ml; B: Control liver sample; C: Control liver sample fortified with DNC at 250 µg/kg; D: Test liver sample containing 300 µg/kg DNC)





**Figure 2.** Representative chromatograms for DNC in feed samples, retention time 14.5 min (A: DNC standard 500 ng/ml; B: Control feed sample; C: Control feed sample fortified with DNC at 8.0 mg/kg; D: Test feed sample containing 7.5 mg/kg DNC)