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Determination of Total Arsenic Residues in Chicken Eggs

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Two levels (50 and 100 ppm) of 3-nitro-4-hydroxyphenylarsonic acid (3-nitro) were fed to different groups of White Leghorn layers for a period of 15 weeks. Arsenic residues in eggs laid by these hens were determined all through the experimental period at almost weekly intervals by a spectrophotometric method sensitive to 0.05 ppm. Contrary to the steadily increased drug intake during the experiment, residues in eggs did not show a continuous accumulation but rather an increase up to a certain level after which it gradually decreased. Arsenic residues determined 2 weeks after the withdrawal of the drug from the feed were negligible.

The organoarsenical compounds such as arsanilic acid and 3-nitro are usually administered in modern-day poultry rations for growth promotion, improvement of feed conversion, better pigmentation, increased egg production, and less mortality. Medication with these compounds could produce residues in edible food products which are usually measured as elemental or inorganic arsenic that might sometimes, and especially when not used at the levels recommended by the Food and Drug Administration (FDA), be a hazard to the public health and thus demands considerable attention.

In this study residual arsenic was determined in eggs laid by hens fed different levels of 3-nitro for a 15-week period and 2 weeks after withdrawal of the drug from the feed.

MATERIALS AND METHODS

Experimental. Forty-eight White Leghorn layers housed in individual wire-floored cages with individual feeders and waterers were used in this experiment. The layers were divided into 12 groups, each group with four birds. The groups were then allocated to three treatments having four groups for each treatment. Medication with 3-nitro was given at the 50 and 100 ppm level in a standard layer type ration. The level permitted by the FDA for layers is 25–50 ppm. The remaining four groups of birds were the control group which received the nonmedicated ration. The feeding of the drug continued for a period of 15 weeks and egg samples from each group were collected at weekly intervals, mainly from those laid on the first 3 consecutive days of the week. About three eggs from each group were pooled for sampling.

Arsenic Analysis. Representative samples of three or four eggs from each group were homogenized in a Waring blender. The homogenate was then left standing for 30 min at 10–15 °C in order to allow escape of the entrapped air. A 20-g sample was then taken for analysis and determination of residual arsenic using the method of George et al. (1973) for dry ashing for the determination of total arsenic in animal tissue.

Table I. Recovery of Arsenic Added to Control Egg Samples[a]

Determinations	ppm As added				
	0	0.1	0.2	0.5	1
1	0.016	0.101	0.192	0.462	0.866
2	0.012	0.089	0.193	0.518	0.898
3	0.003	0.093	0.185	0.472	0.920
4	0.010	0.085	0.190	0.462	0.845
5	0.003	0.095	0.192		0.937
6	0.009				
7	0.006				

[a] Slope = 0.0445; reciprocal slope = 22.5.

Preparation of the Standard Curve. Twenty-gram samples of eggs were spiked with 1 mL of the following arsenic working standard solutions: 0 μg, 2 μg, 4 μg, 10 μg, and 20 μg/mL to get samples containing 0, 0.1, 0.2, 0.5, and 1 ppm As. These were then processed through the dry ashing and distillation as described by the above named procedure (George et al., 1973). The best fitting straight line from ≥4 sets of determinations was obtained for each level of the above concentrations by the method of least squares (AOAC, 1970). The arsenic concentration of an unknown sample was calculated by multiplying the absorbance (540 nm) by the reciprocal slope of the line, discarding the y intercept term.

RESULTS AND DISCUSSION

Recovery of arsenic added to egg samples assayed are presented in Table I. Calculation of residual arsenic in the manner described by using the reciprocal slope of the

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Table II. Arsenic Residues in Chicken Eggs (ppm)[a]

Weeks of feeding	Concentration in diet		
	0 ppm	50 ppm	100 ppm
0	0.002 ± 0.002	0.004 ± 0.003	0.003 ± 0.002
1	0.003 ± 0.003	0.050 ± 0.006	0.111 ± 0.009
2	0.004 ± 0.003	0.089 ± 0.012	0.178 ± 0.013
3	0.005 ± 0.004	0.123 ± 0.011	0.191 ± 0.020
4	0.008 ± 0.000	0.126 ± 0.016	0.240 ± 0.013
5	0.000 ± 0.000	0.133 ± 0.004	0.238 ± 0.019
6	0.019 ± 0.003	0.130 ± 0.007	0.214 ± 0.009
7	0.016 ± 0.005	0.073 ± 0.004	0.169 ± 0.008
9	0.003 ± 0.003	0.064 ± 0.004	0.107 ± 0.003
10	0.003 ± 0.000	0.056 ± 0.002	0.112 ± 0.005
12	0.008 ± 0.007	0.067 ± 0.009	0.116 ± 0.005
15	0.015 ± 0.008	0.069 ± 0.005	0.094 ± 0.006
Withdrawn			
16	0.003 ± 0.002	0.023 ± 0.009	0.035 ± 0.015
17	0.007 ± 0.004	0.011 ± 0.003	0.017 ± 0.007

[a] Each value is the average of four determinations ±SE.

line corrects for any losses during analyses and adjusts to provide essentially a figure which is representative of the average recovery throughout the range of levels used. Average recovery of added As from eggs ranged from about 84 to 94%. The method of calculation includes the native arsenic found in the sample as well as the arsenic contributed by medication. If only arsenic residues from medication are required, then the native arsenic should be subtracted by using control samples.

Results of egg samples analyzed for residual arsenic from the medicated groups and the control are presented in Table II. Calculations made were based on the multiplication of the absorbance of a sample by the reciprocal slope of the line to get x μg of As in a sample of 20 g or $x/20$ ppm As. Very little arsenic was found in eggs laid by hens that received no 3-nitro in their feed. Negligible arsenic also showed in egg samples analyzed prior to the experimental period. Egg samples assayed for the medicated groups showed determinations ranging from 0.050–0.240 ppm depending on the initial rate of inclusion and the feeding period. The FDA permits 0.5 ppm As in eggs (FDA, 1967). Eggs laid by hens receiving 100 ppm 3-nitro in their feed contained the most arsenic at each period, thus indicating a relationship between residue amounts and amounts initially fed. Furthermore, residual arsenic for both of the medicated groups was highest on the fourth, fifth, and sixth week of feeding then the concentration gradually decreased until the end of the feeding period. This could not be explained on the basis of differences in feed consumption since these were not found to differ significantly. The results obtained confirm the work of previous researchers which indicate that the longer the hens received the drug, the less arsenic they transfer to their eggs, indicating that they developed a tolerance (Bruggemann et al., 1963; Evans et al., 1953a,b).

Discontinuation of the medication at the 15th week and analyses of samples 1 week later showed a drastic decrease in residue amounts in both of the medicated groups. Two weeks after the withdrawal of the drug from the feed, residual arsenic was negligible.

The presented data coincide with the general behavior of most feed additives which show residues that increase up to a certain level upon being fed, after which they either remain constant or decrease upon continuous feeding and become negligible once the drug is withdrawn from the feed (Bruggemann et al., 1963; Evans et al., 1953a,b; Kerr et al., 1969).

CONCLUSIONS

The results obtained clearly indicate that there is no continuous accumulation of arsenic residue in eggs, in spite of the length of the feeding period. The highest concentrations of residues were observed on weeks 4, 5, 6 of feeding in both of the medicated groups, then the concentration gradually decreased. The highest amount of residue obtained was 0.240 ppm and the FDA allows up to 0.5 ppm arsenic residues in eggs. Samples analyzed 2 weeks after the withdrawal of the drug from the feed showed negligible residue amounts for both of the medicated groups.

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