Communications

Effect of Arasan-Treated Corn on Laying Hens

During the summer of 1954 a diseaselike condition occurred on at least six farms in Minnesota involving approximately 75,000 hens. Most of these were located on a large breeding farm (*1*), the owner of which appealed to the University of Minnesota animal diagnostic laboratory for assistance.

Birds in full egg production stopped laying normal eggs. Subsequently many soft-shelled eggs were found under the roosts. Most of the few hard-shelled eggs were misshapen. A similar pattern occurs when hens are afflicted with Newcastle disease, infectious bronchitis, or pullet disease, but an attempt to isolate an infectious agent was unsuccessful.

After 2 wk the birds returned toward normal production. However, the same egg-production disturbance soon reappeared. Hens laying 500 eggs daily per 700-bird pen produced 20 to 30 abnormally shaped eggs plus many soft-shelled eggs. With recurrence, the possibility of disease was virtually ruled out, since acute diseases usually impart an immunity to recovered birds.

A home-mixed ration was used on this farm. During the second outbreak, a few of the many pens were changed to a commercial ration. Egg production improved rapidly in these pens. Thus, feed was implicated with the disturbance.

Therefore, an on-the-farm experiment was conducted in which various ingredients were omitted from the normal diet to determine whether a single feed ingredient was responsible. The result indicated that corn was causing the difficulty.

Experiments were then conducted in the university poultry department in which various seed treatments were tested in hen diets. Arasan-SFX had a very pronounced depressing effect on egg production, similar to that produced by the toxic farm rations.

A further experiment (Table 1) compared the performance of a group of hens receiving an implicated farm ration (field ration A) with that of groups receiving Arasan additions to normal diets (*2*). This experiment was performed in batteries with wire-mesh floors to permit daily observation of soft-shelled eggs. The hens receiving field ration A showed only a mild egg-production depression and produced but few soft-shelled eggs. About one-half of the eggs produced were abnormally shaped. This result was similar to that of the group receiving 50 parts per million (ppm) of Arasan; however, egg production was more severely affected by the 50-ppm Arasan diet. Hens receiving 100 to 200 ppm of Arasan ceased laying normal eggs immediately and began producing soft-shelled eggs. In an attempt to show this latter behavior with an implicated farm ration, additional hens were added to the experiment and were fed field ration B. The behavior of this group paralleled that of the group receiving 100 ppm of Arasan.

The various rations used in this experiment were analyzed chemically for tetramethylthiuram disulfide (TMTD), the active fungicidal ingredient of Arasan. Field rations A and B contained the equivalent of about 22 and 104 ppm of Arasan, respectively. Thus the result of the chemical analysis confirmed the biological response presented in Table 1.

Since the Arasan product used in this investigation contained 25 percent of inert ingredients, it was important to determine whether the active fungicidal ingredient also caused the egg-production disturbance. Therefore, a practical grade of TMTD was recrystallized and tested; TMTD was found to be entirely responsible for the effect of Arasan.

The mode of action of TMTD in the metabolism of the hen is obscure. The shell-less eggs are laid at night. This suggests premature expulsion of the egg from the uterus, where the hard shell is normally deposited. Experiments designed to elucidate the metabolic disturbance are in progress.

The tetra*alkyl*thiuram disulfide series of compounds has a wide variety of uses. The methyl derivative is employed as a seed and turf fungicide and is also used widely as an accelerator in the vulcanization process of rubber manufacture. The ethyl derivative is the active component of Antabuse, employed in the treatment of chronic alcoholism in man. The tetra-

Table 1. Effect of Arasan-SFX and implicated farm rations on egg production. Each number without parenthesis represents the number of hard-shelled eggs laid by eight S.C. White Leghorn hens during a 4-day period; each number in parenthesis represents the number of soft-shelled eggs laid during the same period.

Diet	Preexperimental 4 days	Days of experimental period			
		1–4	5–8	9–12	13–16
Control	27	27 (0)	27 (0)	26 (0)	25 (0)
Control plus 10 ppm Arasan	25	27 (0)	22 (2)	22 (1)	23 (1)
Control plus 50 ppm Arasan	23	24 (1)	18 (1)	12 (3)	7 (3)
Control plus 100 ppm Arasan	28	17 (5)	1 (11)	1 (6)	1 (6)
Control plus 200 ppm Arasan	23	8 (16)	0 (11)	0 (12)	0 (6)
Field ration A	24	20 (3)	16 (2)	11 (1)	15 (1)
Field ration B*		15 (9)	1 (8)		

* Field ration B was tested 8 days later than were the other diets. All birds were in good production as judged by previous trap-nest records.

methyl compound is more toxic than the tetraethyl compound for laboratory rats.

A recent report (*3*) dealing with the use of Arasan-DDT-treated seed corn (simultaneous with an epsom-salt flush) in a small farm flock of hens has come to our attention. Egg production was observed to decrease rapidly. However, no conclusions were drawn regarding the causative agent. We are aware of no other published information related to the extreme toxicity of Arasan for hens (*4*).

A sample of corn meal obtained from a farm severely affected during these outbreaks contained 470 ppm of TMTD. Fully treated seed corn contains about 630 ppm of TMTD. The corn meal in field rations A and B contained about 35 and 160 ppm of TMTD, respectively. Thus Arasan-treated seed corn could be diluted heavily with nontreated corn and still produce disastrous results. The label on the container of Arasan-SFX used in this study stated: "The use of this seed for food, feed, or oil purposes is not recommended" (*5*).

Because of the hen's rapid reproductive rate (200 to 300 eggs per year), ease of maintenance, and sensitive reproductive mechanism, it would seem to be an ideal subject for toxicologic studies. Certainly, routine growth studies leave much to be desired in the evaluation of potentially toxic substances.

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References and Notes

1. Ghostley Poultry Farm, Anoka, Minn.
2. The percentage composition of the experimental diet was as follows: ground yellow corn 50, wheat bran 10, wheat middlings 10, alfalfa meal 5, meat and bone scraps 7.5, fish meal 2.5, soybean meal 10, bonemeal 3.0, ground limestone 0.75, iodized salt 1.0, feeding oil (300 D-2250 A) 0.6, and $MnSO_4$ 0.025. The following were added per kilogram; riboflavin 2.75 mg and vitamin B_{12} 3.3 μg. Oyster shell was supplied *ad libitum* for all birds.
3. G. J. Cottier, *Auburn Veterinarian* **10**, 115 (1954).
4. This article is paper No. 3295, Scientific Journal Series, Minnesota Agricultural Experiment Station, We wish to express our appreciation to Norman E. Foster, chief chemist, Minneapolis District, Food and Drug Administration, for his cooperation during this study.
5. Arasan-SFX (Du Pont) contains 75 percent tetramethylthiuram disulfide, which is the active fungicidal ingredient in seed treatment.

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An Application of Statistics

It is commonly stated that one can prove anything by statistics. The mere fact that two variables are significantly correlated by accepted statistical treatment of valid observations does not *ipso facto* prove that the correlation has any biological meaning. In searching for a phenomenon that would illustrate these truisms, I was struck by the fact that months with short names are generally, in the north temperate zone of the continental United States, the warm ones, and those with long names are the cold ones. The short-name months also tend to have more rainfall than the long-name months.

To test whether or not there was a statistically significant correlation between the length of the name of the month and the temperature and precipitation, meteorological data for Chicago, Illinois, were chosen. The data represented the mean monthly temperature and the mean monthly precipitation for that station; the source was *Annual Climatological Summary, 1947*. The statistical procedures employed were taken from F. E. Croxton [*Elementary Statistics with Applications in Medicine* (Prentice-Hall, New York, 1953)]. The regression equation that related the number of letters in the names of the months (Y) and the mean monthly temperature (T) was $Y = 8.46 - 0.047T$. The correlation coefficient was -0.448 ($P = 0.15$). This association was suggestive but not statistically significant. The regression equation that related the number of letters in the names of the months (Y) and the mean monthly precipitation (P) was $Y = 11.92 - 2.10P$. The correlation coefficient was -0.611 ($0.05 < P < 0.025$). This association was significant at the 5-percent level. These associations have proved to be useful teaching examples of what can be done by the application of statistics, for here are significant correlations without *a priori* or *a posteriori* bases.

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Note on a Visible Thermocline

On the afternoon of 5 May 1954 I was exploring the reef off Vaiala, slightly more than ½ mi to the east of the harbor of Apia, Upolu, Western Samoa, in search of a good collecting site for the invertebrates I was studying [under a grant from the Bernice P. Bishop Museum in Honolulu]. I was wearing "skin-diving" equipment, a face plate and swim fins.

The day had been very hot and still, and the surface waters over the ½-mi broad fringing reef were extremely warm, actually hot to the body upon entrance. Moreover, this water was so turbid from suspended matter carried down from the hills in a recent storm that the underwater visibility was less than 3 or 4 ft. There was very weak surf on the reef front.

At the reef front I dived down to explore the bottom, about 30 ft deep. As I went down I was able to detect three layers in the water. The hot turbid layer was 4 or 5 ft deep and sharply delimited from the intermediate layer, which was moderately warm and quite clear. Below the intermediate layer, which extended down to about 15 ft, there was a markedly cooler and clearer layer extending to the bottom.

On the completion of my first dive to the bottom I "coasted" back up, allowing the buoyancy of my body to carry me slowly to the surface while I looked at