

# The Use of Microtracers™ F in Coding the Presence of Coccidiostats in Poultry Feeds - Practical Implications

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In a 1993 article, *Zootechnica* reported on the potential toxicity of various coccidiostats and on their potential to lead to toxicity or illegal tissue residues in poultry meat. This paper also explained how Microtracers F (colored uniformly sized iron particles) had been used to reduce the incidence of feed manufacturing mistakes leading to such problems. While the paper described Microtracers™ and applicable test procedures, it did not discuss the details involved in establishing a quality assurance program nor did it provide any quantitative data from feedmills where tracers were employed on an ongoing basis. This paper provides this information and discusses the implications of such testing to concerns expected to impact the feed manufacturing industry in the future.

## **Poultry Feed Manufacturing:**

In the USA, nearly all broiler feed is manufactured at feedmills where only poultry feed is produced. At some mills, production may not be limited to broiler feed but may also include turkey and breeder feeds. These feedmills I Is often operate nearly 24 hours a day 7 days per week with production capacities of 100 tons/hour or more. Production runs for individual formulas are usually longer than at commercial feedmills manufacturing feeds for many species. The number of formulation changes may also be fewer.

Even though such mills are highly automated, mistakes can and do occur. The coccidiostat may be loaded manually into the wrong bin in a computerized micro-addition system, or someone may make an error in programming the computer or the system itself may malfunction. Feed may bridge and not flow properly in conveyer or elevator legs or in holding bins supposed to be empty.

Discharge gates on a mixer may leak allowing feed ingredients in a second batch to contaminate an earlier batch not discharged from a surge bin below the mixer. While the incidence of such errors is very low, the costs associated may be great and the anxiety may be prohibitive unless the feed manufacturer can implement a "real time" quality assurance program where feed is tested before it leaves the feedmill. This testing must be designed to reduce the incidence of manufacturing errors to as nearly zero as is possible.

## **Micro-Tracer "Real Time" Quality Assurance of Poultry Feed:**

When a Microtracer is included as an ingredient in a coccidiostat premix, it often serves two purposes. In addition to providing feed manufacturers a "quick test" for the medication in their final feeds, it may also identify the premix and feed as proprietary. The tracer is usually formulated to yield 63 particles per 500 grams of feed. Tracer recovery is typically 80% from mash feeds and 65% from pelleted feeds so the feed manufacturer actually expects about 40 tracer particles from analysis of 500 grams of a pelleted feed. The likelihood of finding no tracer when one is expecting 40 particles is

essentially zero if the test is performed correctly. The tracer test involves pouring a feed sample through a "Rotary Detector" laboratory magnetic separator isolating the tracer on a small filter paper, adding a solvent (usually water) to dissolve the dye from the colored iron particles coloring the filter paper. A feed containing the coded coccidiostat will yield a bright ring of color on the filter paper, a feed supposed not to contain the premix should yield no color. Different colored tracers may be used to code different premixes. For a quantitative estimate of the tracer and by inference the medication, one "demagnetizes" the magnetic material and sprinkles it onto a large filter paper wetted with the appropriate developer, usually 50% ethanol, to yield a paper with countable spots.

### **The Microtracer Test - Practical Considerations, Potential Problems:**

1. The sample must be representative of the truckload of feed. The feed should be a composite from various parts of the truckload. Even with great care in sampling, the potential for error is real when analysis of 500 grams of feed is used to characterize 20 metric tons.
2. The feed manufacturer must run a "control positive" each day, testing a feed known to contain the coccidiostat with tracer to be sure a feed positive for the medication yields a positive result for the tracer. For qualitative testing, for most Microtracers, water must be used to develop the tracer color because other developers (i.e. pure ethanol) may yield little or no color. If the wrong solvent is used, a "false negative" result for the tracer will render the test meaningless.
3. The feed manufacturer must establish acceptance/rejection criteria. If one is expecting 40 colored spots from a test and finds 2, should the truckload of feed be considered contaminated and rejected? In such instances, it is probably best to take additional samples from each compartment on the truck and to analyze them. If no sample yields more than one or two spots and a "control positive" yields 30 or 40 spots or a bright ring of color as a qualitative test, then the trace contamination may be tolerable and the feed may be shipped. Each feed manufacturer must establish their own procedures and criteria as to what results are acceptable and what are not.
4. Interpretation of quantitative tracer counts is limited by the uniformity of the tracer, the consistency of tracer recovery at different feedmills and by the variability inherent to the applicable Poisson statistical distribution. The true count of the tracer may vary 10% between lots, the recovery of the tracer may vary 10% between feedmills and if one counts 100 tracer spots this count will have an inherent standard deviation of its square root or 10 and a resulting coefficient of variation of +/-10%. As a practical matter, if one counts 100 tracer spots from a feed sample, this count should allow an estimate of the level of the coded coccidiostat +/- 30-35% with 95% statistical confidence.

### **Actual Data from Three Feedmills:**

#### ***Feedmill #1***

Microtracer F-Red with a specified count of 25,000 particles/gram was formulated in salinomycin to yield 3.6 grams of tracer per metric ton of feed when the formulated level of salinomycin was 60 grams/ton. This would yield a "theoretical" tracer count of 25.6 particles per 280 grams of feed if recovery of the tracer was 100%.

Samples were taken from 208 truckloads of pelleted feed manufactured while the salinomycin/tracer premix was being used. Most but not all these samples were consecutive.

All these samples were analyzed for the tracer and twenty-three were analyzed chemically for salinomycin. The samples analyzed chemically were taken from each feed formula production run in the order these were produced. These formulas called for either 60 grams of salinomycin per ton of feed, 55 grams or none. The samples chosen were biased, however, with many chosen because the tracer result deviated from the feed manufacturer's formulation. A total of 1,336 tracer particles were found in 63 samples formulated with salinomycin at 60 grams/ton. The recovery of the tracer was 82.8% of the formulated level. The recovery of the tracer from 50 feed samples taken from mash feed in the surge bin during this study was 95.7% of theory.

The apparent loss of tracer between the surge bin and the pelleted samples was 13.5%. A total of 583 tracer particles were found in 28 samples formulated with salinomycin at 55 grams/ton with an indicated tracer recovery of 89.3%. A total of 98 tracer particles were found in 117 samples supposed to contain no salinomycin. This represented 4.8% of the total tracer found.

The results indicate that while the chemical assay results for the 23 samples analyzed were more precise in estimating the level of the medication in feeds, the tracer results were as efficacious in qualitatively identifying the drug. For sixteen samples, both the tracer and chemical assays correctly predicted the presence of the drug, for three samples the chemical assay corresponded with the drug formulation while the tracer did not and for four samples the tracer corresponded with the drug formulation while the chemical assay did not. In no case did both the tracer and chemical assay deviate from the formulated medication.

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The level of detection for the chemical assays was 16 grams/ton or 25% the formulated level. Results were thus of little value in estimating contamination levels of the drug. Of the 91 samples formulated with salinomycin at either 60 or 55 grams/ton, 2 yielded tracer counts of 5 or less (1 and 4) compared with an average of 23.5 found in the other 89 samples. These two results may have been "false negative" results for the medication.

Only the sample with a count of 4 was analyzed chemically, this yielding a positive chemical assay at 49.8 grams salinomycin/ton. Of the 117 samples supposed to contain no salinomycin, three yielded counts of 6 or more (6, 8 and 7) all 1/3rd or less tracer than found in the feeds formulated with the medication at 60 grams/ton. These three results would be considered "weak positives" for the medication. Only the sample with a count of 7 was analyzed chemically.

This yielded a positive chemical assay at 56 grams salinomycin/ton. The tracer may have correctly predicted a mistake. The salinomycin/tracer premix was formulated in approximately 60% of the tonnage being produced at the feedmill. Feeds supposed to contain no salinomycin on average contained at 3.0% the formulated level.

## ***Feedmill #2***

Microtracer F-Special Blue was formulated in Nicarbazin premix to yield a theoretical count of 137 particles per kilo of final feed. Samples were taken from 115 truckloads of feed while the Nicarbazin/tracer premix was being used.

All these samples were analyzed for the Microtracer but none were analyzed by chemical assay for medication.

Two samples weighing a total of 863 grams were stated as containing Nicarbazin. One of these samples contained 41 tracer particles in 402 grams the other 38 particles in 462 grams. The tracer recovery was 75.4% of that formulated. 113 samples weighing a total of 52,821 grams were stated as containing no Nicarbazin. These samples together yielded 15 tracer particles. No sample contained more than 2 particles when results were adjusted to an average sample weight of 467 grams. Six of these samples weighing a total of 2,482 grams were of breeder feeds where sequencing and flushing procedures were employed to prevent Nicarbazin from reaching them. These samples contained no tracer particles. Nicarbazin was formulated in approximately 10% of the feed manufactured during the study. Feeds supposed to contain no Nicarbazin on average contained tracer at 0.26% the level formulated. If Nicarbazin had been formulated in a greater proportion of the feed being manufactured, it is likely the level of tracer found in feeds supposed to contain none would have been higher than that found.

### ***Feedmill #3***

This feedmill was owned by the same poultry integrator as "Feedmill #2" but was of a different design.

Microtracer F-Special Blue was formulated as for Feedmill #2 and again 115 feed samples were analyzed for the Microtracer but not chemically for the medication. Five samples stated as containing Nicarbazin all yielded counts of at least 35 particles from sample weights of between 435 and 535 grams.

A total of 201 particles were found in 2,498 grams of feed. The tracer recovery was 68.1 % of that formulated. 10 samples weighing a total of 53,952 grams were stated as containing no Nicarbazin. These samples together yielded 16 tracer particles representing 0.22% of the "theoretical" tracer expected in a feed formulated with Nicarbazin. No sample contained more than 3 particles.

Thirty of these samples weighing a total of 16,306 grams were of breeder feeds. These contained 10 tracer particles or 0.45% of the "theoretical" tracer expected in a feed formulated with Nicarbazin. Again, Nicarbazin was being formulated in approximately 10% of the feed being manufactured.

### **Conclusions:**

The Microtracer results at the two feedmills using Nicarbazin premix with tracer were excellent in qualitatively differentiating feeds formulated with the medication from those supposed to contain none.

The Microtracer results at the feedmill using salinomycin with tracer were not quite as good at differentiating feeds formulated with the medication from those supposed to contain none, though the tracer results were as good as chemical assays in doing so.

Feeds supposed to contain no tracer yielded an average of 3.0% of the tracer expected from feeds formulated with salinomycin at 60 grams/ton.

The level of tracer contamination was higher than at the mills using Nicarbazin.

This may have been because the salinomycin was being formulated in a higher proportion of the feeds being manufactured.

Contamination of tracer into non-target feeds was successfully controlled at one mill where no tracer whatsoever from Nicarbazin was found in breeder feeds.

This was the most recently constructed feedmill and specific sequencing and flushing procedures were being rigorously employed.

### **Implications:**

Coccidiostats that may be toxic if present in non-target feeds at formulated levels or lower appear controllable.

With careful planning, tracer technology should make it possible for feedmills to use limited quantities of specific medications keeping contamination of such medications into non-target feeds at much less than 1% of the formulated level.

Feed additives that can cause illegal tissue residues in meat and poultry seem controllable at levels of concern. With further research, tracer techniques should make it possible to establish objective standards for acceptable contamination of medicated feeds into non-target feeds.

Chemical assays for salinomycin were costly, time consuming and not adequately sensitive to be of value.

### **Bibliography:**

Prevention of Anticoccidial Toxicity Using Microtracer Premix Additive, Zootechnica International, November 1993.

The Use of Colored Iron Particles in Determining Coded Coccidiostats and Antibiotic Premixes in Finished Feeds, AOAC International Forum: Methods for Antibiotic and Drugs in Feeds, San Diego, California, September 1997.