Tecaliman
Center of expertise for a sustainable feed business

i'Tec_H13
August 2006

Keywords: Analysis, Tracer, Laboratory, Mix, Carry-over

Results of a 2005 inter-laboratory ring test on RF-blue lake microtracer

Two years after the first ring test (**i'Tec_H17**) that followed the establishment of the new method for extracting and analysing RF-blue lake microtracer (**i'Tec_H18**), several animal feed research labs implemented this technique. Following on from the first study, and in agreement with Tecaliman's supervisory board, a new ring test was set up to assess analysis performance. The objectives were as follows:

- Carry out blind analyses on a wide range of samples, and then compare the results.
- Vary the matrices

The laboratories of 10 companies took part in blind tests on this circuit:

- Arrivé
- CCPA
- Cooperl
- Evialis
- Glon-Sanders (CRCB)
- Idena
- Inzo
- Primex
- Tecaliman
- Techna

The findings of this ring test were distributed to these laboratories on 8 December 2005, and the companies now wish to disseminate this information throughout the entire trade sector. This is the second ring test carried out on this analysis technique.
Tecaliman also supplemented this research with a study on the free fraction of colorant in microtracer batches.

1. Principle

Each laboratory received a series of 5 samples of known concentrations (4 to 280 ppm) in three different feedstuffs. They also received all the equipment and apparatus required to prepare the colour ranges according to their own methodology (pure microtracer and the three blank feedstuffs).

2. Equipment and apparatus

2.1. Microtracer

The same 100-g batch of microtracer was used for every production.
This batch was divided into 2. One portion was divided into 16 parts in order to send each laboratory a portion of pure microtracer. The other portion was used to make the premixes.

2.2. Feedstuffs

Three feedstuffs were used to make up the samples. These were Pig, Broiler and Dairy Cow.

3. Method

3.1. Mixes

The mixes were produced in a 100-litre pilot blade mixer. The mix was stirred for 2 minutes at 60 rpm.
Table 1 gives the composition of the 5 mixes. The last column shows the theoretical mix concentration.

	Feedstuffs		Microtracer	
	Type	kg	g	ppm
A	Pig	25	0.875	35.0
B	Broiler	25	3.249	130.0
C	DC	25	2.125	85.0
D	Broiler	50	0.206	4.1
E	DC	25	7.002	280.1

Table 1: Mix composition

At Tecaliman, the person who manufactures the mixes and divides them up is not the same person who runs the subsequent analyses.

3.2. Preparing the samples

The samples were successively halved using a sample splitter on the whole mixed batch, giving 16 primary samples. Ten of these samples were sent to the lab; the last six samples were set aside in a cold room.
Each lab received:

- one sample of each mix,
- one sample of each of the three feedstuffs,
- one sample of pure microtracer

3.3. Laboratories

The 10 laboratories were coded anonymously from A to J. The codes differed from those used in the previous study.

3.4. Analyses

Not all the laboratories involved in the ring test used the wet lab technique described in **i'Tec_H18**. This was the case for laboratories H and J. In this ring test, these labs were not hindered by the presence of liquids (fats, molasses, etc.) in the mixes and their recovery rates may therefore be considered substantially similar to those of the alternative method.

4. Results

The following graphs show the raw data from the laboratories in increasing order of mix concentration: D, A, C, B, E. On each graph:

- The baseline value is indicated in black on the left and extends into a dashed line.
- The minimum and maximum values are shown with dark shading.

Looking at these graphs, it becomes immediately apparent that one of the laboratories (F) experienced analysis issues on three separate occasions.

Furthermore, taking all laboratories combined, the deviations between target and actual values are larger at low concentrations.

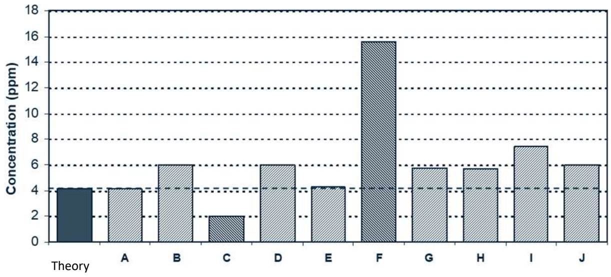

Figure 1: Results from the 10 laboratories for samples D

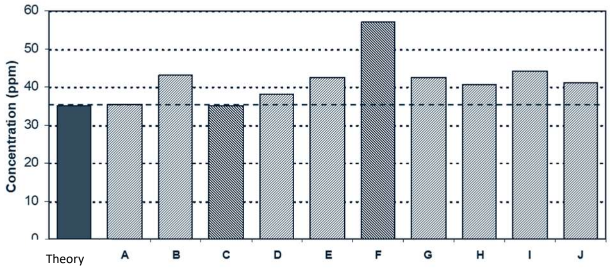

Figure 2: Results from the 10 laboratories for samples A

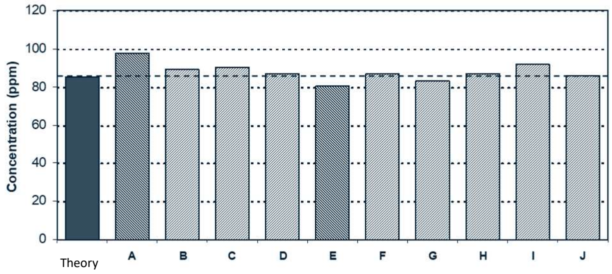

Figure 3: Results from the 10 laboratories for samples C

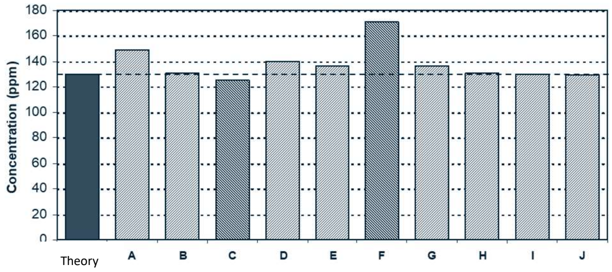

Figure 4: Results from the 10 laboratories for samples B

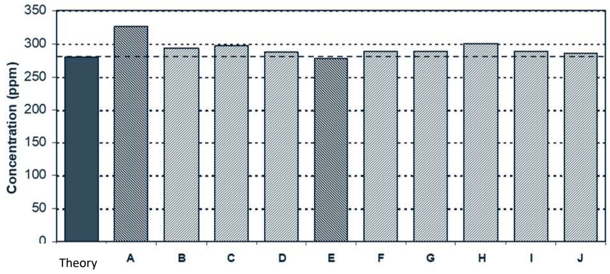

Figure 5: Results from the 10 laboratories for samples E

Statistical processing of the data is used to describe the resulting populations (Table 2) and to study the relationships between a laboratory and a given population via a box plot test (Figure 6).

	D	A	C	B	E
Expected	**4**	**35**	**85**	**130**	**280**
Nb. of values	10	10	10	10	10
Minimum	2.0	35.0	80.4	125.0	277.9
1st quartile	4.3	38.0	86.0	129.9	287.0
Median	5.9	41.7	87.1	133.8	288.6
3rd quartile	6.0	43.0	90.0	140.0	297.0
Maximum	15.6	56.9	98.0	170.8	326.0
Spread	13.6	21.9	17.6	45.8	48.1
Mean	6.3	41.9	87.9	137.9	293.2
CV (standard deviation *100/mean)	57.0	14.7	5.4	9.7	4.5
Estimated variance	12.9	37.7	22.8	179.7	170.9
Estimated standard geometric deviation	3.6	6.1	4.8	13.4	13.1

Table 2: Description of the populations

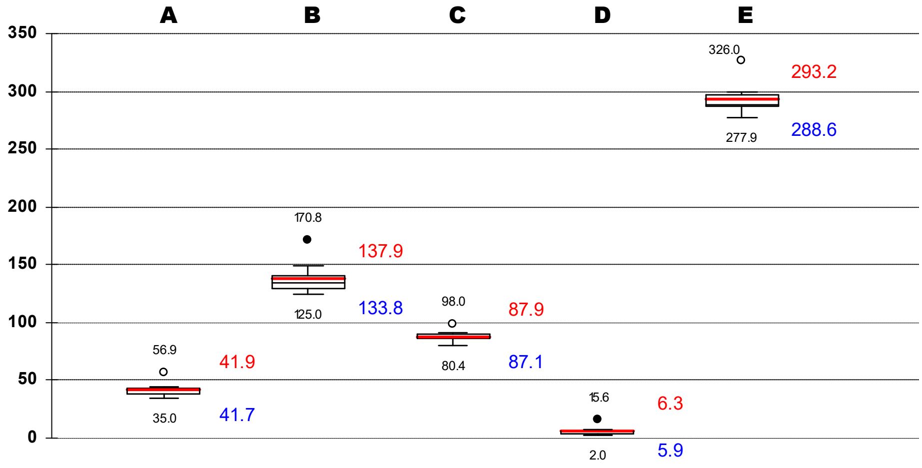

Figure 6: Results of box plot tests for all raw data populations

This test shows that, at every level, the data from a given lab disassociates from the general data population (plot outside the box). While this involves laboratory F for samples A, D and B, it is laboratory A that is involved for samples C and E. All these values are larger than the target value.

After deleting plots outside the overall population (Table 3) and reprocessing the data, the box plot graph then showed that all the values lay within the bounds of the populations (Figure 7).

The descriptive data on these populations (Table 3) demonstrate that the coefficients of variation increased significantly at lower concentrations (A and D).

	D	A	C	B	E
Expected	**4**	**35**	**85**	**130**	**280**
Nb. of values	9	9	9	9	9
Minimum	2.0	35.0	80.4	125.0	277.9
1st quartile	4.2	36.7	84.6	129.5	286.0
Median	5.8	41.0	87.0	131.3	288.5
3rd quartile	6.0	42.8	89.5	138.4	295.5
Maximum	7.5	44.1	91.6	149.0	299.6
Spread	5.5	9.1	11.2	24.0	21.7
Mean	5.3	40.2	86.8	134.3	289.6
CV (standard deviation *100/mean)	30.0	8.3	3.9	5.3	2.3
Estimated variance	2.5	11.1	11.5	51.6	42.8
Estimated standard geometric deviation	1.6	3.3	3.4	7.2	6.5

Table 3: Description of the populations after deleting outliers

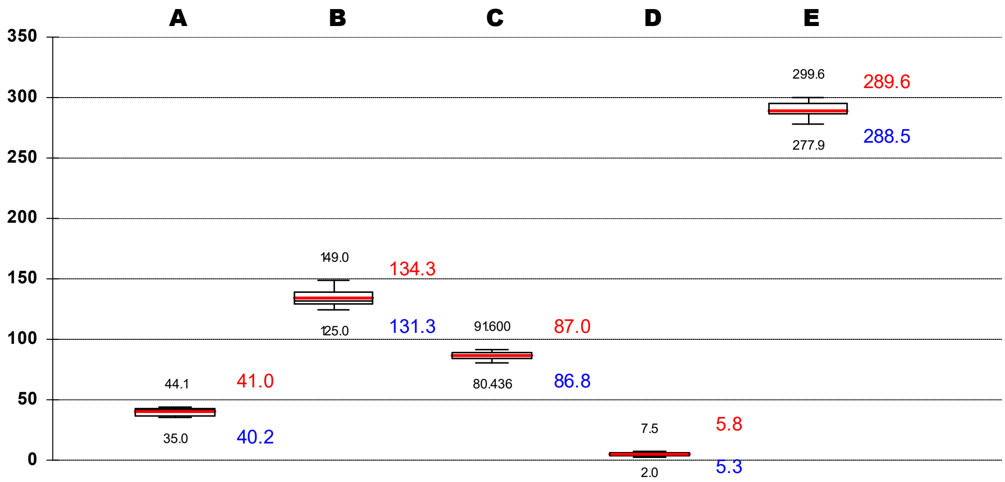

Figure 7: Results of box plot tests on populations after deleting outliers

Plotting the change in the inter-laboratory coefficient of variations against median population concentration (Figure 8) gave a curve similar to that obtained with samples containing decreasing numbers of particles.
When expressed in logs, this data gave a straight line that clearly showed that this is the effect in question (Figure 9)

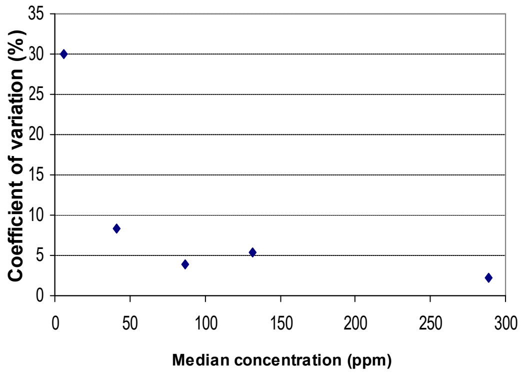

Figure 8: Change in the inter-laboratory coefficient of variations against median population concentration

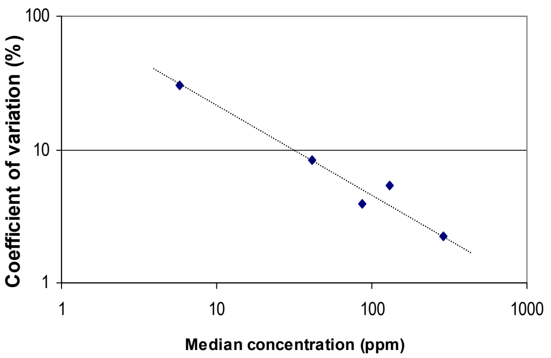

Figure 9: Change in the inter-laboratory coefficient of variations against median population concentration expressed in logs

5. Conclusion

This latest ring test revealed that there are significant discrepancies from one laboratory to the next. A laboratory inspection identified a faulty spectrophotometer, which was then repaired.

This inspection was also used to assess the laboratories' performance with each feedstuff. It appears that this performance was affected not by the difference in matrix, but rather, as logic would suggest, by the number of tracer particles contained in the samples.
If this effect is clearly visible here due to the known number of particles, it would be logical to assume that it can be found in all powdered products.
Various details have been added to the analysis procedures:

- Preparation of test portions by division rather than "tapping" the upper layer of the samples in order to prevent the samples demixing during transportation.
- Study of larger samples at lower concentrations in order to minimise the effect of a decrease in the number of particles.
- Expression of the calibration curve Mass = f(optical density), as the mass has to be predicted by the optical density and not the reverse.

Taken as a whole, the laboratories demonstrated solid performance as pooling their data even made it possible to identify the effect of a physical phenomenon.
This ring test will be continued in 2006 and extended to include use of a microtracer in premixes.

6. Bibliography

i'Tec_H17, 2003. Méthode d'Analyse du microtraceur RF bleu lake après extraction en milieu aqueux – Octobre 2003.