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Comparative study of the mixing behaviour of commercial additives that are subject to approval

1. Objective

The aim of these tests is to test the mixing behaviour of additives in their commercial form. This should provide veterinary services personnel and industrial operators with a better understanding of the dispersion phenomena of these products and justify their possible representativeness as a tracer for assessing the ability of mixer to guarantee dispersion.

2. Materials

2.1. Feed

The basic feed used for mixes is an organic growing finishing feed that does not contain any additives or have any minerals incorporated. Its apparent (bulk) density is 0.636 g/m^3, its median diameter by sieving is 570.3 µm and its angle of repose is 61.2°. This feed is produced in a system that is cleaned in advance with two batches of raw materials.

2.2. Additives

Of the 12 additives tested (Table 1), 10 appear on the positive list, i.e. 3 antibiotics, 4 coccidiostats and 3 trace elements.

Additives	Names	Levels of substances traced (%)
Avilamycin	Maxus 200	18
Flavophospholipol	Flavomycin 40	4.52
Salinomycin	Sacox 120	12.1
Oxytetracycline	Oxytetracycline	88.6
Lasalocid	Avatec 150	16.9
Maduramicin	Cygro 10	0.98
Meticlorpindol	Coyden	25.2
Monensin	Elancoban 200	19.6
Copper	Copper sulphate	26
Manganese	Manganese oxide	61
Selenium	Selenium BMP	1.1
Microtracer	RF-Blue Lake	100

Table 1: List of products used

The list of products traced also includes a medicinal product and an external tracer. The suppliers of products were chosen on the basis of their frequency of use, on completion of a consultation covering premixers. The physical characteristics of these products appear in Table 2.

Products	Angle of repose (°)	Apparent (bulk) density (g/m^3)	Tapped density (g/m^3)	Hausner ratio
Avilamycin	49.6	0.58	0.62	1.06
Flavophospholipol	68.9	0.65	0.83	1.27
Salinomycin	41.0	0.59	0.61	1.05
Oxytetracycline	61.1	0.43	0.59	1.37
Lasalocid	52.8	0.54	0.58	1.06
Maduramicin	46.9	0.55	0.56	1.03
Meticlorpindol	75.9	0.41	0.49	1.18
Monensin	51.6	0.70	0.73	1.03
Copper	41.8	1.41	1.45	1.03
Manganese	72.4	1.09	1.50	1.38
Selenium	47.6	1.52	1.57	1.04
Microtracer	35.5	3.02	3.15	1.04

Table 2: List of products used

2.3. Mixer

A Gondart double ribbon pilot size mixer, with the following technical specifications:

- Spindle speed: 32 rpm
- Outer flight diameter: 0.49 m
- Peripheral linear speed: 0.84 m/s
- Useful volume: 224 l

3. Method

3.1. Testing plan

Three mixes are produced. Three tracers are systematically present in each of the 3 mixes:

- Manganese
- Meticlorpindol

- RF-blue lake microtracer

Each mix also contains 3 other tracers, the mixing behaviour of which is only tested once (Table 3). The use of three separate mixes is considered necessary because of existing analytical incompatibilities between some of these products, in particular the antibiotics analysed by microbiology.

Mix 1	Mix 2	Mix 3
Manganese	Manganese	Manganese
Meticlorpindol	Meticlorpindol	Meticlorpindol
RF microtracer	RF microtracer	RF microtracer
Copper	Selenium	Oxytetracycline
Avilamycin	Flavophospholipol	Salinomycin
Monensin	Maduramicin	Lasalocid

Table 3: Traced products contained in each of the mixes

These tests are carried out at the maximum doses set out in the positive list for all internal tracers, with the exception of flavophospholipol, due to an error in the initial concentration value (Table 4).

3.2. Production of mixes

Before and between each mix, the mixer is systematically rinsed using 50 kg of a poultry feed that does not contain a premix. Deep cleaning is then undertaken using a blow gun and vacuum cleaner.
The mixer is filled as follows: 50 kg of feed + 1 kg of premix as close as possible to the centre of the mixer + 49 kg of feed
After mixing for 5 minutes, the spindle rotation direction is reversed and the hatch is opened, in order to remove the mix in a big-bag.

Products	Incorporation rate (ppm)
Avilamycin	40
Flavophospholipol	2.5 (Typically 25)
Salinomycin	60
Oxytetracycline	500
Lasalocid	125
Maduramicin	5
Meticlorpindol	200
Monensin	125
Copper	175
Manganese	250
Selenium	0.5
RF microtracer	250

Table 4: Incorporation rates

3.3. Sampling

The mix spread on the ground, in the form of a 20 cm mat, is divided into 40 segments (Figure 1). A random sample of approximately 300 g is taken from each of them, at the surface and/or deeper inside. Each of the samples is placed in a numbered 450 ml pot. A random draw of 10 samples then takes place and these are sent to the laboratory. Twenty other samples are drawn at random for microtracer analysis. The last ten samples are kept in reserve.

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40

Figure 1: Sampling grid diagram

The samples are not processed in a specific manner before they are sent to the laboratory. Analyses are carried out in duplicate for each sample and for each tracer. Consequently, the laboratory completely grinds each of the samples, and then remixes them before dividing them in order to provide each analysis unit with two test specimens of each sample. For microtracer analysis, each sample is divided in two using a riffle splitter and the microtracer is then extracted and determined by colorimetry.

3.4. Processing the results

Recovery rates are calculated on the basis of incorporations actually carried out, initial concentrations of the products used (verified by analyses) and by taking account of trace elements provided by the feed.
The analysis results are processed using variance analyses with a factor (sample) based on the random model (Tecaliman, 2001). To assess the significance of results, the variance associated with the analysis process (residual variance) obtained by duplicate analyses of each sample is compared with the variance attributable to the process (homogeneity variance) obtained by the analyses carried out on each sample (variation between samples).

4. Results

4.1. Recovery rates

In general, the recovery rates recorded are above 100 %, which results in an overall mean of 106.5 % (Table 5). By taking account of the "permitted" range of variation in the basic rules, i.e. 70 – 110 % (Tecaliman, 2000b), the data for certain mixes and tracers should, therefore, not be used because of their high recovery rates:

- Meticlorpindol in mix 1
- Avilamycin in mix 1
- Meticlorpindol in mix 2
- Flavophospholipol in mix 2
- Meticlorpindol in mix 3

In the absence of an acceptable argument to explain these recovery rates, none of the assumptions made have been retained.
Similar results were obtained with the microtracer and methyl violet during the course of repeatability trials on previous mixes conducted on industrial sites in 1999 (Tecaliman, 2000a).
Moreover, it is necessary to bear in mind that a proportion of trace elements (manganese, copper and selenium) are supplied by the feed's raw materials. Consequently, of the 300 ppm of manganese analysed at the end, approximately 16 % is provided by the feed. For copper, the proportion provided by the feed is approximately 5.5 % and for selenium, almost 11 %. These proportions in the feed, which are significant for selenium and, above all, for manganese, make it hard to interpret the dispersion of these products, as the effect of the mixer relates to both the additive and one or more raw materials making up the feed.

4.2. Repeatability and homogeneity of mixes

The results appear in Table 5. Three tracers were added to the three mixes at similar concentrations (200 to 300 ppm), which permits a direct comparison of variances. The distributions obtained show that, of these three products, only the external tracer enables significant variations between samples to be identified after each mix, even though these are minor (inter-sample variation is significant where the F calculated is greater than 2.13 for the microtracer and 3.02 for the other products). This appears to tally with the quite low and similar residual variances obtained from one mix to another for this product. Those for manganese are only slightly higher, which enables significant variations between samples to be obtained in the case of mix 3. However, the residual variances for meticlorpindol are variable from one mix to another.
$CV_{homogeneity}$ values obtained in the case of 4 significant variations (microtracer and manganese) are between 2.1 and 3.7 % and CV_{total} values under the same conditions, are between 2.7 and 4.4 %.

Tracers		Recovery rates (%)	Variances					Coefficients of variation		
			Total	Res.	Hom.	F	Signif.	Total	Res.	Hom.
Mix 1	Microtracer	106.7	136.3	40.0	96.3	5.82	Yes	4.4	2.4	3.7
	Manganese	100.4	91.7	48.6	43.1	2.78	No	3.2	2.4	2.2
	Meticlorpindol	118.4	26.4	25.2	1.2	1.09	No	2.2	2.1	0.5
	Avilamycin	125.4	46.6	11.6	35.1	7.07	Yes	15.1	7.5	13.1
	Monensin	92.8	99.8	138.5	-38.7	0.44	No	8.8	10.4	0.0
	Copper	94.6	121.7	37.1	84.6	5.57	Yes	6.1	3.4	5.1
Mix 2	Microtracer	106.4	51.7	21.5	30.1	3.80	Yes	2.7	1.7	2.1
	Manganese	101.6	34.7	49.1	-14.4	0.41	No	2.0	2.3	0.0
	Meticlorpindol	110.5	136.7	162.1	-25.4	0.69	No	5.2	5.7	0.0
	Flavophospholipol	146.6	1.4	1.6	-0.1	0.86	No	29.0	30.0	0.0
	Maduramicin	90.3	0.2	0.2	0.0	1.02	No	9.3	9.3	1.0
	Selenium	91.1	0.0	0.0	0.0	2.95	No	15.5	11.0	10.9
Mix 3	Microtracer	109.1	87.7	37.0	50.7	3.74	Yes	3.4	2.2	2.6
	Manganese	104.3	94.2	39.2	55.0	3.80	Yes	3.2	2.0	2.4
	Meticlorpindol	115.4	57.4	57.1	0.4	1.01	No	3.3	3.2	0.3
	Oxytetracycline	110.0	1763.6	428.2	1335.4	7.24	Yes	7.6	3.8	6.6
	Salinomycin	97.9	7.9	5.3	2.7	2.02	No	4.7	3.9	2.8
	Lasalocid	95.0	52.8	37.5	15.3	1.82	No	5.4	4.6	2.9

Table 5: Results of variance analyses carried out on all the tracers used in the three mixes

Leaving aside the significance of the differences between samples, for all three of these tracers together, the mixer would be assigned CV_{total} values ranging from 2.0 to 5.2 %. This highlights the fact that taking account of the effect of the analytical procedure results in the range of variation on the two sides of the range being limited.

4.3. Mixing behaviour of products

Of these nine additives tested once, only three produce significant variations between samples: avilamycin, copper and oxytetracycline. However, the $CV_{homogeneity}$ values obtained are the highest: 13.1, 5.1 and 6.6 % respectively. These results appear to demonstrate that the mixer is less able to distribute these products than others.

Conducting duplicate analyses reveals that significant variations between samples are only obtained in 7 distributions out of 18. In other words, in 61 % of cases (11/18), the total variation observed cannot safely be attributed to actual differences between samples and, therefore, to the mixer's performance.

5. Conclusions

If the observation of results only related to the CV_{total} values, performances ranging from 2.0 % to 29 %, depending on the tracer used, could be attributed to the mixer.

However, it is certain that a CV of 29 % would inevitably lead to this instrument being called into question while a CV of 2 % would not elicit any comments. This clearly illustrates that the choice of tracer is a key stage in monitoring a mixer's performance.

With the aim of testing a mixer's performance, the following products cannot suitably be selected as tracers, within the framework of current data:

- Meticlorpindol
- Monensin
- Flavophospholipol
- Maduramicin
- Selenium
- Salinomycin
- Lasalocid
-

Manganese does not provide any guarantee of obtaining a significant variation, as this is only obtained in one of three cases. Moreover, a significant proportion of this manganese originates from the feed's raw materials and, therefore, is distributed "naturally" in a homogeneous manner. Copper is, in part, in a similar position, as a proportion of it also originates from raw materials. Obtaining a significant variation between samples with this tracer does not provide a guarantee that this would be the case for all mixes. Furthermore, the $CV_{homogeneity}$ value obtained is one of the three highest.

The use of avilamycin does not appear advisable because of considerable analytical variations and the high $CV_{homogeneity}$ value obtained when the other tracers appear to show that the mixer is effective.

For oxytetracycline, the $CV_{residual}$ value is quite low and the significant variation obtained with this product could allow its use as a tracer to be considered. However, measurements taken lead us to believe that the CV_{total} value or the $CV_{homogeneity}$ value that could be obtained with this product could still be higher than those that would be obtained under similar conditions with the microtracer, the only other remaining potential tracer. Consequently, its use remains a possibility if a database enabling the results to be assessed was established and if a comparison between two measurements was possible by systematically using the same product (same supplier and same physico-technical characteristics) from one test to another.

The microtracer alone, even though it is an external tracer, appears to provide all the guarantees of obtaining valid measurements on the basis of:

- its initial absence in the feed constituting the matrix and, therefore, monitoring its distribution only
- obtaining significant variations between the samples, even if these are minor and, therefore, genuine monitoring of the mixer in question's performance.
- obtaining low $CV_{homogeneity}$ values.

Previous tests conducted in an industrial setting (Tecaliman, 2000a), within the framework of the measurement repeatability study, showed that this tracer could magnify heterogeneity by referring to internal tracers. All of these aspects lead to the conclusion that the use of this tracer provides a guarantee of rapidly and effectively detecting possible drift in the feed homogenisation process.

6. Bibliographic references

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