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Technical rules for assessing the Bacteriolytic efficacy of a pellet mill

These technical rules have been drawn up to validate that, under certain conditions, the pelleting process achieves the desired decontamination targets, i.e. a 3-log reduction in the Enterobacteriaceae population. This reduction is considered sufficient to significantly limit the risk of feedstuffs at the pellet mill output testing positive for Salmonella, possibly deriving from suspect or contaminated raw materials.
A Tecaliman-led programme has demonstrated that it is possible to have feedstuffs pelleted under conditions that enable a 3-log reduction in the Enterobacteriaceae population.
The protocol described in this datasheet will be used to check the efficacy of the selected conditions.

1. FOCUS

The aim is to assess the bacteriolytic efficacy of a pellet mill under a set list of treatment conditions.
This will involve demonstrating that, under these test conditions, the pellet mill enables a 3-log reduction in the Enterobacteriaceae population in a given feedstuff.

2. PRINCIPLE

The method consists in:

- Selecting a pellet mill
- Selecting a feedstuff used to produce one batch
- Defining a set of treatment conditions (packing temperature, maximum flow rates, compression rate, etc.)
- Taking sterile samples before and after treatment (pellet mill output)
- Rapid-cooling the samples under sterile conditions
- Processing the samples and enumerating the Enterobacteriaceae on representative test specimens
- Processing and interpreting the results

3. EQUIPMENT AND APPARATUS

3.1. Tracer

This consists in Enterobacteriaceae that are naturally present in the selected feedstuff. The tracer is not tested for Salmonella due to their low prevalence and the lack of a standardised enumeration procedure.

3.2. Batch

This consists in a given feedstuff that is representative of the type of pelleted feedstuff in the pellet mill tested in the worst-case decontamination scenario(s).

Some well-founded examples of worst-case conditions were detailed in a previous article (Putier F., Rouchouse S., 2020. Mieux appréhender la granulation en nutrition animale dans un objectif de décontamination. IAA, 67, May/June, p. 38-40). These examples include:

- lowest compression rates
- large amount of added vapour
- highest flow rates
- formulations with the highest fat content; less "mineral" (Chicken, Turkey, etc.)

There may be more than one worst-case scenario. If there are several situations where pelleting conditions are less favourable to microbial decontamination, each separate situation will have to be tested.

The tested product batches may be made up of several mixer loads provided that the whole forms the "pelleted batch". The tested batches must be free of any traces of drugs and acidifying agents.

3.3. PELLET MILL

The pellet mill must be fully described and identified both during and after the tests. This involves recording the date of the tests, the equipment used and its identification at the plant. Details on the die must be carefully recorded on the testing date to enable long-term monitoring of the results:

- Condition
- Tonnage
- Number of holes and their diameter

- Compression length

Any recent disinfection operations on the line will also have to be recorded.

3.3.1. Measurements

Packing temperature and vapour pressure will be recorded.

Production throughput will have to be determined in relation to the whole batch.

3.3.2. Testing

Samples are taken at specific times defined prior to pelleting the batch. The operator will have to use a stopwatch to indicate sampling times at the pellet mill output.

The **temperature** of the pelleted feedstuff can be measured at any sampling station, using an adiabatic vessel such as a thermos and a mobile temperature sensor.

Current state-of-the-art means it is very difficult to take in-line measurements of pellet temperature at the pellet mill output. The thermos + temperature sensor technique is practical, but only provides a snapshot of the pellet temperature and may be subject to a slight bias.

3.3.3. Sampling

Samples are taken using sterilised plastic or metal shovels, and then placed in sterile Secure-T Stomacher bags with wire mesh closures.

An alcohol-based disinfecting agent is used to clean all equipment in contact with the samples.

Hot, damp samples at the pellet mill output must be rapidly cooled under sterile conditions that, where possible, mimic those found at industrial plants. It is not recommended to refrigerate these samples either before or during their despatch.

Samples can be repacked before being sent to the lab. This step requires the use of in-line riffle splitters and can be performed by the laboratory if it has the necessary equipment and methods. There is also the option of analysing all the samples, but this would increase test costs.

4. Method

4.1. Batch size

The treated batch must enable a representative line production in order to ensure that all the necessary samples can be taken at the pellet mill output.

4.2. Batch number

One treated batch may correspond to one or more mixer loads.

4.3. Sampling

At least four 50-g samples are taken to obtain satisfactory batch representativeness. Tests carried out by Tecaliman have revealed that Enterobacteriaceae contamination is fairly uniform and that 4 samples is sufficient to obtain an aggregate sample of approx. 200g that is representative of the batch, with a confidence rate of at least 95%.

The initial contamination is determined by taking samples at the mixer or molasser outputs. If the "treated batch" is made up of several loads, the 4 samples will be distributed over the loads as a whole.

Post-pelleting contamination is determined by analysing the samples taken at the pellet mill output. Samples can be taken from a sampling station that is further away but will not, in this case, be solely representative of pellet mill efficacy.

Samples taken at the pellet mill output are individually cooled outside the production circuit. This cooling operation must take care to avoid all possible sources of recontamination.

The equipment used to take and treat the samples must be disinfected and dried prior to use. Samples are taken in a product flow, taking care to vary the direction when cutting the flow for each sample. Care should also be taken to avoid soiling the samples with deposits in the area of the sampling point.

Sampling frequency is defined so as to ensure the samples are spread over all the mixed loads, or over the treated batch, during production throughput at rated speed. This means that the throughput time (flow rate) needs to be estimated at each sampling station prior to running the tests.

Spot samples can be taken at each sampling station to obtain data used to interpret the results:

- Initial physical properties of the feed
- Moisture content and Aw
- Durability/hardness/proportion of fines

4.4. Test performance

Preferably, the tests should not be performed on a line that has been used to treat medicated feed or any product likely to impact on bacterial growth. In such a case, at least two batches free of such products will have to be produced in the same silo bin upstream of the pellet mill before conducting the test in order to minimise any carry-over effect.

The temperature of the product at this sampling station can be measured using a portable temperature sensor and an adiabatic vessel such as a “Thermos”.

4.5. Sample treatment and analysis

Increments sampled at a given sampling point can be grouped and split up under aseptic conditions to minimise the number of analyses.

One portion is held in reserve in positive cold conditions (+4°C) at the splitting location.

Each sample undergoes an Enterobacteriaceae enumeration according to standard ISO 21528-2017 (37°C) with a quantification limit of 10 CFU/g for samples at the mixer output, and with a limit of 1 CFU/g for other samples.

4.6. Expression and interpretation of the results

The tests are characterised by two types of result:

- Performance conditions
- Microbiological results

This involves characterising the performance conditions under which the decontamination results were obtained. This makes it possible (i) to demonstrate that the treatments are systematically performed under these conditions (control conditions), (ii) to define the tolerance limits for these conditions and (iii) to decide which corrective measures to deploy to counter any loss of control.

The following data may be useful for interpreting the results and determining the test conditions:

- Batch size (weighing record(s))
- Overall batch flow rate
- Total treatment time (start to finish of the pelleting batch)
- Sampling times
- Controlled packing temperature
- Temperature at the pellet mill output (possibly)
- Vapour pressure

  Die characteristics (see 3.3.)

The following conditions must be recorded at least:

- Date of the test
- Type of feedstuff
- Treatment temperature (Packing)
- Overall batch flow rate
- Vapour pressure
- Die characteristics

Additional parameters can also be used to interpret the results and to plan corrective or preventive measures:

- The feedstuff's initial physical characteristics (grain size, density, moisture content, etc.)
- Temperature at the pelleting output if this differs from the packing temperature
- Hardness and Durability
- Moisture content and Aw at every sampling point

Enterobacteriaceae enumerations will be interpreted based on:

- initial contamination
- minimum 3-log variation in contamination between the time the product enters the treatment process and the time it exits the pellet mill.

Several, non-exclusive, causes may be considered in the event of inadequate decontamination:

- An initial contamination below 3-log that is insufficient for validating decontamination
- Sample preparation/shipping conditions that diminished their representativeness
- An inadequate treatment scale

The bacteriolytic efficacy of the pellet mill is confirmed if decontamination is greater than or equal to 3-log.

5. Conclusion

The test report must include the following at the least:

- test date
- test conditions
- test results
- list of the types of pelleted feedstuffs generally produced on the line under study, and their targeted treatment conditions (die, preparation temperature, flow rate)
- demonstration that the tested feedstuff(s) correspond(s) to the theoretical worst-case scenario on the line under study. This suggests that this interpretation must make it possible to list in the test report all the feedstuffs that would benefit from the pellet mill's bacteriolytic efficacy.