TOP 7

EN ISO 6498 – Animal Feeding Stuffs – Guidelines for Sample Preparation

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SAMPLING

à take a representative lab sample from a lot

SAMPLE PREPARATION

à prepare a representative test sample from a lab (sub) sample

ANALYSIS

à follow the (microscopical, microbiological, chemical) method protocols

EN ISO 6498 – Animal Feeding Stuffs – Guidelines for Sample Preparation

Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 6498 was prepared by Technical Committee ISO/TC 34, Food products, Subcommittee SC 10, and by Technical Committee CEN/TC 327, Animal feeding stuffs in collaboration.

1 Scope

This International Standard specifies guidelines for the preparation of test samples from laboratory samples of animal feeding stuffs including pet foods mostly quoted from Association of American Feed Control Officials guidelines.

The guidelines are overruled by special instructions and regulations for sample preparation demanded by specific analysis methods for feeding stuffs (e.g. ISO, CEN, IEC).

NOTE This International Standard does not include special guidelines for sample preparation for microbiological analysis of microorganisms like yeasts, bacteria and molds, but for microorganisms which are used as feed additives (probiotics) some important aspects to sample preparation are given.

2 Normative References

ISO 6497:2002, Animal feeding stuffs – Sampling

ISO 664:2008, Oilseeds – Reduction of laboratory sample to test sample

Association of American Feed Control Officials Incorporated (AAFCO), Guidelines for Preparing Laboratory Samples, prepared by : AAFCO Laboratory Methods and Service Committee – Sample Preparation Working Group (Nancy Thiex, Lawrence Novotny, Charles Ramsey, George Latimer, Laszlo Torma, Robert Beine), Second Edition March 2003

Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

3 Principle (1)

All the sample preparation steps depend

- on the different properties of the feedstuffs and
- on the parameters to be analyzed.

In every case the special instructions of the analysis methods concerning sample preparation have to be considered !

3 Principle (2)

The sample preparation guidelines describe the procedure for preparing a sample coming to a laboratory (in general with minimum weight of 0,5 kg) to get a homogeneous test sample (with minimum weight of 100g) with the same constitution, with the same composition and without any contamination.

NOTE In some cases the laboratory sample size could be less than 500 g (i.e. standards of feed additives) but statutory regulations have to be followed and in every case the sample size needs to be homogeneous.

3 Principle (3)

In general the whole laboratory sample is reduced in mass and in particle size to obtain one or more test samples for the analysis of stable and unstable parameters, for microscopy analysis and for reserve.

If the analysis protocol and the intended proceeding of the reserve sample permit it, the laboratory sample should be preferably 'pre-grinded' completely at first to an adequate coarse particle size and then mass reduced to ensure homogeneity of the test samples.

3 Principle (4)

From a test portion (of 0,5g up to 25 g and more) for weighing to feedstuff analysis…

representative results

…should be achieved of the laboratory sample and finally of the whole lot from which the sample was drawn.

3 Principle (5)

Therefore all the steps for sample preparation should be done rather quickly, under convenient and very clean conditions so there could be no degradation of sensitive analytes, no contaminations and no oxidation process due to influences of too high temperatures, daylight, air or from residues of apparatus used or from samples prepared before or simultaneously.

Especially contamination from sample to sample should be prevented.

3 Principle (6)

A loss or a change of moisture during sample preparation should be avoided.

In any case it must be taken into account that for official control results have to be corrected (to origin moisture content, 88 % or 100 % of dry mass).

For feedstuffs with a higher moisture content (dry matter < 85 %) partial drying or freeze drying before mass reduction could be necessary.

3 Principle (7)

For feedstuffs with lumps or particle sizes > 6 mm coarser grinding of the whole laboratory sample to a particle size of < 6 mm before mass reduction / subsampling is absolutely necessary.

3 Principle (8)

The samples have to be stored at every stage of the sample preparation under adequate conditions (e.g. at room temperature, refrigerated, frozen, air-tight, light-protected / dark) to maintain their integrity.

NOTE For microbiological analysis all steps of sample preparation have to be done under aseptic conditions; laboratory samples should not be frozen or heated (>40°C) and not set to vacuum or higher oxygen influence.

4 Definitions – concerning sample (1)

lot
quantity of material that is assumed to be of the same production process and represented by sampling according to the rules of regulation (EC) No. 152/2009.

laboratory sample (lab sample)
sample as prepared (from the lot) for sending to the laboratory and intended for inspection or testing.

4 Definitions – concerning sample (2)

test sample
(sub-) sample prepared from the laboratory sample and from which test portions will be taken.

test portion
quantity of material drawn from the test sample (or from the laboratory sample if both are the same).

reserve sample
left material from the laboratory sample where splitted / subsampled test samples are taken away from and where no further particle size reduction is done.

4 Definitions – concerning parameter (3)

parameters

analytes or constituents or microorganisms for which the feeding stuff is to be analysed by microscopic, (micro-) biological- or chemical procedures.

4 Definitions – concerning parameter (4)

stable parameters

analytes or constituents or microorganisms which do not degrade during sample preparation on common handling or storage at room temperature of 20 °C to 25 °C.

4 Definitions – concerning parameter (5)

unstable parameters

analytes or constituents or microorganisms which degrades during sample preparation on common handling or storage at room temperature of 20 °C to 25 °C because they are volatile, degradable, temperature sensitive or sensitive to light, enzymatic degradation or chemical oxidation.

NOTE Stability of parameters in this context refers only to influences of sample preparation to them like e.g. intensive grinding and not to given minimum durability from producers or from labelling e.g. for a feed (additive).

4 Definitions – parameter classification (6)

	Stable parameters		Unstable parameters	Reason(s) for degradation / change
Nutrients	(Crude) protein, fat, ash, fibre		Moisture	Temperature (volatile)
	Starch, sugar, lactose		Ammonia	Temperature (volatile)
	In vitro tests (e.g. gas production, enzyme soluble organic substance)		Organic acids (e.g. lactic acid, acetic acid, butyric acid, citric acid, fumaric acid, formic acid)	Temperature (volatile)
	Minerals (e.g. Ca, P, Mg, Na, K, Cl)		Unsaturated fatty acids	Air oxidation (might be change to short-chain fatty acids)

4 Definitions – parameter classification (7)

	Stable parameters		Unstable parameters	Reason(s) for degradation / change
Feed additives	Trace elements (e.g. Cu, Zn, Mn, Fe, Se, Co)		Vitamins (e.g. vitamin A, C, D, E)	Temperature, UV-light, air oxidation (sensitive)
	Amino acids (e.g. lysine, methionine, tryptophan)		1,2-propandiol, glycol	Temperature (volatile)
	Enzymes (e.g. phytases, none-starch-polysaccharide enyzmes)		Microorganisms like probiotics (e.g. Saccharomyces cerevisiae, Enterococcus faecium)	Temperature (freezing), pressure (sensitive)

4 Definitions – parameter classification (8)

	Stable parameters		Unstable parameters	Reason(s) for degradation / change
Undesirable substances	Heavy metals (e.g. As, Pb, Cd, Hg)		Mycotoxins (e.g. aflatoxin B_1, deoxynivalenol, fumonisins, ochratoxin A, T-2 and HT-2 toxin, zearalenone, ergot alkaloids)	Mold growth and change of mycotoxins possible at room temperature; UV-light (sensitive –aflatoxin B_1)
	Dioxins and PCB-like Dioxins		Drugs, antibiotics, pesticides	Temperature (sensitive)
			Hydrocyanic acid	Temperature (volatile)
Banned substances	Proteins of animal origin		Banned drugs, banned antibiotics	Temperature (sensitive)
(Other) Micro-organisms			Antibiotics, yeasts, bacteria, molds	Temperature (sensitive), dryness (Anaerobiosis), influx of oxygen

4 Example of feed characteristics (9)

For identification and grouping a laboratory sample to the terms and annexes used within these guidelines some examples of animal feeding stuffs characteristics are given in this document.

NOTE Definitions of animal feeding stuffs are given by legislation worldwide. As an example definitions of European directives and for straight feeds in an alphabetical list from a German committee are mentioned within the bibliography.

4 Example of feed characteristics (10)

dry feeds

feed ingredient or complete animal feed which typically contains not more than 15 % moisture.

NOTE Dry feed pellets are an agglomerated dry feed by a mechanical process.

4 Example of feed characteristics (11)

green fodder

edible parts of plants, other than separated grain, that can provide feed for grazing animals or that can be harvested for feeding, including browse, herbage, and mast.

NOTE Generally, the term refers to more digestible material (i.e. what is called pasturage, hay, silage, dehydrated and green chop) in contrast to less-digestible plant material, known as roughage.

4 Example of feed characteristics (12)

silage

forage preserved in a succulent condition by organic acids produced by anaerobic fermentation of sugars in the forage.

roughage

fibrous, coarsely textured parts of plants.

NOTE Examples for fibrous, coarsely textured parts of plants are stovers, straws, hulls, cobs, and stalks.

4 Example of feed characteristics (13)

hay

the aerial portion of grass or herbage especially cut and cured for animal feeding.

haylage

forage preserved in a succulent condition by organic acids produced by anaerobic fermentation of sugars in the forage with about 45 % moisture.

4 Example of feed characteristics (14)

total mixed ration (TMR)

a single mixture of all feed ingredients (forages, grains, and supplements) that is supplied to an animal for a 24-hour period.

NOTE In practice, the 24-hour allotment of the mixture may be offered in one or more feedings.

4 Example of feed characteristics (15)

by-product

product which remaining during process-procedures for the production of ingredients from plant material.

EXAMPLE Dried destillers grains with solubles (DDGS) from fermentation.

oilseed

any seed from which oil is expressed.

EXAMPLE sunflower seeds

4 Example of feed characteristics (16)

mineral mix

supplementary feed that mainly consists of mineral ingredient in either granular, bead, or small pelleted form and which is as entire mix free flowing.

NOTE Mineral pellets are an agglomerated mineral feed formed by a mechanical process.

4 Example of feed characteristics (17)

premixture

uniform mixture of one or more micro-ingredients with diluent and/or carrier.

NOTE Premixtures are used to facilitate uniform dispersion of the micro-ingredients (i.e. vitamins, probiotics, drugs, antibiotics, and/or pharmaceuticals) in a large mix.

4 Example of feed characteristics (18)

canned pet food

feed product for pets which has been processed, packaged, sealed, and sterilized for preservation in cans or similar containers.

semi-moist feed

meat based feed product for pets or aquatic animals that has been partially dried to prevent microbial decomposition.

NOTE The moisture content may range from 15 % to 40 %. The product generally is in the form of strips or cubes and is designed to be stored at room temperature.

4 Example of feed characteristics (19)

dog chew

meat and skin/peel strips that have been completely dried to a leather-like consistency.

NOTE Dog chews are also known as `rawhide bones´.

4 Example of feed characteristics (20)

range cube and alfalfa hay cubes

agglomerated feed formed by compacting and forcing the mix through die openings by a mechanical process.

NOTE The pellets are about 2 cm of diameter and 5 cm of length (+/- 16 cm3) and may contain molasses; this definition applies also to alfalfa cubes (chopped alfalfa hay) larger than the mentioned dimension.

4 Example of feed characteristics (21)

texturized and sticky feed

mix of assorted grains and commercial feed (generally pelleted) all of which has been treated with a coating of molasses.

NOTE Some of the grains may have been steam heated and / or rolled prior to incorporating into the texturized fee

4 Definitions – sample prep procedure (22)

homogeneity

degree to which a property or a constituent is uniformly distributed throughout a quantity of material.

NOTE Homogeneity may be considered to have been achieved in a practical sense when the sampling error of the processed portion is negligible compared to the total error of the measurement system; since homogeneity depends on the size of the units under consideration, a mixture of two materials may be inhomogeneous at the molecular or atomic level, but homogenous at the particulate level; however, uniform visual appearance does not ensure compositional homogeneity.

4 Definitions – sample prep procedure (23)

partial drying

part of the sample preparation procedure for feedstuff samples with a high moisture content (dry mass < 85 %) in which the sample is carefully dried to allow subsequent sample preparation procedures to be applied like particle size reduction by grinding with a mill.

NOTE 1 Partial drying procedure depends on the heat stability of the parameters (e.g. 70 °C ± 10 °C for drugs and antibiotics trace elements and heavy metals).

NOTE 2 Samples for microbiological analysis should not be dried (at temperatures > 40 °C).

NOTE 3 Partial drying can also be done by freeze drying procedure which is a careful drying process in the vacuum in order to sublimate the moisture.

4 Definitions – sample prep procedure (24)

coarse grinding

a firstly grinding step of the whole sample in cases when the laboratory sample contains large lumps or its particle size is high of about 6 mm before mass reduction.

NOTE Coarse grinding finally is a kind of particle size reduction to ensure homogeneity of the laboratory sample for subsampling purposes.

4 Definitions – sample prep procedure (25)

mass reduction

part of the sample preparation procedure to reduce the mass of a laboratory sample by splitting or subsampling it by the use of (stationary or rotary) dividers or by fractional (alternate) shoveling without changing the consistence of the sample.

NOTE After mass reduction all subsamples should have the same properties like the original laboratory sample.

4 Definitions – sample prep procedure (26)

particle size reduction

part of the sample preparation procedure done by chopping, crushing, cutting, blending (homogenizing), macerating, milling (grinding), pressing, pulverizing to obtain a homogenous test sample for further analysis.

NOTE In general the particle size reduction follows the mass reduction step of the sample preparation procedure with different sieve-size-options to ensure integrity of the test sample(s).

5 Considerations to sample prep errors (1)

Sample preparation steps have been shown
to be some of the largest error of laboratory error.

This error, which is generally overlooked, may be much larger than the error in subsequent analytical procedures.

5 Considerations to sample prep errors (2)

5.1 Subsampling and other errors

Errors deriving from sample heterogeneity may add to the total subsampling error on two levels:

5.1.1 Constitution heterogeneity

5.1.2 Distributional heterogeneity

5 Considerations to sample prep errors (3)

5.1.1 Constitution heterogeneity

On a first level this is a result when not all of the particles of the laboratory sample have the same composition (shape, size, density, etc.).

If a great overall composition-wise difference between the individual fragments exists, the constitution heterogeneity is large, but if the fragments are more homogeneous constitution heterogeneity is lower. The total contribution to heterogeneity is never zero, however, as that would be the case of all fragments being strictly identical.

5 Considerations to sample prep errors (4)

5.1.1 Constitution heterogeneity

Mixing and blending does not change constitution heterogeneity.

The only way to alter the constitution heterogeneity of any given material would be by cominution (crushing/cutting) or by other methods changing the physical properties of a sample. The reduction of the average grain-size is the dominating factor in reducing constitution heterogeneity by such means.

5 Considerations to sample prep errors (5)

5.1.1 Constitution heterogeneity

Therefore a firstly coarse grinding ('pre-grinding') of the whole laboratory sample before subsampling/splitting reduces constitution heterogeneity.

This fundamental subsampling error can be controlled by the appropriate choice of the test sample mass.

Therefore collect enough mass to ensure that all of the particles of different composition are contained in the subsample/split.

The larger the particle size of a material the larger the mass of the subsample must be to minimize error.

5 Considerations to sample prep errors (6)

5.1.2 Distributional heterogeneity

On a second level this is the non-random distribution of particles in the sample, results mainly from the forces of gravity to particles of different densities, sizes and shapes which leads to a grouping and segregation of all particles.

Particles with large differences in size and/or density tend to segregate or stratify heavily, with the smallest and/or densest particles at the bottom of the sample.

5 Considerations to sample prep errors (1)

5.1.3 Other errors

Other errors that arise from sample preparation include losses and gains in analyte concentration from such mechanisms as grinding, excessive heat, loss of fines, contamination, electrostatic separation, etc.

These errors can be large and are usually a result of carelessness or lack of knowledge.

5 Considerations to sample prep errors (1)

5.2 Minimum mass

To properly represent a laboratory sample, the subsample or split must contain adequate mass with view to fundamental subsampling error and maximum particle size.

The amount of mass required depends on the acceptable error in the subsample or split, on the density, heterogeneity and concentration of the analyte particles, and on the largest particle size.

(Calculations with 3 examples - see Annex B)

5 Considerations to sample prep errors (1)

Table 1 — Minimum mass: expected relative standard deviation (RSD) from laboratory subsampling; assumed density = 1 g/cm³

FSE (expected RSD) maximum particle size (d)	15 %	10 %	5 %	2 %	1 %
0,5 mm	0,06 g	0,13 g	0,5 g	3 g	12,5 g
0,75 mm	0,2 g	0,4 g	2 g	10,5 g	42 g
1 mm	0,4 g	1 g	4 g	25 g	100 g
2 mm	4 g	8 g	32 g	200 g	400 g
5 mm	56 g	125 g	500 g	3 130 g	12 500 g

NOTE For materials with densities other than 1 g/cm^3, this table can be modified by multiplying the entries by the density of the material of interest; for example the subsampling of a material with largest particle size of 2 mm, a tolerable subsampling RSD of 5 % and a density of 0,5 g/cm^3 would require 16 g.

5 Considerations to sample prep errors (1)

5.3 Errors associated with splitting techniques

The data in Figure 2 and Table 3 demonstrate the error associated with various splitting techniques:

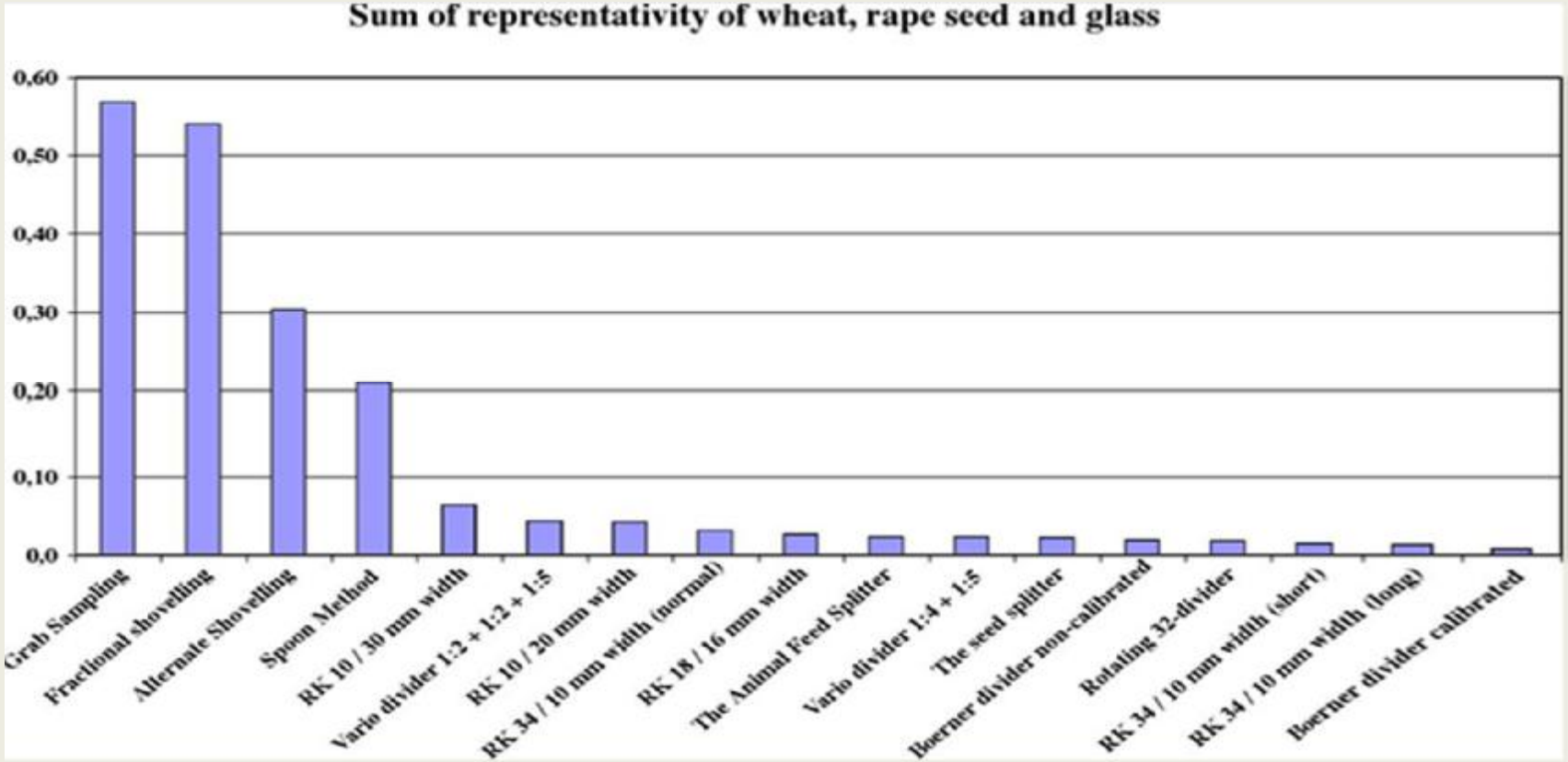
Figure 2 for a model mixture of wheat, rape seed and glass

Table 3 for a model mixture of sand particles

(60% coarse sand with 40% fine sand)

5 Considerations to sample prep errors (1)

5.3 Errors associated with splitting techniques - Figure 2



5 Considerations to sample prep errors (1)

5.3 Errors associated with splitting techniques -Table 3

Coarse+fine sand (60/40) **à** Splitting method A)	number of Increments	standard deviation (s_r) of samples, %	variance $(s_r)^2$, $(\%)^2$	estimated maximum sample error, %
coning & quartering	2	6,81	46,4	22,7
stationary riffling	10 to 12	1,01	1,02	3,4
rotary riffling	> 100	0,125	0,016	0,42
random variation		0,076	0,0058	0,25

A) stationary rifflers with a higher number of increments and less subsampling error are available [6]

6 Safety precautions (1)

The mills for crushing, cutting and grinding have sharp moving blades.

- Never put hands or fingers past the edges of the introduction chamber.
- Never open the mills until they have completely stopped.
- Check to see that safety interlocks on all equipment are operating properly.

Wear appropriate personnel protective equipment as required in the laboratory. Safety is of great importance during the sample preparation phase of the analysis.

6 Safety precautions (2)

Operate the dust ventilation system during dust generation procedures.

To minimize dust, use a vacuum cleaner to clean the hood area, mills, and work area.

Check that all electrical equipment is properly grounded and maintained.

Do not place metal item or aluminum foil in the microwave oven when using it for drying samples.

7 Equipment (1)

7.1 Equipment for sample preparation in general

- Brushes for cleaning grinders, etc.
- Compressed air blower for cleaning
- Vacuum cleaner
- Systems for germ reduction of mills for disinfection and flame treatment

7 Equipment (2)

7.2 Drying systems

- Lyophilization system, forced-air drying oven set at 55 °C ±5 °C and / or microwave oven, household type and / or vacuum oven
- Moisture dish (pan) made of plastic, aluminum, glass, e.g. with • 50 mm diameter, • 40 mm deep

7 Equipment (3)

7.3 Equipment for mass and particle size reduction of 'wet' feeds (e.g. forages, silages)

- Garden pruning clippers for cutting forages or a paper cutter for small sample volumes or a laboratory forage chopper for large volumes and a ceramic cutter especially when of trace elements are of interest
- Cutting mill with 6 mm and 1 mm screens
- Shearing type mill with forage head and 1 mm screen
- Riffle sample splitter, the minimum chute width is to be at least two times the diameter of the largest particle + 5 mm
- Sterile cutter and/or fumigated mill when microbiological analysis of probiotics is of interest

7 Equipment (4)

7.4 Equipment for mass and particle size reduction of 'dry' feeds (e.g. cereals, mineral mixtures, pelleted feeding stuffs)

- Riffle splitter
- Rotary splitter with vibratory feeder
- Shearing grinding mill equipped with 1,0 mm, 0,5 mm and < 0,5 mm – sieves
- Cutting mill with 4 mm to 6 mm screen
- Shearing blending mill (e.g. household coffee mill)

7 Equipment (5)

7.5 Equipment for the storage of samples

- Sterile bottles with air-tight lids (e.g. brown glass bottles for unstable parameters like vitamins) and especially for microbiological purposes
- Wide mouth bottles with screw cap, plastic
- Plastic bags with low germ content, airtight lockable or for setting to vacuum for microbiological purposes
- Refrigerator
- Freezer

8 Procedure (1)

After registration and a check including temperature of a laboratory sample (see 8.1) the homogenization procedure consists in a mass reduction step (see 8.2).

8 Procedure (2)

In the second step the particles in the test samples are reduced to adequate sizes to minimize the subsampling error that arises when the test portion is taken from the test sample. Particle size reduction should be performed without deteriorating the integrity of the substance to be analysed (see 8.3).

8 Procedure (3)

For feedstuffs with higher moisture content (dry mass < 85%) firstly a partial drying below 55 °C - 60 °C could be necessary to grind a subsample by a mill to particle sizes of 1,0 mm for analysis of stable analytes (see 8.4).

8 Procedure (4)

For feedstuffs containing lumps or consisting of particle sizes > 6 mm, firstly a grinding or a chopping to particle sizes below 4 mm to 6 mm could be necessary before subsampling is possible (see 8.5).

8 Procedure (5)

For some fatty and/or sticky feedstuffs (e.g. oilseeds, pet foods, molasses block feed) special sample preparation procedures are useful/necessary (see 8.6).

Finally the samples are stored (see 8.7).

8 Procedure (5)

NOTE Samples taken for routine analysis by NIR should reflect the sample preparation carried out to derive the calibration. By its very nature, NIR requires minimal or no sample preparation and is often used to analyze samples that are either fresh or have been dried and only coarsely chopped. However, when building a calibration it should be recognized that, because spectra may be collected and averaged over large samples it may be necessary to dry, finely grind, and then reduce in mass using a splitter to obtain a subsample suitable for reference analysis. Although the spectra represent an average of a larger sample than used to obtain the reference value this is acceptable practice.

8.1 Sample check

Firstly the laboratory sample will be registered and uniquely identified (e.g. with a unique code number).

Before starting the proper sample preparation procedure some laboratory sample checks have to be done:

- Check of sample constitution
- Check of feeding stuff properties
- Check of substances to be analyzed

8.1.1 Check of sample constitution (1)

Check of sample constitution

- When arriving to the laboratory the sample should have no damages and still should be cooled or frozen if necessary (check of temperature).
- Furthermore the sample protocol should be in accordance to the received sample and all information concerning the sample should be available and complete.

8.1.1 Check of sample constitution (2)

- Deficiencies (e.g. no information about the type of feeding stuff, container of the laboratory sample is opened, the sample protocol does not fit to the sample container) should be documented and subsequently reported to the principal. If possible, the deficiency shall be corrected.

8.1.1 Check of sample constitution (3)

- When this is not possible, and the observed deficiency might affect the analytical result (e.g. when there is not enough sample mass or when there is already mold in the laboratory sample because of too high moisture content or because the sample was not sufficiently cooled during the transport to the laboratory), another sample from the same lot is necessary.

8.1.2 Check of feeding stuff properties (1)

The laboratory sample should be identified for grouping to the feeding stuffs cited within the definitions and categories (see 4.3).

8.1.2 Check of feeding stuff properties (2)

The moisture content of a laboratory sample determines the initial steps of sample preparation:

`wet samples´ with higher moisture content (dry mass <85%) should be prepared as soon as possible or stored at low temperatures, otherwise deterioration starts.

8.1.2 Check of feeding stuff properties (3)

For forages with moisture contents too high for direct grinding (dry mass < 85 %) the whole laboratory sample should be chopped to pieces of about 1 cm.

If necessary, the laboratory sample is subsampled by alternate shovelling and subsequently partially dried.

The above is recommended for stable analytes and for the whole or at least remaining sample for mycotoxin analysis.

8.1.2 Check of feeding stuff properties (4)

For unstable (volatile) analytes (e.g. organic acids, ammonia, hydrocyanic acid, and for GMOs, organic residues) just as for microbiological analysis it is recommended to analyze a test sample as such, without previous drying of the sample.

Alternatively, vacuum drying at low temperatures or freeze-drying could be performed when samples are to be analyzed for non volatile components.

8.1.2 Check of feeding stuff properties (5)

For 'dry feeding stuffs' composed of lumps or with particles larger than 6 mm firstly a coarser grinding of the whole sample (e.g. with a jaw crusher) to particles sizes of 4 mm to 6 mm is recommended before mass reduction / subsampling is initiated.

8.1.3 Check of substances to be analyzed (1)

The number of test samples depends on the analytes to be done.

For the analysis of stable substances, unstable analytes and for microscopy analysis and for microbiological analysis separate test samples should be prepared. The remaining sample is used as reserve (backup).

This enables that for stable analytes the test sample could be reduced to adequate particle sizes at once and subsequently stored at room temperature until further analysis.

8.1.3 Check of substances to be analyzed (2)

Test sample for unstable analytes should be stored at low temperatures and, in order to prevent degradation, they should only be reduced to adequate particle size at the day of analysis (and not long in advance).

For testing the composition of feeding stuffs by microscopy and for (microbiological) analysis of probiotics it is important that no particle size reduction by grinding (milling) is done. Test samples for analysis of probiotics should not be frozen only refrigerated (4 °C to 10 °C).

For mycotoxins and analysis of GMOs by PCR if possible the whole laboratory sample or at least the greater part of the remaining laboratory sample should be used for particle size reduction and then subsampled if necessary.

After mass reduction, in general test samples for stable and unstable analytes of the following particle size classes should be prepared under adequate (temperature) conditions: see table 7 and table 8.

8.1.3 Check of substances to be analyzed (3)

For mycotoxins and analysis of GMOs by PCR if possible the whole laboratory sample or at least the greater part of the remaining laboratory sample should be used for particle size reduction and then subsampled if necessary.

After mass reduction, in general test samples for stable and unstable analytes of the following particle size classes should be prepared under adequate (temperature) conditions: see Table 7 and Table 8.

8.1.3 Check of substances to be analyzed (4)

Table 4 — Recommended particle sizes of test sample(s) for stable analytes and for microscopy

1,0 mm	nutrients (e.g. crude protein, crude fat, crude ash, crude fibre, sugar, lactose) if not grinded with a sieve size of 0,5 mm and minerals, trace elements, heavy metals if not grinded with a sieve size of 0,5 mm or 0,1 mm
0,5 mm	starch, amino acids, methionine hydroxy analogue (MHA)
0,1 mm	in mineral mixes for minerals, trace elements and heavy metals (see EN 15510 : 2007)
no grinding	microscopy analysis (e.g. composition) or NIR/NIT analysis or NMR oil analysis or phytase activity (if not grinded with a sieve size of 1 mm)
cutting to pieces of 1 cm, followed by a reduction to 0,5 mm or 1 mm	in forages for analysis of the corresponding test sample

8.1.3 Check of substances to be analyzed (4)

Table 5 — Recommended particle sizes of test sample(s) for unstable (degradable, volatile, heat-sensitive, microbiological) analytes

1,0 mm	in dry feed for moisture, vitamins, organic acids, propandiol, organic residues like PCBs, OCDs, other pesticides, banned antibiotics, veterinary drugs and mycotoxins
0,5 mm	in dry feed for mycotoxins because of non-uniform distribution within a (laboratory/test) sample if not grinded with a sieve size of 1,0 mm
coarse grinding	for cereals and pressed feeds which do not break down sufficiently in suspension solutions for microbiological analysis
no grinding but soft treating by solving under light pressure	in dry feed - pelleted or as meal - for microbiological analysis (i.e. probiotics)
no grinding	in mineral mixes and premixtures for vitamins, antibiotics, drugs and probiotics when the particle size distribution is sufficient, otherwise grind briefly to 1,0 mm to avoid heat generation
no grinding but cutting to pieces of 1 cm	in forages for analysis of the corresponding test sample with origin moisture content of organic acids, ammonia, hydrocyanic acid, carotene, bacteria, yeasts and moulds
no grinding but macerating with a thermo mixer	in forages for organic residues like pesticides, banned antibiotics, veterinary drugs

8.2 Mass reduction (1)

Laboratory samples can be mass reduced by splitting devices or subsampling.

Mass reduction with the use of rotational dividers or riffle splitters is recommended and the techniques can be used for the reduction of the 100 g test sample to < 1,0 g test portions without serious problems.

8.2 Mass reduction (2)

If it has been established that the mass reduction error is insignificant or if it is not possible to mass reduce with correct mass reduction devices (i.e. rotary or riffle splitters), the mass reduction can be accomplished with subsampling.

With subsampling, anywhere from a single increment to as many as several hundred increments are selected at random from the primary sample to form the subsample.

8.2 Mass reduction (3)

Unfortunately, it is a common practice to take only a few increments. If only a small number of increments are selected, there can be very large subsampling errors due to sample heterogeneity.

The number of increments should not be determined by what is easy, but rather what is acceptable from an error point of view.

Rotary riffle splitting is the most accurate splitting method, coning & quartering is a very poor method and should never be used.

8.2 Mass reduction (4)

If it is known that the material is not segregated, then fewer than 10 increments can be selected. If the material is known or suspected to be heavily segregated, then more than 10 increments should be selected.

Many materials have a wide range of particle sizes when they arrive to the lab and need more increments to properly represent them (consider more than 10 increments).

During the sample preparation of grinding and sieving, the range of particle sizes is reduced and fewer increments can be taken.

8.2 Mass reduction - devices (1)

Riffle splitters

Criteria for the design:

- even number of chutes;
- greater number of chutes is desirable;
- rifflers must be from durable, inert materials (e.g. stainless steel);
- rifflers with bent chutes or any defects must never be used.
- ...

8.2 Mass reduction - devices (2)

Riffle splitters

Criteria for the proper use:

- the riffler should be on a firm, level plane;
- do not feed the sample too fast (the chutes can fill up and overflow)
- fine powders should be fed with care, as they can clog the chutes;
- fines may stick to the splitter due to static electricity; if this occurs and measurement of the fines is important, you may try grounding the riffler or using an anti-static mat.

8.2 Mass reduction - devices (3)

Rotational dividers

Criteria for the design:

- should be made of inert material;
- cutting edge should be radial from center (pie shaped);
- maintain constant speed;
- minimize drop from feeding chute to cutting edge to prevent dust formation;
- fine powders should be fed with care as they can clog the holes.

8.2 Mass reduction - devices (4)

Rotational dividers

Criteria for the proper use:

- use a vibratory feeder to feed the material into the rotary splitter; hand feeding results in uneven feeding rates and therefore non-uniform incremental splitting;

8.2 Mass reduction - devices (5)

Rotational dividers

Criteria for the proper use:

- adjust the feeder rate so that the material flows through the feeder at a continuously slow even rate without overflowing into the rotary splitter; each split (bottle) should contain around 200 increments per split. 50 increments is the recommended minimum of increments per split (bottle); a slower feeding rate results in more increments per split (bottle) and therefore in a more representative subsample;

8.2 Mass reduction - devices (6)

Rotational dividers

Criteria for the proper use:

- after splitting each bottle should contain an equal volume of material; if the volumes are not equal, this indicates that one or more of the splitting chutes became clogged; when the volumes are not equal, then all of the material needs to be recombined together and resplit again.

8.2 Mass reduction - alternate shoveling (7)

It is a very simple splitting technique with following advantages:

- can be implemented in the lab or field;
- does not involve extra equipment (e.g. rifflers);
- has minimal clean up and decontamination requirements;
- any number of splits can be generated;
- has very low sample splitting error.

8.2 Mass reduction - alternate shoveling (8)

The laboratory sample is split into the desired number of samples by collecting increments from the lab sample. The increments from the lab sample are alternately placed into containers or piles to form the split subsamples.

If a sample is to split into two equal subsamples, one split would contain the odd increments and the other subsample would contain the even increments.

If a sample is to be split into three subsamples, the first split would contain increments 1, 4, 7, and so on, the second split would contain increments 2, 5, 8, and so on, and the third split would contain increments 3, 6, 9, and so on.

8.2 Mass reduction - alternate shoveling (9)

NOTE

Towards the end of the splitting process there can be a small amount of fines; it is advisable to reduce the increment size so that the fines are equally apportioned among the splits with at least 10 increments each; if this is not done, it is possible that all fines will end up in only one of the splits.

8.3 Particle size reduction (1)

General methods

- chopping: a material is mechanically cut into smaller parts;
- crushing: applying pressure to fragment larger particles into smaller fragments; specially, variable jaw crushers reduce large, hard samples to 1-15 mm diameter particle size;
- cutting: cutting mills reduce soft to medium-hard and fibrous materials using rotating and stationary cutting knives; reduced size depends on sieves used in combination with mill;

8.3 Particle size reduction (2)

General methods

- blending (homogenizing): materials are broken into smaller parts and blended to make them more uniform in texture and consistency;
- macerating: a soft material is torn, chopped, or cut into smaller pieces;
- milling / grinding: grinding of materials to mechanically reduce particle size is accomplished by cutting, shearing, impacting and attrition using various mills;

8.3 Particle size reduction (3)

Requirements for choosing size reduction equipment

Requirements for suitable size reduction methods differ widely and depend on the sample material.

The equipment should not corrupt the subsequent results of analysis (e.g. cause contamination with trace elements or heavy metals like chrome or nickel from abrasion). Identical results must be achievable in the same lengths of time when the same grinding tools are used.

8.3 Particle size reduction (4)

Considerations for selecting size reduction equipment for a specific application include the following:

- Type of material: How hard is the material? What are the physical and chemical properties? Is the size reduction process affected by heat generation, moisture change or chemical reactions?;
- The initial maximum particle size (e.g. chunks, powder, ...etc.);

8.3 Particle size reduction (5)

- The final desired particle size (mm, µm) and the range of permissible particle sizes;
- Quantity of material to be ground and number of laboratory samples to be processed daily or weekly;
- Amount of time available for size reduction in the overall sample processing;

8.3 Particle size reduction (6)

- Abrasion resistance of the grinding tools: Contamination due to wear of the grinding or cutting elements by the grinding tools is a constant threat and must be absolutely avoided. It is important to select suitable grinding elements that are constructed from materials that will not interfere with the analysis. Typically, particle size reduction tools are made of stainless steel, tungsten carbide, agate, sintered alumina, hard porcelain, and zirconium. A tool with harder surfaces than the lab sample material is desirable and it will minimize contamination;

8.3 Particle size reduction (7)

- Versatility of grinding equipment: Due to the nature of some sample materials, wet grinding may be necessary or the sample must have to be cooled or embrittled during size reduction. Some materials must be ground in an inert atmosphere, with liquid nitrogen, or in a vacuum;
- Requirements for operator time and cleaning equipment. It is impossible to grind laboratory samples without loosing minute amounts of sample because some adheres to the grinding surface. This material is lost during the cleaning.

8.3 Particle size reduction (8)

Types of particle size reduction equipment

Crushers

Reduce particle size by crushing the material. They are generally used to reduce a very large particle size (diameters as large as 150 mm) to 0,5 mm to 1 mm fragments. Other types of mills can then be used to further reduce particle size.

8.3 Particle size reduction (9)

Types of particle size reduction equipment

- Cutting (shearing) mills
- Grinding mills
- Ball mills
- Centrifugal mills
- Disk or burr mills
- Etc.

…..with detailed descriptions!

8.3 Particle size reduction (10)

Maintaining integrity of the laboratory sample

- To minimize moisture loss and preserve the integrity of materials that contain thermally labile or volatile components, heating during the grinding process must be minimized.
- Dry ice sometimes can be added directly to a mortar or ball mill to keep samples cool during grinding (dry ice should be prepared from CO2 that is free from impurities that could contaminate the sample).

8.3 Particle size reduction (11)

Maintaining integrity of the laboratory sample

- Some mills can be fitted with a cooling block to permit the circulation of cool liquid during grinding.
- Pulverizing the material under liquid nitrogen can be performed in a cryogenic mill if lower temperatures are necessary to solidify a material.
- When using cooling agents, it is necessary to avoid condensation of moisture on the material to preserve the integrity of the sample.

8.4 Partial drying (1)

For 'wet' feeds with less than 85 % dry matter (e.g. forages, total mixed rations, but not for liquids), it is necessary to partially dry them prior to fine grinding to analyze stable substances; for unstable substances a partial drying is not possible.

8.4 Partial drying (2)

Partial drying can be done by using either a forced-air oven or a microwave oven. The goal is to dry the feeding stuff while keeping sample temperature below 55 °C – 60 °C so that chemical composition is minimally affected.

Drying at higher temperature (greater than 60 °C) causes chemical changes in the feeding stuff (e.g. protein degradation).

(....)

8.5 Coarse drying

When a 'dry feed' is composed of lumps or its particle sizes are more than 6 mm the whole laboratory sample should be grinded by a jaw crusher or a cutting mill or chopped to particle sizes of 4 mm – 6 mm before mass reduction / subsampling is initiated.

Of course a coarse grinding of the whole laboratory sample to an adequate uniform low(er) particle size before mass reduction is initiated is recommended to ensure homogeneity. But this is a very time consuming procedure and aspects for unstable substances are to consider (e.g. heat generation, grinding at the day of analysis) otherwise deterioration starts.

8.6 Special sample preparation procedures

For samples consisting of high fat, gelatin, and/or molasses some special sample preparation procedures helps to get a representative subsample and fine grinded test samples:

- place the entire laboratory sample in a freezer overnight and prepare the sample in a frozen or chilled state;
- use dry ice during splitting and grinding to keep the sample cold enough to prevent clumping or melting;
- use of blending type mills, blend in intervals of 30 s;
- first coarse grind the whole laboratory sample to pass a 6 mm sieve.

8.7 Storage (1)

Once a representative test sample has been prepared from the laboratory sample, it is essential to maintain its integrity throughout its duration in the laboratory, including all analytical process, reporting of data and the ultimate disposal of any remaining material.

Proper storage may include storage at reduced temperature (refrigeration or freezing), protection from moisture gain or loss, protection from UV light, and so on to avoid the harmful effect of too many microorganisms which can break down the organic compounds.

8.7 Storage (2)

The proper storage conditions will vary among the various types of feed materials and substances which are to analyze.

In deciding upon the proper storage conditions for each material and analyte combination, the laboratories need to consider the effect that composition, matrix interactions, chemical and/or enzymatic activity have on the analyte(s).

Storage and disposal policy should be established and documented within the laboratory to address these issues.

9 Performance tests (quality control)

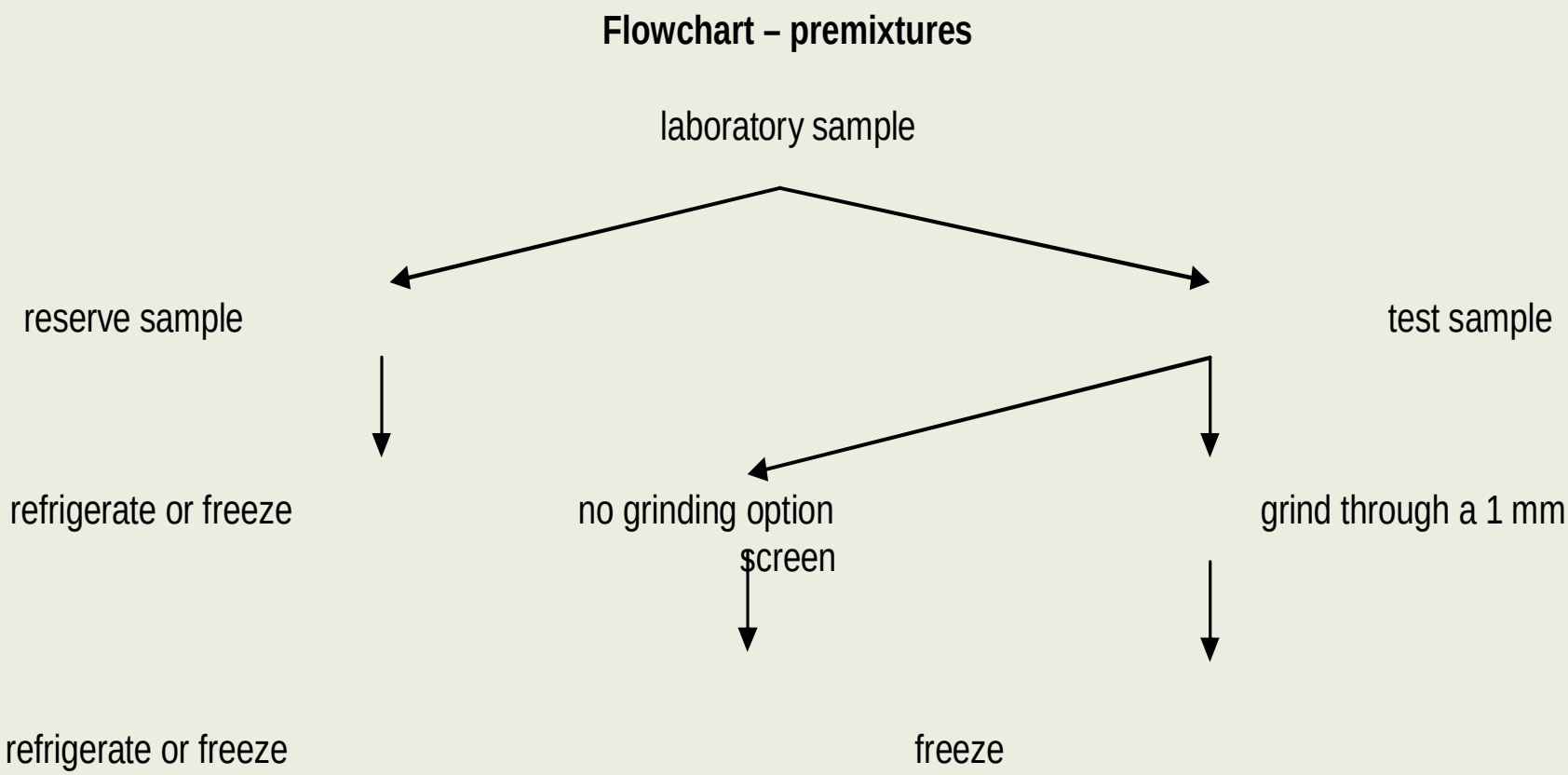
Performance test for mass reduction (splitting)

Performance test for particle size reduction (grinding)

- Grind quality and recovery
- Carryover

Performance test for mixing

10 Flowcharts – e.g. Premixtures



Annex B with Examples to calculate sample preparation error

Table 8 — Minimum mass of a subsample where methionine was added to a mineral mixture

	Minimum sample (g)				
d (mm) / FSE (expected RSD)	*20 %*	*15 %*	*10 %*	*5 %*	*2 %*
0,1	0,002	0,003	0,01	0,02	0,2
0,2	0,01	0,02	0,05	0,2	1
0,5	0,2	0,3	0,8	3	16
1	2	3	6	20	61

Figure 1 — Illustration of definitions concerning 'sample', 'substances', and 'sample preparation procedure'