



S9.75 - Norm for fungal load in feed

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Welcome

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TNO report**TNO 2014 R10671****Norm for fungal load in animal feed**

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1 Introduction

GMP+ International is an independent organization managing the GMP+ Feed Certification scheme, which consists of two modules, GMP+ Feed Safety Assurance (GMP+ FSA) and GMP+ Feed Responsibility Assurance (GMP+ FRA), and is intended for the certification of feed companies active in the feed chain around the world. The GMP+ FSA module has been developed to guarantee feed safety and to reassure consumers about the hygienic way animal feed products are produced, processed, traded, stored and transported. Requirements for feed safety assurance are laid down in the GMP+ standards, which are based on legislation and additional requirements from stakeholders in the market.

One of the criteria of the GMP+ FSAs is that animal feed products that exceed a mould count of 10.000 (10^4) colony forming units per gram (CFU/g) are not allowed directly being delivered to farmers. The norm is implemented as a criterion to reject animal feed batches. The criterion of 10^4 CFU/g, according to the FSAs, is applied to both fresh field crops and crops that still need processing, although processing like ensilaging is known to reduce fungal counts due to oxygen limitations. A situation therefore exists where a company can sell ensilaged products to a farmer, whereas the same product before ensilaging cannot be sold to a farmer while ensilaging occurs normally at farm level. The norm for mould counts by which GMP+ accepts feed crops and products is more stringent than the international guidelines or directives regarding trading of these products. Therefore it is necessary to investigate and objectively evaluate the current GMP+ norm and compare these to the international standard.

A few other issues with regards to the norm for animal crops and feed should be addressed also. At present the norm for maximum mould levels focus on all fungal species and does not distinguish mycotoxin producing from non-mycotoxin forming fungi. Risk assessment of fungal contamination in literature with regards to animal feed and food crops are in general more focused on mycotoxin contamination as is concluded from phase 1 of the project. Thereby the focus of the criteria is specifically on mould and does not set the criteria for yeast. In phase 2 literature and information was evaluated and translated into an advice as to how the current norm and limits for maximum moulds present in animal feed and feed crops can be altered and whether yeast counts should also be taken into account in relation to animal feed safety.

2 Literature assessment

2.1 Risks of fungal contamination in feed

In relation to animal feed and fungal contamination it is usually not the presence of mould or yeast itself that may cause a major health risk. Contamination with non-mycotoxin producing mould species with a maximum level of 1.000.000 CFU/g (10^6) does not lead to high risks in animals, but in some cases can lead to potential energy loss of 5-10% (P. Golob, 2007; Tarr et al., 2006). Mould counts much higher than acceptable in feed ($>10^6/10^7$ CFU/g) only occasionally lead to mycosis. Mould contamination, however, can lead to the production of mycotoxins which can cause severe acute health problems or increase a significant risk to develop disease over time.

Mycotoxin-producing moulds mainly causing problems in feed are *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. (Anonymous, 2009; Anonymous, 2001/2003). The safety control on animal feed is therefore focused globally on the presence and concentration of specific mycotoxins. No clear correlation has yet been observed between levels of the viable counts of moulds and levels of mycotoxin (Tarr, 2006; Whitlow et al., 2008; Garcia et al., 2009). Therefore at present there are no reliable and available predictive models that could calculate or link the health risk in relation to mycotoxins based on the level of mould contamination (Whitlow et al., 2008; Garcia et al., 2009).

2.2 EU regulations

The European Union has not defined maximum levels of mould counts in animal feed. With respect to mycotoxins, maximum levels for Aflatoxin B1 in animal feed were first noted in EU Directive 2002/32/EC¹. In 2011 an amendment on 2002/32/EC became regulation (EC) 574/2011² in which the guideline for maximum levels of Aflatoxin B1 became obligatory for international handling of animal feed within the EU (<http://eur-lex.europa.eu/en/index.htm>). At present, maximum levels in animal feed are only established and described for Aflatoxin B1 since the presence of this mycotoxin in animal feed may also affect human health. For other mycotoxins like Aflatoxin B2, G1, G2 and M1, Ochratoxine A, Patulin, Deoxylivalenol (DON) and Zearalenone no EU regulations have been accepted, but recommendations for maximum levels in animal feed and crops are described in 2006/576/EC³.

¹DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002 on undesirable substances in animal feed

²COMMISSION REGULATION (EU) No 574/2011 of 16 June 2011, amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, *Ambrosia* spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto.

³COMMISSION RECOMMENDATION of 17 August 2006, on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.

2.3 Mould limits outside the EU

With regard to maximum levels of general mould counts, some countries outside the EU show some guidelines but no regulations (Tarr, 2006). In Canada a mould count in feed of 10.000-500.000 (10^4 - $5 \cdot 10^5$) CFU/g is considered being safe. Even animal feed or crops with detected mould viable counts of 500.000-1.000.000 ($5 \cdot 10^5$ - 10^6) CFU/g are still considered relatively safe for farm animals. Only when mould counts exceed 10^6 CFU/g a reduced energy potential of the feed with 5-10% is expected. The FAO and the UN World Food Program have guidelines that indicates a maximum of 100.000 ($=10^5$) CFU/g in dry feed (Tarr, 2006). Also in the US these levels are considered safe (Adams et al., 1993).

2.4 Norm prior processing

Feed crops that function as the basis for animal feed are also analysed for contamination with fungi. Since some feed crops are used for postharvest processing like ensilage, it is likely that the mould viable count will decrease as a consequence of lower pH and lack of oxygen during the ensilage process if the process is optimal (Whitlow et al., 2008). Unfortunately, it would very difficult to set different norms for material used directly or material intended for further processing. Due to the lack of detailed predictive models it is not possible to assess the level of mould contamination in the product unless additional microbiological analysis would have been performed on the end product.

2.5 Norm for yeast contamination

There is no literature or directive that provides information about maximum amounts of yeast in animal feed. Yeast, unlike some mould species, do not produce mycotoxins and therefore do not pose risks in crops or feed to animal health. The only negative effect on health that high counts of yeast may have on animal feed is the potential energy loss of 5- 10% of the nutritional value when counts exceed 10^6 CFU/g as mentioned in §2.1. Another potential risk could be high counts of *Candida krusei* or *Candida albicans* in the product that in some cases may lead to oral and vaginal infections in cattle or mastitis in ruminants (Gaudi et al. 2009). Especially in combination with antibiotic treatment these opportunistic micro-organisms can sometimes cause infection in animals. It is, however, very difficult to prescribe a maximum level of yeasts in wet animal feed like silage products since many yeast species are commonly present during production processes like ensilage.

2.6 Analysis methods

In general, it should be noted that the sampling of animal feed or crops is shown to be very difficult due to the heterogeneous distribution of moulds and toxins throughout the product. In order to determine mould or mycotoxin levels that are representative for the product status it is therefore important to collect multiple samples not just at local spots of the feed volume (Whitlow et al. 2008).

For analysis of the fungal status of feed products various methods can be used:

1) General mould and yeast viability count

The detection methods generally used for enumeration of yeasts and moulds are described in NEN-ISO 21527-1:2008 and NEN-ISO 21527-2:2008. These can be purchased via the NEN website (<http://www.nen.nl>). Method NEN-ISO 21527-1:2008 is in place for feed materials with a water activity (a_w) of less than 0.95. This method can also be applied for materials with a moisture content of 12% or less since a_w in this case will never exceed 0.95. NEN-ISO 21527-2:2008 applies to feed materials with an a_w exceeding 0.95. This method might be applied to feed materials with a much higher moisture content than 12%. In this case the a_w should be checked to verify the correct method.

2) Detection of mycotoxins

The EU describes the laboratory criteria for sampling and detection of mycotoxins in feed in the Commission Regulation (EC) No. 401/2001¹. (<http://eur-lex.europa.eu/en/index.htm>). With regard to the detection of mycotoxins in animal feed, several methods are available. Numerous commercial tests are available that detect mycotoxins qualitatively. The presence of Aflatoxin can also be detected by black-light testing (or UV-Lamp testing) (Anonymous, 2009, Adams et al., 1993). Monoclonal antibodies are also used to detect specific mycotoxins (Anonymous, 2009). Quantitative analysis of mycotoxins can be performed by using High Performance Liquid Chromatography (HPLC) (Anonymous, 2009; <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071435.htm>). This quantitative HPLC method at the moment is the most frequently used method for determination and quantification.

¹ COMMISSION REGULATION (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

3 Conclusions

The criteria and standards as accepted by GMP+ and implemented in their FSAs schedule show a focus on the detection and quantification of mycotoxin as well as mould counts in animal feed and feed crops. EU directive 2006/576/EC¹ and regulation 574/2011/EC² only focus on the levels of mycotoxin in these products in relation to animal health and safety. Thereby Aflatoxin B1 is the only specific mycotoxin that is stated in EU regulation 574/2011/EC, whereas the other mycotoxins like DON, Ochratoxin A, Zearalenon and fumonisins are only stated in EU Directive 2006/576/EC. GMP+ handles maximum levels for these latter mycotoxins as well.

Therefore it can be concluded that there are maximum mould and mycotoxin limits stated in the FSAs that are not applied on an international scale. The FSAs limits are at least as stringent as EU and intercontinental regulations, but often even more strict. This leads to situations where animal feed or crops may be rejected by GMP+ despite the fact that these products could still be traded according to EU regulations.

The presence of yeast in animal feed does not pose a major risk in animal health. However, excessive amounts of *Candida krusei* or *Candida albicans* may cause a mild risk of candidiasis and infections. Thereby yeast, like mould, is a spoilage micro-organism indicating lack of hygiene during processing of dry feed. Therefore a maximum level of yeast in dry food would provide information about the optimal circumstances during production of crops and feed.

Based on these conclusions the norm for animal feed could be altered and might be interpreted or handled in a different way. Recommendations for implementation of alternative norms are described in the next paragraph.

¹COMMISSION RECOMMENDATION of 17 August 2006, on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.

²COMMISSION REGULATION (EU) No 574/2011 of 16 June 2011, amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, *Ambrosia* spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto.

4 Recommendations

For an overview of proposed norm see Appendix I, table 1 and 2.

4.1 Interpretation and alteration of GMP+ FSAs

- Taking into account that EU and intercontinental standards with regards to feed safety are focused on mycotoxins rather than the maximum levels of mould contamination, we conclude that the norm for moulds of 10^4 CFU/g should not be interpreted as a norm for safety. Animal health and safe feeding are not negatively affected by mould counts that do not exceed 10^6 CFU/g. The presence of mould, however, may indicate decreased hygiene during harvest, production, storage or transport. Since mould is a typical spoilage micro-organism, the detection of high mould counts could therefore be handled as a **quality** criterion rather than a **safety** criterion by GMP+. This means that the current norm of 10^4 CFU/g could be increased to 10^6 CFU/g. The quality criteria applied by GMP+ are based on 2005/183/EC¹. Implementing maximum levels of 10^6 CFU/g for mould presence in animal feed would be based on the general requirements for animal feed hygiene and would be in concurrence with Article 4; Article 5 and Article 6 of 2005/183/EC. Based on this regulation the consequence would be that all mould levels in animal feed or crops are accepted in relation to safety. The maximum level of $\leq 10^6$ CFU/g mould count can be applied as a quality and action criterion by GMP+. Companies that show to have mould counts of $\leq 10^6$ CFU/g in their animal crops or feed products are within acceptable hygiene demands. Companies exceeding this maximum level and showing mould counts of $>10^6$ will be summoned to take action in relation to reinsure hygienic production procedures. This action criterion will be applied for all feed materials and products independent of moisture content and destination.
- The maximum levels of Aflatoxin B1 that are stated in the GMP+ FSAs are in line with EU regulation 2011/574/EC². The harmonisation of GMP+ rejection levels of animal feed products should be enforced.
- The maximum levels for mycotoxins DON, Ochratoxin A, T-2, HT-2, Zearalenone and fumonisins that are implemented by GMP+ in the FSAs are not harmonised with the EU directive 2006/576/EC³. This is the case for the parameters that differ between GMP+ and the EU directive 2006/576/EC and also for the maximum levels of mentioned mycotoxins. According to FSAs maximum mycotoxin limits in animal feed or crops are at risk to be rejected by GMP+ while international handling would still be acceptable according to EU guidelines and law. Thereby the possibility exists that 2006/576/EC regulation in the future will be adopted as EU regulation although according to present information from EFSA this is currently not an issue. Taking both arguments into account it is advisable and desirable that GMP+ would implement and harmonise the same parameters and maximum mycotoxin levels that are stated in the 2006/576/EC directive. In general the consequence of this implementation

¹REGULATION (EC) No 183/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 January 2005 laying down requirements for feed hygiene.

²COMMISSION REGULATION (EU) No 574/2011 of 16 June 2011, amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, *Ambrosia* spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto.

³COMMISSION RECOMMENDATION of 17 August 2006, on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.

will lead to a higher maximum level of mycotoxins DON, Ochratoxin A, Zearalenon and fumonisins leading to easier handling within the EU.

- Limits for rye ergot (*Claviceps purpurea*) as described in the GMP+ FSAs are in line with the EU regulation 2011/574/EC¹. Therefore this limit is acceptable and should be enforced.
- The detection and maximum levels of yeast in animal feed or crops are not described by GMP+ nor by EU guidelines or regulations. Some *Candida* species may cause disease in domestic animals. Therefore we propose to harmonise the maximum levels of yeast being present in dry animal feed to be $\leq 10^6$ CFU/g as operational for mould. This could also be adopted as a quality criterion rather than a safety criterion. It is, however, undesirable and impossible to implement this norm for wet feed products. Therefore maximum levels for yeast should become $\leq 10^6$ CFU/g in feed or crops in dry materials corresponding with a maximum moisture content of 12%. Detection can be performed according as described in the next paragraph 4.2 sampling and methods.
- It is undesirable and impossible to set different standards for feed crops before ensilage or other processes. Prediction of quantitative decrease of present fungal is not possible. Therefore we advise not to use a different criterion for animal crops before production and animal feed.

4.2 Sampling & Methods

Mould and yeast count

For the enumeration of mould and yeast GMP+ FSAs still refers to ISO 7954; 1987(E): Microbiology - General guidance for enumeration of yeasts and moulds. The most recent standard for this analysis, however, is NEN-ISO 21527-1:2008 and NEN-ISO 21527-2:2008. We there advise that this should be updated in the GMP+ quality criteria.

Detection of mycotoxins

For detection and quantification of Aflatoxin B1, DON, Zearalenon and Ochratoxin A GMP+ FSAs refers to HPLC methods as well as ELISA and lateral flow analysis. Since there are no EU regulations or guidelines for animal feed analysis these are all common and standard laboratory methods for analysis of these compounds and can be maintained. We do want to mark that EU regulation 2006/401/EC² with criteria for laboratory demands and analysis for sampling and detection of mycotoxins in foodstuff could be a good basis for the GMP+ FSAs.

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6 Signature

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7 Appendix I

To clarify the proposed new norm for mould and yeast in feed all information used from literature, EU regulations 2011/574/EC or EU directive 2006/576/EC is processed in table 1 and table 2.

Table 1: Schedule of the proposed new norm for mould and yeast in feed materials.

Undesirable substance	Products intended for animal feed	Maximum levels CFU/g	Method of determination/analysis	Consequence	Source
Mould	Feed materials ≤12% moisture content or a _w -value ≤0.95	≤10 ⁶ CFU/g	Enumeration NEN-ISO 21527-1:2008	Action limit (quality criterion)	Literature
Mould	Feed materials ≥12% moisture content → a _w -value needs to be checked	≤10 ⁶ CFU/g	Enumeration NEN-ISO 21527-2:2008 (if a _w -value ≥0.95)	Action limit (quality criterion)	Literature
Yeast	Feed materials ≤12% moisture content or a _w -value ≤0.95	≤10 ⁶ CFU/g	Enumeration NEN-ISO 21527-1:2008	Action limit (quality criterion)	Literature
Yeast	Feed materials ≥12% moisture content or a _w -value ≥0.95	none	-	none	Literature

Table 2: Schedule of the newly proposed norm for mycotoxins in feed.

Undesirable substance	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %	Method of determination /analysis	Consequence	Source
Aflatoxin B1	Feed materials Complementary and complete feed with the exception of: Compound feed for dairy cattle and calves, dairy sheep and lambs, dairy goats and kids, piglets and young poultry animals;	0.02 0.01 0.005	HPLC, ELISA, lateral flow analysis	Rejection limit	2011/574/EC

	Compound feed for cattle (except dairy cattle and calves), sheep (except dairy sheep and lambs), goats (except dairy goats and kids), pigs (except piglets) and poultry (except young animals).	0.02			
Rye ergot (<i>Claviceps purpurea</i>)	Feed materials and compound feed containing unground cereals.	1 000	NEN 5393:1999	Rejection limit	2011/574/EC
Deoxylivanelol (DON)	Feed materials (*) Cereals and cereal products (**) with the exception of maize by-products; Maize by-products; Complementary and complete feeding stuffs with the exception of: Complementary and complete feeding stuffs for pigs; Complementary and complete feeding stuffs for calves (< 4 months), lambs and kids.	8 12 5 0.9 2	HPLC, ELISA, lateral flow analysis	Rejection limit	2006/576/EC
Zearalenone	Feed materials (*) Cereals and cereal products (**) with the exception of maize by-products; Maize by-products Complementary and complete feeding stuffs; Complementary and complete feeding stuffs for piglets and gilts (young sows); Complementary and complete feeding stuffs for sows and fattening pigs; Complementary and complete feeding stuffs for calves, dairy cattle, sheep (including lamb) and goats (including kids).	2 3 0.1 0.25 0.5	HPLC, ELISA, lateral flow analysis	Rejection limit	2006/576/EC
Ochratoxin A	Feed materials (*) Cereals and cereal products (**) Complementary and complete feeding stuffs: Complementary and complete feeding stuffs for pigs; Complementary and complete feeding stuffs for poultry.	0.25 0.05 0.1	HPLC, ELISA, lateral flow analysis	Rejection limit	2006/576/EC

Fumonisin B1 + B2	Feed materials (*)		HPLC, ELISA lateral flow analysis	Rejection limit	2006/576/EC
	Maize and maize products (**);	60			
	Complementary and complete feeding stuffs for:				
	Pigs, horses (<i>Equidae</i>), rabbits and pet animals;	5			
	Fish;	10			
Poultry, calves (< 4 months), lambs and kids;	20				
Adult ruminants (> 4 months) and mink.	50				

(*) Particular attention has to be paid to cereals and cereals products fed directly to the animals that their use in a daily ration should not lead to the animal being exposed to a higher level of these mycotoxins than the corresponding levels of exposure where only the complete feeding stuffs are used in a daily ration.

(**) The term 'Cereals and cereal products' includes not only the feed materials listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in part B of the Annex to Council Directive 96/25/EC of 29 April 1996 on the circulation and use of feed materials (OJ L 125, 23.5.1996, p. 35) but also other feed materials derived from cereals in particular cereal forages and roughages.

(***) The term 'Maize and maize products' includes not only the feed materials derived from maize listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in the Annex, part B of Directive 96/25/EC but also other feed materials derived from maize in particular maize forages and roughages.

At GMP+ International, we believe everybody, no matter who they are or where they live, should have access to safe food.

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