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# Categorization of animal feed according to microbiological quality - preferable improvement in the food chain

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**Abstract.** Given the fact that the law on animal feed in Serbia has long been expected and that the current Regulation on the quality of animal feed, which includes microbiological criteria, requires improvement over the years, it is time to choose the best new solutions. The recommendable change that would bring the categorization of animal feed according to more objective and comprehensive criteria is based on the use of the VDLUFA (*Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten*) method. In several European countries, it has become routine, due to the great interest of feed producers and animal breeders in good knowledge of microbiological quality of feed as a guarantee of a wholesome final product. It involves determination of the contents of moulds, yeasts and bacteria while taking into account their potential pathogenicity. Based on the number of microorganisms, divided into seven groups, feed and feedingstuffs are classified into four categories. Classes I to III can be placed on the market, while class IV is not suitable for animal nutrition. More precise, regular determination of microorganisms would also provide a better insight into other common feed-born problems, such as, for instance, the possibility of mycotoxin occurrence.

## 1. Introduction

The link between safe food and feed is now well recognized. In particular, the modern approach to food safety identifies measures to reduce and prevent the entry of hazards in the early stages of the production chain, including primary feed production. It is already well known that a large number of different microorganisms could be present naturally in feed, or could occur as its contaminants [1]. Some microbes are useful and can contribute to feed utilization and animal productivity, or some are purposely added to fight harmful pathogens (examples are probiotics). Nevertheless, feed can also contain undesirable organisms able to affect animal health: bacteria, fungi, viruses, prions, parasites, or their adverse metabolites (toxins and mycotoxins) [2, 3, 4]. EFSA's Panel on Biological Hazards has identified *Salmonella* spp. as a major hazard for microbiological contamination of animal feed, while *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Clostridium* spp. are other possible threats, but animal feed is a far less important source in this regard. However, in terms of transmission of antimicrobial-resistant bacteria or microbial-resistant genes, feed must not be neglected [5].



On the other hand, serious attention must be given even to the presence of saprophytic microorganisms, especially if they are found in large numbers. They can cause organoleptic changes due to the utilization of carbohydrates, and the decomposition of lipids and proteins, thereby reducing the nutritional value of feed. In such situations, changes in the taste and smell of feed are usually evident, and digestive problems occur. Clinically, there is a decline in productivity and impairment of the general health of animals, due to decrease of immunity and weakening of resistance to other hazards [6].

However, complete microbiological analysis is needed to adequately assess feed safety and quality. The Serbian Regulation on the quality of feed [7] prescribes microbiology conditions for feedingstuffs and compound feed in Serbia. However, many comments and questions have emerged since it was published. Due to several professional objections, there has long been a need to amend it. In the European Union, the criteria for microbiological quality of feed are based on the principles of the Codex Alimentarius [8] and are in line with Regulation (EC) No 183/2005 laying down requirements for feed hygiene [9] and Regulation (EC) no 767/2009 [10]. The purpose is to establish a feed safety system for food producing animals that covers the entire food chain, taking into account relevant aspects of animal health and the environment, in order to minimize risks to consumers' health. The HACCP system in the feed industry and the application of good practices contribute to feed safety. However, national regulations can vary, and there are several European countries that have raised the hygienic standards of animal feed based on the VDLUFA (*Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten*) method. It has become routine, due to the great interest of feed producers and animal breeders in good knowledge of microbiological quality of feed, as a guarantee of a wholesome final product [11, 12]. Therefore, this method could be a guideline in the modernization of feed hygiene and feed legislation in Serbia. In addition, mentioning the category of the microbiological quality within the feed declarations would increase the competitiveness of the products on the market.

The aim of this paper is, based on the European experience, to give a description and advantages of the categorization of animal feed according to microbiological quality using the VDLUFA method, as a proposal for improving the Serbian feed system and current feed regulation.

## **2. VDLUFA (*Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten*) method description**

According to the official VDLUFA website, it represents an association of German agricultural analytical and research institutes established with the aim of achieving harmonised professional work, bringing together analytical and research institutes (LUFA), centres for dairy science and vocational education, university institutes, federal and other research and testing centres. VDLUFA's focus is both on the development of methods and on quality assurance in the field of agricultural sciences, in addition to providing a platform for applied agricultural research. The Department of Microbiology of Animal Feed at VDLUFA, since 1981, governed research on a systematic approach to determine microbiological quality according to the contents of moulds, yeasts and bacteria. The method for such categorization of feed is described in Methods book III. Detailed instruction is given within the four standard operating procedures (SOPs):

- SOP No 28.1.1 for enumeration of microorganisms using solid culture media [13] describes media and their recipes. In the main, they are identical to those in ISO standards. The difference is that the sample for bacteria count should be cultured on tryptose agar with triphenyltetrazolium chloride (TTC). After three days of incubation at 30°C, depending on their metabolic activity, bacteria reduce TTC to red formazan. In this way, otherwise colourless colonies become yellow, orange (saprophytes), red or pink (most often contamination indicators). The number of moulds is determined on nutrient agar, dichloran-rose-bengal-chloramphenicol agar (DRBC) and dichloran 18% glycerol agar (DG 18). In contrast to ISO standards, in this method both nutrient agars are used, irrespective of the water activity ( $a_w$ ), while taking into account the counts from the agar on which more colonies of yeasts and moulds are grown. This SOP gives basic procedural rules to determine germ numbers (colony forming

units = cfu) in samples of feed additives, premixtures, single and compound feedstuffs. It describes quality requirements for working equipment and general methodical steps.

- SOP No 28.1.2 for enumeration of bacteria, yeasts and *Dematiaceae* moulds (blackness fungi) in feeds [14] prescribes the counting technique, which is done as described in ISO 21527-1:2011 [15] and ISO 21527-2:2011 [16] and ISO 4833:2014 [17]. Detected colonies can be diagnostically differentiated as indicator microorganisms. In this case, the method provides data with regard to the description of feed quality and unspoiltness as defined by legal terms.
- SOP No 28.1.3 is for identification of bacteria, yeasts and moulds as product-typical or spoilage indicating microorganisms (IM) [18]. The microorganisms, distinguishable on the basis of their colony morphology, microscopic features, and further tests, are identified as 19 product-typical or spoilage-indicating indicator microorganisms. Indicator microorganisms with the same microbiological significance are allocated to seven microorganism groups.
- SOP 28.1.4 for microbiological quality assessment using orientation values [19] describes fundamental rules directing the analysis and evaluation of germ numbers (cfu) of bacteria, yeasts, moulds and *Dematiaceae* (blackness fungi) in feed. It shows whether a feed is unspoiled or has developed signs of degradation in gradual stages compared with normal quality.

### 3. Current vs. VDLUFA approach

Microorganisms naturally colonize feed in various ways. Certain species can be found on plant materials dominating at the time of harvest (collective term: field flora or primary flora). Feedingstuffs of animal origin, on the other hand, show relatively low germ numbers (relict flora) because of processing. Current Serbian regulation on the quality of feed [7] regulates certain pathogenic bacteria in Article 102: *Salmonella*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus* and a category named “other microorganisms” which is not clear and not precise enough. Article 101 gives maximal permitted levels of saprophytic bacteria and yeasts and moulds in feedingstuffs and compound feed. In feedingstuffs of plant origin, the maximum allowed number of mesophilic aerobic bacteria is 12,000,000 cfu/g and yeasts and moulds 200,000 cfu/g. Feedingstuffs of animal origin are also mentioned, although there is another regulation for the hygiene of processed by-products [20], so this information is confusing to interpret. Regarding mash compound feed, regulation [7] provides only partial possibility to distinguish microbiological quality, without any determination of microbial species and based on the number of microorganisms in feed for two categories of animals: “young” (mesophilic aerobic bacteria 3,000,000 cfu/g and yeasts and moulds 50,000 cfu/g) and “adult” (mesophilic aerobic bacteria 5,000,000 cfu/g and yeasts and moulds 200,000 cfu/g). Special values are also prescribed for pelleted mixtures (mesophilic aerobic bacteria 2,000,000 cfu/g and yeasts and moulds 20,000 cfu/g).

The VDLUFA method categorizes feed in accordance with its microbiological quality in a more objective and comprehensive manner, taking into account the type of feed and what animal category it is intended for. It also includes whether the feed mixture is subjected to heat treatment (pelleting) and, most importantly, what species of microorganisms are found in the mash. Therefore, it is crucial whether there are saprophytes or indicator microorganisms, since orientation values for pollution indicators (especially yeasts and *Mucorales* moulds) are lower than for saprophytic microorganisms. Table 1 shows orientation values (OV) by the VDLUFA method [19] as are included in the Croatian regulation on feed safety [21] and previously published by Nesic et al. [22].

**Table 1.** VDLUFA orientation values (OV) for the Quality Class I feed (desirable quality) [19, 21, 22]

Groups of indicator microorganisms (IM)	Mesophilic aerobic bacteria x 10 <sup>6</sup> cfu/g			Moulds x 10 <sup>3</sup> cfu/g		Yeasts x 10 <sup>3</sup> cfu/g	
	I	II	III	IV	V	VI	VII
	<b>Orientation values (OV)</b>						

<b>Feedingstuffs</b>							
Flour and grits from oilseeds extraction	1	1	0.1	10	20	1	30
Oil cake from compression of oilseeds	1	1	0.1	10	20	2	30
Meal and branches, except wheat and rye branches	5	1	0.1	50	30	2	50
Wheat and rye branches	8	1	0.1	50	50	2	80
Corn (grain and wholemeal)	2	0.5	0.05	20	30	5	60
Wheat and rye (grain and wholemeal)	5	0.5	0.05	30	20	2	30
Barley (grain and wholemeal)	20	1	0.05	40	30	2	100
Oat (grain and wholemeal)	50	1	0.05	200	50	2	200
Hay	30	2	0.15	200	100	5	150
Straw	100	2	0.15	200	100	5	400
Silage	0.4	0.2	0.03	5	5	5	1000
Haylage	0.2	0.2	0.01	5	5	5	200
<b>Mash compound feed for</b>							
broilers	3	0.5	0.1	30	20	5	50
laying hens	5	1	0.1	50	50	5	50
piglets	5	0.5	0.1	30	20	5	50
breeding and fattening pigs	6	1	0.1	50	50	5	80
calves	2	0.5	0.1	30	20	5	50
dairy cows and breeding and fattening cattle	10	1	0.1	50	50	5	80
<b>Pelleted compound feed for</b>							
broilers	0.5	0.1	0.05	5	5	1	5
laying hens	0.5	0.5	0.05	5	10	1	5
piglets	0.5	0.1	0.05	5	5	1	5
breeding and fattening pigs	1	0.5	0.05	5	10	1	5
calves	0.5	0.5	0.05	5	5	1	5
dairy cows and breeding and fattening cattle	1	0.5	0.05	5	10	1	5
horses	0.5	0.5	0.01	2	6	1	5
rabbits	0.2	0.2	0.01	1	3	1	2

Quality Class I (desirable quality) includes feed for which it is determined that the number of indicator microorganisms (IM) does not exceed the established orientation value (OV) as given in Table 1. Quality class II (reduced quality) includes feed in which detected number of IM is up to five times greater than the established OV. Quality class III (poor quality) includes feed for which determined number of IM is 5 to 10 times above the established OV. Quality class IV (not acceptable for animal feeding) includes feed for which it is established that the number of IM is 10 times higher than the OV set out in Table 1. Feed placed on the market and used in animal nutrition must meet the criteria for classification into class I to III, in accordance with the parameters laid down in Table 1. Animal feed that is based on parameters from this table listed in grade IV will not be suitable for animal feeding.

**Table 2.** VDLUFA groups of indicator microorganisms (IM) [18]

Group	Significance	IM group	Indicator microorganisms (IM)
Aerobic, mesophilic bacteria	Product-typical	1	Yellow pigmented bacteria <i>Pseudomonas/ Enterobacteriaceae</i> , other bacteria (e.g. coryneform bacteria)
	Spoilage indicating	2	<i>Bacillus</i> spp. <i>Staphylococcus/Micrococcus</i>
		3	<i>Streptomyces</i>
Moulds and <i>Dematiaceae</i> (blackness fungi)	Product-typical	4	<i>Dematiaceae</i> <i>Verticillium</i> spp. <i>Acremonium</i> spp. <i>Fusarium</i> spp. <i>Aureobasidium</i> spp. Other product-typical moulds
	Spoilage indicating	5	<i>Aspergillus</i> spp. <i>Penicillium</i> spp. <i>Scopulariopsis</i> spp. <i>Wallemia</i> spp. Other spoilage-indicating moulds
		6	<i>Mucorales</i> moulds ( <i>Mucor</i> spp., <i>Rhizopus</i> spp.)
		7	All yeasts ( <i>Candida</i> , <i>Rhodotorula</i> )

According to the VDLUFA method, indicator microorganisms (IM) are divided into seven groups depending on the extent to which they can affect the animal health, as shown in Table 2. Thus, in groups 1 and 4 are microorganisms specific for contamination in the field and the number of which decreases with storage. In groups 2 and 5 are microorganisms that multiply in storage, while in groups 3 and 7 are those which might impair animal health. In a separate group are *Mucorales* moulds, which produce plum mycelium in larger quantities than other genera, thus, inhibiting the growth of other moulds, which has to be taken into account in assessing microbiological quality [12].

Although good knowledge of microorganisms growing on different media for their proper identification and distribution into appropriate groups is needed, the advantages of the VDLUFA categorization are encouraging. It is a far more detailed approach than in current Serbian regulation, and which gives a more complete picture of the actual microbiological situation. However, whether such a feed could harm the health of animals or of consumers, or whether it represents a danger to the natural balance in addition to the reduction in quality, can only be examined by a risk analysis for the specific case under review. This could involve, for instance, further examinations, e.g. for pathogens or toxic substances (e.g. mycotoxins). The examination for specific pathogenic microorganisms, such as e.g. *Salmonella*, *Escherichia coli*, *Listeria* and *Clostridium perfringens*, is therefore not the subject of this procedure and has to be done additionally. The assessment of a feed with regard to a risk-free feeding is principally not subject of this operating procedure, but is an indicator of its microbiological quality.

#### 4. Conclusion

Microbiological categorization of animal feed based on VDLUFA method, according to the years of experience within several European countries, could also be beneficial for the assessment of feed in Serbia. It can provide a benchmark in the modernization of feed hygienic standards and feed legislation, while the data on the category of microbiological quality, if mentioned on feed declarations, would increase the competitiveness of the products on the market. This approach also offers the possibility to

reduce and prevent the entry of hazards in the early stages of the food production chain. More precise, regular determination of microorganisms could provide a better insight into other common feed-borne problems, such as the possibility of mycotoxin occurrence. All in all, it would be a positive step forward, therefore, advisable and preferable.

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