

## **ASSESSMENT OF THE MICROBIOLOGICAL QUALITY OF FEED USING THE *VERBANDS DEUTSCHER LANDWIRTSCHAFTLICHER UNTERSUCHUNGS UND FORSCHUNGSANSTALTEN* (VDLUFA) METHOD**

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### Abstract

*Introduction.* The *Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten* (VDLUFA) is an association of German agronomic, analytical and scientific institutes dealing with various types of research in the field of agronomy and the development, adaptation and validation of analytical methods. This association has established an approach for determining the microbiological quality of feed based on the contents of molds, yeasts and bacteria by taking into account not only their total numbers (cfu/g), but also their potential pathogenicity. According to the degree of the potential pathogenicity, microorganisms are divided into seven groups. Based on the number of microorganisms in the seven groups, feed and feedingstuffs are classified into four categories according to microbiological quality.

*Materials and Methods.* This method for categorization of feed is described in *Methods Book III*. Detailed instruction is given within the four Standard Operating Procedures (SOPs).

*Results and Conclusions.* The VDLUFA method of assessing animal feed and feedingstuffs is a far more detailed approach that gives a more complete picture of the actual microbiological status of feed than the approach currently used in Serbia. Feed placed on the market to be used in animal nutrition should meet the criteria for classification into classes I to III, in accordance with the parameters laid down in the method. Animal

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feed that belongs to class IV is not suitable for animal feeding. This method could be a useful guideline in the modernization of hygienic standards for feed in Serbia in the future.

**Key Words:** feed quality, saprophytes, yeasts and molds

## INTRODUCTION

A wide range of microbes occurs naturally on or as contaminants of feed (D'Mello, 2004). Some species can have beneficial effects on feed utilization and animal productivity, or are added to reduce the harmfulness of pathogens (i.e. feed probiotics). However, feed can contain organisms able to negatively affect animal health: bacteria, fungi, viruses, prions, parasites, or their adverse metabolites, toxins and mycotoxins (Nesic et al., 2014; Nesic, 2018). The EFSA Panel on Biological Hazards identified *Salmonella* spp. as the major hazard for microbial contamination of animal feed. *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Clostridium* spp. are other hazards for which feed is regarded a far less important source. In addition, antimicrobial resistant bacteria or antimicrobial resistance genes can be transmitted via feed (EFSA, 2008).

If saprophytic microorganisms are present in large numbers, they can cause organoleptic changes in feed due to the utilization of carbohydrates, as well as the decomposition of lipids and proteins, thereby reducing the nutritional value of feed. In accordance with changes in the taste and smell of feed, consumption by the animals is often reduced, and consequently, this interferes with digestion. Decline in production results, while impairment of immunity and the general health condition of the animals can be noted (Markovic et al., 2010; Pavlovic et al., 2019).

However, for adequate and complete assessment of the microbiological quality of feed, it is necessary to carry out microbiological analysis. Nonetheless, even in the European Union, the criteria for the microbiological quality of feeds are not clearly defined. In Serbia, the Regulation on the Quality of Feed (Serbia, 2010) prescribes the hygienic conditions for feed and compound feedingstuffs, although there has been discussion and a long-standing need for change to this legislation, as several errors have been noted since it was published. Therefore, the VDLUFA (*Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten*) method could be a guideline in the modernization of hygienic standards for feed in Serbia in the future. The VDLUFA method is used in the EU German-speaking countries, Slovenia and Croatia in routine work because of the great interest of feed producers and animal breeders in reliable knowledge of the microbiological quality of feed as a guarantee of a wholesome final product (Gafner, 2012; Zadavec et al., 2015). In addition, including the microbiological quality category on the feed declaration should increase the competitiveness of the product on the market.

The aim of this paper is to give a description and advantages of the VDLUFA method and to indicate the possibility of its implementation in the Serbian feed control system.

## MATERIALS AND METHODS

The VDLUFA is an association of German agronomic, analytical and research institutes dealing with various types of research in the field of agronomy and the development, adaptation and validation of analytical methods. The Department of Microbiology of Animal Feed at VDLUFA has, since 1981, directed research on a systematic approach for determining the microbiological quality of feed based on the contents of molds, yeasts and bacteria. The method for this feed categorization is described in the Methods Book III. Detailed instruction is given within the four Standard Operating Procedures (SOPs):

1. No 28.1.1. (VDLUFA, 2012a) is a SOP for enumeration of microorganisms using solid culture media, which describes the culture media (with recipes) to be used. Basically, they are the same as those in ISO standards. One difference is that the total bacteria count is conducted using 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. This leads to otherwise colorless colonies becoming yellow, orange (saprophytes), red or pink (most often contamination indicators). The number of molds is determined on nutrient agar, dichloran-rose-bengal-chloramphenicol agar (DRBC) and dichloran 18% glycerol Agar (DG 18). Unlike ISO standards, this method employs nutrient agar irrespective of its water activity (*aw*), while also taking into account the values from the agars designed for yeast and mold growth, on which greater numbers of yeast and mold colonies are seen.
2. No 28.1.2. (VDLUFA, 2012b) is a SOP for enumeration of bacteria, yeasts and molds. Enumerations are conducted as described in ISO 21527-1:2011, ISO 21527-2:2011 and ISO 4833:2014.
3. No 28.1.3. (VDLUFA, 2012c) is a SOP for identification of bacteria, yeasts and molds as product-typical or spoilage indicator microorganisms.
4. No 28.1.4. (VDLUFA, 2012d) is a SOP for the assessment of the microbiological quality of feed using orientation values. This instruction describes fundamental rules directing the analysis and evaluation of viable numbers (colony forming units = cfu) of bacteria, yeasts, molds and *Dematiaceae* (blackness fungi) in feeds and feedingstuffs.

To establish orientation values, firstly for important dry single and compound feeds, a study project was supported by the German Federal Ministry for Food, Agriculture and Consumer Protection (BMELV) and trade associations of the animal feed sector, and was accordingly realized by examining routine samples taken during official monitoring of animal feedingstuffs by 13 laboratories. A total of 3277 specimens in 12 feed categories and in 15 German federal states were examined within the framework of this project using the VDLUFA method during January 1994 to February 1996. The data obtained made it possible to specify upper levels (orientation values) of viable numbers for the seven groups of microorganisms. Orientation values were also drawn up for the feed types not covered by the project, if analogies existed,

taking special features of these feeds into account. These results were reported by the Feed Microbiology Working Group of the Feedingstuffs Section of the VDLUFA to the BMELV in 1999. Following supplementary suggestions, an extended report was accepted from the state representatives for feed law and the feed industry in 2002. The existing orientation values for cereals (grain and ground grain) were amended by evaluation of new data obtained during 1999-2010. During 1999-2010, orientation values were derived for additional dry feeds. For the first time, data were also collected and evaluated for moist feeds.

## RESULTS AND DISCUSSION

Feed is naturally colonized by microorganisms in various ways. Specific species of microorganisms dominate plant materials at harvest time (collective term: field flora or primary flora). Feedingstuffs of animal origin, on the other hand, contain relatively low numbers of viable microorganisms (relict flora) as a consequence of processing. According to the VDLUFA method, microorganisms are divided into seven groups depending on the extent to which they can affect the animal's health (Table 1). Thus, groups 1 and 4 contain microorganisms specific for contamination in the field and which decrease during storage. Groups 2 and 5 contain microorganisms that multiply during storage, while groups 3 and 7 contain microorganisms that might impair animal health. The order of Mucorales molds produce plum mycelium in larger quantities than other mold genera, and so inhibit the growth of other molds and have to be taken into account in the assessment of feed's microbiological quality; they are separated in group 6 (Zadravec et al., 2015).

**Table 1.** Distribution of microorganisms into seven groups according to the VDLUFA (*Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten*) method for studying animal feedingstuffs

Groups	Microorganisms
1	Yellow pigmented bacteria ( <i>Erwinia</i> spp.), <i>Pseudomonas/Enterobacteriaceae</i> , saprophytic <i>coryneform</i> bacteria
2	Indicator microorganisms: <i>Bacillus</i> spp., <i>Staphylococcus</i> , <i>Micrococcus</i>
3	Indicator microorganisms: Streptomycetes
4	Saprophytic molds (field molds): <i>Dematiaceae</i> , <i>Verticillium</i> spp., <i>Acremonium</i> spp., <i>Fusarium</i> spp., <i>Aerobasidium</i> spp.
5	Indicator microorganisms (storage molds): <i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Scopulariopsis</i> spp., <i>Wallemia</i> spp.
6	Indicator microorganisms: Mucorales molds ( <i>Mucor</i> spp., <i>Rhizopus</i> spp.)
7	Indicator microorganisms: Yeasts ( <i>Candida</i> , <i>Rhodotorula</i> )

The VDLUFA method categorizes the microbiological quality of feed in an objective manner, taking into account the type of feed and the animal category for which the feed is intended. Also, whether the feed mixture is subjected to heat treatment (pelleting) and what species of microorganisms are found in the mixture are accounted for. The presence of saprophytes or indicator microorganisms is important, since orientation values for contamination indicators (especially yeasts and molds in the

order Mucorales) are lower than for saprophytic microorganisms. Table 2 shows orientation values according to the VDLUFA method as applied in the Croatian feed safety regulation (Croatia, 2016).

**Table 2.** Orientation values for allowed levels of indicator microorganisms to classify Quality Class I animal feedingstuffs according to the VDLUFA (*Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten*) method

Indicator microorganisms (IM)	Mesophilic aerobic bacteria x 10 <sup>6</sup> cfu/g			Molds x 10 <sup>3</sup> cfu/g			Yeasts x 10 <sup>3</sup> cfu/g
	1	2	3	4	5	6	7
<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>Compound feed</b>							
Feed mixtures for							
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 feed</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 2. Quality class II (reduced quality) includes feed in which the detected number of IM is up to five times greater than the established OV. Quality class III (poor quality) includes feed for which the determined number of IM is 5 to 10 times above the established OV. Quality class IV feed is not acceptable for animal feeding and contains feed for which it is established that the number of IM is 10 times higher than the OV set out in Table 2. Feed placed on the market and used in animal nutrition must meet the criteria for its classification into classes I to III, in accordance with the parameters laid down in Table 2. Animal feed that is classified as class IV is not suitable for animal feeding.

By interpreting the microbiology results according to the current Serbian regulation, as shown in Table 3, which gives maximum permitted levels of saprophytic bacteria and yeasts and molds in feed, it is possible to distinguish the quality by taking into account the number of microorganisms and category of animal as “young” or “adult”, without any determination of microbial species. For pelleted mixtures, special values are prescribed (Serbia, 2010).

**Table 3.** Maximum permitted levels of saprophytic bacteria, yeasts and molds allowed in animal feed and feedingstuffs according to Serbian legislation (Serbia, 2010)

Feed and feedingstuffs	Mesophilic aerobic bacteria [cfu/g]	Yeasts and molds [cfu/g]
Feedingstuffs of plant origin	12 000 000	200 000
Feed mixtures for young animals	3 000 000	50 000
Feed mixtures for adult animals	5 000 000	200 000
Pelleted feed	2 000 000	20 000

Although expert knowledge of the microorganisms able to proliferate on the different media is required to properly identify them and distribute them into appropriate groups, the advantages of the VDLUFA categorization method are numerous. It is a far more detailed approach than that in the current legislation, and one that gives a more complete picture of the actual microbiological status of the feeds. EU legislation does not prescribe the exact criteria for microbiological quality of feed, but it does enable each member state to implement their own national standards at the national level. In those countries where the VDLUFA method has already been applied for many years, it has been confirmed that feed producers and animal breeders are invested in better knowing the microbiological quality of animal feed as a guarantee of good product.

## CONCLUSION

Based on the experience of several EU member states in the long-term application of VDLUFA method in their feed safety systems, we conclude that implementation of

this method for assessing the microbiological quality of animal feeds and feedingstuffs would also give positive results in Serbia. It would be a guideline for the modernization of hygienic feed standards in the future, and for the categorization, on feed declarations, of animal feed and feedingstuffs according to their microbiological quality. It seems very likely this would increase the competitiveness of the products on the market.

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### **Authors contributions**

KN - Collection of material and writing; MP - Comparison with actual microbiological methods; SI - Discussion on the topic.

### **Competing interests**

The authors declare that they have no competing interests

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## PROCENA MIKROBIOLOŠKOG KVALITETA HRANE ZA ŽIVOTINJE PRIMENOM VDLUFA METODE

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### Kratak sadržaj

*Uvod.* VDLUFA (*Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten*) je udruženje nemačkih agronomskih analitičkih i naučnih instituta koje se bavi različitim vrstama istraživanja u oblasti agronomije i razvoja, adaptacije i validacije analitičkih metoda. Za određivanje mikrobiološkog kvaliteta hrane za životinje utvrdili su pristup baziran na sadržaju plesni, kvasaca i saprofitskih bakterija, uzimajući u obzir ne samo njihov broj (cfu/g), već i njihovu potencijalnu patogenost. Prema stepenu potencijalne patogenosti, mikroorganizme su podelili u sedam grupa, a na osnovu broja mikroorganizama u određenim grupama, hranu za životinje svrstavaju u četiri kategorije mikrobiološkog kvaliteta.

*Materijal i metode.* Metoda za VDLUFA kategorizaciju hrane za životinje opisana je u knjizi metoda III, a detaljna uputstva data su u okviru četiri standardne operativne procedure (SOP).

*Rezultati i zaključak.* VDLUFA je daleko detaljniji pristup koji daje potpuniju sliku stvarnog mikrobiološkog statusa hrane za životinje. Hrana koja se stavlja u promet i koristi u ishrani životinja mora da ispunjava kriterijume za klasifikaciju od I do III



razreda, u skladu sa parametrima utvrđenim u metodi. Hrana za životinje koja pripada klasi IV nije pogodna za ishranu životinja i ne sme se koristiti. Ova metoda bi mogla u budućnosti da posluži kao smernica u modernizaciji higijenskih standarda za hranu za životinje u Srbiji.

**Ključne reči:** kvalitet hrane, saprofiti, kvasci i plesni