Products for Shaping the Gut Microbiome – Regulatory Opportunities and Challenges

The gut microbiome is meant to influence various compartments, organs and functions of the body. At the same time, the microbiome may be influenced by many factors such as the physiological state and the diet, but also by substances such as medicines. In animals, feed and feed additives can influence the landscape of microorganisms present in the gastrointestinal tract. In diseased animals, specifically designed products may beneficially affect the gut flora. Any of these products have to meet different provisions and fit into regulatory requirements of the legislation in the territory before being placed on the target market. Depending on the product, its intended use and classification, different regulatory pathways for feed materials, feed additives or veterinary medicinal products are defined in the European Union, as described in more detail hereafter. These different regulatory pathways provide different opportunities, but also challenges for the product on the market. Other factors such as quality requirements, distribution channels and market opportunities also have to be taken into consideration for a successful development. This article focuses on the regulatory options available and the different opportunities for such products.

Regulatory Requirements for Feed

One of the simplest ways to influence an animal's microbiome is the diet. Regulation (EC) No 178/2002 defines feed as 'any substance or product, including feed additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals'. Feed materials are products of vegetable or animal origin with the principal purpose of meeting the nutritional needs of animals. Whoever wants to place any feed material on the market has to comply with Regulation (EC) 767/2009 where the EU requirements are stated. In brief, any feed material has to be safe and should not have any direct adverse effect on both the environment and animal welfare, following also the provisions set in Regulation (EC) 178/2002. Furthermore, any feed product has to be sound, genuine, unadulterated, fit for its purpose and of merchantable quality, as per Regulation 767/2009, and has to have a nutritional value. The characteristic of the production of feed has to be simple, limited to include low-end extraction processes. The feed business operator selling the feed has to reside within the European Union. Any claim associated with the new product has to be substantiated, either by own research or based on scientific literature. Regulation (EU) No 68/2013 defines the catalogue of feed materials, listing a description and compulsory declarations for all nutritional ingredients currently accepted as feed in the EU.

The best-known representatives of microbiome-influencing feed materials to date are prebiotics, for example fructans and oligosaccharides. These selectively fermented fibres influence specific changes in the composition and activity of the gastrointestinal microbiota, thus conferring benefits upon maintaining or supporting host health. They pass the first part of the gastrointestinal tract unaffected by gastric acid or enzymes, but can be fermented by microorganisms present in the intestine[1]. Typical examples for fibres considered as prebiotics listed in the catalogue of feed material are barley and pea fibre.

Feed is certainly the easiest, fastest and most cost effective regulatory pathway to the market (see Table 1 and Table 2). However, there are certain limitations as health claims cannot be made for such products influencing the microbiome and the product has to fall under the definition of feed; the manufacturing must be simple and may not include any complex process and there must be a nutritional purpose of such feed.

	Veterinary Medicinal Product	Feed Additive
Legislation	VMP: Directive 2001/82/EC as amended, from 28Jan2022: Regulation (EC) 2019/6. Medicated feed: Regulation (EC) 2019/4	Regulation (EC) 1831/2003
Administrative documentation	SPC, product literature, critical expert reports	Label
Quality	GMP, fully validated manufacturing process, stability, validated analytical methods	Feed manufacturing process (FAMI-QS), validated analytical methods
Safety	Tox package, target animal safety, user safety, environmental safety, consumer safety	Tox package, target animal safety, user/**worker** safety, environmental safety, consumer safety
Efficacy	Dose finding, dose confirmation and field studies	3 efficacy studies *in vivo*, if claim for zootechnical FA

FPA: food-producing animal; GMP: good manufacturing practice; FAMI-QS: Feed Additives and Premixtures Quality System; SPC: summary of product characteristics

Table 1: Differences in dossier requirements in the EU

	Veterinary Medicinal Product	Feed Additive	Feed Material
Quality of (active) ingredient	GMP (Pharma)	FAMI-QS	Feed grade
Production	GMP (Pharma)	FAMI-QS	Feed mill (cert. to QS, GMP+)
Dossier	Electronic submission	Electronic submission	N/A
Evaluation by	CVMP (EMA)	FEEDAP (EFSA)	Label by national competent authority
Benefit-risk evaluation	EMA	EU Commission	N/A
Decision and/or MA	EU Commission	EU Commission	N/A
Estimated costs for development	≤1-100 Mio €	≤0.5-10 Mio €	≤0.1Mio €
Average time to market	Ca. 5-10 years	2-5 years	<0.25 year

CVMP: Committee for Medicinal Products for Veterinary Use; EFSA: European Food Safety Authority; EMA: European Medicines Agency; FAMI-QS: Feed Additives and Premixtures Quality System; FEEDAP: Panel on Additives and Products or Substances used in Animal Feed; GMP: good manufacturing practice; N/A: not applicable; QS: Qualität und Sicherheit

Table 2: Overview of product requirements

Regulatory Requirements for Feed Additives

To date, many products used for modulating the gut microbiome will fall under the definition of a feed additive. Regulation (EC) No 1831/2003 states that these are any substances, microorganisms or preparations (that are not feed material or pre-mixtures) added to feed or to water in order to perform one or more of the following functions: They favourably affect the characteristics of feed, animal products, colour of ornamental fish and birds, animal production, performance or welfare, or environmental consequences of animal production. Furthermore, they may satisfy the nutritional needs of animals or have a coccidiostatic or histomonostatic effect. Based on the feed additive's functions and properties it will be allocated to one of the five existing categories. Feed additives modifying the microbiome most likely fall within the definition of technological (substances added to feed for a technological purpose) or zootechnical additives (affecting favourably the performance of animals in good health or the environment). These categories are further divided into functional groups. A list of authorised feed additives is published in Annex I of Regulation (EC) No 1831/2003, the Register of Feed Additives.

Live organisms, often called probiotics when administered orally in adequate doses to animals, may provide a benefit to the animals. These include the majority of the registered gut microbiome modulating feed additives. They fit perfectly in the description of a zootechnical feed additive, more precisely in the functional groups 'gut flora stabilisers' or 'physiological condition stabilisers'. The latter one was just introduced in June 2019. Authorised microorganisms listed in the Register of Feed Additives include *Saccharomyces cerevisiae, Bacillus spp., Enterococcus faecium, Pediococcus acidilactici* and *Lactobacillus acidophilus*, amongst others. Other probiotics may belong to the category of technological additives as they primarily exert a technological effect on the feed, but not on the animal.

For a new probiotic intended to be placed on the market as feed additive, there is a marketing authorisation procedure defined involving the European Commission (EC) and the European Food Safety Authority (EFSA). The manufacturing of the feed additive has to follow the principles of good quality standards, e.g. FAMI-QS. As the first step of the process to obtain a marketing authorisation, the applicant compiles the application dossier, consisting of data on quality, safety and efficacy for the animal species and category the product is intended to be used in. The dossier has to be submitted to the EC that informs EFSA. A complete technical dossier prepared by the applicant has also to be provided to EFSA for scientific evaluation. EFSA, after a thorough review and evaluation, provides the scientific opinion on the new feed additive to the EC, the legal body for authorising new feed additives. If approved by the standing committee at the EC after a positive scientific evaluation by the FEEDAP panel of EFSA, the marketing authorisation is granted for 10 years, and subsequently a re-assessment is required.

The structure and content of the dossier are defined in Regulation (EC) No 1831/2003 and Regulation (EC) No 429/2008. The applicant suggests the category and functional group of the feed additive for the new probiotic. The selection is not limited to one option, but has to be chosen based on Regulation (EC) No 1831/2003 by the applicant. Independent of whether the feed additive is a microorganism or not, the animal species including categories and age groups has to be stated as listed in Regulation (EC) No 429/2008. Each individual microorganism that is part of the feed additive has to be identified by its name and taxonomic classification and thoroughly evaluated for its potential quality, safety and effect. The applicant has to establish and validate appropriate analytical methods for all ingredients including the microorganism, and such methods are evaluated by the European Union Reference Laboratory.

Another important topic to be addressed is any potential genetic modification of the microorganisms intended to be used in feed additives and thus released into the environment. Directive 2001/18/EC states whether an organism may be defined as genetically modified. In order to remain transparent, the genetic modification has to be described and a unique identifier – alphanumeric in line with Regulation (EC) No 65/2004 – has to be given.

Independent of the nature of a feed (additive), contaminants and impurities have to be monitored assuring compliance with Directive 2002/32/EC. Probiotics are live (micro) organisms, and the focus lies on a potential microbiological contamination. Freedom of at least *Salmonella*, enterobacteriaceae, yeast and filamentous fungi has to be shown. Depending on the fermentation medium and excipients, further testing for mycotoxins, heavy metals and arsenic may be required. The same applies if the source of the microorganism is an animal. Probiotics are intended to influence the animal's gut flora in a positive way, but sometimes microorganisms may exert unexpected and unwanted properties. Therefore, the absence of toxins and virulence factors has to be proven.

Fifteen years ago, EFSA simplified the evaluation of biological agents by a public list of microorganisms that are considered to be safe, and the qualified presumption of safety (QPS) status was introduced. The following aspects are examined during the evaluation of the organisms: the definition of the taxonomic group defining its identity, the available body of knowledge, potential safety issues, and the intended use. In case there are no safety issues posed, the microorganisms can be granted QPS status. In some cases, the status is subject to certain conditions, such as the restriction of use for production processes only. Organisms with this status are exempt from a repeated complete safety assessment. The list of QPS strains is publicly available and regularly updated by EFSA. For every new feed additive, safety has to be proven for the target species, the consumer (in case it is fed to food-producing animals), workers, users and the environment. In case the probiotic is a microorganism on the QPS list and thus safe in humans, safety studies in the target species, on consumer and environmental safety can be omitted. However, if this is not the case, genotoxicity and mutagenicity studies as well as a sub-chronic oral toxicity study are required. To prove the chosen claim or effect of a zootechnical additive, at least three independent *in vivo* studies on efficacy must be provided demonstrating a significant effect when using the lowest proposed dose. If the new additive is intended to affect the performance of animals, long-term studies are required. Studies on both safety and efficacy should be performed using appropriate quality standards such as Good Laboratory Practice in accordance with Directive 2004/10/EC, VICH-GCP or similar well-recognised quality assurance standards. The requirements on animal welfare as defined in Directive 2010/63/EU have to be followed when conducting any studies. Technical guidance on study design and duration is provided by EFSA as there are many things to be considered based on the wide variation of target species, category, weight and age, as well as effects. With Regulation (EU) No 2019/1381 a comprehensive tool has been just recently introduced to improve transparency, independence during the evaluation of the dossier, and risk communication. For this purpose, from 27 March 2021, applicants will have to

register any study in a public database before the start of the study if it shall be used for the registration of a feed additive.

Based on the current European regulations on feed additives, the majority of products actively shaping the microbiome will most likely fall under the definition of a feed additive and thus require a high-quality, well-defined manufacturing process, a well-proven safety profile and effectiveness, if any claim is made (see Table 1 and Table 2).

Regulatory Requirements for Veterinary Medicinal Products

At the beginning of this article, three tools were mentioned to influence the gut microbiome. While the requirements for feed material are quite distinct, the data requirements for feed additives are similar to those for veterinary medicinal products (VMP), if you compare the dossier requirements and registration process. However, the purpose of VMPs is to treat or to prevent a disease in animals, make a medicinal diagnosis or to restore, correct or modify a physiological function, all claims that are forbidden to be made by any feed or feed additive. In both humans and animals, dysbiosis (microbial imbalance), as a first step of appearance of clinical disease, is associated with a wide range of disorders including gastrointestinal-related diseases such as inflammatory bowel disease[2], post-weaning diarrhoea, or clostridiosis. Up to now, antibiotics have been the first choice in the treatment of such diseases, but increasing concerns about antimicrobial resistance have led to an increased search for alternatives. While high-level feeding of ZnO, exerting a medicinal effect, will be banned shortly due to environmental concerns, low level ZnO has primarily nutritional value only. Beyond others, potential new products in focus of scientific research are faecal microbiota transplants (FMT)[3], competitive exclusion (CE) products[4] and bacteriophages[5].

Companies have different options to obtain a marketing authorisation for a VMP in the EU: via the centralised (all EU Member States), the decentralised (certain EU Member States) or the national (one EU Member State) procedure. As for feed additives, a dossier containing data on quality, safety and efficacy for each intended animal species is required. The dossier has to be submitted for scientific evaluation either to the European Medicines Agency (EMA) for centralised applications or to national competent authorities in case of decentralised and national applications. In case of a centralised procedure, the EMA provides an opinion to the EC. The EC adopts the decision on the product and grants or refuses the marketing authorisation after consultation in the standing committee. For decentralised and national authorisations, the national authorities grant or refuse the authorisation. The respective dossier requirements are currently defined in Directive 2001/82/EC as amended by Directive 2004/28/EC and 2009/09/EC. The manufacturing of veterinary medicinal products and the respective active substances used in the production have to follow the principles of Good Manufacturing Practice (GMP) that are described in Directive 91/412/EEC. The control of the quality standards is ensured by the requirement to follow the European Pharmacopoeia monographs whenever possible. This affects the quality part of dossiers for pharmaceuticals, and the quality, safety and efficacy part of an immunological VMP dossier. Similar to feed additives, safety for the target animals, consumer (if applicable) and user, as well as for the environment, has to be demonstrated by the applicant. Pharmacological, toxicological, residues and safety tests need to be performed in compliance with Good Laboratory Practice (GLP), following Directive 2004/9/EC and Directive 2004/10/EC. Any study used within a dossier has to comply with Directive 2010/63/EU on animal welfare. Another parallel to the dossier for feed additives is that genetically modified organisms are of particular focus and additional specific data has to be provided. The assessment of environmental risks should be in line with Directive 2001/18/EC and Regulation (EC) 726/2004. Any claim made on efficacy and field safety has to be proven in dedicated pre-clinical and clinical studies in the target animal species, intended indication and testing the route of administration with the product representative for the final composition to be marketed. Any clinical field study has to comply with the principles of Good Clinical Practice (GCP) (VICH Guideline 9) and must be representative for the territory intended to be used.

In December 2019, Regulation (EC) 2019/6 was published, defining requirements to obtain marketing authorisations for veterinary medicinal products, where the application is submitted from 22 January 2022. Regulation (EC) 2019/4 explains the requirements for medicated feed. Regulation 2019/6 defines for the first time the term "novel" therapies. These definitions include products like stem cell-derived products, bacteriophages, products based on nanoparticle, and gene or antisense technologies. Such products will require authorisation using the centralised procedure. While quality, safety and efficacy requirements for such products are challenging to define and to implement, and legal requirements for such veterinary medicinal products are missing in the current legislation (Directive 2001/82/EC), more guidance by EMA/CVMP and the EC is urgently expected. Nevertheless, previous authorisations of a monoclonal antibody for dogs and a stem cell product for horses demonstrate that novel therapy products can be authorised even under the current legislation. Novel therapies based on the new legal framework were the subject of earlier articles in this journal[6,7].

If an applicant wishes to make health claims for the product shaping the microbiome, the pathway via a VMP will be mandatory. To achieve the marketing authorisation as a VMP will be at least as challenging as for a feed additive; higher standards are usually with the manufacturing of the product, while safety and efficacy may be very similar (see also Table 1 and Table 2 for comparison).

Opportunities and Challenges of Different Regulatory Pathways

Depending on the product, its mechanism of action and intended claim, different regulatory routes to market are available. Bacteriophages demonstrate this principle very well. These viruses are omnipresent in the environment and target bacteria in a species-specific manner. Being explicitly mentioned in the new veterinary regulation (EC) 2019/6, an authorisation of a bacteriophage as VMP targeting pathogenic bacteria in the gastrointestinal tract will certainly be possible. As for a variety of other orally delivered products, a marketing authorisation for bacteriophages as feed additive is also possible, depending on the targeted effect and claim intended to be made. Table 1 compares the dossier requirements for veterinary medicinal products and feed additives. The advantages or disadvantages of regulatory pathways are outlined in Table 2. Any applicant is advised to carefully consider other impacts as well, first of all the potential market opportunities when deciding on any pathway. While VMPs are usually due to prescription, feed material and feed additives may rather have to follow the route of feed when marketed in food-producing animals. In pets, different alternatives appear possible.

Summary and Conclusion

There are many products that may influence the gut microbiome; based on the current legislation, there are only three options: feed material, feed additives and veterinary medicinal products. Nevertheless, a product may either be possible to assign to one of these options or may also possibly work in any of them. The mode of action (MoA), the presentation and the associated claim(s) may help to classify such product. It is of high strategic importance to consider in the early phase of development which regulatory pathway may be the best for both the product and the applicant. It is of utmost importance to carefully consider all aspects such as market size, quality requirements of production, potential distribution channels and partners available for development and commercialisation. For some products, the MoA already may clearly define the pathway; for others, various pathways may be an option. While for feed, no registration is required, the regulation of feed additives and veterinary medicinal products require registration based on a well-prepared dossier and time-consuming thorough evaluation by competent authorities. It is typical in these high risk: – high investment markets, that the registration of both feed additives and VMPs are likely to carry intrinsic and extrinsic risks and require high investment. While "novel" VMPs have already been successfully registered and the new legislation for VMPs initiates the generation of further guidance, an updated feed additive regulation is currently discussed in Europe. As long as the gaps in guidance are not closed, any applicant will have to ensure it aligns its plans with experts and keeps close contact with the competent authorities in order to de-risk the approach to the market for products shaping the microbiome of animals.

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