fermentation

Editorial
In Vitro Fermentation

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The rumen of ruminants, as well as the colon of monogastric animals, are inhabited by over one trillion bacteria, fungi, and protozoa, and these are emerging as critical regulators in dietary micronutrients and animal health. Gut and rumen microbes can regulate host nutrition, metabolism, and immune-related functions by fermenting indigestible nutrients into absorbable metabolites, as well as by increasing energy metabolism and supplying essential vitamins. Due to the fact that large, in vivo animal trials increase the use of animals in research as well as the experimental time and cost, and the fact that this is not in line with the standard of the three R's in animal research—reduce, refine, and replace—in vitro fermentation methodology has now been widely developed and used in animal nutrition research in order to more efficiently investigate the interactions between host metabolism and microbiota of the gut and rumen.

In vitro fermentation includes simulated rumen in vitro fermentation, in vitro gas production, in vitro microbial fermentation, in vitro enzyme fermentation, combined two-step fermentation, etc. Furthermore, over the years, in vitro fermentation has allowed us to evaluate the value of a large number of feeds for both ruminants and monogastric animals such as swine. This not only enriches the feed database, but also makes an important contribution to the development and utilization of microbial resources and the improvement of the feed utilization rate. Therefore, this Special Issue (SI) is focused on the latest research on the application of in vitro fermentation methodology in domestic animals. This SI consists of 15 publications (14 research articles and 1 review article) with various applications, all intended to evaluate the utilization rate of animal feeds such as ramie, corn and corn straw, sorghum straw, wheat straw, apple pomace, chicory, and lucerne. In addition, the potential usage of exogenous probiotics, such as *Aspergillus oryzae*, *Lactiplantibacillus plantarum*, *Bacillus licheniformis*, *Enterococcus avium*, *Streptococcus lutetiensis*, *Streptococcus equinus*, *Lactobacillus buchneri*, and *Pediococcus pentosaceus* during in vitro fermentation is also evaluated Publications in this SI also highlight some other aspects, such as the impact of feed processing methods, the ruminal degradability of mycotoxin deoxynivalenol, and dynamic variations in rumen bacteria during in vitro fermentation. Moreover, this SI covers a variety of livestock fermentation models, such as cows, goats, sheep, pigs, and poultry.

One of the most important applications of in vitro fermentation in animal husbandry is to evaluate the usefulness of feeds. In this SI, Xu et al. [1] investigated the nutritional value of ramie silage and its consequences for chewing activity, rumen fermentation, and enteric methane emissions in goats. The authors demonstrated that ramie can be an alternative forage resource to stimulate chewing activity and reduce CH_4 emissions in ruminants by comparing it with corn stover silage. Meanwhile, Wang et al. [2] also investigated the optimum proportion of sorghum straw and ammoniated wheat straw (S:AWS) in ruminant diets, and recommended that the ratio of S:AWS should be 8:2. Ruminants have been shown to effectively utilize nutrients in feed through both in vitro and in vivo models. Furthermore, Han et al. [3] compared the effects of corn processing methods such as grinding, extrusion, and steam flaking on the starch properties, nutrient profiles, fermentation

Citation: Wang, M. In Vitro Fermentation. *Fermentation* **2023**, *9*, 86. https://doi.org/10.3390/fermentation9020086

Received: 16 January 2023
Accepted: 18 January 2023
Published: 19 January 2023

parameters, and molecular structure of corn, and suggested that extrusion was probably the most effective processing method for corn due to its higher starch gelatinization and lower starch crystallinity. Moreover, endophytic fungal infection is the major reason for the intoxication of animals caused by drunken horse grass. Ma et al. [4] recommended that the ratio of endophyte-free *Achnatherum inebriants* in sheep fattening diet range from 25% to 50%, and that the maximum proportion not exceed 75%. Dufourny et al. [5] also found that apple pomace can be used as a dietary strategy to influence bacterial changes in the intestine by stimulating the growth of bacteria which have been identified as next-generation probiotics. This was accomplished using an in vitro piglet gastrointestinal model. Iqbal et al. [6] found that chicory and lucerne supplementation in feed could be beneficial for poultry by exhibiting inhibitory effects against pathogenic microorganisms and positively modulating the cecal microbiota, as well as enhancing the production of short-chain fatty acids (SCFAs), thus contributing to improved nutrition.

With the development of in vitro microbial culture techniques and next-generation high-throughput sequencing techniques, there is increasing evidence of the merits of probiotics as additives in animal diets. For example, Hu et al. [7] found that strains of *Enterococcus avium*, *Streptococcus lutetiensis*, and *Streptococcus equinus* could be used as potential probiotics and silage strains in cows. Furthermore, Wang et al. [8] also reported that mixed fermentation of *Lactobacillus buchneri*, *Lactobacillus plantarum*, and *Pediococcus pentosaceus* could be applied in corn silage preparation for ruminant feeding practices. In addition, Huang et al. [9] found that mixed fermentation of tea residue by *Lactiplantibacillus plantarum* and *Bacillus licheniformis* was able to ameliorate chemical composition, reduce bacterial community diversity, and improve the rumen degradation rate in vitro. Their study suggested that these strains may be used to develop feed fermented with tea residue. It is also worth noting that Guo et al. [10], by means of a rumen simulation technique system, reported that dietary *Aspergillus oryzae* increased the growth rate of certain rumen bacteria that digest fiber or utilize lactate in the presence of severe subacute rumen acidosis. Finally, Wei et al. [11] also reviewed the advances of functional bacteria in rumen, such as *Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes,* as well as the application of micro-encapsulation fermentation technology in ruminants, in this SI.

Excluding the evaluations of animal feeds and probiotics, this SI also reported some other aspects related to in vitro fermentation. For example, Wang et al. [12] found that dietary vitamin B_{12} supplementation may compromise the performance and health of dairy cows by affecting SCFA production in rumen. Zhang et al. [13] investigated the effects of deoxynivalenol (DON) produced by Fusarium fungi on rumen fermentation and DON degradability under different forage levels, and authors found that DON addition decelerated the fermentation process, inhibited microbial fermentation in terms of decreased SCFAs, and shifted the fermentation pattern to non-glycogenic acid and methane production. Meanwhile, Shi et al. [14] provided new insight into the capabilities of the rumen microbiome, including microbe-derived intracellular and extracellular proteins, using a new approach named metaproteomics. Lastly, Wei et al. [15] explored the dynamic variations of rumen fermentation characteristics and bacterial community composition during an in vitro fermentation study lasting 24 h. It is interesting to find that both rumen fermentation characteristics and the composition of the bacterial community were dynamic. The authors also provided a reference for decision-making regarding the sampling time point during in vitro fermentation.

In short, this SI highlighted that the states of the substrates, microorganisms, enzymes, and fermentation are important elements of in vitro fermentation, and that the regulation of equipment in vitro fermentation also needs to be guaranteed. This SI provides innovative research results and perspectives on in vitro fermentation with nutrients, new feed resources, and probiotics.

Funding: This study was supported by grants from the Natural Science Foundation of China (31672446), Special agricultural science and technology innovation project (NCG202232), Key Program (2021ZD07, SKLSGIHP2021A03) of the State Key Laboratory of Sheep Genetic Improvement and Healthy Production and Priority Academic Program Development of Jiangsu Higher Education Institutions, China.

Acknowledgments: The editor thanks Yongkang Zhen, all of the authors and the editorial staff who contributed to the success of this Special Issue.

Conflicts of Interest: The authors declare no conflict of interest.

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