



2024年第3期总4期

# 饲料用酶工程

## 本期导读

### ▶ 政策法规

1. 欧洲食品安全局发布《与饲料中存在麦角生物碱有关的动物健康风险报告》
2. 欧洲食品安全局发布《食品和饲料生产和加工环境中微生物危害的持久性报告》
3. 欧洲食品安全局发布《饲料中脱毒过程评估指南》

### ▶ 前沿资讯

1. 华中农业大学猪肠道微生物参考基因集及核心优势细菌功能解析研究取得进展
2. 华中农业大学在江汉鸡肠道微生物组解析和益生菌筛选中取得新进展

### ▶ 学术文献

1. 基于里氏木霉多sgRNA加工平台的高效CRISPRCas9基因组编辑系统，用于菌株改良和酶生产
2. 重组酶介导的酵母启动子和终止子重组基因表达组合优化
3. 对茴香醛通过靶向细胞结构和ap1调控抗氧化系统抑制黄曲霉感染和黄曲霉毒素的合成
4. 饲料中原花青素对鲤鱼抗氧化能力、肌肉营养、脂质代谢和肠道微生物

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## 物群影响的多组学分析

### 5. 利用机器学习加速生物催化发现： 酶工程、发现和设计的范式转变

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2024年3月1日

## ➤ 政策法规

### 1. Risks for animal health related to the presence of ergot alkaloids in feed (欧洲食品安全局发布《与饲料中存在麦角生物碱有关的动物健康风险报告》)

简介: The European Commission requested EFSA to provide an update of the 2012 Scientific Opinion of the Panel on Contaminants in the Food Chain (CONTAM) on the risks for animal health related to the presence of ergot alkaloids (EAs) in feed. EAs are produced by several fungi of the Claviceps and Epichloa genera. This Opinion focussed on the 14 EAs produced by *C. purpurea* (ergocristine, ergotamine, ergocornine,  $\alpha$ - and  $\beta$ -ergocryptine, ergometrine, ergosine and their corresponding 'inine' epimers). Effects observed with EAs from *C. africana* (mainly dihydroergosine) and *Epichloa* (ergovaline/-inine) were also evaluated. There is limited information on toxicokinetics in food and non-food producing animals. However, transfer from feed to food of animal origin is negligible. The major effects of EAs are related to vasoconstriction and are exaggerated during extreme temperatures. In addition, EAs cause a decrease in prolactin, resulting in a reduced milk production. Based on the sum of the EAs, the Panel considered the following as Reference Points (RPs) in complete feed for adverse animal health effects: for pigs and piglets 0.6 mg/kg, for chickens for fattening and hens 2.1 and 3.7 mg/kg, respectively, for ducks 0.2 mg/kg, bovines 0.1 mg/kg and sheep 0.3 mg/kg. A total of 19,023 analytical results on EAs (only from *C. purpurea*) in feed materials and compound feeds were available for the exposure assessment (1580 samples). Dietary exposure was assessed using two feeding scenarios (model diets and compound feeds). Risk characterisation was done for the animals for which an RP could be identified. The CONTAM Panel considers that, based on exposure from model diets, the presence of EAs in feed raises a health concern in piglets, pigs for fattening, sows and bovines, while for chickens for fattening, laying hens, ducks, ovines and caprines, the health concern related to EAs in feed is low.

来源: European Food Safety Authority

发布日期: 2024-01-23

全文链接: <http://agri.nais.net.cn/file1/M00/03/6B/Csgk0WXgQbKAQHanADC101U4ybk769.pdf>

### 2. Persistence of microbiological hazards in food and feed production and processing environments (欧洲食品安全局发布《食品和饲料生产和加工环境中微生物危害的持久性报告》)

简介: *Listeria monocytogenes* (in the meat, fish and seafood, dairy and fruit and vegetable sectors), *Salmonella enterica* (in the feed, meat, egg and low moisture food sectors) and *Cronobacter sakazakii* (in the low moisture food sector) were identified as the bacterial food safety hazards most relevant to public health that are associated with persistence in the food and feed processing environment (FFPE). There is a wide range of subtypes of these hazards involved in persistence in the FFPE. While some specific subtypes are more commonly reported as persistent, it is currently not possible to identify universal markers (i.e. genetic determinants) for this trait. Common risk factors for persistence in the FFPE are inadequate zoning and hygiene barriers; lack of hygienic design of equipment and machines; and inadequate cleaning and disinfection. A well-designed environmental sampling and testing programme is the most effective strategy to identify contamination sources and detect potentially persistent hazards. The establishment of hygienic barriers and measures within the food safety management system, during implementation of hazard analysis and critical control points, is key to prevent and/or control bacterial persistence in the FFPE. Once persistence

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is suspected in a plant, a ‘seek-and-destroy’ approach is frequently recommended, including intensified monitoring, the introduction of control measures and the continuation of the intensified monitoring. Successful actions triggered by persistence of *L. monocytogenes* are described, as well as interventions with direct bactericidal activity. These interventions could be efficient if properly validated, correctly applied and verified under industrial conditions. Perspectives are provided for performing a risk assessment for relevant combinations of hazard and food sector to assess the relative public health risk that can be associated with persistence, based on bottom-up and top-down approaches. Knowledge gaps related to bacterial food safety hazards associated with persistence in the FFPE and priorities for future research are provided.

来源: European Food Safety Authority

发布日期:2024-01-19

全文链接: <http://agri.nais.net.cn/file1/M00/03/6B/Csgk0WXgP1aAJsy2AHZPfQ8-Uew526.pdf>

### **3. Guidance for the assessment of detoxification processes in feed (欧洲食品安全局发布《饲料中脱毒过程评估指南》)**

简介: Directive 2002/32/EC of the European Parliament and of the Council, in its article 3, provides that the use of products intended for animal feed which contain levels of undesirable substances exceeding the maximum levels laid down in Annex I of that Directive cannot be placed in the European market. It is possible to use acceptable detoxification processes on these products in order to conform with the provisions of Annex I of that Directive. Commission Regulation (EU) 2015/7862 establishes the acceptability criteria for detoxification processes to ensure that the detoxified feed does not endanger animal and public health and the environment and that the characteristics of the feed are not adversely altered by the detoxification process. The Panel on Contaminants in the food chain (CONTAM Panel) has assessed a series of requests for feed detoxification processes since the entry into force of the Regulation (EU) 2015/786 on 1 July 2017. Frequently the information provided is insufficient for the Panel to come to conclusions if the detoxification process fulfils the criteria set in the legislation and additional clarifications are necessary which prolong the risk assessment process. To help the feed business operators in the preparation and submission of the necessary information for the evaluation of feed detoxification processes according to the Regulation, the CONTAM Panel, in its Plenary meeting on 15 December 2022, identified the need for guidance. The guidance will be provided in a statement addressing the implementation of the criteria for the acceptance of detoxification processes applied to products intended for animal feed, based on the Panel's experience gained during the last years while working under the provisions of the abovementioned Regulation.

来源: European Food Safety Authority

发布日期:2024-01-10

全文链接: <http://agri.nais.net.cn/file1/M00/10/3D/Csgk0EGJjG-AGPLxAAJiGly6Ekg971.pdf>

## 前沿资讯

### **1. 华中农业大学猪肠道微生物参考基因集及核心优势细菌功能解析研究取得进展**

简介: 近日, The ISME Journal 杂志在线发表了华中农业大学农业微生物资源发掘与利用全国重点实验室、教育部动物育种与健康养殖前沿科学中心、湖北洪山实验室晏向华教授课题组的研究成果, 论

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文题为“Characterizing core microbiota and regulatory functions of the pig gut microbiome”。该研究构建了猪肠道微生物参考基因集，筛选到猪肠道核心优势细菌，并解析其对宿主代谢的调控作用机制，对实施肠道菌群干预改善猪生长与肠道健康具有重要意义。研究人员综合运用宏基因组学和细菌16S rDNA扩增子测序技术系统解析了7个品种猪（即杜×（长×大）猪、藏猪、莱芜猪、沙子岭猪、从江香猪、环江香猪和宁乡猪）共计56头断奶仔猪和56头育肥末期猪的肠道微生物区系组成。鉴定了11418273个非冗余肠道微生物基因，通过与已发表文献的非冗余肠道微生物基因进行整合，本研究构建了包含17020160个非冗余基因的猪肠道微生物基因集，通过对宏基因组组装数据进行分箱，共重构获得了4910个非冗余原核微生物基因组。研究表明我国地方猪肠道微生物的营养物质（包括脂类、氨基酸、碳水化合物和核苷酸）代谢能力和能量代谢能力比杜×（长×大）商品猪更强。杜×（长×大）商品猪肠道微生物的抗生素抗性基因丰度比中国地方猪更高，研究发现品种和日龄是影响猪肠道微生物区系组成和功能的关键因素。综合评估微生物的出现率、微生物相对丰度和猪生长阶段，利用宏基因组学和细菌16S rDNA扩增子测序共鉴定出3种猪肠道核心优势细菌（*Phascolarctobacterium succinatutens*、*Prevotella copri*和*Oscillibacter valericigenes*），结果表明灌服上述3种核心优势细菌可显著增加无菌小鼠的器官指数（包括心脏、脾脏和胸腺），降低胃肠道的长度，增强肠道上皮屏障功能，增加肠道的隐窝深度，同时上述3种核心优势细菌可显著改变无菌小鼠的营养物质代谢过程（包括初级胆汁酸的生物合成、苯丙氨酸、酪氨酸和色氨酸的生物合成、苯丙氨酸的代谢等）。综上，本研究系统性解析了7个品种猪的肠道微生物的组成与功能，筛选出猪肠道微生物核心优势细菌并证明了其对无菌小鼠器官指数、肠道屏障功能、肠道黏膜形态和营养物质代谢的关键调控作用，为实施肠道菌群干预改善猪生长与肠道健康提供理论依据和新资料。

来源：华中农业大学

发布日期：2024-02-29

全文链接：<http://agri.nais.net.cn/file1/M00/03/6B/Csgk0WXgQ1aALS2dAA-aHRPAmvM436.pdf>

## 2. 华中农业大学在江汉鸡肠道微生物组解析和益生菌筛选中取得新进展

简介：华中农业大学生命科学技术学院益生菌智造创新团队赵述淼副教授课题组在国际学术期刊Microbiome上发表题为“Metagenome-assembled genome reveals species and functional composition of Jianghan chicken gut microbiota and isolation of *Pediococcus acidilactic* with probiotic properties”的研究论文，研究采用宏基因组测序技术揭示江汉鸡肠道微生物群物种和功能组成以及分离了具有益生菌特性的乳酸片球菌。研究团队利用宏基因组测序技术分析了长江流域附近四个城市江汉鸡肠道微生物的物种和功能组成。结果表明，江汉鸡肠道菌群的细菌种类组成与其他鸡品种相似，其中Phocaeicola和Bacteroides是最丰富的细菌属。LEfSe分析显示荆州样品与其他三组样品之间的物种组成和功能特征存在显著差异。功能注释表明，江汉鸡肠道菌群以代谢基因为主，其中与碳水化合物代谢相关的基因数量最多。发现了多个抗生素抗性基因（ARG），并且ARG的组成与集约化笼养鸡相似，表明抗生素广泛存在于江汉鸡的肠道微生物群中。结合宏基因组的研究结果，从鸡肠道中分离、鉴定出了829株微生物，并进行了大规模的益生性评价，其中3株乳酸片球菌（*P. acidilactic*）表现出较强的益生潜力。这些发现提供了对江汉鸡独特肠道微生物群的深入了解，并强调了益生菌株在养殖业的潜在应用前景。

来源：华中农业大学

发布日期：2024-02-18

全文链接：<http://agri.nais.net.cn/file1/M00/10/3D/Csgk0EGJkF6Ab0qKABVexwgHmYA208.pdf>

## 学术文献

### 1. An efficient CRISPR-Cas9 genome editing system based on a multiple sgRNA processing platform in *Trichoderma reesei* for strain improvement and enzyme production (基于里氏木霉多sgRNA加工平台的高效CRISPR-Cas9基因组编辑系统, 用于菌株改良和酶生产)

**简介:** Background The CRISPR/Cas9 technology is being employed as a convenient tool for genetic engineering of the industrially important filamentous fungus *Trichoderma reesei*. However, multiplex gene editing is still constrained by the sgRNA processing capability, hindering strain improvement of *T. reesei* for the production of lignocellulose-degrading enzymes and recombinant proteins. Results Here, a CRISPR/Cas9 system based on a multiple sgRNA processing platform was established for genome editing in *T. reesei*. The platform contains the arrayed tRNA-sgRNA architecture directed by a 5S rRNA promoter to generate multiple sgRNAs from a single transcript by the endogenous tRNA processing system. With this system, two sgRNAs targeting *cre1* (encoding the carbon catabolite repressor 1) were designed and the precise deletion of *cre1* was obtained, demonstrating the efficiency of sgRNAs processing in the tRNA-sgRNA architecture. Moreover, overexpression of *xyr1-A824V* (encoding a key activator for cellulase/xylanase expression) at the *ace1* (encoding a repressor for cellulase/xylanase expression) locus was achieved by designing two sgRNAs targeting *ace1* in the system, resulting in the significantly enhanced production of cellulase (up to 1- and 18-fold on the Avicel and glucose, respectively) and xylanase (up to 11- and 41-fold on the Avicel and glucose, respectively). Furthermore, heterologous expression of the glucose oxidase gene from *Aspergillus niger* ATCC 9029 at the *cbh1* locus with the simultaneous deletion of *cbh1* and *cbh2* (two cellobiohydrolase coding genes) by designing four sgRNAs targeting *cbh1* and *cbh2* in the system was acquired, and the glucose oxidase produced by *T. reesei* reached 43.77 U/mL. Besides, it was found the ER-associated protein degradation (ERAD) level was decreased in the glucose oxidase-producing strain, which was likely due to the reduction of secretion pressure by deletion of the major endogenous cellulase-encoding genes. Conclusions The tRNA-sgRNA array-based CRISPR-Cas9 editing system was successfully developed in *T. reesei*. This system would accelerate engineering of *T. reesei* for high-level production of enzymes including lignocellulose-degrading enzymes and other recombinant enzymes. Furthermore, it would expand the CRISPR toolbox for fungal genome editing and synthetic biology.

**来源:** Biotechnology for Biofuels and Bioproducts

**发布日期:** 2024-02-11

**全文链接:** <http://agri.nais.net.cn/file1/M00/10/3D/Csgk0EGJhFyAOWPZADETceOnPFk153.pdf>

### 2. Combinatorial optimization of gene expression through recombinase-mediated promoter and terminator shuffling in yeast (重组酶介导的酵母启动子和终止子重组基因表达组合优化)

**简介:** Microbes are increasingly employed as cell factories to produce biomolecules. This often involves the expression of complex heterologous biosynthesis pathways in host strains. Achieving maximal product yields and avoiding build-up of (toxic) intermediates requires balanced expression of every pathway gene. However, despite progress in metabolic modeling, the optimization of gene expression still heavily relies on trial-and-error. Here, we report an approach for in vivo, multiplexed Gene Expression Modification by

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LoxPsym-Cre Recombination (GEMbLeR). GEMbLeR exploits orthogonal LoxPsym sites to independently shuffle promoter and terminator modules at distinct genomic loci. This approach facilitates creation of large strain libraries, in which expression of every pathway gene ranges over 120-fold and each strain harbors a unique expression profile. When applied to the biosynthetic pathway of astaxanthin, an industrially relevant antioxidant, a single round of GEMbLeR improved pathway flux and doubled production titers. Together, this shows that GEMbLeR allows rapid and efficient gene expression optimization in heterologous biosynthetic pathways, offering possibilities for enhancing the performance of microbial cell factories.

来源: Nature Communications

发布日期:2024-02-07

全文链接: <http://agri.nais.net.cn/file1/M00/10/3D/Csgk0EGJhr0AQXTfABeYwvp2gzA770.pdf>

### **3. p-Anisaldehyde suppresses *Aspergillus flavus* infection and aflatoxin biosynthesis via targeting cell structure and Ap1-regulatory antioxidant system (对茴香醛通过靶向细胞结构和ap1调控抗氧化系统抑制黄曲霉感染和黄曲霉毒素的合成)**

简介: Aflatoxin, mainly produced by *Aspergillus flavus*, is one of the most notorious mycotoxin for its toxicity and carcinogenicity. Despite extensive efforts, effective strategies to control *A. flavus* and AFB1 contamination remain elusive. Here, we investigate the potential of p-anisaldehyde (AS), an aldehyde derived from plant essential oils, as a natural antifungal agent against *A. flavus* and its ability to modulate AFB1 biosynthesis. We found that AS exhibited broad-spectrum antifungal activities against *Aspergillus* spp. and effectively inhibited *A. flavus* asexual development, AFB1 production, and pathogenicity. AS treatment disrupted the cell surface structure and membrane integrity, as observed by scanning electron microscopy and PI staining. RNA-sequencing analysis revealed that AS significantly altered the expression of genes involved in redox homeostasis, plasma membrane function, and cell cycle progression. Further investigation demonstrated that AS induced a reduction in mitochondrial membrane potential ( $\Delta\psi_m$ ) and accumulation of reactive oxygen species (ROS), leading to cell cycle arrest at the G2/M phase. The AS-induced ROS accumulation was found to be mitigated by the superoxide dismutase-mediated antioxidant system, which is regulated by transcriptional factor Ap1. Notably, the Ap1-regulatory ROS detoxification system was also found to be involved in *A. flavus* pathogenicity and AFB1 production. Overall, these findings provide valuable insights into the inhibitory mechanism of AS against *A. flavus*, paving the way for its potential application as a natural strategy to mitigate AFB1 contamination in both food and agriculture crops.

来源: Food Frontiers

发布日期:2024-01-31

全文链接: <http://agri.nais.net.cn/file1/M00/10/3D/Csgk0EGJ190AM3dXADVWdBLa0Vk190.pdf>

### **4. Multi-Omics Analysis to Understand the Effects of Dietary Proanthocyanidins on Antioxidant Capacity, Muscle Nutrients, Lipid Metabolism, and Intestinal Microbiota in *Cyprinus carpio* (饲料中原花青素对鲤鱼抗氧化能力、肌肉营养、脂质代谢和肠道微生物群影响的多组学分析)**

简介: Proanthocyanidins (Pros), a natural polyphenolic compound found in grape seed and other plants, have received significant attention as additives in animal feed. However, the specific mechanism by which Pros

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affect fish health remains unclear. Therefore, the aim of this study was to investigate the potential effects of dietary Pro on common carp by evaluating biochemical parameters and multi-omics analysis. The results showed that Pro supplementation improved antioxidant capacity and the contents of polyunsaturated fatty acids (n-3 and n-6) and several bioactive compounds. Transcriptomic analysis demonstrated that dietary Pro caused an upregulation of the sphingolipid catabolic process and the lysosome pathway, while simultaneously downregulating intestinal cholesterol absorption and the PPAR signaling pathway in the intestines. Compared to the normal control (NC) group, the Pro group exhibited higher diversity in intestinal microbiota and an increased relative abundance of *Cetobacterium* and *Pirellula*. Furthermore, the Pro group had a lower Firmicutes/Bacteroidetes ratio and a decreased relative abundance of potentially pathogenic bacteria. Collectively, dietary Pro improved antioxidant ability, muscle nutrients, and the diversity and composition of intestinal microbiota. The regulation of lipid metabolism and improvement in muscle nutrients were linked with changes in the intestinal microbiota.

来源: Antioxidants

发布日期: 2023-12-12

全文链接: <http://agri.nais.net.cn/file1/M00/03/6B/Csgk0WXgOdyAaW9RAErVnf0bSC4293.pdf>

## **5. Accelerating Biocatalysis Discovery with Machine Learning: A Paradigm Shift in Enzyme Engineering, Discovery, and Design (利用机器学习加速生物催化发现: 酶工程、发现和设计的范式转变)**

简介: Emerging computational tools promise to revolutionize protein engineering for biocatalytic applications and accelerate the development timelines previously needed to optimize an enzyme to its more efficient variant. For over a decade, the benefits of predictive algorithms have helped scientists and engineers navigate the complexity of functional protein sequence space. More recently, spurred by dramatic advances in underlying computational tools, the promise of faster, cheaper, and more accurate enzyme identification, characterization, and engineering has catapulted terms such as artificial intelligence and machine learning to the must-have vocabulary in the field. This Perspective aims to showcase the current status of applications in pharmaceutical industry and also to discuss and celebrate the innovative approaches in protein science by highlighting their potential in selected recent developments and offering thoughts on future opportunities for biocatalysis. It also critically assesses the technology's limitations, unanswered questions, and unmet challenges.

来源: ACS Catalysis

发布日期: 2023-10-26

全文链接: <http://agri.nais.net.cn/file1/M00/03/6B/Csgk0WXgPOmAXRRPAFQm0JPEIHE659.pdf>