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摘要

全球监测报告显示 72%的饲料和饲料原料受到一种以上霉菌毒素的污染。来自亚洲的饲料样品中真菌毒素污染率（82%）高于来自欧洲和美洲的饲料样品（40%）。我国 2022 年饲料原料及配合饲料普遍受多种霉菌毒素污染，其中粕类、玉米及副产物、小麦和麸皮易被黄曲霉毒素 B1（AFB1）和脱氧雪腐镰刀菌烯醇（DON）污染。霉菌毒素已经被鉴定出了 400 多种，其中 AFB1 和 DON 对饲料和饲料原料危害最为严重。家畜采食霉变饲料，出现如饲料转化率低、生长速度缓慢、免疫力下降和繁殖障碍等症状。因此，研发安全高效降解饲料中霉菌毒素的产品迫在眉睫。本研究旨在分离筛选出能高效降解 AFB1 和 DON 的益生菌及中药组分，并研究其在不同条件下对 AFB1 和 DON 的降解率，又进行了肉鸡的体内试验，来探究益生菌及益生菌配合中药水提液在体内对 AFB1 和 DON 的降解效果。主要研究内容如下：

（1）降解 AFB1 及 DON 菌株和中药组分的筛选　本试验以香豆素和氧化苯乙烯为 AFB1 和 DON 的替代物，从淤泥、腐叶、动物粪便、牛羊瘤胃液及实验室保存的菌种中进行降解毒素益生菌的初步筛选，共获得十一株能在初筛平板上良好生长的菌株。以保肝解毒为筛选标准，通过查阅资料初步筛选出山楂、白术、茵陈、蒲公英四味中药作为备用药。采用 ELISA 试剂盒检测这十一株菌株对 AFB1 和 DON 的降解率，最终选择一株枯草芽孢杆菌 ECL1.2 为备用菌株，该菌株为实验室保存的益生菌，前期已对其生物学特性进行了研究。该菌株对 AFB1 和 DON 的降解率分别为 82.77%、49.49%。另外 ELISA 试剂盒检测单味中药在体外对 AFB1 和 DON 的降解，结果显示这几味中药均无体外降解效果，因其有保肝解毒功效，后期将其与益生菌配合做动物体内降解试验。

（2）菌株 ECL1.2 降解毒素条件的优化　利用试验一筛选出的菌株 ECL1.2，以对 AFB1 和 DON 降解率为依据，探索其在不同温度（30℃、37℃、40℃）、时间（12 h、24 h、48 h）、pH（pH4、pH7、pH9）、菌浓度（1×10^5 CFU/mL、1×10^6 CFU/mL、1×10^7CFU/mL）对 AFB1 和 DON 降解率的影响。在不同温度下，37℃时菌株 ECL1.2 对 AFB1 和 DON 降解率最高；在不同时间下，菌株 ECL1.2 作用 24 h 对 AFB1 和 DON 降解率最高；在不同 pH 下，菌株 ECL1.2 在 pH7 条件下对 AFB1 和 DON 降解率最高；在不同菌浓度下，当菌株 ECL1.2 浓度为 1×10^6 CFU/mL 对 AFB1 和 DON 降解率最高。菌株 ECL1.2 对 AFB1 和 DON 最佳降解条件为：温度 37 ℃、作用 24 h、初始 pH 7.0、菌浓度 1×10^6 CFU/mL。

（3）菌株 ECL1.2 和中药水提液对肉鸡饲料中 AFB1 和 DON 解毒效果的研究　试验采用单因子试验设计，挑选了 180 只 1 日龄健康、体重接近、雄性 AA 肉鸡，分为前期（1~21 天）和后期（21~42 天）两个阶段进行饲养。按体重分为 6 个组，每组 3 个重复，每个重复 10 只鸡。试验分组：A 组（基础日粮）、B 组（试验日粮，即添加发霉玉米和玉米喷浆粉使 AFB1 和 DON 含量分别达到 70 和 1000 μg/kg）、C 组（试验日粮+1×10^6 CFU/g ECL1.2）、D 组（试验日粮+2×10^6CFU/g ECL1.2）、E 组（试验日粮+0.05%中药水提液（山楂:茵陈:白

术:蒲公英=4:2:2:1））、F 组（试验日粮+1×10^{6}CFU/g ECL1.2+0.05%中药水提液（山楂:茵陈:白术:蒲公英=4:2:2:1））。试验结束后通过测定肉鸡生长性能、营养物质代谢率、肉鸡血清、肝脏、胸肌及粪便中 AFB1 和 DON 含量、免疫器官指数和消化器官相对长度、血清生化指标、肝脏和肠道组织切片及肠道内容物微生物区系等相关指标，探究益生菌 ECL1.2 和中药水提液在体内对毒素的作用效果。结果显示与试验 B 组相比，试验 C、D、E 和 F 组均能有效缓解毒素对肉鸡生长的抑制作用，显著增加平均日增重（*P*<0.05），料重比和平均日采食量显著降低（*P*<0.05），试验 F 组（益生菌搭配中药水提液组）效果最佳，且试验 C、D、E 和 F 组显著降低肉鸡死亡率；试验 C、D、E 和 F 组显著提高了肉鸡对粗蛋白、粗脂肪和钙的代谢率（*P*<0.05）；试验 C、D、E 和 F 组粪便中 AFB1 和 DON 含量显著降低（*P*<0.05），21d 和 42d 血清中，各试验组间 AFB1 含量无显著差异（*P*>0.05），而试验 C、D、E 和 F 组 DON 含量显著降低（*P*<0.05），试验 C、D、E 和 F 组肝脏和胸肌中 AFB1 和 DON 含量显著降低（*P*<0.05）；试验 C、D、E 和 F 组改善了 AFB1 和 DON 对肝脏器官指数的影响；试验 C、D、E 和 F 组显著降低了血清中谷草转氨酶（AST）、谷丙转氨酶（ALT）和碱性磷酸酶（ALP）活性（*P*<0.05）；试验 F 和 C 组能有效缓解 AFB1 和 DON 引起的肝细胞脂肪变性、坏死和弥漫性水样变性，试验 D 和 E 组有轻微的水样变性；试验 C、D、E 和 F 组能有效缓解 AFB1 和 DON 对肠道黏膜的损伤，空肠绒毛高度和绒毛高度与隐窝深度的比值显著增加（*P*<0.05），隐窝深度显著降低（*P*<0.05）。通过对盲肠内容物微生物区系进行分析，与试验 B 组相比，试验 C、D 和 F 组提高了 Chao1 指数、Simpson 指数、Shannon 指数和 Observed species 指数，各组 OUT 数目增大，提高了肠道菌群多样性和丰度；对比各组盲肠内容物在门水平和属水平菌群结构，发现益生菌 ECL1.2 和中药水提液在门水平上主要通过增加疣微菌门相对丰度，在属水平上增加乳杆菌相对丰度，从而增加肠道有益菌，改变肠道微生物组成，调节肠道菌群。不同处理的试验组中，试验 F 组（益生菌搭配中药水提液）效果最佳。与试验 B 组相比，试验 F 组门水平上疣微菌门、互养菌门丰度明显升高，拟杆菌门丰度明显降低；属水平上巴恩斯氏菌属、乳杆菌属明显升高，粪杆菌属丰度明显降低。与试验 A 组相比，试验 F 组门水平疣微菌门丰度明显升高，拟杆菌门、互养菌门丰度明显降低；巴恩斯氏菌属、乳杆菌属丰度明显升高，粪杆菌属丰度明显降低。综合来看体内试验结果对比显示，试验 F 组即菌株 ECL1.2 搭配中药水提液组对于肉鸡生长性能提高及体内毒素的降解效果最佳。本研究为益生菌及益生菌与中药配合在肉鸡体内降解 AFB1 和 DON 的应用提供了理论基础及试验依据。

关键词：黄曲霉毒素 B1；脱氧雪腐镰刀菌烯醇；枯草芽孢杆菌；毒素降解；肉鸡试验

Screening of probiotics and Chinese herbal compounds for decomposition of AFB1 and DON

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ABSTRACT： Global monitoring reports show that 72% of feed and feed ingredients are contaminated by more than one mycotoxin. The contamination rate of mycotoxins in feed samples from Asia (82%) was higher than that in feed samples from Europe and the Americas (40%). In 2022, feed ingredients and formulated feeds in China are generally contaminated by a variety of mycotoxins. Among them, meals, corn and by-products, wheat and bran are easily contaminated by aflatoxin B1 (AFB1) and deoxynivalenol (DON). More than 400 mycotoxins have been identified, among which AFB1 and DON are the most harmful to feed and feed ingredients. Livestock feed on moldy feed, such as low feed conversion rate, slow growth rate, decreased immunity and reproductive disorders and other symptoms. Therefore, it is urgent to develop safe and efficient products to degrade mycotoxins in feed. The purpose of this study was to isolate and screen probiotics and traditional Chinese medicine components that can efficiently degrade AFB1 and DON, and to study their degradation rates of AFB1 and DON under different conditions. The in vivo experiment of broilers was carried out to explore the degradation effect of probiotics and probiotics combined with traditional Chinese medicine water extract on AFB1 and DON in vivo. The main research contents are as follows :

(1) Screening of AFB1-degrading and DON-degrading strains and traditional Chinese medicine components In this study, coumarin and styrene oxide were used as substitutes for AFB1 and DON. Preliminary screening of toxin-degrading probiotics was carried out from sludge, rot leaves, animal feces, rumen fluid of cattle and sheep, and strains preserved in the laboratory. Eleven strains that can grow well on the preliminary screening plate were obtained. With liver protection and detoxification as the screening criteria, four traditional Chinese medicines, hawthorn, atractylodes, artemisia capillaris and dandelion, were preliminarily selected as standby medicines by consulting the data. The degradation rates of AFB1 and DON by these 11 strains were detected by ELISA kit. Finally, a strain of *Bacillus subtilis ECL1.2* was selected as the backup strain, which was a probiotic preserved in the laboratory. Its biological characteristics have been studied in the early stage. The degradation rates of AFB1 and DON were 82.77% and 49.49%, respectively. In addition, ELISA kit was used to detect

the degradation of AFB1 and DON by single Chinese medicine in vitro. The results showed that these Chinese medicines had no degradation effect in vitro. Because of their hepatoprotective and detoxification effects, they were later combined with probiotics for in vivo degradation test.

(2) Optimization of the conditions for degrading toxins by strain ECL1.2 Based on the degradation rate of AFB1 and DON, the effects of different temperatures (30°C, 37°C, 40°C), time (12 h, 24 h, 48 h), pH (pH4, pH7, pH9) and bacterial concentration (1×10^5 CFU/mL, 1×10^6 CFU/mL, 1×10^7 CFU/mL) on the degradation rate of AFB1 and DON were explored. At different temperatures, the degradation rate of AFB1 and DON by strain ECL1.2 was the highest at 37°C. At different times, the degradation rate of AFB1 and DON by strain ECL1.2 was the highest at 24 h. At different pH, strain ECL1.2 had the highest degradation rate of AFB1 and DON at pH7. Under different bacterial concentrations, the degradation rate of AFB1 and DON was the highest when the concentration of strain ECL1.2 was 1×10^6 CFU/mL. The optimal degradation conditions of AFB1 and DON by strain ECL1.2 were as follows : temperature 37 °C, action time 24 h, initial pH 7.0, and bacterial concentration 1×10^6 CFU/mL.

(3) Study on the detoxification effect of strain ECL1.2 and water extract of traditional Chinese medicine on AFB1 and DON in broiler feed In this experiment, a single factor experimental design was used to select 180 1-day-old healthy, close-weight, male AA broilers, which were divided into two stages: early stage (1~21 days) and late stage (21~42 days). They were divided into 6 groups according to body weight, with 3 replicates in each group and 10 chickens in each replicate. Experimental groups : group A (basal diet), group B (experimental diet, that is, adding moldy corn and corn spray powder to make AFB1 and DON content reach 70 and 1000 μg/kg, respectively), group C (experimental diet + 1×10^6 CFU/g ECL1.2), group D (experimental diet + 2×10^6 CFU/g ECL1.2), group E (experimental diet + 0.05% Chinese medicine water extract (hawthorn : capillaris : atractylodes : dandelion = 4 : 2 : 2 : 1)), group F (experimental diet + 1×10^6 CFU/g ECL1.2 + 0.05% Chinese medicine water extract (hawthorn : capillaris : atractylodes : dandelion = 4 : 2 : 2 : 1)). At the end of the experiment, the growth performance, nutrient apparent digestibility, AFB1 and DON contents in serum, liver, breast muscle and feces, immune organ index and relative length of digestive organs, serum biochemical indexes, liver and intestinal tissue sections and intestinal content microflora of broilers were measured to explore the effect of probiotics ECL1.2 and water extract of traditional Chinese medicine on toxins

in vivo. The results showed that compared with the experimental group B, the experimental groups C, D, E and F could effectively alleviate the inhibitory effect of toxins on the growth of broilers, significantly increase the average daily gain (P<0.05), and the F/G ratio and average daily feed intake were significantly reduced (P<0.05). The experimental group F (probiotics with traditional Chinese medicine water extract group) had the best effect, and the experimental groups C, D, E and F significantly reduced the mortality of broilers; groups C, D, E and F significantly increased the digestibility of crude protein, crude fat and calcium in broilers (P<0.05). The contents of AFB1 and DON in feces of group C, D, E and F were significantly decreased (P<0.05), while the content of DON in group C, D, E and F was significantly decreased (P<0.05). The contents of AFB1 and DON in liver and breast muscle of group C, D, E and F were significantly decreased (P<0.05). Groups C, D, E and F improved the effects of AFB1 and DON on liver organ index. The activities of aspartate aminotransferase(AST), alanine aminotransferase(ALT) and alkaline phosphatase (ALP) in serum were significantly decreased in groups C, D, E and F (P<0.05). Groups F and C could effectively alleviate hepatocyte steatosis, necrosis and diffuse watery degeneration caused by AFB1 and DON, while groups D and E had slight watery degeneration. Group C, D, E and F could effectively alleviate the damage of intestinal mucosa caused by AFB1 and DON. The villus height and the ratio of villus height to crypt depth of jejunum were significantly increased (P<0.05), and the crypt depth was significantly decreased (P<0.05). By analyzing the microflora of cecal contents, compared with group B, group C, D and F increased Chao1 index, Simpson index, Shannon index and Observed species index, increased the number of OUT in each group, and improved the diversity and abundance of intestinal flora. Comparing the microbial structure of cecal contents in each group at the phylum level and the genus level, it was found that probiotics ECL1.2 and traditional Chinese medicine water extract mainly increased the relative abundance of *Verrucomicrobia* at the phylum level, and increased the relative abundance of *Lactobacillus* at the genus level, thereby increasing intestinal beneficial bacteria, changing intestinal microbial composition, and regulating intestinal flora. Among the experimental groups with different treatments, the experimental group F (probiotics combined with traditional Chinese medicine water extract) had the best effect. Compared with the experimental group B, the abundance of *Verrucomicrobia* and *Syntrophobacteria* in the experimental group F was significantly increased, and the abundance of *Bacteroidetes* was significantly decreased.