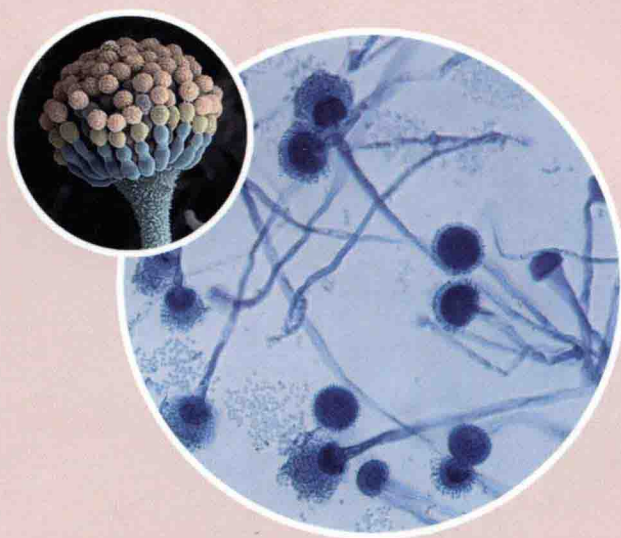


Scientific Research Monographs

Biodegradation of Mycotoxins in Swine Feed

霉菌毒素对猪危害及生物降解法研究

Chief Editor Liu Di



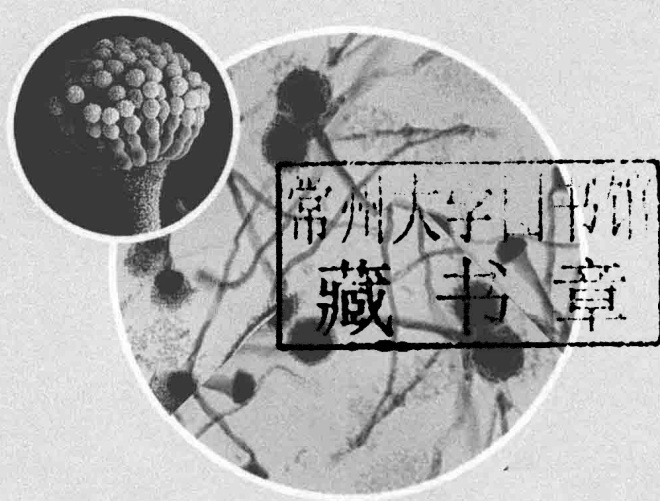
中国农业科学技术出版社

Scientific Research Monographs

Biodegradation of Mycotoxins in Swine Feed

霉菌毒素对猪危害及生物降解法研究

Chief Editor Lin Di



中国农业科学技术出版社

图书在版编目 (CIP) 数据

霉菌毒素对猪危害及生物降解法研究 = Biodegradation of mycotoxins in swine feed: 英文 / 刘娣主编. —北京: 中国农业科学技术出版社, 2014. 9

ISBN 978 - 7 - 5116 - 1787 - 3

I. ①霉… II. ①刘… III. ①饲料 - 真菌毒素 - 生物降解 - 研究 - 英文 IV. ①S816.3

中国版本图书馆 CIP 数据核字 (2014) 第 190826 号

责任编辑 朱 绯 李 雪
责任校对 贾晓红

出 版 者 中国农业科学技术出版社
北京市中关村南大街 12 号 邮编: 100081
电 话 (010) 82109704 (发行部) (010) 82106626 (编辑室)
(010) 82109703 (读者服务部)
传 真 (010) 82106626
网 址 <http://www.castp.cn>
经 销 者 新华书店北京发行所
印 刷 者 北京京华虎彩印刷有限公司
开 本 787 mm × 1 092 mm 1/16
印 张 9
字 数 108 千字
版 次 2014 年 9 月第 1 版 2014 年 9 月第 1 次印刷
定 价 48.00 元

现代农业产业技术体系建设专项 (CARS-36)
科技部国际合作项目 “天然抗霉菌毒素饲料添加剂
生产技术的引进与开发” (2011DFA30760)
资助出版

Biodegradation of Mycotoxins in Swine Feed

Editorial Board

Chief Editor :

Liu Di Heilongjiang Academy of Agricultural Science

Deputy Chief Editor :

Feng Yanzhong Heilongjiang Academy of Agricultural Science

Reviewer :

Qin Guoqing InnoTech Nutrition Solutions

Editorial Board (In alphabetical order) :

Chen Heshu Heilongjiang Academy of Agricultural Science

Feng Yanzhong Heilongjiang Academy of Agricultural Science

He Xinmiao Heilongjiang Academy of Agricultural Science

Liu Di Heilongjiang Academy of Agricultural Science

Liu Huanqi Qingdao Agricultural University

Qin Guoqing InnoTech Nutrition Solutions

Ren Qingna Qingdao Agricultural University

Wang Wentao Heilongjiang Academy of Agricultural Science

Xu Huilian International Nature Farming Research Center

Zheng Shimin Northeast Agricultural University

《霉菌毒素对猪危害及生物降解法研究》

编著委员会

主 编 刘 娣 黑龙江省农业科学院

副主编 冯艳忠 黑龙江省农业科学院

主 审 秦国庆 加拿大奥特奇公司

编 委 (按姓氏拼音顺序排序)

陈赫书 黑龙江省农业科学院

冯艳忠 黑龙江省农业科学院

何鑫淼 黑龙江省农业科学院

刘 娣 黑龙江省农业科学院

刘焕奇 青岛农业大学

秦国庆 加拿大奥特奇公司

任庆娜 青岛农业大学

王文涛 黑龙江省农业科学院

徐会连 日本自然农法研究所

郑世民 东北农业大学

PREFACE

Research shows that 95% of mycotoxins in the presence of animal feed have been produced in the field, especially in wet or humid weather. Crops in harsh climatic conditions the occurrence of mycotoxins and its concentrations tend to be higher. Mycotoxins may occur during the storage of feedstuff or feed product. Currently, more than 200 mycotoxins are known in the presence of animal feed. In China, the most common and most harmful mycotoxins to hog industry are mainly aflatoxins, T-2, zearalenone, in addition to fumonisin and ochratoxin.

Pigs in all stages of production are very sensitive to mycotoxins. Mycotoxins can suppress immune function in pigs, resulting in decreased resistance to disease, inducing chronic infection and lower therapeutic response; furthermore, it has caused huge losses in hog industry. China is situated in Asia with high occurrences of mycotoxins, the southern, eastern, and northern usually present more rainfalls. In harvest seasons, crops in the field under humid are prone to be contaminated by mold, especially aflatoxin widespread in corn, peanut meal and other feed ingredients. Deterioration of feedstuff not only irritates the health of pigs, but also ultimately affects human health. In recent years, moldy corn produced in northeastern China appeared to climb; potentially it may significantly damage productivity and efficiency of pigs.

The approach of mycotoxin detoxification is of particular importance. In comparison with the detoxification processes of mycotoxins, biodegradation is more preferable, so the study of mycotoxins, especially aflatoxins is necessary. We launched a joint program with other experts from Canada and Japan, conducted trials including biodegradation of mycotoxins applied probi-

otic fermentation and inhibition of lactic acid to *Aspergillus* parasitical. The research led to the development of a natural anti-mycotoxin feed additive, and evaluated the efficacy of this additive.

The authors would like to summarize and publish the results for your reference. The authors want to raise the attention of mycotoxins, in addition to take preventive measures.

Due to our knowledge and experience, there might be error or mistakes in this book. The authors will much appreciate your comments.

Editorial Board

CONTENTS

1 Introduction	1
1.1 Mycotoxin research progress	1
1.1.1 Types of mycotoxins	2
1.1.2 The hazards of mycotoxins on livestock	5
1.2 The impact of mycotoxins on pigs	9
1.2.1 The impact of aflatoxins on pigs	10
1.2.2 The impact of zearalenone on pigs	10
1.2.3 The impact of DON on pigs	12
1.2.4 The impact of T-2 toxin on pigs	12
1.2.5 The impact of ochratoxin on pigs	13
1.2.6 The impact of fumonisin on pigs	14
1.3 Mycotoxin contamination in feed	14
1.3.1 The type of mycotoxin and the effect in feed	15
1.3.2 The limits of mycotoxins in the feed	16
1.3.3 Reasons for mycotoxin contamination in feed	17
1.4 Feed mycotoxin detoxification method	17
1.4.1 Physical detoxification	18
1.4.2 Chemical detoxification	19
1.4.3 Enzyme detoxification	20
1.4.4 Biological detoxification	21
1.5 Purpose and significance and experiment plan of the research	22
1.5.1 Purpose and significance of the research	22
1.5.2 Experiment plan of the research	23
2 Material and Method	24
2.1 Material	24

2 Biodegradation of Mycotoxins in Swine Feed

2.1.1	Strains	24
2.1.2	The main chemical agent and biologic agent	25
2.1.3	Medium preparation	26
2.1.4	The main equipment	28
2.1.5	The main experimental animal	29
2.2	The main experimental content and methods	29
2.2.1	Screening of probiotics fermentation conditions and quality evaluation	30
2.2.2	The inhibitory effect of probiotics on the growth of <i>Aspergillus parasiticus</i> and its influencing factors	34
2.2.3	The inhibition effects of Anti-mycotoxin plants on <i>Aspergillus</i>	36
2.2.4	Method and technology of probiotics on zymolysis anti-mycotoxin	41
2.2.5	Probiotics zymolite detection and feeding effects experiment	41
2.2.6	Probiotics zymolite effect on glycolysis of mycotoxin in finisher feed	42
2.2.7	Probiotics zymolite effect on glycolysis of mycotoxin in sow feed	50
2.2.8	Establish addition of probiotics zymolite in regular feed and feeding effects	56
2.3	Data processing	57
3	Results and analysis	58
3.1	Screening of probiotics fermentation conditions and determination of the shelf life	58
3.1.1	Screening of probiotics fermentation conditions	58
3.1.2	The determination of the shelf life of probiotics	61
3.2	Quality evaluation of probiotics	63
3.2.1	Determination of organic acids content in probiotics	63
3.2.2	Determination of amino acids in probiotics	63
3.2.3	Determination of inorganic composition of probiotics	64
3.2.4	Determination of heavy metals content of probiotics	65
3.3	The inhibitory effect of probiotics on the growth of <i>Aspergillus parasiticus</i> and its influencing factors	66
3.3.1	The effects of probiotics on spores activity of <i>Aspergillus parasiticus</i>	66
3.3.2	Probiotics effects on activity of <i>A.parasiticus</i> spores at different pH	69
3.3.3	Probiotics effects on activity of <i>A.parasiticus</i> spores at different density	69
3.3.4	The impact of probiotics on the toxin production of <i>A.parasiticus</i>	70
3.4	Anti-mycotoxin plants inhibition effect on <i>Aspergillus parasiticus</i> growth	71

3.4.1	Screening of anti-mycotoxin plant	71
3.4.2	Anti-mycotoxin plants inhibition on A.parasiticus growth	71
3.4.3	The animal experiment of anti-mycotoxin plants	72
3.5	Method and technology of probiotics zymolysis anti-mycotoxin plants	73
3.6	Probiotics zymolite detection and feeding effects experiment	73
3.6.1	The traits of probiotics zymolite	73
3.6.2	Probiotics effect on the performance of piglets and its side effect	74
3.7	Probiotics zymolite effect on glycolysis of mycotoxin in finisher feed	75
3.7.1	Mycotoxin detection in the basal diet	75
3.7.2	Pig growth status	75
3.7.3	Incidence detection	76
3.7.4	Common disease diagnosis of pigs and effects of Probiotics zymolite adding feed on pigs	81
3.8	Probiotics zymolite effect on glycolysis of mycotoxin in sow feed	91
3.8.1	The impact of probiotics zymolite on sow reproductive performance	91
3.8.2	Sow antibody detection	92
3.9	Establish addition of probiotics zymolite in regular feed and feeding effects experiment	93
3.9.1	Detection of mycotoxin in normal basal diet	93
3.9.2	Survey of piglets growth performance	93
3.9.3	Piglets antibody detection	94
4	Discussion	95
4.1	Selection and optimize of probiotic fermentation condition	95
4.1.1	The impact of temperature on the fermentation of probiotics	95
4.1.2	The impact of pH on the fermentation of probiotics	96
4.1.3	The impact of inoculation amount on the fermentation of probiotics	96
4.2	Mycotoxin contamination of pig feed	97
4.3	The impact of mycotoxins on pig immunity	98
4.4	Probiotics zymolite degradation of mycotoxins in pig feed	99
5	Conclusion	102
6	Innovation	104
	References	105
	APPENDIX I Market Hog Vaccination Program	126
	APPENDIX II Sow Vaccination Program	127
	Attachment	128

1 Introduction

1.1 Mycotoxin research progress

Any fungus living on a substrate grown as cotton wool, fluffy or cobweb-like mycelium are collectively known as molds. Molds exist in two forms in nature, namely the field mold and storage mold. The occurrence of mold can be in the field of harvest or under storage conditions. Molds can survive the winter and can breed under cold conditions^[1]. Mold in feed will continue to consume the nutrients resulting in the consumption of nutrients and mold contaminated feed have poor sensory properties which will seriously affect the palatability. Mold will produce mycotoxins and other secondary metabolites in the process of contaminating feed. these toxins have strong side effects on animals. Livestock will appear stunted growth, reduced reproductive performance, immune function decline even if the content is very low in the feed. Currently three categories are known to produce mycotoxins, namely *Aspergillus*, *Penicillium* and *Fusarium*. These mycotoxin produced by fungi include *Aflatoxin*, *Ochratoxions*, *Fumonisin*, *Zearlenone* and *Trichothecenes*. *Trichothecenes* include DON, T-2 and DAS.

Feed ingredients, food, meat, DDGS were analyzed (Table 1 – 1) from 731 different regions worldwide by BIOMIN company in 2011. As can be seen from the data, fungal toxins have occurred and existed in Asia, South America, North America, Europe, Africa and other regions. The most serious contamination occurred in Asia and the mycotoxin contamination rate

in Asia is higher than that in North America. Mycotoxins contents in Asia samples are several times to dozens of times higher than those in North America. Meanwhile, changes of the mycotoxins contents in the same feed samples were tested by BIOMIN company from July to September, 2010. The results showed that mycotoxin content increased from July to September, 2010, indicating that mycotoxin increased 30% to 62% in only three months and the fast changing speed is shocking^[2].

1.1.1 Types of mycotoxins

Mycotoxins are poisonous substances produced by the fungus. Mycotoxins can be found in the soil and plants, including cereals, forage and silage. Mycotoxins can be formed at the time of harvesting and can also be formed on harvested crops under unfavorable storage conditions. These mycotoxins can cause impacts on feed and livestock^[3,4].

Table 1 – 1 BIOMIN's Asia and North America mycotoxin survey report in the first quarter of 2011

Toxin type	Asia				North America			
	Sample amount	Pollution rate (%)	Average content (μg/kg)	Max-D (μg/kg)	Sample amount	Pollution rate (%)	Average content (μg/kg)	Max-D (μg/kg)
Afla	267	36	94	677	14	14	2	2
ZON	272	51	511	511	17	35	197	506
DON	266	59	1 768	1 768	17	47	577	1 141
FUM	267	45	1 717	1 717	10	60	1 167	2 088
OTA	260	32	12	12	10	30	30	2

1.1.1.1 Aflatoxin

Aflatoxin (Afla) is highly toxic and highly carcinogenic toxins and is produced by the *Aspergillus flavus* (*Aspergillus*) microorganisms. Aflatoxin is a compound made up of two unequal dihydro-nitrofurantoin-ring, both are derivatives of furanocoumarins (Difuranocoum). Aflatoxin in contaminated food are only 4 species aflatoxins B₁, B₂, G₁ and G₂. The nomination of these mycotoxins is mainly based on the different colors they emitted under ultraviolet light. The color of AFB₁ and AFB₂ emitted is blue light while the color emitted by AFG₁ and AFG₂ is yellow-green. Almost all aflatoxin have the primary toxicity to animals liver in which

AFB₁ is the most toxic which is 10 times more toxic than the potassium cyanide and 68 times more toxic than arsenic. The order of the aflatoxin according to their toxicity from strong to weak is B₁, G₁, B₂ and G₂. AFB₁ has been identified as a class I carcinogen by IARC for human and livestock. Aflatoxin contamination in the daily feed is mainly AFB₁ which accounts for the largest amount and the most toxic. Therefore, AFB₁ is used as indicators for feed contamination of aflatoxin by the quality of supervision in our country^[5].

Aflatoxins are very stable and their toxicity can only be destroyed at a temperature of 268 – 269°C. it is quite stable in acidic and neutral solution and it won't dissolve in acid solution until pH 1 – 3. It can be quickly decomposed to form a sodium salt in alkali solution of NaOH in pH 9 – 10 and the fluorescence also will disappear. But the process is reversible and it will restore the original structure under acidic conditions. Aflatoxin can be completely destroyed by 5% sodium hypochlorite solution, Cl₂, NH₃, SO₂ and other strong oxidants^[6].

1. 1. 1. 2 Trichothecenes

Trichothecenes is the secondary metabolites produced by *Fusarium* at low temperatures. It is widespread in nature and is more common in long-term storage of food crops. It will easily lead to serious illness and even death if eating by mistake. Trichothecenes are divided into four sub-categories, in which Class A and Class B are most important. Class A of trichothecene produced by *Fusarium oxysporum* and *Fusarium* pears, including T-2 toxin, HT-2 toxin, fusaric acid (NEO) and diacetoxymercury marqueter ene alcohol (DAS). Class B trichothecene produced mainly by yellow *Fusarium graminearum* and *Fusarium* which include bacteria enolase deoxynivalenol toxin (deoxynivalenol, DON) and its three-acetyl or 15-acetyl derivatives, snow rot *Fusarium enolase* (NIV) and *Fusarium ketene-X* (Fusarenon-X, FX)^[2]. T-2 toxin of type A and DON of type B are the most common toxins.

Trichothecenes are toxic to both humans and animals and can cause acute and chronic diseases which include vomiting, diarrhea, skin irritation, feed refusal, nausea, neurological disorders and abortion^[7]. Furthermore, high doses of trichothecenes can accelerate apoptosis of leukocytes^[8].

1. 1. 1. 3 Zearalenone

Zearalenone (abbreviation ZEA), also known as F-2 toxin was first isolated from corn