PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

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ANA SILVIA DE LARA PIRES BATISTA GOMES

Resistência das cultivares de milho transgênico às micotoxinas e seus efeitos na alimentação animal - revisão sistemática e metanálise

(Resistance of transgenic maize cultivars to mycotoxins and their effects on animal feed – systematic review and metanalysis)

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Orientador: Prof. Dr. Saulo Henrique Weber

Coorientador: Prof. Dr. Fernando Bittencourt Luciano

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Curitiba, 30 de junho de 2023.

Caroline Nocera Bertton
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RESUMO GERAL

O crescimento acelerado da população mundial tem aumentado a demanda por alimentos de origem animal, gerando um crescimento na produção de aves, suínos, bovinos e peixes. Os parâmetros produtivos dessas espécies incluem ganho de peso, qualidade da carne, produção de ovos e quantidade e qualidade do leite, altamente influenciados pela nutrição. A compreensão do consumo de alimentos é essencial para otimizar o desempenho animal, no entanto, a contaminação dos alimentos com fungos e a presença de micotoxinas representam um desafio para a produção animal. As micotoxinas são compostos tóxicos produzidos por fungos e podem causar diversos efeitos negativos na saúde animal, principalmente as aflatoxinas, fumonisinas, desoxinivalenol e zearalenona. Essas micotoxinas são difíceis de serem eliminadas dos alimentos e sua contaminação é especialmente preocupante no milho, um dos principais componentes das rações animais. Uma das formas para reduzir as quantidades dessas toxinas em plantas é a utilização de transgênicos que além de apresentar desempenho produtivo maior, também possuem qualidade nutricional equivalente às variantes não transgênicas. Portanto, esta dissertação visa comparar o efeito do milho transgênico em relação ao milho convencional na produção de micotoxinas e no desempenho de diferentes espécies animais, por meio de revisão sistemática e metanálise. Para isto, esta dissertação foi divida em duas revisões sistemáticas, uma sobre o efeito do milho transgênico na quantidade de micotoxinas, com realização de metanálise; e uma segunda revisão sobre o efeito do milho transgênico na produção animal. Por meio dos dados obtidos nas revisões pode-se concluir que o milho transgênico possui efeito benéfico sobre a produção de micotoxinas, apresentando uma redução de mais de 50% na quantidade de micotoxinas em comparação com o não transgênico. Ainda, ambos apresentam equivalência nutricional para alimentação animal, sem efeitos nocivos para os animais, como diarreia e alergias, e com mesmo desempenho produtivo.

Palavras-chave: milho transgênico; micotoxinas; alimentação animal; revisão sistemática; metanálise

ABSTRACT

The accelerated growth of the global population has led to an increased demand for animal-origin foods, resulting in a growth in the production of poultry, swine, cattle, and fish. The productive parameters of these species include weight gain, meat quality, egg production, and quantity and quality of milk, all of which are highly influenced by nutrition. Understanding food consumption is essential to optimize animal performance; however, food contamination with fungi and the presence of mycotoxins pose a challenge to animal production. Mycotoxins are toxic compounds produced by fungi and can cause various negative effects on animal health, particularly aflatoxins, fumonisins, deoxynivalenol, and zearalenone. These mycotoxins are difficult to eliminate from food, and their contamination is especially concerning in corn, one of the main components of animal feed. One way to reduce the levels of these toxins in plants is through the use of transgenics, which not only exhibit higher productive performance but also have nutritional quality equivalent to non-transgenic variants. Therefore, this dissertation aims to compare the effect of genetically modified corn versus conventional corn on mycotoxin production and the performance of different animal species through systematic review and meta-analysis. To achieve this, the dissertation is divided into two systematic reviews: one focusing on the effect of genetically modified corn on mycotoxin levels, including meta-analysis, and a second review on the effect of genetically modified corn on animal production. Based on the data obtained from these reviews, it can be concluded that genetically modified corn has a beneficial effect on mycotoxin production, demonstrating a reduction of over 50% in mycotoxin levels compared to non-genetically modified corn. Furthermore, both types of corn exhibit nutritional equivalence for animal feeding, without harmful effects such as diarrhea and allergies, and with comparable productive performance.

Keywords: transgenic maize; mycotoxins; animal feed; systematic review; metanalysis

CAPÍTULO 1

INTRODUÇÃO E CONTEXTUALIZAÇÃO

O crescimento acelerado da população mundial entre o século XX e os dias atuais trouxe um desafio para a produção animal, aumentar a produção de alimentos de forma a atender a população mundial [1]. O aumento populacional e de renda causou aumento na demanda por proteína animal global desde os anos 60, com crescimento rápido de produção de aves, suínos, bovinos e peixes [2]. Desta forma, torna-se necessário o estudo abrangente de tais espécies animais em relação a fatores que podem afetar sua produção, entre eles: a utilização de milho transgênico na alimentação animal; a contaminação dos alimentos com fungos e a produção de micotoxinas. Estudos já foram realizados para comparar milho transgênico com não transgênico em relação às quantidades de micotoxinas [3], assim como em relação aos efeitos tanto de transgênicos [4] como das micotoxinas [5] à produção animal. Entretanto, o efeito conjunto da concentração de micotoxinas em cultivares de milho transgênico no desempenho zootécnico de espécies animais de interesse econômico foi realizado em apenas dois artigos em suínos [6, 7], até o presente momento. Assim, não é possível tirar conclusões a respeito da utilidade da transgenia sobre a produtividade animal frente às micotoxicoses.

O milho transgênico apresenta uma produtividade maior de grão por hectare comparado ao milho convencional [8], ainda existem indícios de que possua capacidade de reduzir a presença de fungos em relação ao milho convencional [9, 10], e que não possui efeitos nocivos ao animal [11]. Desta forma, é necessária uma análise sistemática e abrangente da produção de micotoxinas entre transgênicos e não transgênicos, bem como da sua interferência em animais de produção e em seu desempenho produtivo.

Portanto, esta dissertação tem como objetivo comparar o efeito do milho transgênico em relação ao milho convencional na quantidade de micotoxinas totais e de quatro micotoxinas isoladamente (aflatoxina, fumonisina, deoxinivalenol e zearalenona); bem como comparar o efeito do milho transgênico na alimentação e produção animal em quatro espécies de importância econômica (aves, suínos, bovinos e peixes).

1.1. MICOTOXINAS

Micotoxinas são compostos metabólicos secundários tóxicos de baixo peso molecular produzidos por alguns gêneros de fungos, sendo os principais Alternaria, Aspergillus, *Fusarium e Penicillium* [12, 13]. Atualmente já foram identificadas mais de 300 micotoxinas, as quais apresentam inúmeros riscos para saúde humana e animal. Outro fato importante de ser destacado é que essas substâncias são muito difíceis de serem eliminadas dos alimentos por suas características químicas que as fazem resistir as etapas de processamento [14]. As principais micotoxinas que afetam alimentos destinados para humanos e animais são as aflatoxinas, desoxinivalenol, fumonisinas, ocratoxina, patulina e zearalenona [15].

As micotoxinas são responsáveis por diversos sintomas quando ingeridos, o que gera impacto econômico negativo. Entre esses possíveis sintomas estão: efeitos agudos, distúrbios nervosos; alteração de conteúdo nutricional; alteração em função endócrina e neuroendócrina; supressão do sistema imune; câncer; diminuição de taxa de concepção, edema pulmonar, leucoencefalomalácia, entre outros [16, 17].

Aflatoxinas (AFL) são prevalentes em cereais e são produzidas por certas espécies de Aspergillus, principalmente *Aspergillus parasi*ticus e *Aspergillus flavus*. Tais toxinas causam extrema preocupação por apresentarem propriedades mutagênicas, carcinogênicas e teratogênicas, sendo a aflatoxina B1 o composto carcinogênico natural mais potente que se tem conhecimento [18, 19]. Devido ao fato de serem altamente resistentes ao calor, processos como cozimento e outros processamentos térmicos destroem uma pequena ou nenhuma porção do contaminante. Em animais apresenta efeitos crônicos e agudos, entre eles a redução de absorção de nutrientes, anorexia e imunossupressão [13].

As fumonisinas (FUM) são produzidas por espécies de *Fusarium* e foram dadas como possíveis carcinógenos para humanos. Além disso, estão relacionadas a problemas em animais, tais como: problemas cardíacos, edema pulmonar (suínos), leucoencefalomalácia (equinos), redução no desenvolvimento, diarreia e redução no ganho de peso [19].

O desoxinivalenol, conhecido como DON, é produzido por diversas espécies de *Fusarium*, podendo aparecer em combinação com outras micotoxinas ou sozinho. É a micotoxina mais frequente, com maior ocorrência em grãos, e causa fitotoxicidade para plantas [20, 21]. Nos animais causa hemato e imunotoxicidade, anorexia, supressão de ganho de peso, vômitos, entre outros [16].

A produção de zearalenona (ZEA) pode ocorrer tanto isoladamente como em concomitância com DON , por também ser produzida por fungos do gênero Fusarium [22]. É rapidamente absorvida após administração oral, com efeito dose-dependente [22]. Entre os efeitos de seu consumo nos animais de produção estão: redução da fertilidade e ovulação, aumento da incidência de abortos efeitos nas glândulas mamárias, entre outros [23, 24].

As micotoxinas contaminam uma diversidade de cereais utlizados na alimentação animal, entre elas milho, trigo, soja e sorgo, podendo gerar micotoxicose quando há o consumo de rações com contaminação [25].

1.2. TRANSGÊNICOS

As rações animais correspondem a cerca de 70% dos custos da produção [26]. A alimentação animal consiste em uma série de ações, com o objetivo de permitir a manutenção e produção por meio da provisão de nutrientes adequados e balanceados. Já a nutrição animal envolve desde o processo de apreensão e ingestão até a absorção de nutrientes, sendo o suprimento de elementos químicos necessários para as reações biológicas acontecerem [27, 28]. A alimentação pode ser composta por alimentos volumosos, concentrados ou a combinação destes, sendo os volumosos alimentos com teor de fibra bruta superior a 18% na MS, destacando – palhas, fenos, [27]. Os alimentos concentrados são divididos em energéticos (nível de proteína bruta menor que 18% na MS), tendo como exemplos o milho, sorgo e trigo; e proteicos (nível de proteína bruta maior que 18% na MS), destacando a soja, canola e girassol [27]. Desta maneira, tanto alimentação quanto nutrição animal são temas de grande importância para a produção, pois cada animal requer critérios específicos de alimentação para o bom desenvolvimento e desempenho, sendo necessárias rações específicas para cada espécie [27].

Um dos principais grãos que compõe as rações é o milho, que pode ser fornecido em até 50% da ração para bovinos e 80% para suínos e aves, e é fornecido em grão, silagem ou milho desintegrado com palha e sabugo, sendo estes alguns exemplos [28]. O milho tem alto valor nutritivo, sendo uma fonte importante de energia, carboidratos e aminoácidos

para aves e suínos, além de ser rico em carotenoides [26]. No entanto, um dos problemas para a produção do milho que reduzem sua qualidade para utilização na alimentação animal e humana é a contaminação por fungos [29].

Uma das formas de mitigação das micotoxinas é o uso de adsorventes na alimentação, no entanto, esses compostos podem trazer efeitos indesejados para os animais quando consumidos, como imunossupressão, redução na produtividade, alterações celulares e interação com medicamentos [30]. Além disso, os adsorventes podem apresentar quantidades de metais pedados e outros compostos que podem induzir toxicidade nos animais [30]. Desta maneira, uma forma para evitar a produção de micotoxinas nas plantas, especialmente no milho, é a utilização de transgênicos, que são um tipo de Organismo Geneticamente Modificado (OGM), o qual recebeu material genético exógeno e pode passar a expressar uma característica de interesse [31]. Existem diversos tipos de milho transgênico, com finalidades de transgenia diferentes, como: resistência a insetos [32], tolerância a herbicidas [33], produção de compostos [34, 35], resistência a fungos [36], entre outros. As mais utilizadas são a transgenia com finalidade de obter resistência a insetos [32] e tolerância a herbicidas [33]. Alguns transgênicos foram transformados para apresentar características de resistência ao crescimento de fungos fitopatogênicos produtores de micotoxinas [36]. Além disso, essas cultivares apresentam produtividade e quantidade de nutrientes equivalente às variantes não-transgênicas [3].

1.3. PRODUÇÃO ANIMAL

Tanto a alimentação adequada quanto a contaminação de rações são fatores que interferem nas espécies de produção animal [27, 28].

Os dois principais grupos de produção de aves são: frango de corte e galinhas poedeiras. O frango de corte é a base da produção de carne de aves e é muito consumido mundialmente [37]. Com o avanço da tecnologia e melhoramento genético foi possível obter um crescimento acelerado dos animais, alcançando o máximo de suas principais características produtivas (alta taxa de crescimento, ganho de peso e maior quantidade de carne no peito) em menor tempo [37]. Já as aves poedeiras possuem como característica principal a facilidade de confinamento, utilizando ventilação e luz artificial, além de uma grande quantidade de animais em espaço reduzido [38]. As principais características produtivas das poedeiras são a produção, qualidade, peso e composição dos ovos [38]. Para obter essa maior produção, é necessária dieta com formulação adequada de nutrientes [37].

Os parâmetros produtivos para gado de corte são o ganho de peso, qualidade da carne e rendimento de carcaça, enquanto para o gado leiteiro são quantidade e qualidade de leite [39, 40]. Apesar da seleção e melhoramento genéticos terem papel importante na eficiência, a dieta ainda é um fator de influência para a produção [39].

Os suínos também são altamente afetados pela dieta fornecida, sendo os custos totais das dietas entre 60 e 70% dos custos totais da produção [41]. Os principais parâmetros de desempenho dos suínos são o ganho de peso, conversão alimentar, rendimento de carcaça e qualidade da carne [41]. Para alcançar os melhores resultados para esses parâmetros, o melhoramento genético é muito utilizado em conjunto com a nutrição [42]. A compreensão do consumo de ração com o objetivo de ganho de peso dos animais é muito importante, pois interfere desde o ganho de peso diário até o teor de gordura nos tecidos [41].

Entre os animais de produção, os peixes apresentam a maior variedade de espécies e habitats e, ao mesmo tempo, com maiores dificuldades de controlar a variabilidade de disponibilidade dos animais [43]. Dentre a diversidade de espécies pertencentes ao grupo, a *rainbow trout*, apresenta compatibilidade com a maioria dos climas e condições climáticas [44] e um de seus parâmetros produtivos principais são os carotenoides, obtido através das dietas, podendo ser aumentado através de dietas ricas nesses compostos [45]. Outra espécie, a tilápia do Nilo (*Oreochromis niloticus*), é a mais produzida e importante comercialmente [46]. Um dos aspectos positivos de sua produção é que sua manipulação genética para promover produtividade é relativamente fácil, com o principal aspecto de desempenho o ganho de peso [46]. No entanto, um dos problemas enfrentados em sua produção, assim como das outras espécies animais, é a confiabilidade dos alimentos a serem fornecidos [46].

1.4. REVISÃO SISTEMÁTICA E METANÁLISE

Revisão sistemática (RS)_consiste na sintetização de evidências provenientes de todas as pesquisas relevantes disponíveis sobre uma questão de pesquisa, área do conhecimento ou fenômeno específico [47]. Ela utiliza de um processo de revisão de literatura abrangente, passível de reprodução e imparcial, que sintetiza, localiza e avalia um conjunto de informações provindas de todos os estudos científicos sobre o tema para obter uma visão confiável e geral do assunto abordado [47].

Existem variados tipos de RS, sendo eles: revisões de efetividade, experimentais (qualitativas), de custos/avaliação econômica, de prevalência e/ou incidência, de acurácia de testes diagnósticos, de etiologia e/ou risco, de opiniões de especialistas/políticas, psicométricas, prognósticas e metodológicas sistemáticas [48]. Elas variam em relação ao objetivo de pesquisa e ao formato da questão, como por exemplo os formatos PICO (*Population, Intervention, Control, Outcomes*), PIRD (*Population, Index Test, Reference Test, Diagnosis of Interest*) e PEO (*Population, Exposure, Outcome*) [48].

Quando apropriado, uma RS pode apresentar métodos estatísticos, as metanálises, permitindo apresentar o desfecho de forma mais precisa pelo aumento do tamanho amostral [47]. Elas combinam os dados de todos os estudos de acordo com a similaridade de tratamentos e metodologias, permitindo explorar o tamanho "real" do efeito da intervenção [47]. Algumas vantagens da realização de metanálise são: responder questões não levantadas pelos estudos individuais, melhorar a precisão e resolver controvérsias de estudos aparentemente contraditórios ou gerar hipóteses novas [49]. Os princípios básicos utilizados são: realização de processo em duas fases, onde primeiro é calculada uma estatística resumida de cada estudo e depois uma estimativa combinada com a média ponderada dos efeitos; a combinação de estimativas pode estimar se os efeitos são aleatórios ou fixos; o erro padrão é utilizado para obter um intervalo de confiança e valor de p; pode incorporar avaliação para determinar se a variação dos estudos isolados é compatível com a aleatória ou se é grande o suficiente para apontar inconsistências; no entanto, a falta de dados é um problema a ser ponderado quando na realização da metanálise [49].

O método do inverso da variância é um dos métodos de análise, utilizado tanto para variáveis contínuas como dicotômicas [49]. Alguns tipos de metanálise são: de efeito fixo, de efeito aleatório, para desfechos dicotômicos, método de Mantel-Haenszel, método de Peto e metanálise de efeitos raros [49].

Existem casos em que não deve ser realizada metanálise, como quando são encontrados apenas dois estudos com dados de efeito opostos, de maneira que os achados não devem ser comparados [47]. Nos casos de vários estudos apresentando resultados divergentes deve-se utilizar ferramentas estatísticas para avaliar o grau de similaridade entre os efeitos encontrados, como teste para heterogeneidade e inconsistência [47].

Com o tempo, ocorreram mudanças em relação a terminologia de RS ou metanálises, mudando o nome de QUORUM para PRISMA, consistindo em um checklist de 27 itens e um fluxograma de quatro etapas, com o objetivo de abranger a ambos e auxiliar os autores a melhorar seus relatos [50]. Seu enfoque é em ensaios clínicos randomizados, mas é possível utilizá-lo como base para revisões de qualquer tipo de pesquisa, em especial avaliação de intervenções [50].

CAPÍTULO 2

Artigo científico submetido no periódico *Food and Chemical Toxicology*

Resistance of transgenic maize cultivars to mycotoxin production – Systematic Review and Meta-analysis.

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ABSTRACT

In food, approximately 25% of grain contamination is caused by fungi, some of which are mycotoxin producers that can cause adverse effects when consumed. Maize has been engineered for insect resistance and herbicide tolerance. This systematic review compared the quantities, by means of meta-analysis, of four mycotoxins (AFL, FUM, DON, ZEA) between genetically modified (GM) and conventional maize kernels. This manuscript was prepared following the PRISMA method, and search was performed using PubMed, Web of Science, Scopus, Google Scholar, and CAPES journals databases. Analyses were performed using the RevMan v.5.4 software. Transgenic maize showed a decrease in total amount of mycotoxins by 54% ($p<0.001$) compared to that of conventional maize. A similar result was observed for fumonisins, with 56% ($p<0.001$) less mycotoxins in GM maize. The amounts of AFL and ZEA were lower in GM maize than in conventional ones by 49% ($p = 0.02$) and 51% ($p <0.001$), respectively. However, the opposite result was observed for the amount of DON, with an increase in mycotoxin quantity by 6% ($p<0.001$) in genetically modified maize compared to that in conventional maize. Nevertheless, these results and the results of ZEA were deemed inconclusive due to the amount of research and sample units used. We conclude that the use of transgenic maize reduces over 50% of the amount of mycotoxins, mainly fumonisin and aflatoxin. Further studies involving ZEA and DON are needed to confirm their reduction in transgenic maize, with a larger sample size.

Keywords: Aflatoxin; Deoxynivalenol; Fumonisin; Zearalenone

1. INTRODUCTION

Fungi contaminate approximately 25% of grains consumed by humans and animals [1]. Fungi of the genera *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* produce secondary metabolites, including mycotoxins, low molecular weight toxic compounds [1].

Over the past few decades, more than 300 mycotoxins have been identified, which pose risks to human and animal health, such as nervous disorders, pulmonary edema, alteration of endocrine functions, and cancer [1,2]. The main mycotoxins contaminating animal feed products are aflatoxin (AFL), deoxynivalenol (DON), fumonisin (FUM), ochratoxins, zearalenone (ZEA), and patulin [3]. Elimination of these substances from food is difficult because of their chemical characteristics that confer resistance to the processing stages [4]. A loss of approximately 40 million dollars in maize owing to FUM contamination has been estimated in the US [5]. For these reasons, several countries have legislation regarding the most common types of mycotoxins, determining limits on the maximum amounts allowed in products for animal consumption (Table 1).

Table 1. Maximum permitted concentration of mycotoxin in grains and derived products in major maize producers.

Country/Region	**Mycotoxin**	**Quantity (μg/kg)**
Argentina [6]	AFL	20
Brazil [7]	AFL	50
China [8]	AFL	50[1]
	FUM	60000
	ZEA	500
Europe Union [9]	AFL	20
	FUM	60000
	DON	12000[1]
	ZEA	3000[1]
United States [10]	AFL	20[2]
	FUM	5000[2]
	DON	5000[2]

[1]Maize products only; [2]minimum value referring to maize in the effective legislation for the region, which may have higher values depending on the intended use.

In general, mycotoxins ingested by humans and animals, particularly dairy cattle, poultry, horses, pigs, and fish, can cause acute toxicity (hemorrhages, abortions, gastroenteritis), nervous disorders, alterations in nutritional content, alterations in endocrine and neuroendocrine function, suppression of the immune system, cancer, decreased conception rate, pulmonary edema, and leukoencephalomalacia, with consequent negative economic impacts [11, 12].

The main-field mycotoxins are FUM, DON, and ZEA, which are mainly produced by *Fusarium* and affect maize [13]. DON can concomitantly occur with ZEA, and pest control can minimize the production of these toxins [14]. Regarding contamination during storage, the most common mycotoxin is AFL produced by *Aspergillus* and *Penicillium*. AFL is considered the most significant risk factor for human and animal contamination and is described as a class 1 carcinogenic compound [2, 13]. Its appearance in the field can also occur, to a lesser extent, in plants damaged by drought or pests [14].

One possible method to mitigate mycotoxins in grains is to produce transgenic plants that resist the growth of phytopathogenic fungi and can consequently inhibit the growth of toxigenic fungi. The term transgenic refers to a type of genetically modified organism that has received exogenous genetic material and can express a desired trait [15]. Maize, soybeans, oats, rice, sorghum, and barley stand out among the important grains in animal feed [16]. Maize is the second highest-produced grain in the world, with approximately 80% of its production destined for animal consumption [17] and has transgenics for different purposes, including resistance to insects [17], herbicides [18], and cultivars resistant to *Fusarium* [19, 20]. In addition, these cultivars have productivity and nutrient contents similar to those of the non-transgenic varieties [21]. This investment in cultivar development prevents the growth of fungi and, consequently, the production of mycotoxins [22].

Studies have compared transgenic and non-transgenic maize in terms of the amount of mycotoxin produced [23, 24]; however, no general view on which types of transgenics are effective in mitigating mycotoxins and which mycotoxins are deleterious has been presented. Therefore, the present systematic review aims to analyze the effects of transgenic maize in mitigating FUM, AFL, DON, and ZEA, through a meta-analysis. The research question used for this was, "Do genetically modified maize cultivars have the ability to mitigate mycotoxins compared to conventional cultivars?".

2. METHODS

This systematic review was conducted using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [25], following the PICO acronym standard: population, intervention, control, and outcome.

The concentrations of FUM, AFL, DON, and ZEA in genetically modified and conventional maize grains were analyzed, with maize as the population and mycotoxin quantity as the outcome. Once this systematic review aims to evaluate the ability of transgenic methods in maize to mitigate economically relevant mycotoxins, the comprehensive search was performed using the following search protocol:

Population: maize OR corn OR *Zea mays*

AND

Intervention: transgenic OR transgenic plant OR OGM

AND

Control: conventional maize OR conventional corn

AND

Outcome: mycotoxin OR fungal toxin

The search was conducted using PubMed, Web of Science, Scopus, Google Scholar, and the CAPES (Brazilian Higher Education Personnel Improvement Coordination) journals portal. Initial search yielded 2597 articles. English was the language used for the search. Only articles published in scientific journals using Qualis A1 to B2 (according to CAPES classification) or SCIMago Q1 and Q2 were included in the search.

From the articles selected using the search protocol, the title, year, journal, journal quality, and database were recorded.

The exclusion criteria were as follows: i) Duplicate articles (same article in different databases); ii) title (articles without maize as a population, evaluating pathogens and not mycotoxins, or not containing transgenics); iii) abstract (articles that did not have mycotoxins as an outcome, did not contain transgenic maize, or were literature or systematic reviews); and iv) methodology (absence of: average mycotoxin levels, described quantification techniques and description of samples used).

After applying the exclusion criteria, 42 articles were selected (Figure 1), for which an extraction form was applied, containing the analyzed mycotoxin, amount of mycotoxin, unit of quantification, sample value, methodology for quantification, region where experiments were conducted, crop mode and season of planting and harvesting, cultivated hybrids, type of transgenics, and proteins and genes used, when described.

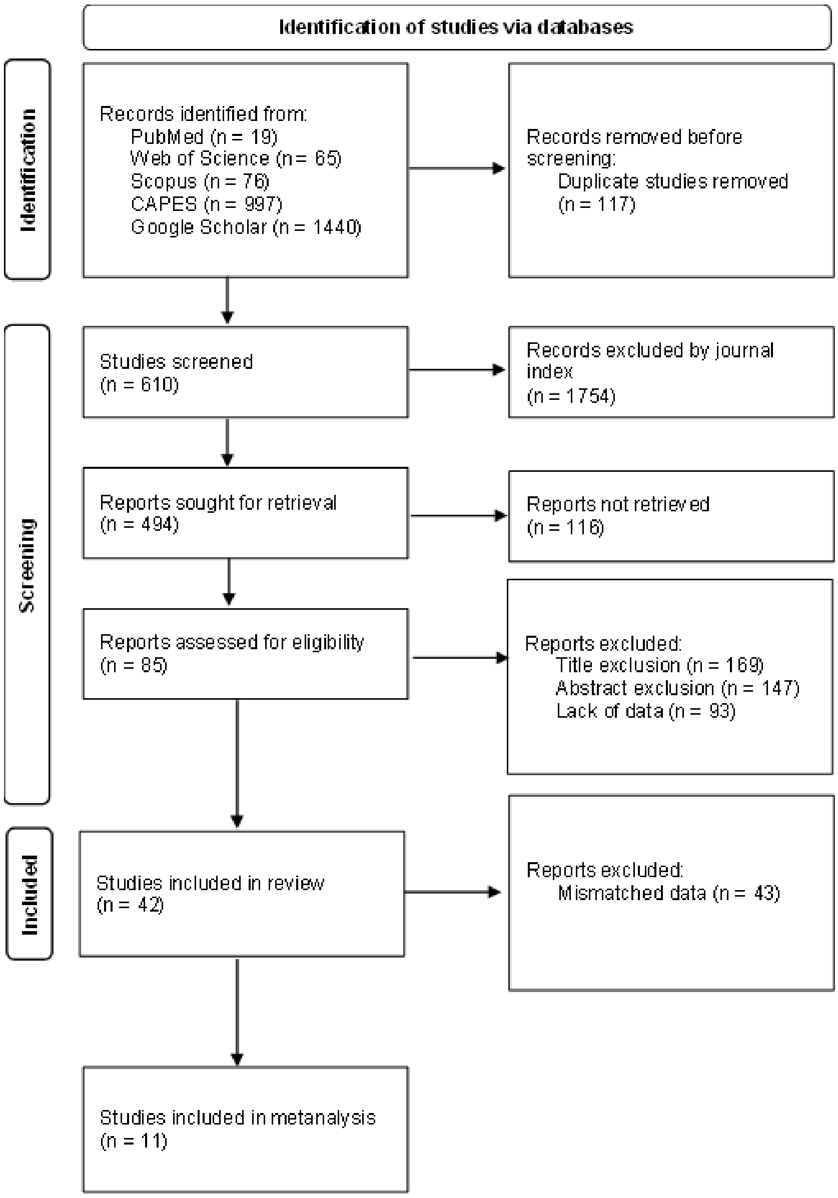

Figure 1. Flow diagram of study selection based on PRISMA [26].

Articles with compatible methods for quantifying mycotoxins were considered suitable for extraction and those that presented the evaluated maize hybrids and described the geographic region and execution of the experiments, listed the evaluated mycotoxins and their quantities, described the proteins used in the process and genes that conferred resistance, and provided the sample size (n) of cultivars used.

The risk of analysis bias was performed according to the Cochrane method [27]. Risk can be low, high, or uncertain and includes five points: sample allocation, blinding, incomplete or selective outcomes, sample size, and journal quality were considered. Low risk is considered for sample allocation when a random selection or random number generator is used to select the samples, and high risk is considered when the selection was performed. Regarding blinding, a low risk is considered when the test is double-blind, and a high risk is considered when it occurs without blinding, which may be owing to evaluation bias or the placebo effect. Outcomes present low risk when no loss of samples is observed or the loss is justified and high risk in the case of many unexplained losses. A low risk for sample size is associated with numerous samples, whereas a high risk is associated with few samples. Finally, the journal quality is evaluated, with a low risk for Q1 and Q2 journals and a high risk for Q4 or non-indexed journals.

Statistical Analysis

Data were compiled and organized for meta-analysis using Review Manager v.5.4 [28]. The amount of mycotoxin (mg/kg) was compared between conventional and transgenic maize using the standard mean difference with 95% confidence intervals and a fixed-effect model. Heterogeneity was evaluated using I^2 and Cochran's Q tests, and the overall effect between treatments was analyzed using the Z-test. A p-value < 0.05 was considered statistically significant. Analyses were conducted for both the total amount of mycotoxins (evaluating the four mycotoxins together) and each mycotoxin individually.

The results were classified as conclusive or inconclusive based on inverse variance analysis and sample size. Conclusive results were described as having significantly different values, as well as high sample size intensity. Positively conclusive results were those where transgenic grains had relatively low level of mycotoxin than had non-transgenic grains and were negatively conclusive when transgenic grains had relatively high levels of mycotoxin than had non-transgenic grains. Inconclusive results were those that did not show significant agreement (i.e. opposite results regarding the mean values of transgenic and non-transgenic maize) or had low sample size intensity.

Figures and images for visual summarization were prepared using the Lucidchart software [29].

3. RESULTS

This session was divided into general analyses and analyses of mycotoxin quantity, encompassing total analysis of all mycotoxins together and each toxin individually. Regarding mycotoxin concentration, the groups were divided according to the average quantity of mycotoxins in non-transgenic maize.

3.1. GENERAL

Based on the selection criteria and subsequent application of the exclusion criteria by title, 325 articles were selected. A total of 178 articles were selected based on the selection criteria by the abstract and methodology. After selection, 42 articles [30–71] were included in the systematic review.

The US had the highest number (17) of studies, followed by Brazil, Italy, Germany, and Spain with five, four, three, and three studies, respectively. In addition, two studies were conducted in Argentina and France, and isolated studies were conducted in Canada, Hungary, Japan, Kenya, Mexico, the Philippines, South Africa, and Republic of Korea.

Regarding the location and planting season, 76% (31) samples were cultivated in the field, and 61% (25) were cultivated in the summer. Other forms of cultivation included greenhouse (four cases), growth chamber, glasshouse, or material collection after harvest (two cases each); and one case was not reported. Regarding season, studies were also registered with planting or harvesting occurring in spring (eight cases) and autumn (10 cases), in addition to reporting temperature in the case of growth chambers, and eight cases without information on the season or cultivation temperature.

A total of 273 conventional and transgenic hybrids were analyzed (between 1 and 43 in each study), within a total of 1,449 analyzed samples. The hybrids studied were conventional maize, crosses, genetically modified organisms, and transgenic plants. In terms of quantity, 19% articles (eight cases) presented only one evaluated hybrid; 23.8% (ten cases) presented two hybrids; 11.9% (five cases) contained three hybrids; 23.8% contained four to ten hybrids; 21.4% (nine cases) evaluated more than ten hybrids, and one case (2.4%) did not describe the hybrids used.

The number of samples analyzed per article ranged between 1–480, with an average of 35, consisting of grain pools, ground grains, or maize residues (leaves, stems, and ears with and without grains).

The purpose of genetic modification was mainly to confer resistance to insects in 20 cases (48%), followed by herbicide resistance in 11 cases (26%), transformations for Aspergillus flavus resistance in five cases (12 %), and antibiotic resistance in two cases (5%). Of the total number of studies, 31% (13) did not present data on the technique or function of genetic modification (Table 2).

Table 2. Number of articles with each transgenic

Transgenic objective	Number of articles
Insect resistance/tolerance	19
Herbicide resistance/tolerance	11
Aspergillus flavus resistance	5
Antibiotic resistance	2
Not reported	14

The mycotoxins analyzed in this review were AFL, FUM, DON, and ZEA; however, ochratoxin and nivalenol were also evaluated in some of the studies included in this review. Based on the collected data, a high number of studies were found for FUM (67%), followed by AFL (45%), DON (26%), and ZEA (21.4%) (Figure 2). Three articles conducted analysis of the quantity of mycotoxins in the four mycotoxins, observing both their isolated presence and their concomitant presence. Three other articles evaluated FUM and AFL, four evaluated DON, FUM, and ZEA, two evaluated AFL and DON, and one evaluated DON and ZEA. No article evaluated AFL together with DON or ZEA, and likewise, no article evaluated FUM together with DON or ZEA.

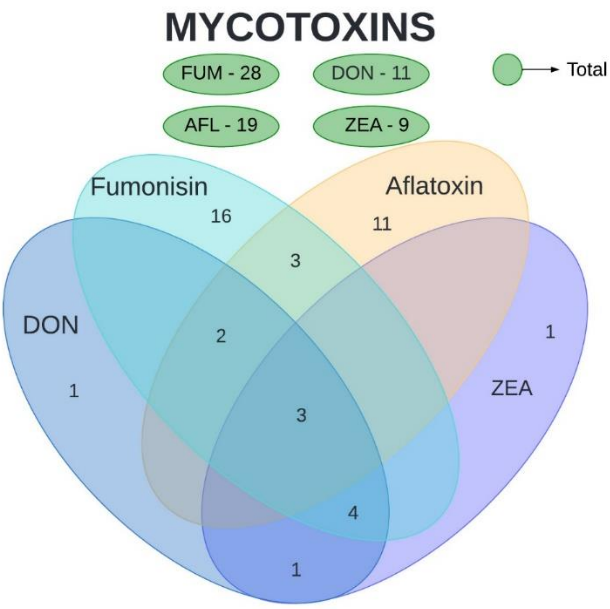

Figure 2. Number of articles evaluating mycotoxin concentration.

The main methods used for quantification of mycotoxins were liquid chromatography with fluorescence detection (FLD), enzyme-linked immunosorbent assay (ELISA), and liquid chromatography coupled with mass spectrometry (MS/MS), which were used in 22, 15, and 14 studies, respectively. In addition, rapid one-step assay (ROSA), VICAM AflaTest, Fungi-Plex multiplex mycotoxin assay kit, FluoroQuant AflaTest, and Ridascreen Fast Test were used. Six articles did not describe the quantification methodology. In some cases, more than one test was used in the same study to evaluate one or more mycotoxins (Figure 3 and Table 2).

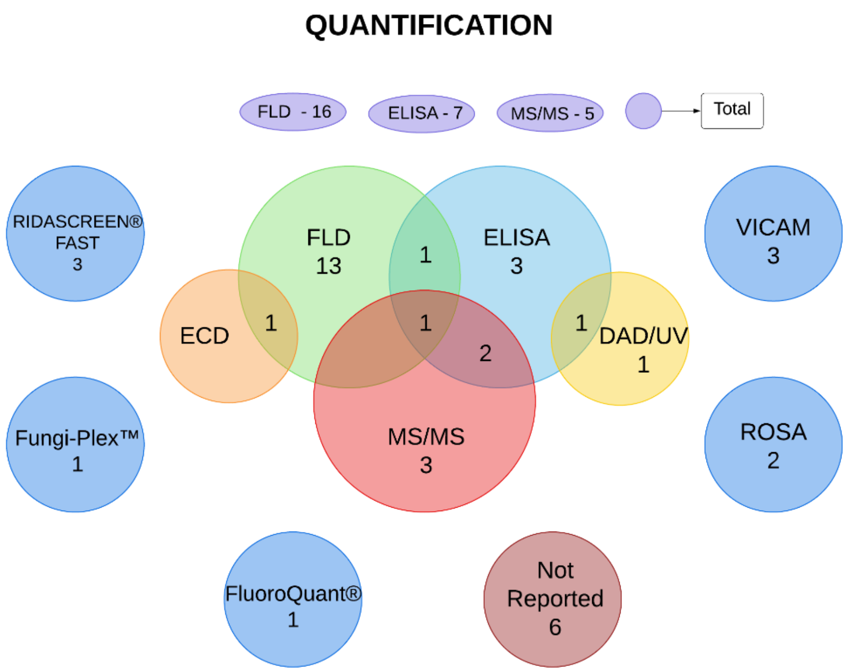

Figure 3. Methods of mycotoxin quantification used in the selected articles. The figure was created using Lucidchart software. DAD/UV, Diode Array Detector/Ultraviolet; ECD, Electron-capture Dissociation; ELISA, Enzyme-Linked Immunosorbent Assay; FLD, Fluorescence Detection; MS/MS, Tandem Mass Spectrometry; ROSA, Rapid One Step Assay.

Subsequently, the mean values, standard deviations, sample sizes, and p-values of the analyses were obtained (Table 3). Some studies have assessed more than one mycotoxin, and multiple experiments have been conducted in the same study. Therefore, eleven studies contained a total of 28 experiments.

Table 3. Comparison of the amounts of mycotoxins between genetically modified (GM) and non-GM maize in 11 studies containing 28 experiments

		NT	T	
Analysis	Articles[2]	$\bar{X}$ ± SD (n)	$\bar{X}$ ± SD (n)	p
Total	11	4.63 ± 0.56 (764)	2.13 ± 0.26 (674)	< 0.001
Total[1]	11	2.59 ± 0.38 (508)	1.68 ± 0.20 (482)	< 0.001
FUM	8	6.07 ± 0.74 (571)	2.69 ± 0.33 (508)	< 0.001
FUM[1]	8	2.59 ± 0.38 (508)	1.77 ± 0.20 (482)	< 0.001
AFL	4	0.15 ± 0.03 (61)	0.08 ± 0.03 (34)	0.020
DON	2	0.70 ± 0.04 (90)	0.74 ± 0.06 (90)	< 0.001

ZEA	1	0.01 ± <0.01 (42)	0.01 ± <0.01 (42)	< 0.001

$\bar{X}$: mean (mg/Kg), SD: standard deviation (mg/Kg), N: number of samples, P: p-value - inverse analysis of variance, NT: non-transgenic maize, T: transgenic maize, [1]: there were discrepant results in the groups for the analyses, which were removed from the analysis in order to evaluate possible interference of these data in the results, [2]: quantity of articles.

Eleven articles were included in the meta-analysis, containing 28 experiments, all evaluated transgenic hybrids having resistance to insects, with six out of 11 (55%) being resistant only to insects, four out of 11 (36%) also presenting resistance to herbicides, and one (9%) expressing resistance to herbicides and antibiotics (Table 4). Therefore, resistance to insects might also contribute to the decrease in mycotoxin quantities in transgenic grains.

Table 4. Data for mycotoxins and the purpose of transgenics from the articles included in the meta-analysis.

Article ID	**Transgenic objective**	**Mycotoxin**
ABBAS, et al., 2005 [30]	Insect resistance	AFL, FUM
ABBAS, et al., 2008 [31]	Insect resistance	AFL
ACCINELLI, et al., 2014 [32]	Insect resistance and herbicide resistance	AFL
BORDINI, et al., 2019 [41]	Insect resistance and herbicide resistance	FUM
BOWERS, et al., 2014 [42]	Insect resistance	FUM
DOWD, 2001 [47]	Insect resistance and herbicide resistance	FUM
FOLCHER, et al., 2010 [49]	Insect resistance	FUM, DON, ZEA
HERRERA, et al., 2010 [54]	Insect resistance	FUM
NAEF, et al., 2006 [59]	Insect resistance, herbicide resistance, and antibiotic resistance	DON
ROCHA, et al., 2016 [64]	Insect resistance	FUM
WEAVER, et al., 2017 [69]	Insect resistance and herbicide resistance	AFL, FUM

Of the 28 experiments, eight showed relatively high amounts of mycotoxins in transgenic plants, with three out of eight (38%) [42, 49] showing resistance to insects only and the other five (62%) [32, 47, 59, 69] showing resistance to insects and herbicides.

The results obtained in the meta-analysis were shown in the forest and funnel plots presented in the supplementary material, where it was possible to observe the symmetry of the results and the low dispersion. The funnel plot is used for assessing publication bias, thus observing the tendency for statistically significant studies to be more likely to be published [72]. Additionally, the dispersion of points indicates the precision of the studies, where studies with higher precision exhibit lower dispersion, while studies with lower precision exhibit higher dispersion [72].

3.2. TOTAL

A significant difference ($p<0.001$) was observed in the total amounts of FUM, AFL, DON, and ZEA in 1438 samples between non-transgenic (n=764) and transgenic (n=674) maize (figure S1 and S2). To understand the discrepancies and similarities in detail between the studies developed in 28 experiments [30, 31, 32, 41, 42, 47, 49, 54, 59, 64, 69], three groups were considered: Averages < 10 mg/kg, between 10–100 mg/kg, and > 100 mg/kg.

The first group consisted of 15 experiments (54%) [30, 31, 32, 42, 47, 49, 69] with mean amounts of mycotoxins ranging between 0.02–6 mg/kg, containing at least one experiment for each evaluated mycotoxin. Of the 15 experiments, 14 (93.3%) [30, 31, 42, 47, 49, 69] used grain samples taken from ears harvested from the field between summer and autumn, whereas one experiment [32] used samples of subsequently inoculated maize residue. Five out of fifteen experiments (33%) evaluated FUM [42, 47, 69]; six (40%) evaluated AFL [30, 31, 32, 69]; two (14%) [49] evaluated DON; and two (14%) [49] evaluated ZEA; here, 10 experiments [30, 31, 32, 49, 69] used high-performance liquid chromatography with mass spectrometry (HPLC–MS/MS) for quantification, and five (33%) [42, 47] used HPLC with FLD (HPLC–FLD). Regarding the mean amounts of mycotoxins, 6 experiments (40%) [31, 42, 47, 49] presented a higher amount of mycotoxins in transgenic plants than in non-transgenic plants; one (7%) [69] presented similar values for both groups; and 8 (53%) [30, 32, 42, 47, 49, 69] presented lower means in transgenic plants than in non-transgenic plants; this group was positively conclusive.

The second group consisted of five experiments (18%) [30, 42, 54, 59] with means ranging between 3–50 mg/kg, where three experiments (60%) evaluated FUM [30, 42, 54]; and two (40%) evaluated DON [59]; with all samples taken from ears collected in the field between spring and autumn. Immunoassay detection methods were used in three (60%) experiments (ELISA in two [59] and ROSA in one [54]), and HPLC–FLD was used in the other two experiments (40%) [30, 42]. In four out of five experiments [30, 42, 54, 59], the mean amounts of mycotoxin were lower in transgenic than in non-transgenic maize. Opposite results were observed in the other one [59]. Despite the opposite results observed in these studies, considering the sample size and methodological quality described in the articles, it can be concluded that this result is conclusive, this means, the amount of mycotoxins in transgenic maize was lower than in non-transgenic maize.

The third group consisted of eight experiments (29%) [30, 41, 49, 64], with means ranging between 5–820 mg/kg. All experiments involved FUM analysis using HPLC–FLD for quantification. All mean amounts of mycotoxins were lower in the transgenic group than in the non-transgenic group, thereby rendering this group positively conclusive.

Among the 28 experiments analyzed, three (11%) [30] presented discrepant values for mycotoxin, prompting a new analysis without those experiments. Therefore, the total number of experiments decreased from 28 to 25 [30, 31, 32, 42, 47, 49, and 69], and that of the first group decreased from 15 to 12 articles. In this analysis, the first group had six experiments (50%) [31, 42, 47, 49] with higher amounts of mycotoxins in transgenic groups than in non-transgenic groups; one (8%) [69] showed equal mean amounts for both groups; and five (42%) [32, 42, 47, 49, 69] showed lower mean amounts in transgenic groups than in non-transgenic groups, thereby rendering the results of this group inconclusive. No changes were observed in the other two groups, and the results showed a significant difference ($p<0.001$) in mycotoxin amounts with a positive and conclusive outcome (figure S3 and S4).

3.3. FUM

A significant difference ($p<0.001$) in the amount of FUM was observed between transgenic ($n=508$) and non-transgenic ($n=571$) maize in 16 experiments [30, 41, 42, 47, 49, 54, 69], accounting for 57% of 28 experiments (figure S5 and S6). To understand the discrepancies and similarities among the studies in detail, three groups were considered: mean values < 10 mg/kg, between 10–200 mg/kg, and > 200 mg/kg.

The first group was composed of five experiments (31%) [42, 47, 69], with mean amounts of FUM ranging from 0.5–4.8 mg/kg, extracted from ear samples directly harvested from the field, and quantified by HPLC–FLD. Three [47, 69] of the five experiments (60%) analyzed AFL along with FUM. Regarding the amount of FUM, two (40%) experiments [42, 47] showed higher mean values in transgenic groups than in non-transgenic groups, two (40%) [42, 47] showed lower mean values in transgenic groups than in non-transgenic groups, and one (20%) [69] presented same mean value for both groups, being inconclusive.

The second group, with five of the 16 experiments (31%) [30, 42, 49, 54], had mean values of mycotoxin ranging between 3–130 mg/kg. Three experiments (60%) [30, 49] used HPLC–MS/MS as the quantification method, one (20%) [42] used HPLC–FLD, and one (20%) used ROSA [54]. Two experiments (40%) [42, 54] evaluated FUM only, whereas three (60%) [30, 49] evaluated other mycotoxins. All experiments showed higher mean values in non-transgenic groups than in transgenic groups; therefore, this group was positively conclusive.

The third group consisted of six of the 16 experiments (38%) [30, 41, 64] with average FUM levels ranging between 150–820 mg/kg in samples of ears harvested in the field. Three of the six experiments (50%) [30] evaluated AFL along with FUM, and the other three [41, 64] evaluated FUM only. All experiments used HPLC–MS/MS to detect mycotoxins. All experiments showed lower averages in transgenic groups than in non-transgenic groups; therefore, this group was positively conclusive.

Among the 16 analyzed experiments, three (19%) [30] showed highly different mycotoxin levels, resulting in a new analysis without these experiments. Therefore, the total number of experiments decreased from 16 to 13 [30, 41, 42, 47, 49, 54, and 69], and that of group three decreased from six to three experiments [41, 64]. Group three showed lower averages in transgenic groups than in non-transgenic groups, rendering the results of this group positively conclusive. No change for the other two groups was observed, and the results showed a significant difference ($p<0.001$) in mycotoxin levels, having a positively conclusive outcome (figure S7 and S8). Therefore, based on the evaluated samples, the amount of FUM in GM maize was lower than that in non-GM maize.

3.4. AFL

In the meta-analysis, a difference ($p = 0.02$) was observed in the amount of AFL between non-transgenic ($n = 61$) and transgenic ($n = 34$) maize in six of the 28 experiments [30, 31, 32, 69], comprising 21% of the total experiments (figure S9 and S10). To better understand the similarities and discrepancies between studies, two groups were formed: those with average doses < 1 mg/kg and those with average doses ≥ 1 mg/kg.

The first group consisted of three out of six experiments (50%) [30, 32, 69] with average amounts of mycotoxin ranging between 0.04–0.4 mg/kg, quantified through fluorescence detection. Two out of three experiments (67%) were conducted with ear samples directly harvested from the field [30, 69], and one (33%) used samples of maize residues (ears with and without grains, leaves, and stems) collected from the field and inoculated with A. flavus isolates. Two experiments (67%) evaluated FUM and AFL, and all samples were collected during late summer/early autumn. Two experiments (67%) showed higher average amounts in transgenic maize than in non-transgenic maize, thereby presenting a negatively conclusive result for this group.

The second group contained three (50%) [30, 31] with average amounts ranging between 1–4 mg/kg. All experiments were conducted with ear samples collected directly from the field in the same manner as in the previous group, during late summer/early autumn. Fluorescence-based quantification and analysis of one or more mycotoxins along with AFL were unanimously used. All experiments in this group showed lower mycotoxin amounts in transgenic maize than in non-transgenic maize, resulting in a positively conclusive result for this group.

In Group 1, the amount of AFL was higher in transgenic maize than in non-transgenic maize, whereas the opposite trend was observed in Group 2. Although a significant effect was observed in the meta-analysis, the results were considered to be inconclusive.

3.5. DON

In the meta-analysis, a significant difference ($p<0.001$) was observed in the levels of DON between transgenic ($n=90$) and non-transgenic ($n=90$) maize, with four out of 28 (14%) [49, 59] experiments showing average DON levels between 2–43 mg/kg (figure S11 and S12).

Four experiments were conducted using field-harvested ear samples. Two experiments (50%) [59] evaluated FUM and ZEA in addition to DON and quantified them using HPLC–MS/MS, whereas the other two (50%) [49] quantified DON only using immunochromatographic ELISA. Of the four experiments, two (50%) [49] showed higher average levels of mycotoxins in transgenic maize than in non-transgenic maize; one (25%) [59] showed equal levels in both groups, and one (25%) [59] showed lower levels of mycotoxins in transgenic maize than in non-transgenic maize.

Therefore, despite the significant effect observed in the meta-analysis, the results were considered to be inconclusive.

3.6. ZEA

In the meta-analysis, a highly significant difference ($p<0.001$) was observed in the amount of ZEA between non-transgenic ($n=42$) and transgenic ($n=42$) maize in two out of 28 experiments (7%) [49], with mean values ranging between 0.02–0.4 mg/kg (figure S13 and S14).

Both experiments were conducted in France, with maize ears harvested in the field during summer. Mycotoxins were quantified using mass spectrometry (MS/MS). Other mycotoxins were also evaluated using the same samples, and the mean levels of mycotoxins were lower in transgenic maize than in non-transgenic maize.

Therefore, despite a significant effect observed in the meta-analysis, the results were inconclusive owing to the number of experiments.

Based on these results, a significant difference ($p<0.05$) in the levels of mycotoxins was observed during the evaluation of total and individual mycotoxins. These findings indicate that, except for DON, higher amounts of mycotoxins were present in conventional maize than in transgenic maize.

4. DISCUSSION

In the analysis of overall mycotoxin content, transgenic maize exhibited a significant reduction by 54% ($p<0.001$) with respect to conventional maize. Similar outcomes have been observed for the levels of FUM, with 56% reduction ($p<0.001$) in GM maize. Additionally, the levels of AFL and ZEA were lower in GM maize than in conventional maize, with reductions of 49% ($p = 0.02$) and 51% ($p<0.001$), respectively. Conversely, DON levels showed a significant increase by 6% ($p<0.001$) in GM maize with respect to those in conventional maize. Despite the high occurrence of AFL during storage, evidences support its production in the field, which may cooccur with the production of other mycotoxins, such as FUM, DON, and ZEA, in the field, allowing for an equivalent comparison of the effects of transgenic maize for all analyzed mycotoxins [14]. These results are supported by the forest and funnel plots obtained from the meta-analysis. Observing the result of the forest plot is important because, in the absence of bias, the distribution of points on the graph is symmetric around the central line, while in the presence of bias, this dispersion of points is wider and asymmetric [72]. Additionally, the asymmetry of the funnel plot can be influenced not only by bias but also by the precision of the studies used [72].

The higher relative difference found in the quantities of mycotoxins was observed in the group with higher concentrations of fumonisin. This demonstrates that specifically in the group that causes the highest economic losses, the best results were observed. The results presented in groups with higher means showed a relatively greater reduction compared to groups with lower means, which may be associated with the fact that insect damage levels are higher. However, there are no studies that have evaluated the correlation between different insect infestation levels and the production of mycotoxins. In order to establish this correlation, a controlled study would be necessary, with different insect infestation levels, to assess the extent of damage to maize plants and subsequently measure the amount of mycotoxins produced.

Damage by insects, drought, and other factors that can cause tissue damage in plants are among the factors that serve as gateways for fungal contamination and subsequent mycotoxin production [14, 73]. The use of transgenic maize allows the reduction of target insects in the field without affecting other insect populations [74]. This is owing to the use of genes, including cry1A and cry1Ab, which are toxic to specific insect species [75]. These genes belong to Bacillus thuringiensis, the most common insertion in transgenic maize, and are toxic to insects, particularly those in the Coleoptera, Diptera, and Lepidoptera families that are harmful to maize and other crops [75]. When the proteins produced by cry1A and cry1Ab are ingested by insects, they are dissolved by proteases and the alkalinity of their intestines and form toxic crystals that result in inflammation, starvation, and eventual death of the insects [76]. Insects from the three main families mentioned above cause damage to plants by chewing, which primarily affects roots, leaves, and stems [77]. These insects act as vectors of infections, and the damage they cause paves the way for contamination by pathogens, mainly fungi and bacteria [77].

In this way, once insect-resistant transgenic maize reduces the number of insects, it may subsequently reduce damage to maize, allowing for reduced fungal contamination and low mycotoxin production [73], which is in line with the findings of the present study, providing evidence for the reduction of mycotoxins in insect-resistant transgenic maize kernels.

Regarding herbicide-resistant transgenic maize, the effect of herbicides on fungal contamination has not been observed; however, the lack of use of herbicides impacts the production of mycotoxins because certain weed species can facilitate fungal growth, which can subsequently contaminate maize [78]. In maize, the use of herbicides (acetochlor, sulcotrione, metribuzin, and pendimethalin) reduces weed competition and decreases competition for nutrients, water, and light, allowing an increase in the percentage of carbohydrates in grains without leaving residues in plants [79]. Glyphosate reduces weed competition and entails plant growth; however, its use is toxic to plants, affecting the accumulation of stalk dry mass and plant height, causing high accumulation and growth at low doses, but the reverse effect is observed at high doses [80]. Notably, the level of toxicity in plants exposed to glyphosate is dose-dependent. At a dose of 72 g·ha-1, low levels of toxicity (1.379) is noticed. When glyphosate is combined with phosphite (3 L·ha-1), the level of toxicity in plants increase up to 16.3% at a dose of 72 g·ha-1 glyphosate, leading to a significant reduction in plant height by 54% [80]. Given these circumstances, the use of herbicide-resistant transgenic maize prevents weed competition, thereby reducing the proliferation of fungi that use weeds as vectors and consequently preventing contamination by fungi and subsequent production of mycotoxins.

In the included articles in this systematic review, there was different geographical region where the experiments were conducted. It was observed a greater number of studies in the Northern Hemisphere compared to the Southern Hemisphere, where differences in temperature, precipitation, and other factors that influences mycotoxin production are significant. The relation of this environmental parameters were analyzed and confirmed in a systematic review that studied the distribution of mycotoxins worldwide [81]. Temperature plays an important role in fungal contamination and mycotoxin production [81, 82]. The optimal temperature for AFL, FUM, DON, and ZEA ranges between 25 and 30°C; however, mycotoxin production can occur to a lesser extent at temperatures between 4 and 37°C [82, 83]. This variation suggests that in locations with higher temperatures, mycotoxin production will be greater due to the ideal temperatures for their production and the growth of the fungi that produce them.

5. CONCLUSION

Based on the results obtained from the systematic review and meta-analysis, it can be concluded that: i) for the total amount of mycotoxins, fumonisin and aflatoxin genetically modified maize produces a lower amount of mycotoxin compared to non-genetically modified maize (positively conclusive); ii) for zearalenone and deoxynivalenol, the results are inconclusive.

We conclude that the use of transgenic maize reduces over 50% of the amount of mycotoxins, mainly fumonisin and aflatoxin. Further studies involving zearalenone and deoxynivalenol are needed to confirm their reduction in transgenic maize, with a larger sample size.

6. ACKNOWLEDGEMENTS

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7. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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CAPÍTULO 3

Artigo científico a ser submetido

Effects of transgenic maize in animal production – a systematic review.

ABSTRACT

Maize is a highly nutritious food widely used in animal feed and has a variety of genetically modified plants that express different traits, ranging from insect resistance to compound production. This systematic review compared the effects of transgenic maize on the feeding of four economically important production animal species: poultry, swine, cattle, and fish. The review was conducted following the PRISMA method, using the PubMed, Web of Science, Scopus, Google Scholar, and CAPES journals databases. The main performance parameters of each species were evaluated, including weight gain, feed intake, carcass yield, meat quality, egg and milk quality and quantity, and tissue coloration. Transgenic maize showed nutritional equivalence for use in feed compared to non-transgenic maize. No difference in animal performance was found between those treated with transgenic and non-transgenic maize. However, significant differences were observed in cases where maize enriched with carotenoids or producing phytase was used, with higher levels of carotenoids and increased phosphorus absorption in animals consuming transgenic maize. Based on these results, it can be concluded that the use of transgenic maize in animal feed not only maintains productivity but also does not have adverse effects on the animals.

Keywords: transgenic maize; animal feed; broiler; swine; cattle; fish.

1. INTRODUCTION

Maize is a plant that was selectively bred by humans through cross-pollination 10,000 years ago, making it one of the earliest human innovations [1]. In the 1960s, pesticides containing Bacillus thuringiensis (Bt) were used in maize, which were later replaced by Bt transgenic maize grains [1]. Transgenics are genetically modified organisms that have received genetic material from another species to express a desired characteristic, such as insect resistance, herbicide tolerance, and production/enrichment of compounds like phytase and carotenoids, among others [2]. In the 1990s, field studies and commercialization of transgenic crops began, making it the fastest-adopted technology for cultivation worldwide [3]. Transgenic maize has a field yield that is approximately 10% higher than conventional maize, and its characteristics of herbicide and insecticide resistance allow for a reduction in the use of these compounds, thus decreasing costs [3]. Additionally, maize is an important ingredient in animal feed, constituting 50% or more of the diet (depending on the species), and plays a crucial role in the nutrition of production animals [4].

Animal nutrition encompasses the intake and all processes leading to nutrient absorption, tailored to each species, and is essential for their development and productive performance [4, 5]. In the production chain, poultry, cattle, pigs, and fish are the most economically significant production animals [6]. These species have performance parameters directly linked to their productivity, including weight gain, feed intake, and the quality of meat, milk, and eggs [7, 8, 9, 10].

Analyses of the effects of transgenic maize on animal feeding, compared to non-transgenic maize, have been conducted separately for each species [11, 12, 13], necessitating a comprehensive evaluation across multiple species. Therefore, the objective of this review is to present and compare the effects of transgenic maize and conventional maize on production and health of poultry, pigs, cattle, and fish.

2. METHODS

In the present study, an analysis was conducted on the effect of using transgenic maize in animal feeding, following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) method [14] and using the PICO framework (Population, Intervention, Control, and Outcome). The research was conducted using the PubMed, Web of Science, Scopus, Google Scholar, and CAPES (Brazilian Higher Education Personnel Improvement Coordination), with English as the language for the searches. Only articles published in scientific journals with a Qualis ranking of A1 to B2 (according to CAPES classification) or Q1 and Q2 (SCImago Journal Rank) were included in the searches.

Information sources and searches

The searches were performed using the following terms:

Population: "animal"

AND

Intervention: "transgenic maize" OR "transgenic corn"

AND

Control: "conventional maize" OR "conventional corn"

AND

Outcome: "animal feed"

The final search was conducted on October 10, 2022, and the listed articles were analyzed by two independent reviewers. Duplicates were removed, and the titles and abstracts were evaluated. The title, year, journal, journal Qualis ranking, and database were recorded. At the end, the lists were compared, and any discrepancies were discussed.

Inclusion and exclusion criteria

The review included scientific articles written in English that presented the effect of transgenic maize on the zootechnical performance and health of production animals. Exclusion criteria were as follows: duplicate articles (same article in different databases); articles that did not focus on pigs, cattle, poultry, horses, or fish as the population; articles that did not involve transgenic maize; articles that did not provide data on animal production; literature or systematic reviews; and articles that did not provide the required information for data extraction.

Data collection, analysis, and outcomes

After applying the exclusion criteria based on the abstracts, 61 articles (Figure 1) were selected for data extraction using the extraction form. The extracted data included the following: year of the experiment; maize hybrid; purpose of the transgenic trait; geographic region; animal species; zootechnical data (life phase, weight gain, feed conversion); cellular and tissue changes; food safety; phytase; carotenoids; sample type; and sample value.

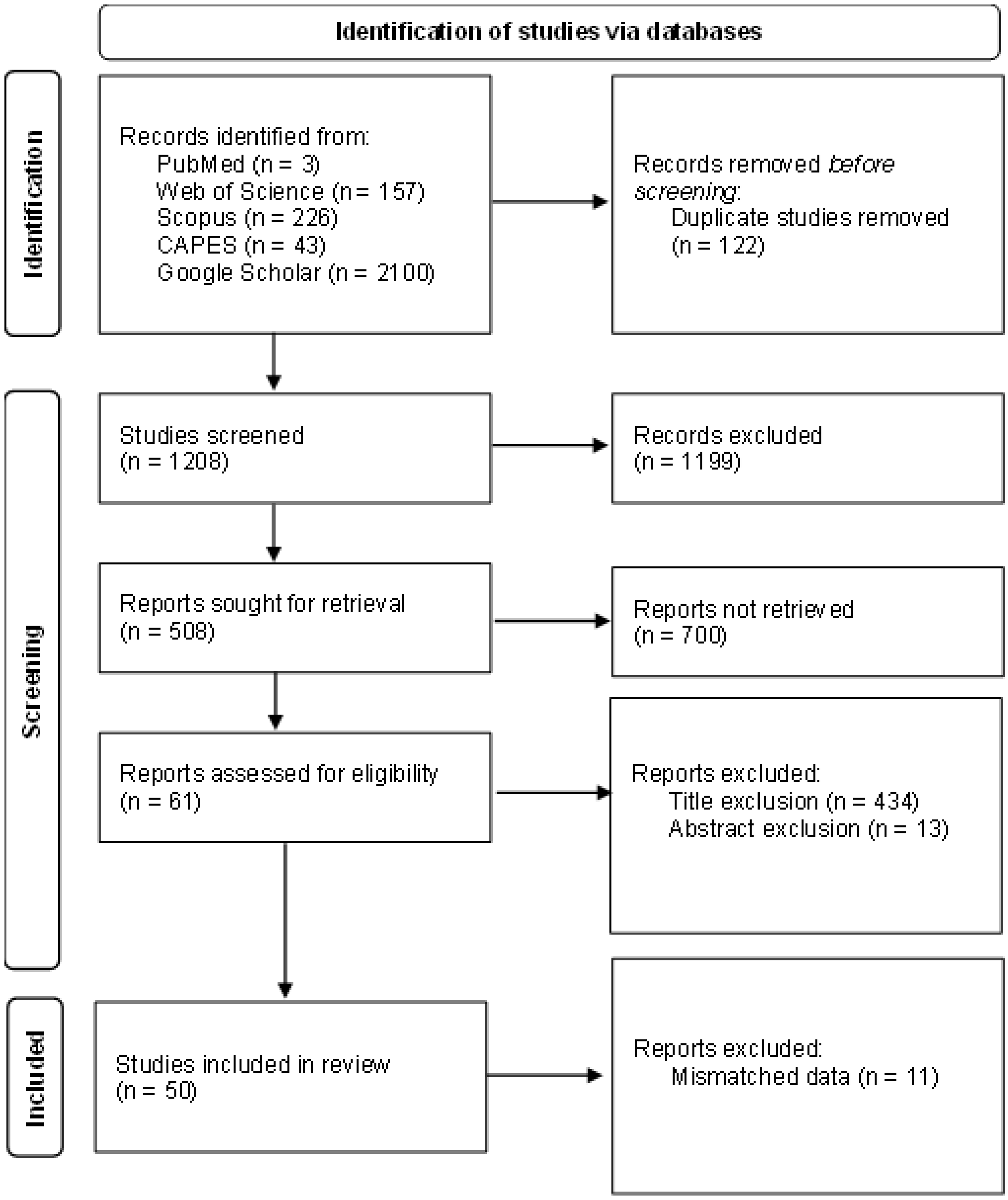


Figure 1. Flow diagram of study selection based on PRISMA [14]. The diagram shows the process of article retrieval following the inclusion and exclusion criteria, starting from the number of articles in the initial search and ending with the final number of articles retained in the review.

Bias risk analysis

Bias risk analysis was performed according to the Cochrane method [15], which can be low, high or uncertain and takes into consideration 5 points: drawing of samples; blinding; incomplete or selective outcomes; sample intensity and quality of the journal. For sample drawing, the risk was considered low when a draw or random number generator was used to choose the samples, and high when a choice was made. Regarding blinding, the risk was low when the test was double blind and high when it was unblinded, which could be due to the evaluator's bias or placebo effect. The outcomes were low risk when there were no sample losses or the loss was justified, and high risk when there were many losses without justification. The low risk for sample intensity is in the presence of a large number of samples, while the high risk is in the presence of few samples. Finally, journal quality is evaluated with Q1 and Q2 journals as low risk and Q4 journals or no index as high risk.

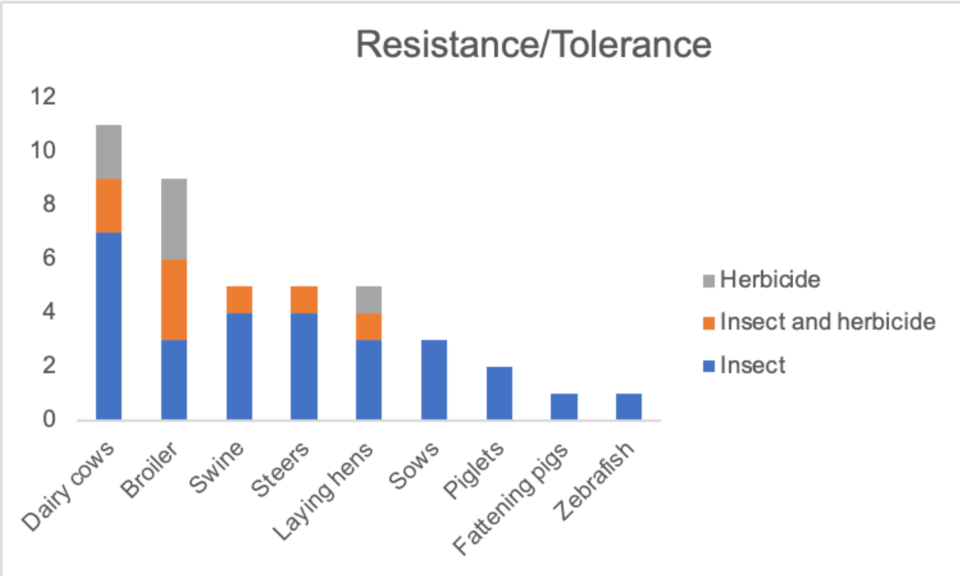
3. RESULTS

Based on the selection criteria and after applying the exclusion criteria based on titles, 508 articles were obtained. After selecting 61 articles based on the selection criteria from the abstracts, a further selection based on methodology was made, resulting in 50 articles [16 - 65] that were included for the data extraction phase.

In the initial search, the term generated results that were not within the scope of this review. Although this term was included in the initial search, it was later changed to "animal feed" during the course of the work.

All the articles provided information about the animal species studied and the purpose of using transgenic maize cultivars, with the majority aimed at conferring insect resistance to maize [16, 18, 22, 23, 25, 26, 27, 29, 30, 31, 32, 33, 34, 45, 53, 54, 55, 57, 58, 59, 60, 61, 65]. The search yielded articles with transgenic maize exhibiting traits such as insect and herbicide resistance, increased phytase production, carotenoids, β-glucanase, β-lactamase, and enrichment with vitamin E. Figure 2 shows the proportion of each species in relation to the purpose of transgenesis.

A.



B.

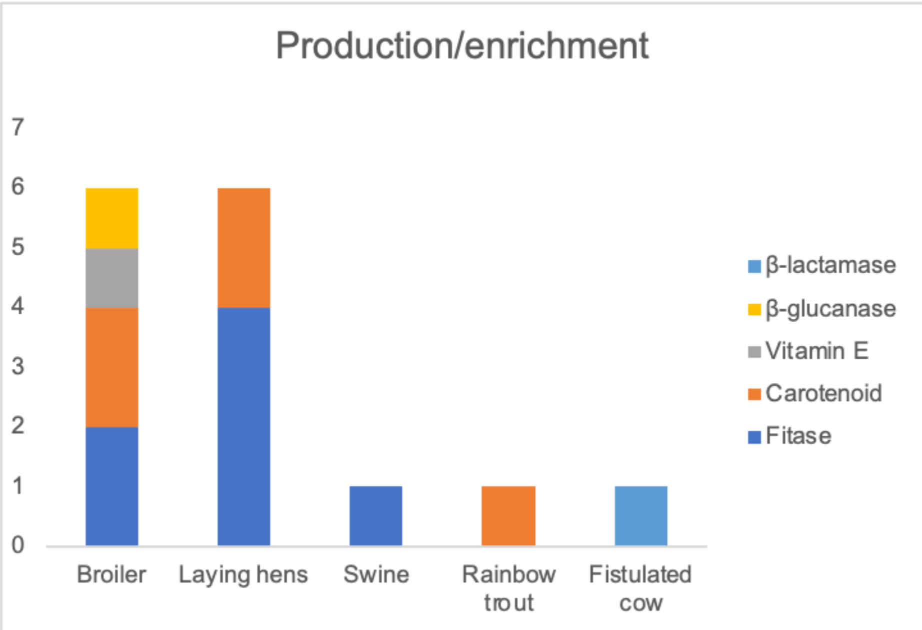


Figure 2 - Connection between the purposes of transgenesis and the animal species studied. Figure A refers to studies with transgenic maize hybrids with resistance or tolerance, and Figure B refers to studies in which there was transgenesis for production or enrichment of maize hybrids.

Bromatological analyses (moisture, ether extract, protein, fiber, ash, and amino acids) were performed in sixteen articles to evaluate the compositions of transgenic maize grains compared to conventional maize (table 1) [17, 18, 21, 22, 23, 37, 40, 41, 43, 47, 48, 49, 51, 56, 59, 65].

Table 1. Comparison of bromatological analyses between transgenic and non-transgenic maize grains.

	NT	T		NT[1]	T[1]	
%	$\bar{X}$ ± SD	$\bar{X}$ ± SD	p	$\bar{X}$ ± SD	$\bar{X}$ ± SD	p[1]
Moisture	14,66 ± 8,16	14,21 ± 6,33	0,43	12,74 ± 1,56	12,79 ± 1,22	0,46
Ether extract[2]	3,82 ± 0,85	3,82 ± 0,92	0,50	3,55 ± 0,40	3,61 ± 0,63	0,41
Protein	9,84 ± 4,05	10,18 ± 3,89	0,40	8,42 ± 1,48	8,85 ± 1,35	0,21
Fiber[3]	5,30 ± 6,69	5,41 ± 7,03	0,48	3,94 ± 3,60	3,98 ± 3,76	0,49
Ash	2,18 ± 2,02	2,21 ± 2,06	0,48	1,50 ± 0,77	1,54 ± 0,81	0,46

$\bar{X}$, mean (%); SD, standard deviation (%); p, *p*-value using t-test; NT, non-transgenic maize; T, transgenic maize; [1]: results after remove discrepant values (2 papers); [2]: only eleven articles presented the ether extract values; [3]: one paper had no fiber values.

Through bromatological analyses, it is possible to observe that there is no significant difference between genetically modified and non-genetically modified grains. Among the sixteen articles, two [37, 59] presented discrepant data compared to the others, as it had a lower quantity of grain (23% and 38%) in the treatment compared to the other studies (over 50%), resulting in a new analysis excluding such data. Nevertheless, no significant difference was observed between genetically modified and non-genetically modified grains, demonstrating nutritional equivalence between cultivars without negative impact on animal performance parameters (weight gain, feed intake, carcass yield, laying rate, egg weight, and quality).

The presence of transgenic DNA in animal tissues was assessed in two articles [27, 29]. One article evaluated the presence of allergies resulting from the consumption of transgenic maize by pigs and found no allergies in animals that consumed the treatment with transgenic maize.

In transgenic grains designed for phytase and carotenoid production, bromatological composition analyses indicated alterations only in relation to the target compounds. Transgenics with higher phytase production showed greater phosphorus bioavailability in tissues and increased phosphorus absorption without adverse effects on the animals [24, 35, 44, 62, 63]. Similarly, transgenics with higher carotenoid production increased the amount of carotenoids and significantly enriched tissue coloration [28, 50, 51, 52].

The geographic region where the experiments were conducted was indicated in 75% (38/51) of the articles, with the majority of these studies being conducted in the United States of America (19), followed by China (eight), Spain (six), Poland (three), Ireland (two), Italy (one), Germany (one), Japan (one), France (one), and Canada (one).

Nine groups were formed according to the animal species to compare specific performance parameters. For rainbow trout and zebrafish, only one article was evaluated for each species, and the results were described without comparison.

Broiler

For this systematic review, 14 articles [17, 18, 20, 24, 27, 28, 38, 46, 47, 49, 52, 54, 63, 64] were included, evaluating performance parameters such as weight gain (g), daily weight gain (g/day), tissue coloration, feed intake, and carcass yield in broiler chickens. The weight gain and daily weight gain of the animals did not show significant differences between those treated with transgenic maize and non-transgenic maize [17, 18, 27, 28, 46, 47, 49, 63]. Tissue coloration resulting from carotenoid consumption showed significant differences in tissue coloration (breast, eyelids, feathers, comb, skin, and fat) between transgenic and non-transgenic groups [28, 52]. Feed intake was evaluated in nine articles [18, 24, 27, 28, 38, 47, 49, 63, 64], and in seven of these articles, no significant difference was observed between animals treated with transgenic and non-transgenic maize. Two articles showed significant differences, with better performance in animals that consumed a diet with transgenic maize [24, 63]. Carcass yield was evaluated in six articles [17, 18, 38, 46, 47, 49], with no significant difference between transgenic and conventional maize. One article [20] evaluated diets containing only 2% transgenic maize, without significant difference in weight gain.

Laying hens

In eleven articles [35, 36, 41, 44, 48, 50, 51, 54, 57, 62, 65], weight gain, egg quality, laying rate, egg weight, feed intake, the effect of phytase consumption, and tissue coloration in laying hens were evaluated. Weight gain was assessed in six studies [35, 41, 48, 50, 51, 65], showing no significant difference between transgenic and non-transgenic maize. Four articles [41, 48, 62, 65] evaluated egg quality and feed intake, finding no significant differences between treatments. Laying rate was analyzed in four articles [48, 51, 62, 65], which did not find significant differences in performance between transgenic and non-transgenic maize. No significant difference was found in egg weight between animals treated with transgenic and non-transgenic maize [41, 48, 51, 62, 65]. The effects of phytase were evaluated in four articles [35, 36, 44, 62], showing higher phosphorus bioavailability and no harmful effects on the animals, with two studies showing significantly lighter yolk coloration in the transgenic group [35, 44]. Two articles used phytase extracted from transgenic maize instead of the grain [35, 36]. Two studies evaluated the effect of transgenic maize enriched with carotenoids [50, 51], showing significant differences in yolk coloration (using the YolkFanTM color scale) [50]. Fragments of transgenic DNA were found in the digesta samples from the gizzard and duodenum, but without causing significant differences in animal performance [57]. One study [54] evaluated histopathological changes in tissues (liver, kidney, pancreas, duodenum, jejunum, skeletal muscle, and spleen), finding no significant differences between the treatments with and without transgenic maize.

Swine

Six articles were included in the present review [25, 43, 54, 55, 56, 60], where performance parameters such as weight gain [56] and carcass yield [56] were analyzed for pigs. When comparing transgenic maize with conventional maize, no significant differences were found. In one study [25], the presence of maize DNA and Cry1Ab protein in the gastrointestinal content of pigs was analyzed, finding fragments of DNA from both transgenic and conventional maize, without affecting the performance of the animals. A study [43] evaluating the digestibility of phytase and phosphorus demonstrated that phytase does not interfere with the digestibility of other compounds and improves the bioavailability of phosphorus for absorption. An article [54] presented analyses of histopathological changes, resulting in the absence of adverse effects from the transgenic maize. In one study [55], the physicochemical alterations in pigs were analyzed, and no significant differences were found between the treatments, emphasizing the safety of consuming insect-resistant cultivars. An analysis of the immune response was conducted in one study [60], observing no significant differences between transgenic and conventional maize, and no residues of transgenic DNA or fragments of Cry1Ab protein were found in the tissues or white blood cells.

Sows and piglets

Three articles [22, 58, 61] evaluated performance factors such as weight gain [58, 61] and feed intake [58] for sows and piglets, finding no significant differences between transgenic and conventional maize. One article emphasized that transgenic maize does not affect the body composition of sows during gestation and lactation [61]. Three articles [22, 58, 61] assessed the cellular changes caused by the consumption of transgenic maize, with results showing no significant differences and the absence of transgenic DNA fragments. One article evaluated only piglets regarding the presence of maize

DNA, finding small fragments in the tissues, mainly in the blood, liver, and kidney [45].

Dairy Cattle

Nine articles [16, 21, 23, 30, 31, 32, 37, 40, 53] were included in the review, evaluating parameters such as weight gain, milk production, milk composition, and feed intake for dairy cows. Weight gain was analyzed in eight articles [16, 21, 23, 30, 31, 32, 37, 40], with no significant difference found between transgenic and non-transgenic maize. Milk production was assessed in seven studies [16, 21, 23, 31, 32, 37, 40] without significant differences found. Eight articles [16, 21, 23, 30, 31, 32, 37, 40] evaluated milk composition and found no significant differences between the treatments. In one study [30], significantly higher quantities of lactose and SNF were obtained in milk produced by cows treated with transgenic maize. Feed intake was evaluated in five articles [21, 30, 32, 37, 40], with no significant difference found between animals fed transgenic and conventional maize. One article reported a significantly higher consumption of silage [16], and another article showed significantly higher dry matter intake [23] in animals treated with transgenic maize. A study [53] evaluated the degradation of Cry1Ab protein in the digesta, observing a significant reduction of 44% in protein concentration.

Beef Cattle

Five articles [26, 32, 33, 39, 54] were included in this review, evaluating performance parameters such as weight gain [32, 39], feed intake [32, 39], and carcass yield [39] for beef cattle, with no significant differences between transgenic and conventional maize. Two articles [25, 33] analyzed the presence of maize DNA fragments and Cry1Ab protein in the rumen of fistulated cattle, finding DNA fragments in the rumen fluid. An article assessing histopathological changes in tissues (liver, kidney, spleen, pancreas, duodenum, jejunum, and skeletal muscle) found no significant difference between transgenic maize and conventional maize [54]. In one study, the consumption of transgenic and conventional maize was compared, and significant increases were observed in dry matter intake, daily weight gain, and feed efficiency [32].

Fish

In an article analyzing Rainbow trout [19], transgenic maize oil enriched with astaxanthin, a type of carotenoid, was used, resulting in an increase of over 60% in astaxanthin content in the incorporated carotenoids and a significant enhancement in fillet coloration. In an article evaluating transgenic maize DNA in zebrafish [29], residues of DNA derived from feed were found in tissues (intestines, muscle, and liver) without significant differences between the treatments.

4. DISCUSSION

The nutritional equivalence evaluated in 16 out of the 50 articles included in this review, as well as the information on grain equivalence in the remaining articles, raises concerns about the potential adverse effects on both animal health and production [30]. Such equivalence allows producers to take advantage of the benefits of using transgenic maize in production without concerns about affecting the animals [39]. Additionally, the insect-resistant transgenic grain reduces the amount of damage caused by insects, improving grain quality and preventing fungal contamination [66]. Prevention of fungal contamination assists in reducing the production of mycotoxins, which cause toxicosis in animals [67, 68, 69, 70]. Furthermore, some adsorbents used to minimize the effects of mycotoxins end up having negative effects on animal nutrition as well, as they also form bonds with nutrients, hindering proper absorption by the animal [71]. Therefore, the use of genetically modified organisms allows for the elimination of such compounds, enabling animals to have full access to nutrients.

Regarding nutrition, a balanced diet according to the species is necessary for proper growth, and the required time for evaluating performance varies for each species, as well as the duration of experiments to assess the effects of transgenic maize on animals (Table 2) [5]. In addition, the equivalence between the transgenic and non-transgenic maize grains used in the experiments can be observed, because no significant difference is obtained for the parameters: moisture, ether extract, crude protein, crude fiber and ash percentages.

Animal species	**n (mean)**	**experiment time (min - max) [days]**
Broiler	9298 (581)	40 (14 - 48)
Laying hens	3888 (353)	96 (20 - 210)
Swine	496 (35)	58 (9 - 140)
Dairy cows	296 (21)	108 (21 - 750)
Beef cattle	347 (58)	88 (70 - 109)
Fish	150 (75)	32 (14 - 49)

Table 2. Relationship between animal species, sample size (n), and experiment duration. The table displays the n and mean values for each species, as well as the average experiment duration in days, including the minimum and maximum timespan reported in the included articles of the review.

Furthermore, the considerable variation in experiment duration observed in laying hens, swine, and dairy cows, with no adverse effects resulting from the use of transgenic maize in shorter periods [30, 43], also occurred in longer periods [53, 58], as well as between breeding animals and piglets [22, 60, 61]. These findings support the safety of using transgenic crops in animal feed and nutrition.

5. CONCLUSION

It can be stated that there are no differences in the use of transgenic maize compared to conventional maize on animal production or health. This conclusion allows for the selection of characteristics with better productivity for cultivation, without concern that these choices will negatively impact the performance and health of animals that consume this crop.

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CAPÍTULO 4

CONCLUSÃO/ IMPACTO E PERSPECTIVAS FUTURAS

Por meio das revisões realizadas nesta dissertação foi possível demonstrar que o milho transgênico apresenta quantidade de fumonisina e aflatoxina reduzidas em mais de 50% em relação ao milho convencional. Também foi possível concluir que o milho transgênico apresenta composição nutricional equivalente ao milho convencional, gerando os mesmos resultados de desempenho quando consumidos por animais de produção.

Além disso, foram identificadas lacunas e desafios com necessidade de abordagem futura. Essas áreas potenciais de pesquisa incluem estudos com desoxinivalenol e zearalenona com intensidade amostral maior, visando testar se as suas quantidades também são reduzidas em milho transgênico. Também há necessidade de mais estudos avaliando os efeitos de milho transgênico na alimentação de peixes, com espécies diferentes e maior quantidade de amostras, pela escassez de estudos realizados com essa espécie.

No contexto mais amplo, os resultados desta pesquisa podem potencialmente auxiliar na escolha dos cultivares de milho por parte do produtor, uma vez que a planta transgênica apresenta maior capacidade produtiva por unidade de área, com redução de quantidade de micotoxinas e sem efeitos adversos aos animais.

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ANEXOS

SUPPLEMENTARY MATERIAL - RESISTANCE OF TRANSGENIC MAIZE CULTIVARS TO MYCOTOXIN PRODUCTION – SYSTEMATIC REVIEW AND METANALYSIS

FOREST PLOT AND FUNNEL PLOT OF METANALYSES

Total

Figure S1. Forest plot for the analysis of overall mycotoxin quantity, with all experiments included

	Convencional			Trangênico				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
ABBAS 2005 AFL 99	0.215	0.049	17	0.197	0.085	4	0.0%	0.02 [-0.07, 0.10]
ABBAS 2005 AFL E98	0.227	0.046	17	0.196	0.086	4	0.0%	0.03 [-0.06, 0.12]
ABBAS 2005 AFL H98	0.0266	0.0175	15	0.0036	0.0018	14	0.1%	0.02 [0.01, 0.03]
ABBAS 2005 FUM 01	48.1	4.2	17	38.2	6.1	4	0.0%	9.90 [3.60, 16.20]
ABBAS 2005 FUM 99	45.6	6	17	39.6	4.2	4	0.0%	6.00 [0.99, 11.01]
ABBAS 2005 FUM E98	3.32	0.6	15	2.09	0.25	14	0.0%	1.23 [0.90, 1.56]
ABBAS 2005 FUM H98	36.35	3.45	14	42.35	6.25	4	0.0%	-6.00 [-12.39, 0.39]
ABBAS 2008 AFL	0.0562	0.0056	1	0.1354	0.008	1	0.0%	-0.08 [-0.10, -0.06]
ACCINELLI 2014 AFL	1.08	0.27	2	0.43	0.13	4	0.0%	0.65 [0.25, 1.05]
BORDINI 2019 FUM	0.5	0.19	1	0.54	0.27	5	0.0%	-0.04 [-0.48, 0.40]
BOWERS 2014 FUM 08	6.6	8.62	2	1.52	2.03	4	0.0%	5.08 [-7.03, 17.19]
BOWERS 2014 FUM 09	0.67	1.08	3	0.28	0.47	6	0.0%	0.39 [-0.89, 1.67]
BOWERS 2014 FUM 10	0.76	0.93	3	0.47	0.59	6	0.0%	0.29 [-0.86, 1.44]
DOWD 2001 FUM 98	0.211	0.0259	5	0.109	0.0143	5	0.0%	0.10 [0.08, 0.13]
DOWD 2001 FUM 99	5.93	0.039	80	4.48	0.05	80	0.0%	1.45 [1.44, 1.46]
FOLCHER 2010 DON 05	3.19	0.038	80	2.76	0.037	80	0.0%	0.43 [0.42, 0.44]
FOLCHER 2010 DON 06	0.673	0.02557	49	0.405	0.04	9	0.0%	0.27 [0.24, 0.30]
FOLCHER 2010 FUM 05	0.52	0.035	24	0.52	0.033	24	0.0%	0.00 [-0.02, 0.02]
FOLCHER 2010 FUM 06	1.79	0.11	24	1.25	0.11	24	0.0%	0.54 [0.48, 0.60]
FOLCHER 2010 ZEA 05	0.8	0.071	6	0.8	0.071	6	0.0%	0.00 [-0.08, 0.08]
FOLCHER 2010 ZEA 06	0.043	0.011	6	0.053	0.013	6	0.0%	-0.01 [-0.02, 0.00]
HERRERA 2010 FUM	6.12	0.28208	21	0.26562	0.02489	21	0.0%	5.85 [5.73, 5.98]
NAEF 2006 DON 02	5.62	0.31717	21	0.42508	0.05437	21	0.0%	5.19 [5.06, 5.33]
NAEF 2006 DON 03	0.238805	0.01224	21	0.97561	0.10295	21	0.0%	-0.74 [-0.78, -0.69]
ROCHA 2016 FUM T1	0.113576	0.01248	21	0.18569	0.0102	21	0.1%	-0.07 [-0.08, -0.07]
ROCHA 2016 FUM T2	0.01895	0.00193	21	0.009373	0.00066	21	7.7%	0.01 [0.01, 0.01]
WEAVER 2017 AFL	0.00347	0.0005	21	0.001567	0.00031	21	92.0%	0.00 [0.00, 0.00]
WEAVER 2017 FUM	1.17	0.62	240	0.99	0.3	240	0.0%	0.18 [0.09, 0.27]
Total (95% CI)			**764**			**674**	**100.0%**	**0.00 [0.00, 0.00]**

Heterogeneity: Chi² = 63994.66, df = 27 (P < 0.00001); I² = 100%
Test for overall effect: Z = 24.99 (P < 0.00001)

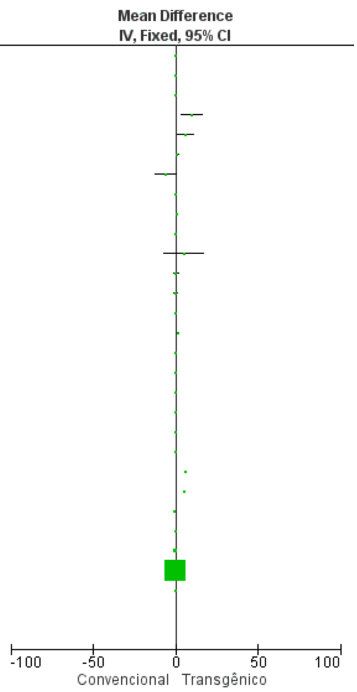
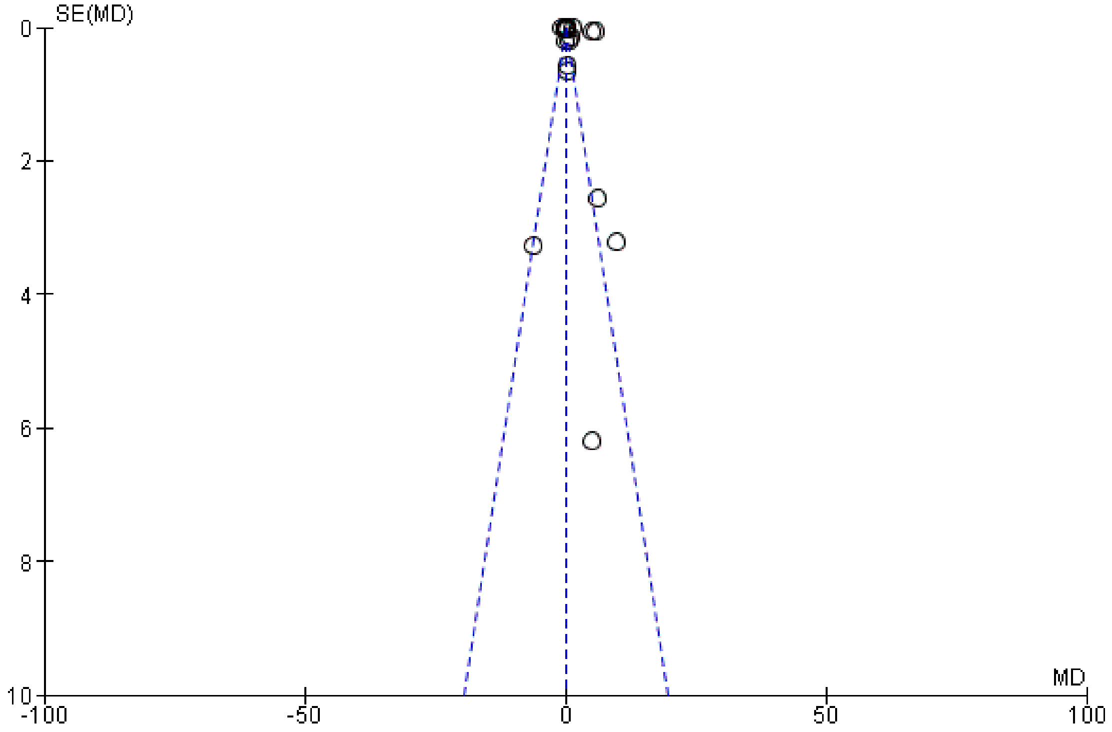


Figure S2. Funnel plot for the analysis of overall mycotoxin quantity with all experiments included



Total[1]

Figure S3. Forest plot for the analysis of overall mycotoxin quantity, without the three experiments with mycotoxin amounts

Study or Subgroup	Convencional Mean	SD	Total	Trangênico Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
ABBAS 2005 AFL 99	0.215	0.049	17	0.197	0.085	4	0.0%	0.02 [-0.07, 0.10]
ABBAS 2005 AFL E98	0.227	0.046	17	0.196	0.086	4	0.0%	0.03 [-0.06, 0.12]
ABBAS 2005 AFL H98	0.0266	0.0175	15	0.0036	0.0018	14	0.1%	0.02 [0.01, 0.03]
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BOWERS 2014 FUM 08	6.6	8.62	2	1.52	2.03	4	0.0%	5.08 [-7.03, 17.19]
BOWERS 2014 FUM 09	0.67	1.08	3	0.28	0.47	6	0.0%	0.39 [-0.89, 1.67]
BOWERS 2014 FUM 10	0.76	0.93	3	0.47	0.59	6	0.0%	0.29 [-0.86, 1.44]
DOWD 2001 FUM 98	0.211	0.0259	5	0.109	0.0143	5	0.0%	0.10 [0.08, 0.13]
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FOLCHER 2010 FUM 05	0.52	0.035	24	0.52	0.033	24	0.0%	0.00 [-0.02, 0.02]
FOLCHER 2010 FUM 06	1.79	0.11	24	1.25	0.11	24	0.0%	0.54 [0.48, 0.60]
FOLCHER 2010 ZEA 05	0.8	0.071	6	0.8	0.071	6	0.0%	0.00 [-0.08, 0.08]
FOLCHER 2010 ZEA 06	0.043	0.011	6	0.053	0.013	6	0.0%	-0.01 [-0.02, 0.00]
HERRERA 2010 FUM	6.12	0.28208	21	0.26562	0.02489	21	0.0%	5.85 [5.73, 5.98]
NAEF 2006 DON 02	5.62	0.31717	21	0.42508	0.05437	21	0.0%	5.19 [5.06, 5.33]
NAEF 2006 DON 03	0.238805	0.01224	21	0.97561	0.10295	21	0.0%	-0.74 [-0.78, -0.69]
ROCHA 2016 FUM T1	0.113576	0.01248	21	0.18569	0.0102	21	0.1%	-0.07 [-0.08, -0.07]
ROCHA 2016 FUM T2	0.01895	0.00193	21	0.009373	0.00066	21	7.7%	0.01 [0.01, 0.01]
WEAVER 2017 AFL	0.00347	0.0005	21	0.001567	0.00031	21	92.0%	0.00 [0.00, 0.00]
WEAVER 2017 FUM	1.17	0.62	240	0.99	0.3	240	0.0%	0.18 [0.09, 0.27]
Total (95% CI)			**701**			**648**	**100.0%**	**0.00 [0.00, 0.00]**

Heterogeneity: $Chi^2 = 63923.40$, df = 23 ($P < 0.00001$); $I^2 = 100\%$

Test for overall effect: $Z = 24.99$ ($P < 0.00001$)

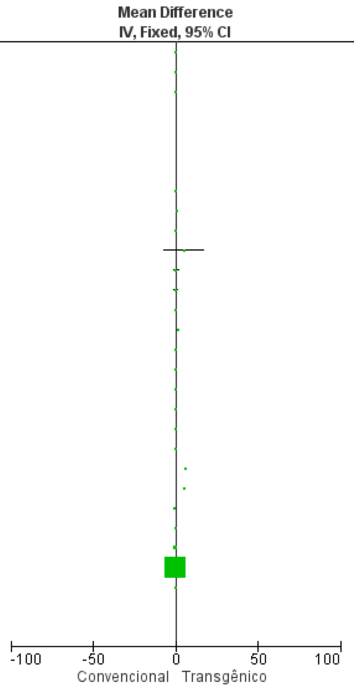
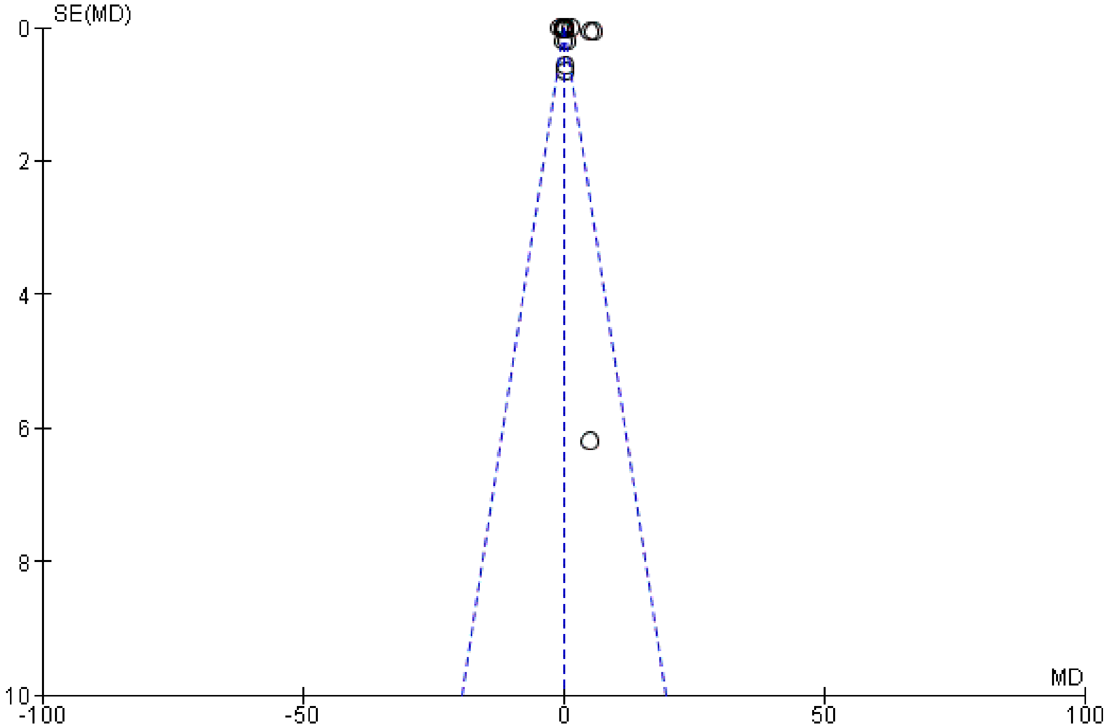


Figure S4. Funnel plot for the analysis of overall mycotoxin quantity, without the three experiments including mycotoxin amounts



Fumonisin

Figure S5. Forest plot for the analysis of fumonisin quantity, with all experiments included

	Convencional			Trangênico				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
ABBAS 2005 FUM 01	48.1	4.2	17	38.2	6.1	4	0.0%	9.90 [3.60, 16.20]
ABBAS 2005 FUM 99	45.6	6	17	39.6	4.2	4	0.0%	6.00 [0.99, 11.01]
ABBAS 2005 FUM E98	3.32	0.6	15	2.09	0.25	14	0.1%	1.23 [0.90, 1.56]
ABBAS 2005 FUM H98	36.35	3.45	14	42.35	6.25	4	0.0%	-6.00 [-12.39, 0.39]
BORDINI 2019 FUM	1.08	0.27	2	0.43	0.13	4	0.0%	0.65 [0.25, 1.05]
BOWERS 2014 FUM 08	0.5	0.19	1	0.54	0.27	5	0.0%	-0.04 [-0.48, 0.40]
BOWERS 2014 FUM 09	6.6	8.62	2	1.52	2.03	4	0.0%	5.08 [-7.03, 17.19]
BOWERS 2014 FUM 10	0.67	1.08	3	0.28	0.47	6	0.0%	0.39 [-0.89, 1.67]
DOWD 2001 FUM 98	0.76	0.93	3	0.47	0.59	6	0.0%	0.29 [-0.86, 1.44]
DOWD 2001 FUM 99	5.93	0.039	80	4.48	0.05	80	36.0%	1.45 [1.44, 1.46]
FOLCHER 2010 FUM 05	3.19	0.038	80	2.76	0.037	80	51.5%	0.43 [0.42, 0.44]
FOLCHER 2010 FUM 06	0.673	0.02557	49	0.405	0.04	9	9.5%	0.27 [0.24, 0.30]
HERRERA 2010 FUM	0.8	0.071	6	0.8	0.071	6	1.1%	0.00 [-0.08, 0.08]
ROCHA 2016 FUM T1	6.12	0.28208	21	0.26562	0.02489	21	0.5%	5.85 [5.73, 5.98]
ROCHA 2016 FUM T2	5.62	0.31717	21	0.42508	0.05437	21	0.4%	5.19 [5.06, 5.33]
WEAVER 2017 FUM	1.17	0.62	240	0.99	0.3	240	0.9%	0.18 [0.09, 0.27]
Total (95% CI)			**571**			**508**	**100.0%**	**0.82 [0.81, 0.83]**

Heterogeneity: $Chi^2 = 24982.07$, $df = 15$ ($P < 0.00001$); $I^2 = 100\%$
Test for overall effect: $Z = 192.43$ ($P < 0.00001$)

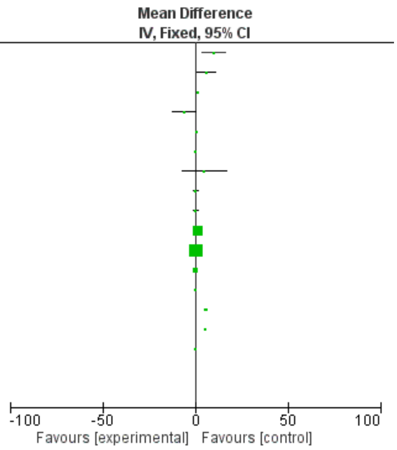
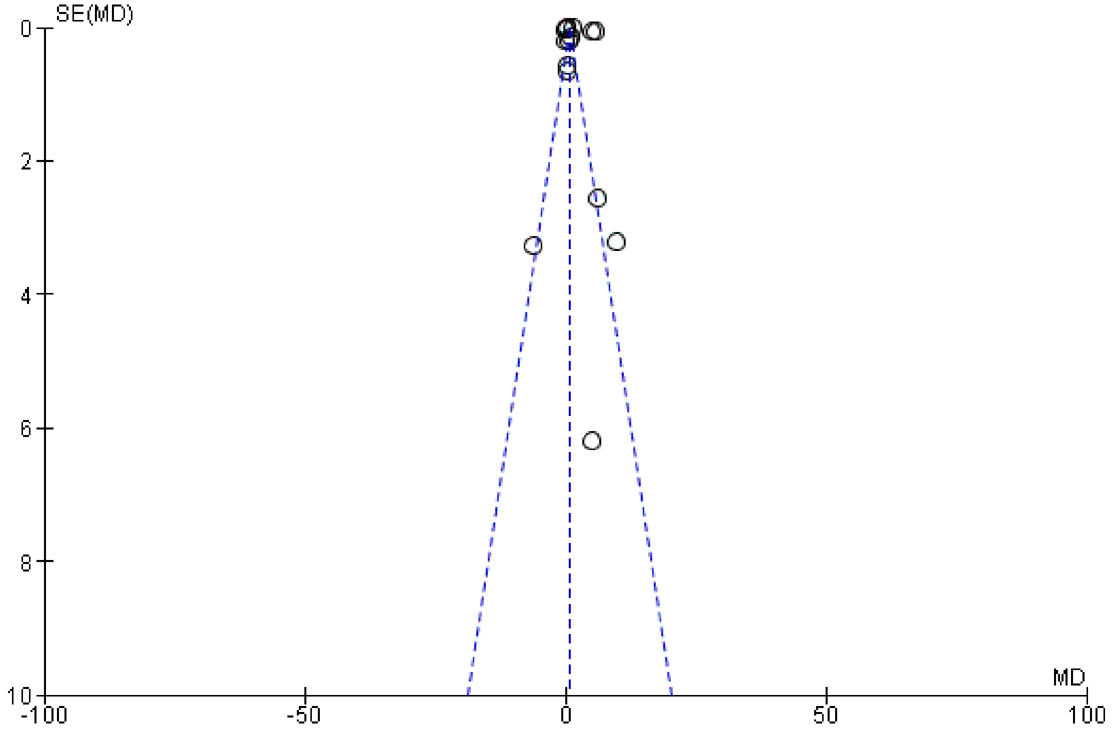


Figure S6. Funnel plot for the analysis of fumonisin quantity, with all experiments included



Fumonisin[1]

Figure S7. Forest plot for the analysis of fumonisin quantity without three experiments including mycotoxin amounts

	Convencional			Trangênico				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
ABBAS 2005 FUM 01	48.1	4.2	17	38.2	6.1	4	0.0%	9.90 [3.60, 16.20]
ABBAS 2005 FUM 99	45.6	6	17	39.6	4.2	4	0.0%	6.00 [0.99, 11.01]
ABBAS 2005 FUM E98	3.32	0.6	15	2.09	0.25	14	0.0%	1.23 [0.90, 1.56]
ABBAS 2005 FUM H98	36.35	3.45	14	42.35	6.25	4	0.0%	-6.00 [-12.39, 0.39]
BORDINI 2019 FUM	1.08	0.27	2	0.43	0.13	4	0.0%	0.65 [0.25, 1.05]
BOWERS 2014 FUM 08	0.5	0.19	1	0.54	0.27	5	0.0%	-0.04 [-0.48, 0.40]
BOWERS 2014 FUM 09	6.6	8.62	2	1.52	2.03	4	0.0%	5.08 [-7.03, 17.19]
BOWERS 2014 FUM 10	0.67	1.08	3	0.28	0.47	6	0.0%	0.39 [-0.89, 1.67]
DOWD 2001 FUM 98	0.76	0.93	3	0.47	0.59	6	0.0%	0.29 [-0.86, 1.44]
DOWD 2001 FUM 99	5.93	0.039	80	4.48	0.05	80	36.1%	1.45 [1.44, 1.46]
FOLCHER 2010 FUM 05	3.19	0.038	80	2.76	0.037	80	51.5%	0.43 [0.42, 0.44]
FOLCHER 2010 FUM 06	0.673	0.02557	49	0.405	0.04	9	9.5%	0.27 [0.24, 0.30]
HERRERA 2010 FUM	0.8	0.071	6	0.8	0.071	6	1.1%	0.00 [-0.08, 0.08]
ROCHA 2016 FUM T1	6.12	0.28208	21	0.26562	0.02489	21	0.5%	5.85 [5.73, 5.98]
ROCHA 2016 FUM T2	5.62	0.31717	21	0.42508	0.05437	21	0.4%	5.19 [5.06, 5.33]
WEAVER 2017 FUM	1.17	0.62	240	0.99	0.3	240	0.9%	0.18 [0.09, 0.27]
Total (95% CI)			**508**			**482**	**100.0%**	**0.82 [0.81, 0.83]**

Heterogeneity: $Chi^2 = 24959.66$, $df = 11$ ($P < 0.00001$); $I^2 = 100\%$
Test for overall effect: $Z = 192.31$ ($P < 0.00001$)

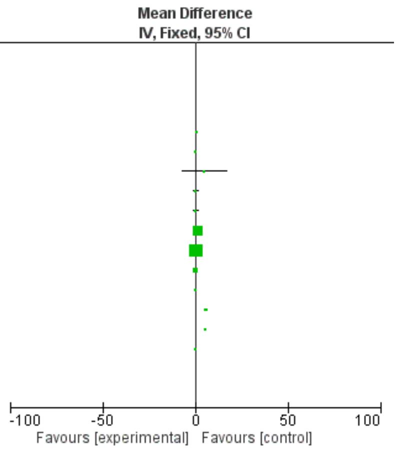
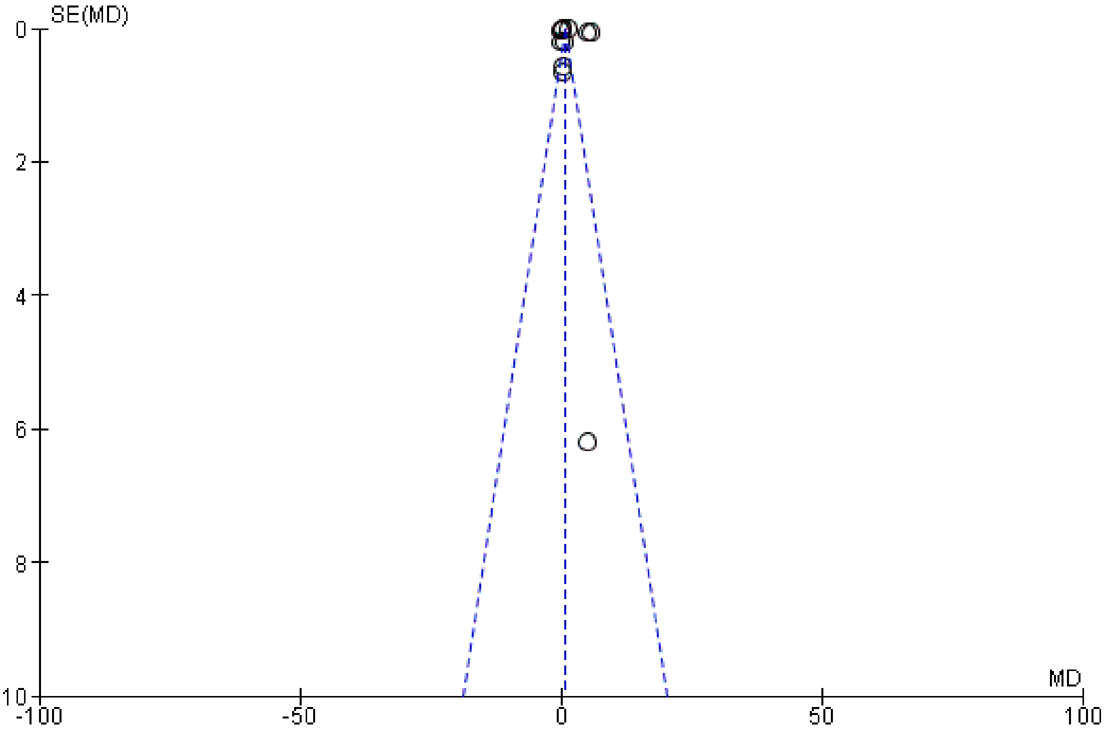


Figure S8. Funnel plot for the analysis of fumonisin quantity without three experiments including mycotoxin amounts



Aflatoxin

Figure S9. Forest plot for the analysis of aflatoxin quantity

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Fixed, 95% CI
ABBAS 2005 AFL 99	0.215	0.049	17	0.197	0.085	4	0.6%	0.02 [-0.07, 0.10]
ABBAS 2005 AFL E98	0.227	0.046	17	0.196	0.086	4	0.6%	0.03 [-0.06, 0.12]
ABBAS 2005 AFL H98	0.0266	0.0175	15	0.0036	0.0018	14	56.1%	0.02 [0.01, 0.03]
ABBAS 2008 AFL	0.0562	0.0056	1	0.1354	0.008	1	12.1%	-0.08 [-0.10, -0.06]
ACCINELLI 2014 AFL	0.211	0.0259	5	0.109	0.0143	5	6.6%	0.10 [0.08, 0.13]
WEAVER 2017 AFL	0.043	0.011	6	0.053	0.013	6	24.0%	-0.01 [-0.02, 0.00]
Total (95% CI)			**61**			**34**	**100.0%**	**0.01 [0.00, 0.01]**

Heterogeneity: Chi² = 148.13, df = 5 (P < 0.00001); I² = 97%
Test for overall effect: Z = 2.33 (P = 0.02)

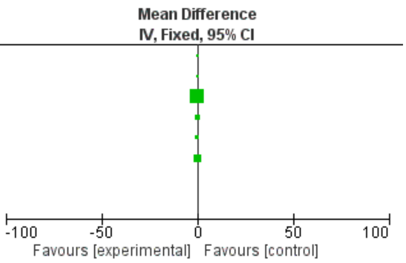
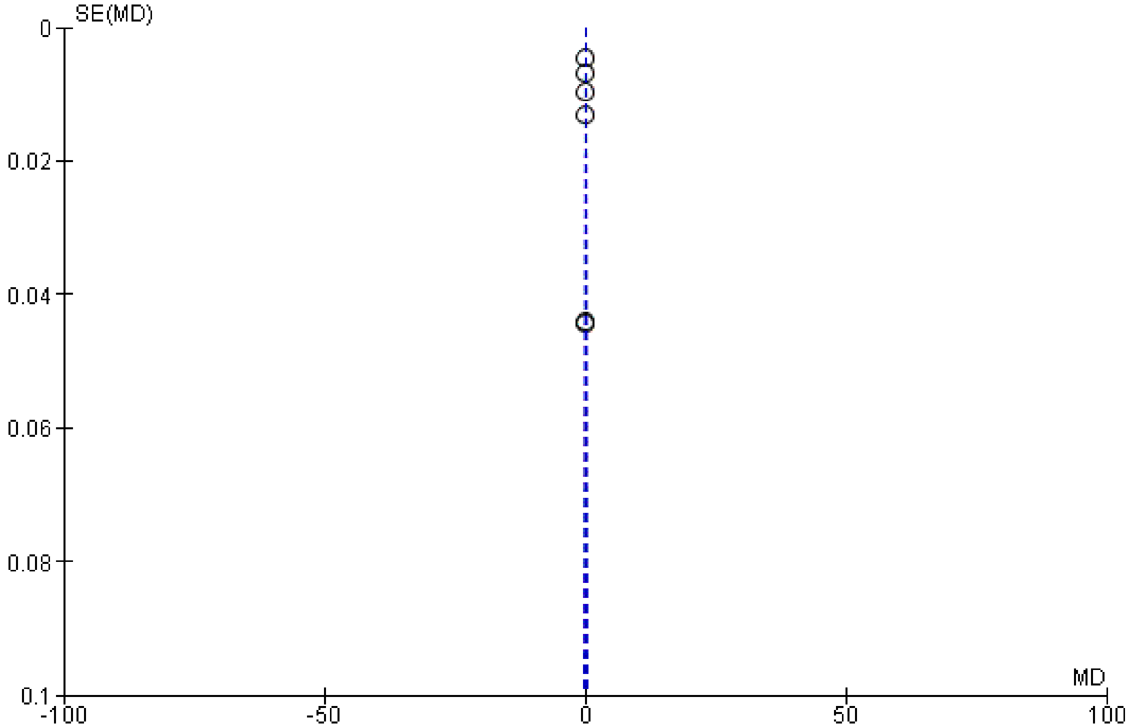


Figure S10. Funnel plot for the analysis of aflatoxin quantity



Deoxynivalenol

Figure S11. Forest plot for the analysis of deoxynivalenol quantity

Study or Subgroup	Convencional Mean	Convencional SD	Convencional Total	Transgênico Mean	Transgênico SD	Transgênico Total	Weight	Mean Difference IV, Fixed, 95% CI
FOLCHER 2010 DON 05	0.52	0.035	24	0.52	0.033	24	11.0%	0.00 [-0.02, 0.02]
FOLCHER 2010 DON 06	1.79	0.11	24	1.25	0.11	24	1.1%	0.54 [0.48, 0.60]
NAEF 2006 DON 02	0.238805	0.01224	21	0.97561	0.10295	21	2.1%	-0.74 [-0.78, -0.69]
NAEF 2006 DON 03	0.113576	0.01248	21	0.18569	0.0102	21	85.9%	-0.07 [-0.08, -0.07]
Total (95% CI)			**90**			**90**	**100.0%**	**-0.07 [-0.08, -0.07]**

Heterogeneity: Chi² = 1288.69, df = 3 (P < 0.00001); I² = 100%
Test for overall effect: Z = 21.94 (P < 0.00001)

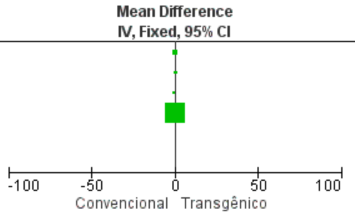
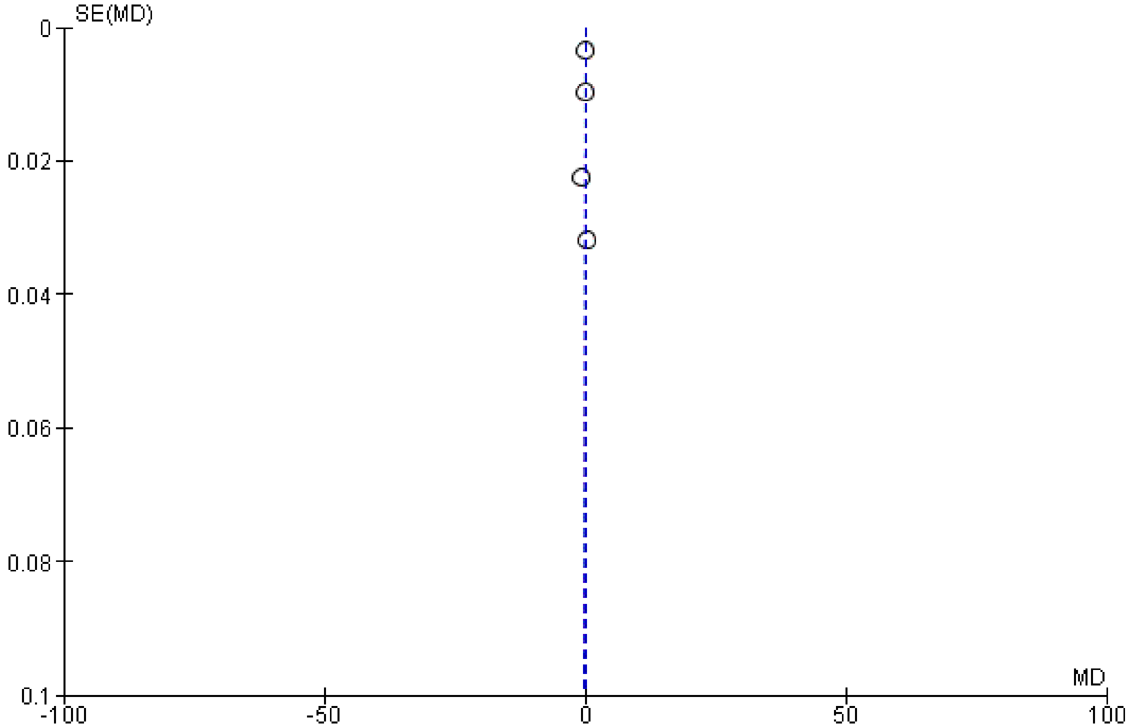
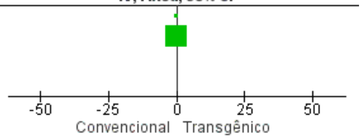


Figure S12. Funnel plot for the analysis of deoxynivalenol quantity



Zearalenone

Figure S13. Forest plot for the analysis of zearalenone quantity



	Convencional			Trangênico				Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI	IV, Fixed, 95% CI
FOLCHER 2010 ZEA 05	0.01895	0.00193	21	0.009373	0.00066	21	7.7%	0.01 [0.01, 0.01]	
FOLCHER 2010 ZEA 06	0.00347	0.0005	21	0.001567	0.00031	21	92.3%	0.00 [0.00, 0.00]	
Total (95% CI)			**42**			**42**	**100.0%**	**0.00 [0.00, 0.00]**	
Heterogeneity: Chi² = 274.42, df = 1 (P < 0.00001); I² = 100%									
Test for overall effect: Z = 20.21 (P < 0.00001)									

Figure S14. Funnel plot for the analysis of zearalenone quantity