

UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA

Juliano Kobs Vidal

**CLASSIFICAÇÃO DE HÍBRIDOS DE MILHO POR
CARACTERÍSTICAS PRODUTIVAS, NUTRICIONAIS E
MICOTOXICOLÓGICAS PARA NUTRIÇÃO ANIMAL**

Santa Maria, RS
2024

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ANIMAL**

Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Medicina Veterinária Preventiva, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Medicina Veterinária**.

Orientador: Prof. Dr. Carlos Augusto Mallmann

Santa Maria, RS
2024

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Vidal, Juliano
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CARACTERÍSTICAS PRODUTIVAS, NUTRICIONAIS E
MICOTOXICOLÓGICAS PARA NUTRIÇÃO ANIMAL / Juliano Vidal.-
2024.

55 p.; 30 cm

Orientador: Carlos Augusto Mallmann
Coorientadora: Fernanda Silveira Flores Vogel
Tese (doutorado) - Universidade Federal de Santa
Maria, Centro de Ciências Rurais, Programa de Pós
Graduação em Medicina Veterinária, RS, 2024

1. milho transgênico 2. nutrição animal 3. micotoxinas
4. composição nutricional 5. indústria avícola I. Augusto
Mallmann, Carlos II. Silveira Flores Vogel, Fernanda
III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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Veterinária**.

Aprovada em 22 de março de 2024.

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Santa Maria, RS
2024

AGRADECIMENTOS

Agradeço primeiramente a Deus por me abençoar a cada passo da minha vida, sempre me mostrando o caminho certo a seguir!

À minha família, por todo o apoio, confiança e exemplo de honestidade e humildade, especialmente à minha mãe Eideli e minha avó Engeli. Aos meus irmãos Juarez, Jardel e Simone, pela companhia e amizade durante toda a minha infância. Aos meus tios e tias, especialmente Mareli e Vanderlei, que estiveram ao meu lado em todas as minhas escolhas e que, por muitas vezes, assumiram o papel de pais. Amo vocês demais!

À minha noiva, amiga e companheira Vandrieli, por sempre estar ao meu lado, apoiar-me nos meus projetos e por me proporcionar o melhor presente da minha vida, nossa filha Ágata! Amo vocês para sempre!

Aos meus amigos que considero irmãos: Michael, Rafael, Bruno A., Lúcio, Mateu, Marton (in memoriam) e Ícaro, obrigado pelos churrascos, momentos de descontração e amizade. Vocês são incríveis!

Ao Prof. Carlos Augusto Mallmann, que me auxiliou, incentivou, desafiou e ajudou imensamente na minha formação profissional, não só durante o doutorado, mas também durante toda a graduação e mestrado. Obrigado pela confiança, conselhos e apoio.

Ao Adriano Mallmann, que contribuiu muito para o meu crescimento profissional, obrigado pelos teus ensinamentos, paciência e principalmente pela tua amizade!

A todo o pessoal do LAMIC, aos pós-graduandos, bolsistas e residentes, pela ajuda em cada discussão científica, conversa e descontração. Principalmente ao Carlos, Cristina, Cristiane, Mara Luciane, Denize, Francis, Dima, Luara e a Profa. Lu, por todos os momentos que compartilhamos juntos. Levarei vocês sempre em meu coração!

A toda a equipe do CEAN / Adisseo, que me apoia e incentiva diariamente, principalmente à Cristiane, Washington, Adriana, José H., Karine, Jéssica e Rariellen, meu muito obrigado!

À Universidade Federal de Santa Maria por me permitir fazer parte do seu corpo docente.

Aos professores do Programa de Pós-graduação em Medicina Veterinária, pela ajuda neste período da minha formação.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo apoio financeiro durante o primeiro ano do doutorado. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES/PROEX) – Código de Financiamento 001.

Muito obrigado!!

RESUMO

CLASSIFICAÇÃO DE HÍBRIDOS DE MILHO POR CARACTERÍSTICAS PRODUTIVAS, NUTRICIONAIS E MICOTOXICOLÓGICAS PARA NUTRIÇÃO ANIMAL

AUTOR: Juliano Kobs Vidal
ORIENTADOR: Dr. Carlos Augusto Mallmann

Esta tese teve como objetivo avaliar a influência da transgenia nas características produtivas, nutricionais e micotoxicológicas de diferentes híbridos de milho produzidos no Brasil. A pesquisa foi desenvolvida a partir de dois estudos principais. No primeiro estudo, foram coletadas 150 amostras de 50 híbridos de milho em experimento de campo. A análise de variância foi realizada e os híbridos foram agrupados pelo teste de Scott-Knott, com um nível de significância de 5%. A maioria das variáveis apresentou diferenças entre os híbridos de milho ($P < 0,05$), exceto fósforo disponível, grãos avariados e contaminações por desoxinivalenol (DON) e zearalenona (ZEA) ($P > 0,05$). Foram observadas correlação positiva ($P < 0,05$) entre produtividade e custo de formulação da ração ($r = 0,35$), e negativa ($P < 0,05$) entre a produtividade e níveis de proteína bruta ($r = -0,42$), treonina digestível (Tre dig.) ($r = -0,42$) e metionina + cistina (Met + Cis) dig. ($r = -0,42$). O custo da formulação de rações apresentou correlação positiva ($P < 0,05$) com fumonisinas (FUM: FB₁ + FB₂) ($r = 0,33$) e ZEA ($r = 0,29$), e correlação negativa ($P < 0,05$) com proteína bruta ($r = -0,74$). No segundo estudo, investigou-se o efeito de três importantes tecnologias transgênicas de milho — VT PRO3[®], PowerCore[®] ULTRA e Agrisure[®] Viptera 3 — nas características de campo, composição nutricional e contaminação por micotoxinas de 215 amostras de diferentes híbridos de milho, em três anos de avaliação: 2020, 2021 e 2022. O rendimento da cultura foi superior no VT PRO3[®] (9029 kg/ha em 2020, 5085 kg/ha em 2021, 9411 kg/ha em 2022) comparado ao PowerCore[®] ULTRA (8591 kg/ha em 2020, 4166 kg/ha em 2021, 8806 kg/ha em 2022) ($P < 0,05$). Os níveis de FUM foram significativamente maiores no VT PRO3[®] (1180 µg/kg em 2020, 1657 µg/kg em 2021, 2566 µg/kg em 2022) comparados ao PowerCore[®] ULTRA (280,8 µg/kg em 2020, 414,0 µg/kg em 2021, 990,6 µg/kg em 2022) ($P < 0,05$). A energia metabolizável aparente (AME_n) foi significativamente maior ($P < 0,05$) no VT PRO3[®] em todos os anos. Quanto aos aminoácidos, em 2020, isoleucina, leucina e fenilalanina totais e digestíveis foram maiores no PowerCore[®] ULTRA comparado ao VT PRO3[®] ($P < 0,05$). Em 2021, todos os aminoácidos totais e digestíveis foram significativamente diferentes entre as tecnologias de milho ($P < 0,05$), com concentrações mais altas no PowerCore[®] ULTRA. Em 2022, a tecnologia PowerCore[®] ULTRA teve uma maior concentração da maioria dos aminoácidos totais e digestíveis do que o VT PRO3[®] ($P < 0,05$), com exceção de lisina total ($P = 0,1480$) e triptofano digestível ($P = 0,0909$). A tecnologia Agrisure[®] Viptera 3 foi avaliada apenas em 2020, onde apresentou resultados intermediários para a maioria das variáveis estudadas. Os estudos demonstraram que existem variações nas propriedades produtivas, nutricionais e micotoxicológicas dos híbridos de milho investigados, evidenciando a necessidade de uma abordagem holística que leve em conta não só o rendimento, mas também a qualidade nutricional e a segurança alimentar ao selecionar variedades transgênicas para a produção de milho.

Palavras-chave: milho transgênico, nutrição animal, micotoxinas, composição nutricional, indústria avícola.

ABSTRACT

CLASSIFICATION OF MAIZE HYBRIDS BASED ON PRODUCTIVE, NUTRITIONAL, AND MYCOTOXIN CHARACTERISTICS FOR ANIMAL NUTRITION

AUTHOR: Juliano Kobs Vidal
ADVISOR: Dr. Carlos Augusto Mallmann

This thesis aimed to evaluate the influence of transgenics on the productive, nutritional, and mycotoxin characteristics of different corn hybrids produced in Brazil. The research was developed based on two main studies. In the first study, 150 samples from 50 corn hybrids were collected in a field experiment. Variance analysis was performed, and the hybrids were grouped by the Scott-Knott test, with a significance level of 5%. Most variables showed differences among the corn hybrids ($P < 0.05$), except for available phosphorus, damaged grains, and contamination by deoxynivalenol (DON) and zearalenone (ZEA) ($P > 0.05$). Productivity presented a positive correlation ($P < 0.05$) with feed formulation cost ($r = 0.35$), and negative correlations ($P < 0.05$) with crude protein ($r = -0.42$), digestible threonine (Dig. Thr) ($r = -0.42$), and digestible methionine + cystine (Met + Cys) ($r = -0.42$). The cost of feed formulation showed a positive correlation ($P < 0.05$) with fumonisins (FUM: FB₁ + FB₂) ($r = 0.33$) and ZEA ($r = 0.29$), and a negative correlation ($P < 0.05$) with crude protein ($r = -0.74$). In the second study, the effect of three important transgenic corn technologies — VT PRO3[®], PowerCore[®] ULTRA, and Agrisure[®] Viptera 3 — on field characteristics, nutritional composition, and mycotoxin contamination was investigated in 215 samples of different corn hybrids over three years: 2020, 2021, and 2022. Crop yield was higher in VT PRO3[®] (9029 kg/ha in 2020, 5085 kg/ha in 2021, 9411 kg/ha in 2022) compared to PowerCore[®] ULTRA (8591 kg/ha in 2020, 4166 kg/ha in 2021, 8806 kg/ha in 2022) ($P < 0.05$). Total FUM levels were significantly higher in VT PRO3[®] (1180 µg/kg in 2020, 1657 µg/kg in 2021, 2566 µg/kg in 2022) compared to PowerCore[®] ULTRA (280.8 µg/kg in 2020, 414.0 µg/kg in 2021, 990.6 µg/kg in 2022) ($P < 0.05$). The apparent metabolizable energy balance (AME_n) was significantly higher ($P < 0.05$) in VT PRO3[®] in all years ($P = 0.0007$ in 2020, $P = 0.0014$ in 2021, $P = 0.0001$ in 2022). Regarding amino acids, in 2020, total and digestible isoleucine, leucine, and phenylalanine were higher in PowerCore[®] ULTRA compared to VT PRO3[®] ($P < 0.05$). In 2021, all total and digestible amino acids were significantly different between the corn technologies ($P < 0.05$), with higher concentrations in PowerCore[®] ULTRA. In 2022, PowerCore[®] ULTRA technology had a higher concentration of most total and digestible amino acids than VT PRO3[®] ($P < 0.05$), except for total lysine ($P = 0.1480$) and digestible tryptophan ($P = 0.0909$). The Agrisure[®] Viptera 3 technology was assessed only in 2020, having intermediate results for most of the variables studied. The studies demonstrated that there are variations in the productive, nutritional, and mycotoxin properties of the corn hybrids investigated, highlighting the need for a holistic approach that considers not only yield but also nutritional quality and food safety when selecting transgenic varieties for corn production.

Keywords: transgenic corn, animal nutrition, mycotoxins, nutritional composition, poultry industry.

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1 INTRODUÇÃO

Nos últimos anos, a percepção sobre a qualidade do milho sofreu uma transformação marcante, impulsionada por novas demandas e avanços na pesquisa. O milho, anteriormente considerado uma simples *commodity*, agora é reconhecido em uma abordagem mais abrangente, levando em conta a diversidade de cultivares disponíveis, cada uma com propriedades específicas que atendem a uma diversidade de necessidades, tanto humanas quanto animais.

A alimentação animal, representando cerca de 70% dos custos de produção de aves e suínos, tem impulsionado a busca por dietas que não apenas maximizem o desempenho animal, mas também considerem cuidadosamente a composição dos alimentos e os riscos associados às micotoxinas. Nesse contexto, a seleção criteriosa dos ingredientes torna-se essencial para assegurar a qualidade dos grãos.

Além dos desafios relacionados à garantia da qualidade sanitária dos cereais, é constante a preocupação com a presença de micotoxinas, compostos orgânicos produzidos por fungos como *Aspergillus*, *Penicillium* e *Fusarium*, que afetam não apenas a segurança alimentar, mas também a saúde animal e humana (BENNET; KLICH, 2003; RICHARD, 2007). Por ser um ingrediente rico em energia e nutrientes, o milho é frequentemente afetado por micotoxinas como fumonisinas, aflatoxinas e desoxinivalenol (OLIVEIRA *et al.*, 2017). Para lidar com esse desafio, métodos tradicionais de análise de micotoxinas são eficazes, porém demorados e custosos. A espectroscopia de reflectância no infravermelho próximo (NIRS) surge como uma promissora alternativa, permitindo predições de micotoxinas em grãos de milho de forma rápida e precisa (TYSKA, *et al.*, 2022). O Brasil destaca-se como o segundo maior produtor e exportador de milho do mundo (USDA, 2024), com grande parte da produção destinada à alimentação animal. A contaminação do milho por micotoxinas é uma preocupação importante, especialmente em climas tropicais e subtropicais, onde diversas espécies de fungos produtores de micotoxinas podem se desenvolver (MALLMANN *et al.*, 2019).

A qualidade nutricional do milho, incluindo seu potencial nutricional e susceptibilidade à contaminação por micotoxinas, é um fator importante a ser considerado na seleção de genótipos de milho. Assim, a caracterização detalhada do milho é fundamental para otimizar seu uso na indústria de rações (MALLMANN *et al.*, 2019) e no desenvolvimento de novas variedades de milho

Apesar do progresso no melhoramento genético do milho, que tem se concentrado principalmente na alta produtividade, resistência ao acamamento e resistência a alguns patógenos, a resistência à fusariose e a susceptibilidade à contaminação por micotoxinas são características ainda negligenciadas. Surpreendentemente, nas safras 2020, 2021 e 2022, 82%, 99% e 96% dos híbridos, respectivamente, não possuíam informação quanto à resistência à fusariose (EMBRAPA, 2020; EMBRAPA, 2021; EMBRAPA, 2022). Além disso, a ausência de híbridos de milho certificados quanto à qualidade nutricional e à susceptibilidade ao ataque dos principais fungos produtores de micotoxinas destaca uma lacuna significativa no mercado.

Assim, é essencial a avaliação e conhecimento sobre a qualidade de híbridos de milho destinados à nutrição animal, levando em consideração as características desejadas: alta produtividade, alto teor nutricional com digestibilidade dos nutrientes e menor susceptibilidade à contaminação por micotoxinas. Este estudo objetivou avaliar diferentes híbridos de milho com base em características produtivas, nutricionais e micotoxicológicas visando otimizar sua utilização na nutrição animal.

2 REVISÃO BIBLIOGRÁFICA

2.1 MILHO: PRODUÇÃO, IMPORTÂNCIA E MELHORAMENTO GENÉTICO

Quatro países são responsáveis por 70% da produção mundial de milho, sendo eles: EUA, China, Brasil e Argentina, respectivamente (USDA, 2024). Por ser um cereal rico em sua composição química, o milho é utilizado na alimentação humana e na produção de ração animal. Cerca de 70% do milho utilizado no Brasil é destinado ao consumo animal (EMBRAPA, 2022). Esse cereal é o principal ingrediente na fórmula de ração de aves e suínos, sendo considerado um ingrediente energético pela sua composição nutricional (STRINGHINI *et al.*, 2000).

Por se tratar de uma das principais culturas do Brasil, são feitos muitos investimentos no desenvolvimento de novos híbridos de milho, assim como em melhoramento genético para aumentar a produtividade deste cereal. Contudo não há diferenciação de preços para o milho por seu teor nutricional ou índice de contaminação por micotoxinas. Nos últimos anos houve um decréscimo considerável no número de híbridos ofertados ao mercado. Na safra 2010/2011 foram ofertados ao mercado 498 diferentes cultivares (EMBRAPA, 2020), já para a safra 2020/2021 foram ofertados 98 diferentes cultivares, uma redução de 80% em 10 anos (EMBRAPA, 2021). Isso se deve ao fato de disponibilidade de sementes remanescentes de safras anteriores no mercado e a fusão de algumas empresas desenvolvedoras de híbridos de milho. Por outro lado, algumas empresas apenas fizeram uma atualização de alguns dos seus híbridos para uma versão mais nova de um evento transgênico (EMBRAPA, 2021).

Dentre as cultivares geneticamente modificadas disponibilizadas no mercado brasileiro nos últimos anos, as de maior ocorrência apresentavam os eventos transgênicos VT PRO3[®], PowerCore[®] ULTRA e Agrisure[®] Viptera 3. São, consistentemente, os eventos mais encontrados nos híbridos de milho produzidos no Brasil e representaram juntos 46% das tecnologias híbridas disponíveis em 2020, 64% em 2021 e 56% em 2022 (EMBRAPA, 2022; EMBRAPA, 2021; EMBRAPA, 2020).

O *Bacillus thuringiensis* (Bt) é uma bactéria que produz proteínas que são toxinas letais para insetos específicos, mas consideradas seguras para humanos, animais e plantas (EMBRAPA, 2009). Os genes Bt são inseridos em plantas de milho para conferir resistência a certos insetos-praga. Estes genes codificam proteínas cristalinas (Cry) que, quando ingeridas pelos insetos, se ligam a receptores específicos, causando perfurações nas paredes intestinais. Isso leva à paralisia e eventual morte do inseto. As proteínas Cry são específicas para certos grupos de insetos, como é o exemplo das lagartas (*Lepidoptera*), permitindo um controle

direcionado das pragas e minimizando o impacto sobre insetos benéficos e outros organismos que não atacam a cultura do milho (EMBRAPA, 2009).

A tecnologia VT PRO3[®] tem a presença de três genes Bt (Cry1A.105, Cry2Ab2 e Cry3Bb1). De acordo com a fabricante, esses genes conferem resistência a insetos-praga (*Spodoptera frugiperda*, *Diatraea saccharalis*, *Helicoverpa zea*, *Elasmopalpus lignosellus* e *Diabrotica speciosa*), além de tolerância ao herbicida glifosato (MONSANTO, 2023). A tecnologia PowerCore[®] ULTRA carrega quatro genes Bt (Cry1F, Cry1A.105, Cry2Ab2 e Vip3Aa20), que, de acordo com a fabricante, conferem resistência a diferentes insetos-praga (*S. frugiperda*, *D. saccharalis*, *Helicoverpa armigera*, *H. zea*, *Elasmopalpus lignosellus*, *Agrotis ipsilon*, *S. eridania* e *S. cosmioides*), além de tolerância ao herbicida glifosato (CORTEVA AGRISCIENCE, 2023). A tecnologia Agrisure[®] Viptera 3 possui três genes Bt (Cry1Ab, Vip3Aa20 e Cp4-EPSPS), que, de acordo com a fabricante, conferem resistência a vários insetos-praga (*S. frugiperda*, *D. saccharalis*, *H. zea*, *E. lignosellus* e *A. ipsilon*) e tolerância ao herbicida glifosato (SYNGENTA, 2023).

Em uma revisão sobre melhoramento genético, características morfológicas e densidade de plantio das culturas do milho, Silva *et al.* (2021) evidenciaram o principal objetivo ao promover o melhoramento genético do milho: produtividade. Segundo os autores, o melhoramento genético, tem ocasionado o desenvolvimento de cultivares com maior potencial produtivo, de ciclo variados, arquitetura mais ereta e porte baixo, o que garante maior resistência ao acamamento de plantas e facilita a sucessão com outras culturas e mecanização. A abordagem sobre qualidade nutricional dos grãos produzidos não foi realizada na revisão acima citada.

2.2 VARIABILIDADE NUTRICIONAL DO MILHO

Devido a sua composição rica em carboidratos, o milho é considerado um alimento energético para as dietas humana e animal. Sua composição média em base seca é 72% de amido, 9,5% proteínas, 9% fibra e 4% de óleo (BICUDO *et al.*, 2006). Apesar de apresentar baixo teor de proteína e aminoácidos em sua composição, o milho tem papel importante na fórmula de ração de aves e suínos pelo seu alto teor de inclusão (STRINGHINI *et al.*, 2000).

A composição detalhada dos nutrientes do milho pode ser facilmente encontrada em tabelas de composição média como nas Tabelas Brasileiras Para Aves e Suínos (ROSTAGNO *et al.*, 2017). Porém, é fundamental manter uma avaliação constante da variabilidade nutricional desse ingrediente, uma vez que sua composição pode ser afetada por uma série de

fatores, incluindo condições climáticas, tipo e variedade de grãos, origem, métodos de armazenamento e processamento (EYNG *et al.*, 2009).

Alguns estudos foram conduzidos para avaliar a interferência da genética, entre outros fatores, na variabilidade nutricional do milho. Em um estudo conduzido no Brasil, Mallmann *et al.* (2015) investigaram 43 híbridos de milho, observando uma faixa de variação de 7,40 a 8,82% na concentração de proteína bruta (PB) e de 3.183 a 3.295 kcal kg⁻¹ de energia metabolizável para aves corrigida para balanço de nitrogênio (EMA_n). Da mesma forma, em uma pesquisa realizada por Vieira *et al.* (2007), envolvendo 45 híbridos de milho, foi identificada uma variação na concentração de PB de 6,85 a 10,08%, e na EMAN, uma oscilação de 2.997 a 3.459 kcal kg⁻¹. Isso evidencia a importância do monitoramento constante da composição nutricional do milho, bem como a busca por híbridos que forneçam altas produtividades atrelado a composição nutricional e as demais características desejáveis e já bem estabelecidas.

2.4 USO DA ESPECTROSCOPIA NO INFRAVERMELHO PRÓXIMO NA PREDIÇÃO NUTRICIONAL DO MILHO

A análise por espectroscopia no espectro próximo do infravermelho (NIRs) é uma abordagem ágil para a avaliação precisa de amostras de alimentos. Os procedimentos utilizando o infravermelho próximo demandam mínima preparação de amostra e recursos humanos (McMULLIN; MIZAIKOFF; KRSKA, 2015). O espectrofotômetro emprega princípios matemáticos na análise química, conhecida como quimiometria. Essa abordagem combina espectroscopia e análise computacional de dados.

A faixa do espectro eletromagnético no infravermelho próximo abrange de 750 a 2500 nm. A tecnologia NIRS oferece uma alternativa rápida, de aplicação simples e não destrutiva em comparação aos métodos tradicionais de caracterização. Além disso, tem encontrado ampla aplicação em diversos setores, incluindo indústrias, instalações de recebimento de grãos, fábricas de ração e portos (RAMBO; FERREIRA; AMORIM, 2016).

2.6 MICOTOXINAS NO MILHO

Produtos de origem vegetal estão sob risco de infestações por fungos. Dentre estes estão algumas linhagens de fungos filamentosos produtores de micotoxinas, as quais são metabólitos secundários tóxicos. Esses fungos têm ocorrência mundial, predominando em climas tropicais e subtropicais, onde seu desenvolvimento é favorecido por fatores como condições de umidade e temperatura (MALLMANN; DILKIN, 2007). Além da produção

desse contaminante tóxico a humanos e animais, esses fungos causam deterioração do grão, reduzindo consideravelmente seu valor químico e nutricional (HERMANNNS *et al.*, 2006).

Segundo a Organização das Nações Unidas para a Alimentação e Agricultura (FAO), cerca de 25% das cultivares produzidas no mundo apresentam alguma micotoxina. As perdas mundiais desses produtos devido a esses metabólitos tóxicos estão na casa de 1 bilhão de toneladas por ano (FAO, 2015).

As principais micotoxinas encontradas em cereais são as aflatoxinas (AFLA), produzidas por *Aspergillus spp.*; ocratoxinas, produzidas por *Penicillium spp.*; desoxinivalenol (DON); toxina T-2; zearelenona (ZEA); ergotoxinas; e fumonisinas (FUM), produzidas por *Fusarium spp.* (ABRUNHOSA *et al.*, 2012). Esses fungos podem se desenvolver e produzir metabólitos secundários tanto em nível de campo (pré-colheita) quanto de estoque e armazenamento (pós-colheita) (ROCHA *et al.*, 2012).

Esses compostos possuem diversos efeitos deletérios à saúde humana e animal, como por exemplo, efeitos anabolizantes, estrogênicos, carcinogênicos, mutagênicos e teratogênicos. São muitos os fatores que levam a uma maior ou menor prejudicialidade quando ocorre um quadro de micotoxicose. Alguns efeitos podem estar diretamente relacionados com o tipo de micotoxina ingerida, dose, frequência de exposição, raça, sexo, idade, fatores ambientais, manejo e condições nutricionais (MALLMANN *et al.*, 2017).

A contaminação por micotoxinas dos ingredientes utilizados onera o custo final da ração para aves e suínos, seja pela inclusão de aditivo antimicotoxina (AAM) ou de nutrientes sintéticos devido à baixa disponibilidade nos ingredientes naturais, causados pela presença de fungos nos grãos (HERMANNNS *et al.*, 2006). Vidal *et al.* (2016) encontraram uma diferença de R\$ 15,00 por tonelada na fórmula de ração para frangos de corte a partir dos dados nutricionais e micotoxicológicos do milho utilizado nas fórmulas. Mallmann *et al.* (2019), concluíram que a composição micotoxicológica do milho foi influenciada pelo híbrido utilizado. No estudo foram avaliados 26 diferentes híbridos onde a tecnologia Viptera apresentou menor concentração de FUM, maiores percentuais de proteína bruta, metionina digestível, cistina digestível e treonina digestível e, conseqüentemente, a fórmula de ração de frangos de corte apresentou custo mais baixo.

2.6.1 Fumonisin

As FUM constituem um grupo de micotoxinas descoberto em 1988 com produção constatada por *Fusarium verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. nygamai*,

F. anthophilum e *F. Napiniforme*. Os *F. verticillioides* e *F. proliferatum* são considerados os principais produtores (FIGUEIRA *et al.*, 2003).

As FUM mais abundantes de incidência natural são do grupo B, produzidas pela maioria das cepas de *Fusarium verticillioides*. Dentro dessa família predominam as fumonisinias B₁ (FB₁), B₂ (FB₂) e B₃ (FB₃), porém a FB₁ constitui mais de 75% do total (TORRE-HERNÁNDEZ *et al.*, 2014). As concentrações de FUM no milho são influenciadas por fatores como temperatura, umidade, estresse causado pela seca e precipitação durante períodos da pré-colheita e colheita. Além disso, grãos atacados por insetos podem apresentar maiores níveis. A atividade de água (Aw) favorável para a produção desta micotoxina é de 0,92 a 0,98, com temperatura entre 30 e 35 °C (IARC, 2012).

A FB₁, está relacionada com a ocorrência de leucoencefalomalácia em cavalos e edema pulmonar em suínos (DUARTE-VOGEL; VILLAMIL-JIMÉNEZ, 2005). Além disso, está relacionada com alterações da resposta imune produzindo diminuição da viabilidade dos linfócitos em pintinhos e imunossupressão em suínos (PESTKA; BONDY, 1994). A FB₁ foi classificada no grupo 2B da Agência Internacional de Pesquisa de Câncer (IARC) como um possível cancerígeno em humanos, principalmente relacionada ao câncer de esôfago (IARC, 2012).

Mallmann *et al.* (2017) encontraram uma positividade de 81% em amostras de milho analisadas no período de 2008 a 2017. Os autores verificaram uma contaminação média de 1.990 µg/kg e uma média de 2.454 µg/kg considerando apenas as amostras contaminadas.

2.6.2 Aflatoxinas

As principais AFLA conhecidas são B₁, B₂, G₁ e G₂ (AFB₁, AFB₂, AFG₁ e AFG₂). Esses metabólitos secundários são produzidos por cepas de fungos do gênero *Aspergillus*, principalmente das espécies *A. flavus* e *A. parasiticus* (HUONG *et al.*, 2016). A IARC classificou a AFB₁ como cancerígena – grupo I (IARC, 2012).

As AFLA afetam o metabolismo dos carboidratos, lipídeos, ácidos nucleicos e proteínas. Além disso, possuem efeito mutagênico, teratogênico, carcinogênico e hepatotóxico (ELLIS *et al.*, 2009). Em frangos de corte as AFLA afetam a função hepática pelo aumento dos níveis séricos de aspartato amino-transferase, e quando combinadas com outras micotoxinas ocorre um efeito aditivo (ELIANA *et al.*, 2010). Em todas as espécies animal causam diminuição da imunidade, redução do ganho de peso, desordens digestivas, hepatopatias, anorexia, ataxia, tremores e podem levar a óbito (MALLMANN *et al.*, 2017).

Mallmann *et al.* (2017) encontraram uma positividade de 47% de AFLA em amostras de milho analisadas no período de 2008 a 2017. Os autores verificaram uma contaminação média de 9 µg/kg e uma média de 19 µg/kg considerando apenas as amostras contaminadas.

2.6.3 Desoxinivalenol

O DON é uma das principais micotoxinas do grupo dos Tricotecenos (TCT). Os TCTs são produzidos por fungos do gênero *Fusarium*, principalmente *F. graminearum* e *F. tricinctum* e formam um grupo químico de metabólitos, com a mesma estrutura básica. A produção de DON se dá em baixas temperaturas, variando entre 6 e 24 °C. Sua ocorrência é significativa em culturas de inverno como arroz, aveia, centeio, cevada e trigo (MALLMANN *et al.*, 2017).

Os suínos apresentam a maior sensibilidade a DON, seguidos pelas aves. As intoxicações por DON provocam recusa de alimentos, vômito, redução da conversão alimentar e diarreia. Mallmann *et al.* (2017) encontraram uma positividade de 21% em amostras de milho analisadas no período de 2008 a 2017. Os autores verificaram uma contaminação média de 78 µg/kg e uma média de 367 µg/kg considerando apenas as amostras contaminadas.

2.6.4 Zearalenona

A ZEA é uma micotoxina que ocorre em praticamente todos os cereais, especialmente em culturas de inverno, como aveia, cevada, trigo, centeio e milho, contaminadas por fungos do gênero *Fusarium*, principalmente *F. graminearum*. Essa micotoxina é produzida em temperatura de 24 a 26 °C, com Aw de 0,90 (DILKIN, 2002; IANAMAKA; OLIVEIRA; TANIWAKI, 2010).

Considerada uma micotoxina estrogênica, a ZEA possui afinidade com os receptores de estrogênio (17-β-estradiol), atuando como um disruptor endócrino. Em humanos a ingestão de ZEA tem sido associada à apresentação de puberdade precoce em meninas e ao aumento do tamanho dos órgãos reprodutores em crianças (DUARTE-VOGEL; VILLAMIL-JIMÉNEZ, 2005). Em suínos os principais sinais clínicos e lesões causadas pela ZEA são a síndrome de hiperestrogenismo (vulvovaginite) e *splayleg* em leitões recém-nascidos (MALLMANN *et al.*, 2017).

Mallmann *et al.* (2017) encontraram uma positividade de 50% em amostras de milho analisadas no período de 2008 a 2017. Os autores verificaram uma contaminação média de 91 µg/kg e uma média de 180 µg/kg considerando apenas as amostras contaminadas.

2.7 REGULAMENTAÇÃO DE MICOTOXINAS NO BRASIL

A legislação para os limites máximos tolerados de micotoxinas em alimentos no Brasil está estabelecido pela Agência Nacional de Vigilância Sanitária (ANVISA) na Instrução Normativa - IN nº 160, de 1º de julho de 2022 (ANVISA, 2022) (Tabela 1).

Tabela 1. Limites máximos toleráveis (LMT) de micotoxinas em milho indicados na IN nº 160, de 1º de julho de 2022.

Micotoxina	Alimento	LMT (µg/kg)	Situação
Aflatoxinas (B ₁ +B ₂ + G ₁ +G ₂)	Milho, milho em grão inteiro, partido, amassado ou moído, farinhas ou sêmolos de milho	20	Em vigência
Fumonisinias (B ₁ +B ₂)	Milho em grão para posterior processamento	5000	Em vigência
Zearalenona	Milho em grão e trigo para posterior processamento	400	Em vigência
Desoxinivalenol	Milho em grãos para posterior processamento	2000	Em vigência

Fonte: Agência Nacional de Vigilância Sanitária (ANVISA, 2022).

3 HIPÓTESES CIENTÍFICAS

Hipótese 1:

Há efeito do híbrido sobre a produtividade de milho cultivado.

Hipótese 2:

Há efeito do híbrido sobre a composição nutricional do milho.

Hipótese 3:

Há efeito do híbrido sobre a contaminação por micotoxinas no milho.

Hipótese 4:

Há efeito do híbrido sobre o custo da ração para frangos de corte.

Hipótese 5:

Há correlação estatística entre as variáveis nutricionais, produtividade, contaminação por micotoxinas e custo da ração para frangos de corte para diferentes híbridos de milho.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Avaliar diferentes híbridos de milho com base em características produtivas, nutricionais e micotoxicológicas visando otimizar sua utilização na nutrição animal.

4.2 OBJETIVOS ESPECÍFICOS

Avaliar a produtividade de diferentes híbridos de milho sob as mesmas condições de cultivo.

Avaliar a composição nutricional de diferentes híbridos de milho.

Investigar a presença e a concentração de micotoxinas em diferentes híbridos de milho.

Correlacionar a produtividade com a composição nutricional e a contaminação por micotoxinas para diferentes híbridos de milho.

Publicar uma tese de doutorado, artigos e resumos em publicações nacionais e internacionais.

5 ARTIGO 1

Este capítulo é apresentado em formato de artigo de pesquisa denominado “Productivity, nutritional composition, and presence of mycotoxins in different corn hybrids.” submetido em 2023 ao periódico *Ciência Rural*. O artigo foi aceito para publicação em abril de 2024.

1 **Productivity, nutritional composition, and presence of mycotoxins in different corn**
2 **hybrids**

3 **Avaliação da produtividade, composição nutricional e presença de micotoxinas em**
4 **diferentes híbridos de milho**

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11
12 **ABSTRACT**

13 An experiment was conducted to assess the crop yield, nutritional composition, and
14 mycotoxin contamination in different corn hybrids. The impact of these variables on the feed
15 formulation cost of starter diets for broilers was also evaluated. A total of 150 samples from
16 50 corn hybrids was obtained from a field experiment. Nutrients were predicted by NIRS and
17 mycotoxins were analyzed by HPLC-MS/MS. Data were submitted to analysis of variance
18 and corn hybrids were grouped using Scott-Knott test at 5% significance. Pearson correlation
19 analysis was performed among the main variables. Most of the variables were different
20 among corn hybrids ($P < 0.05$), with exception of available phosphorus (Av. P), damaged
21 grains, and DON and ZEA contaminations ($P > 0.05$). Crop yield had a positive correlation
22 ($P < 0.05$) with feed formulation cost ($r = 0.35$) and a negative correlation ($P < 0.05$) with crude
23 protein (-0.42), digestible (dig.) Thr (-0.42), and dig. Met+Cys (-0.42). The feed cost
24 correlated positively ($P < 0.05$) with FUM (0.33) and ZEA (0.29), and negatively ($P < 0.05$)
25 with crude protein (-0.74). Different corn hybrids vary in their productivity, nutritional

26 content and mycotoxins contamination. It was demonstrated that an increase in the crop yield
27 might be related to a reduction in corn nutritional content and quality, resulting in an increase
28 in feed formulation cost.

29 **Keywords:** corn hybrid, aflatoxin, fumonisin, NIRS, amino acids.

30

31 **RESUMO**

32 Um experimento foi conduzido para avaliar a produtividade, a composição nutricional e a
33 contaminação por micotoxinas em diferentes híbridos de milho. Essas medidas também foram
34 correlacionadas com o impacto do custo do milho na formulação de ração inicial para frangos
35 de corte. Um total de 150 amostras de 50 híbridos de milho foram obtidas em experimento de
36 campo. As variáveis nutricionais foram preditas via NIRS e as micotoxinas foram analisadas
37 por HPLC-MS/MS. Os dados foram submetidos à análise de variância e os híbridos
38 agrupados pelo teste de Scott-Knott a 5% de significância. Correlação de Pearson foi
39 realizada entre as principais variáveis. A maioria das variáveis foi diferente entre os híbridos
40 de milho ($P < 0,05$), com exceção de fósforo disponível, grãos avariados e contaminações por
41 DON e ZEA ($P > 0,05$). A produtividade teve correlação positiva ($P < 0,05$) com o custo de
42 formulação da ração ($r = 0,35$) e correlação negativa ($P < 0,05$) com proteína bruta (-0,42), Tre
43 digestível (dig.) (-0,42) e Met+Cis dig. (-0,42). O custo da formulação de rações apresentou
44 correlação positiva ($P < 0,05$) com FUM (0,33) e ZEA (0,29), e negativa ($P < 0,05$) com
45 proteína bruta (-0,74). Diferentes híbridos de milho variam em produtividade, conteúdo
46 nutricional e contaminação por micotoxinas. Foi demonstrado que um aumento no rendimento
47 à campo pode estar relacionado com redução no conteúdo nutricional e na qualidade do
48 milho, resultando em um aumento no custo da formulação de ração.

49 **Palavras-chave:** híbrido de milho, aflatoxina, fumonisinas, NIRS, aminoácidos.

50

51 INTRODUCTION

52 Due to its nutritional value, corn (*Zea mays* L.) is one of the main ingredients used in
53 the diets for poultry worldwide. Brazil is one of the largest corn producer and exporter
54 countries (USDA, 2021), and, according to ABIMILHO (2023), about 50% of the corn
55 produced in Brazil in the 2019/2020 harvest was used for animal consumption, reaching more
56 than 54% in the 2022/2023 harvest. The grain is globally traded as a commodity because it is
57 a homogeneous product from which is expected small variability in its characteristics
58 (PRATES, 2007) and its price is regulated by the supply and demand principle. However,
59 high prices scenarios have an impact on the cost of feed formulations, affecting the whole
60 animal production chain, and, consequently, meat prices.

61 The natural composition of corn is susceptible to mycotoxin contamination. The
62 contamination by mycotoxins depends on several factors such as seed genetics, growing
63 location, soil fertility, climate conditions, and pre- and post-harvest handling (FAO, 2024).
64 The main mycotoxin-producing fungi found in corn are *Aspergillus* and *Fusarium*, which can
65 produce mycotoxins from the groups of aflatoxins, fumonisins, and trichothecenes,
66 respectively (MALLMANN; MALLMANN, 2020). Therefore, it is important to provide
67 current information on how some of these factors can impact the characteristics of corn and;
68 consequently, its utilization. Furthermore, it is important to consider the use of antimycotoxin
69 additive (AMA) when identifying mycotoxin contamination, seeking tested products with
70 guaranteed efficacy for the groups of mycotoxins and animal species being worked with.

71 In the 2020/2021 harvest, 98 new corn cultivars were introduced in the Brazilian
72 market (EMBRAPA, 2021); however, technical improvement of corn has primarily focused
73 on developing high-yield hybrids with resistance to lodging and pathogens. There are no
74 nutritionally certified hybrids available in the market, and there is limited information
75 regarding resistance to fungal attacks and potential mycotoxins contamination.

76 In this context and considering that corn is the primary cereal in the global animal
77 nutrition chain, the nutritional composition and the quality of corn hybrids should therefore be
78 considered in the genetic selection and improvement processes. Therefore, this study assessed
79 the productivity, nutritional composition, and presence of mycotoxins in different corn
80 hybrids cultivated in the same harvest in Brazil, focusing on poultry nutrition. Information on
81 nutrients composition and mycotoxins contamination from each corn hybrid was also
82 considered to formulate starter diets for broilers and to evaluate the impact of these variables
83 on feed formulation cost.

84

85 **MATERIALS AND METHODS**

86 *Field experiment*

87 The experiment was conducted at the Agricultural Research Center (24°37'18"S;
88 53°18' 20"W; 580 m altitude) of the Cooperativa Agroindustrial Consolata (COPACOL), in
89 Cafelândia city, western region of the Paraná State, Brazil. The soil was classified as
90 dystroferric red latosol. In February 2020, 50 commercial and pre-commercial corn hybrids
91 were cultivated in a consolidated no-tillage system under the same agro-climatic conditions
92 and soil type. The identification of corn hybrids was done numerically (from 1 to 50) and the
93 commercial names have been withheld to ensure confidentiality. The experiment was design
94 in a randomized blocks design with three replicates of each corn hybrid. Experimental plots
95 consisted of four corn rows with a spacing of 68 cm, having 2.72 m in width by 10 m in
96 length. Crop phytosanitary management was conducted according to the technical
97 recommendations on pest and weed control. The soil fertilization was conducted according to
98 chemical analyses, employing a base fertilization of 300 kg/ha of 10-15-15 (NPK).
99 Harvesting was performed in July using a Wintersteiger combine® experimental plot
100 harvester-classic model, where the two central lines of each plot were harvested, totaling 13.6

101 m² of useful plot. The mass of grains and gravimetric moisture content were automatically
102 determined by the Easy Harvest weighing system (Grain Gage®) coupled to the harvesting
103 system. The crop yield of the experimental plots was calculated in kg/ha and adjusted for 13%
104 moisture. Grains that were damaged were classified according to MAPA recommendations
105 (BRASIL, 2011) and the percentage of damaged grains was obtained by the equation: [weight
106 of damaged grains (g)/weight of the sample (g)]* 100.

107

108 *Quantification of mycotoxins by high-performance liquid chromatography coupled to tandem*
109 *mass spectrometry (HPLC-MS/MS)*

110 After harvesting, samples were dried in a forced-air oven at 55 °C for 12 h and
111 prepared for mycotoxins and nutrients analyses. Mycotoxins quantification was conducted in
112 the Laboratory of Mycotoxicological Analyses at Federal University of Santa Maria, Brazil.
113 Analytical standards for aflatoxins (AFLA) AFB1, AFB2, AFG1, AFG2, fumonisins (FUM)
114 B1 (FB1) and B2 (FB2), deoxynivalenol (DON), and zearalenone (ZEA) were purchased
115 from Sigma Aldrich (St Louis, MO, USA). Methanol, acetonitrile, formic acid, and
116 ammonium acetate (HPLC grade) were purchased from JT Baker (Center Valley, PA, USA).
117 Ultrapure water was obtained from a Milli-Q Water Purification System. The limits of
118 detection (LOD) and quantification (LOQ) (in µg/kg) for the assessed mycotoxins were,
119 respectively: 0.4 and 1 for AFB1; 0.6 and 1 for AFB2, AFG1 and AFG2; 10 and 125 for FB1;
120 20 and 125 for FB2; 3 and 20 for ZEA; and 50 and 200 for DON.

121 Samples of 1 kg were milled at 1 mm using an ultra-centrifugal mill (RETSCH®,
122 model ZM 200), homogenized and then analyzed for mycotoxins presence. Analyses of
123 AFLA, FUM, DON and ZEA were performed according to MALLMANN et al. (2020). For
124 AFLA, a 5 g sample was mixed with 20 mL acetonitrile–water solution (84:16, v/v) and
125 shaken on a shaking table for 60 min. The resulted extract was spun (Eppendorf 5804R) at

126 2,500 rpm, 20 °C, for 5 min, and then 60 µL was diluted with 840 µL methanol–water (1:1,
127 v/v) solution. For FUM, 3 g of sample was mixed with 15 mL acetonitrile–water solution
128 (1:1, v/v) and vortexed for 20 min in an orbital shaker. The extract was spun at 2,500 rpm, 20
129 °C, for 5 min, and then 20 µL was diluted in 980 µL acetonitrile–water–formic acid solution
130 (50:40:10, v/v/v). For DON and ZEA, a 3 g sample was mixed with 24 mL methanol–water
131 (70:30, v/v) solution and vortexed for 20 min using an orbital shaker. The resulted extract was
132 spun at 2,500 rpm, 20 °C, for 5 min, and then 40 µL was diluted in 960 µL methanol–water–
133 ammonium acetate solution (90:9:1, v/v/v). For the four mycotoxins, 20 µL of the diluted
134 solution was injected into a 1200 Series Infinity HPLC instrument (Agilent, Palo Alto, CA,
135 USA) coupled to a 5500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA,
136 USA) equipped with an electrospray ionization source in positive mode. Chromatographic
137 separation for AFLA and FUM was performed at 30 °C using an Eclipse XDB-C8 column
138 (4.6 × 250 mm, 5 µm particle diameter) (Agilent) and for DON an ZEA at 40 C with a Zorbax
139 SB-C18 column (4.6 × 150 mm, 5 µm) (Agilent).

140

141 *NIRS nutritional predictions*

142 For predictions by near infrared spectroscopy (NIRS), samples were milled through a
143 0.5 mm sieve in an ultra-centrifugal mill (RETSCH®, model ZM 200). Samples were then
144 placed in plastic bags and left for 15 min to reach room temperature (between 18 °C and 22
145 °C) and humidity (between 40% and 60%). Subsequently, manual homogenization of each
146 sample was performed for two min in a plastic bag using circular movements. Nutritional
147 predictions were performed by reading the spectra of the samples in a Bruker® instrument,
148 model Tango-R, with a wavelength range of 3,952 - 11,536 cm⁻¹, using the calibration curves
149 from the AMINONRG® and AMINONir® programs (Evonik Nutrition & Care GmbH,
150 Hanau, Germany). The following variables were predicted: dry matter (DM) (%), crude

151 protein (CP) (%), ether extract (EE) (%), ash (%), total phosphorus (TP) (%), phytic
152 phosphorus (PP) (%), total and digestible (dig.) amino acids (AA, %) for poultry, and
153 apparent metabolizable energy (AMEn) (kcal/kg) for poultry. For study and comparison
154 purposes, all values were adjusted to 87% DM basis.

155

156 *Feed formulation and AMA inclusion in the diet*

157 Average nutrient composition and mycotoxins contamination were calculated for each
158 corn hybrid and used to calculate the cost of feed formulation of starter diets for broilers (8 to
159 21 days of age) using each hybrid as the corn source in the formula. The starter diet was used
160 in this investigation due to the high susceptibility to mycotoxins that broilers present at this
161 phase (MALLMANN & MALLMANN, 2020). Feeds were formulated using the PFR
162 spreadsheet (UNESP, SP, Brazil) and following recommendations of ROSTAGNO et al.
163 (2017) for standard-high performance male broilers. Costs were obtained from market prices
164 in the region of the study in November 2020 (Table 1). The inclusion of the AMA was
165 calculated proportionally to mycotoxins concentration levels of each corn hybrid and based on
166 the Mycotoxins Risk algorithm (MALLMANN & MALLMANN, 2020). The inclusion of
167 AMA was adjusted as follows: 2.5 kg of AMA/t of feed for every 28 µg/kg of AFLA + 2.5 kg
168 of AMA/t of feed for every 10,000 µg/kg of FUM.

169

170 *Statistical analyses*

171 Data were analyzed using the Statgraphics® software (Statgraphics Centurion 15.2.11,
172 Manugistics Inc., Rockville, MD, USA). Different variables were submitted to analysis of
173 variance and the Scott-Knott test was used to group hybrids with similar characteristics at 5%
174 significance. To summarize the information in a smaller set of results that can be easily
175 observed, Pearson correlation analysis was conducted among the main variables.

176 **RESULTS AND DISCUSSION**

177 Corn is one of the most produced cereals in the world, with a global production
178 exceeding 1.1 billion tons in the 2020/2021 harvest season (USDA, 2021). Genetic
179 improvement in the major corn-producing countries has led to a significant increase in
180 productivity per cultivated area. Results of field traits and mycotoxins contamination of the
181 corn hybrids are presented in table 2. For crop yield, the 50 corn hybrids were divided into 4
182 distinct groups ($P<0.05$) with an average productivity of 7,407 kg/ha. The difference between
183 the most and the least productive hybrid was 2,935 kg/ha. The average crop yield in the
184 current study is 34% higher than the Brazilian average in the 2019/2020 harvest, which was
185 5,510 kg/ha, and 28% superior than the global average of 5,780 kg/ha (USDA, 2021).

186 SILVA et al. (2021) stated that the objective of genetic improvement in corn is mainly
187 focused on productivity. Currently, the nutritional quality of the grain is not typically
188 considered in a genetic improvement program. In the present study, different cultivars of corn
189 were grown under conditions similar to Brazilian production reality, and correlations among
190 productivity and other variables related to corn quality and feed production cost were
191 assessed. Interestingly, there was a positive correlation ($P<0.05$) between crop yield and the
192 final cost of the starter feed for broilers, suggesting that an increase in grain productivity
193 might be related to an increase in the feed cost. This could be explained by the negative
194 correlation ($P<0.05$) of crop yield with CP ($r = -0.42$), dig. Met+Cys (-0.42), and dig. Thr ($-$
195 0.42), nutrients that have an impact in the feed formulation cost. Such findings are in
196 agreement with previous studies conducted by DUVICK (2005) and ALVAREZ-IGLESIAS
197 et al. (2021), where the negative correlation among grain productivity and nutrients content,
198 especially crude protein, was observed.

199 The percentage of damage grains and the average contamination of DON and ZEA
200 was not different among the corn hybrids ($P>0.05$) whereas contaminations of AFLA and

201 FUM were affected by corn technology ($P<0.05$). FUM was the most prevalent mycotoxin,
202 affecting 68% of the crop, with a contamination average of 197 $\mu\text{g}/\text{kg}$. In addition to the
203 nutritional composition of the corn, humidity and temperature conditions are factors that may
204 have influenced this result. The average of AFLA, ZEA and DON were 0.045 $\mu\text{g}/\text{kg}$, 4.08
205 $\mu\text{g}/\text{kg}$, and 9.11 $\mu\text{g}/\text{kg}$, respectively. The prevalence of FUM observed herein corroborates
206 with previous studies (MALLMANN et al., 2019; SIMÕES et al., 2023) and highlights the
207 current need for reliable information on susceptibility of corn hybrids to *Fusarium*. In the
208 2019/2020 crop season, 90% of the corn hybrids available in the Brazilian market lacked data
209 on fusariosis resistance (EMBRAPA, 2021).

210 The inclusion of AMA in the diet was based on technical recommendations and was
211 directly proportional to the contamination of grains by mycotoxins. The average inclusion of
212 AMA was 0.34 kg/ton of feed, with a maximum of 1.79 kg/ton (Table 3). As expected, the
213 cost of the feed increased as the AMA was added in the diet, and a positive correlation
214 ($P<0.05$) was observed between the feed cost and contamination by FUM (0.33) and ZEA
215 (0.29).

216 Results on nutrients concentration are presented in table 3. Contents of starch,
217 EE, AME_n, CP, dig. Lys, dig. Thr and dig. Met+Cys were different among ($P<0.05$) corn
218 hybrids. The average AME_n content was 3,294 kcal/kg with a difference of 81 kcal/kg
219 between the minimum and maximum values. The average CP was 7.8% with a difference
220 higher than 30% between the minimum and maximum values. Such findings are in agreement
221 with previous research (COWIESON, 2005; DOZIER et al., 2011) and reinforce the need to
222 monitor these nutrients in corn, due to the high variability observed and the economic impact
223 it generates.

224 In order to summarize the results from the present experiment, only the first three
225 limiting AA for poultry are presented; however, all the essential AA established by the NRC

226 (1994) were predicted by NIRS and used in the feed formulations (Table 1). Feed formulation
227 cost presented a negative correlation ($P<0.05$) with CP (-0.74), dig. Lys (- 0.66), dig.
228 Met+Cys (-0.73), and dig. Thr (-0.78). This indicates that the lower the level of CP and dig.
229 AA, the higher the cost of the feed, due to the increased inclusion of synthetic amino acids in
230 the formula to supply the AA requirements. In the period of the study, the synthetic amino
231 acids used had costs per kilogram ranging from R\$ 3.89 to R\$ 8.89. These findings are in line
232 with a study conducted by MALLMANN et al. (2019), which reported that FUM, and CP had
233 a significant effect on the final cost of feed for broiler.

234

235 **CONCLUSION**

236 Based on the results obtained, we can conclude that the genetic development of corn and
237 modern agricultural techniques have significantly contributed to increasing its productivity.
238 However, the presence of mycotoxins in the grains represents a challenge, raising feed
239 production costs. The variation in corn nutrient levels highlights the importance of closely
240 monitoring its quality. Our findings underscore the ongoing need for research to improve
241 resistance to mycotoxins and the nutritional quality of corn, aiming to ensure sustainability
242 and profitability in animal feed production. Additionally, it is crucial to provide information
243 on the resistance of different corn varieties to diseases such as fusariosis, to assist farmers in
244 choosing the most suitable varieties. This study offers valuable insights to enhance efficiency
245 and safety in animal feed production.

246

247 **CONFLICTS OF INTEREST**

248 The authors have no conflict of interest regarding the content of this article.

249

250 **AUTHORS' CONTRIBUTIONS**

251 All authors contributed equally for the conception and writing of the manuscript. All
252 authors critically revised the manuscript and approved the final version.

253

254 **ACKNOWLEDGMENTS**

255 The authors acknowledge the Copacol cooperative for providing the experimental field
256 and assisting in the project's development. J.K. Vidal, C.T. Simões, and L.M.L. Schlösser are
257 grateful to the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES),
258 Brazil – Finance Code 001, for providing their graduate scholarship.

259

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317

318 Table 1 - Nutritional requirements, ingredients, and costs used in feed formulations for
 319 broilers starter diets.

Nutrients and energy requirements ¹	Unit	Starter diet	Ingredients	R\$/kg
AME _n ²	kcal/kg	3,050	Corn	0.81
Crude Protein	%	23.3	Soybean meal, 46%	2.39
Dig. Met	%	0.51	Meat and bone meal, 45%	1.68
Dig. Met + Cys	%	0.93	Soybean oil	6.18
Dig. Lys	%	1.25	DL-hydroxy-Methionine 88%	8.89
Dig. Thr	%	0.83	L-Lysine HCl (55% lysine)	3.89
Dig. Trp	%	0.22	L-Threonine 98.5%	6.43
Dig. Arg	%	1.34	Limestone	0.31
Dig. Gly + Ser	%	1.84	Dicalcium phosphate	2.72
Dig. Val	%	0.96	Salt	0.43
Dig. Ile	%	0.84	Vitamin and mineral premix ³	23.07
Dig. Leu	%	1.34	Antimycotoxin additive	7.80
Ca	%	0.88		
Available P	%	0.42		
Na	%	0.22		

320 ¹Nutrients and energy requirements according to Rostagno et al. (2017) recommendations for standard-high performance
 321 male broilers.

322 ²AME_n: Apparent metabolizable energy for birds, corrected for nitrogen balance (kcal/kg).

323 ³Composition per kilogram of feed: iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium,
 324 0.25 mg; vitamin A, 9,000 IU; vitamin D3, 2,500 IU; vitamin E, 20 IU; vitamin K3, 2,5 mg; thiamine, 2 mg; riboflavin, 6
 325 mg; pyridoxine, 3.8 mg; cyanocobalamin, 0.015 mg; pantothenic acid, 12 mg; niacin, 35 mg; folic acid, 1,5 mg; biotin, 0.1
 326 mg.
 327

328 Table 2 - Damaged grains, crop yield, aflatoxins (B₁+B₂+G₁+G₂), fumonisins (B₁+B₂),
 329 zearalenone and deoxynivalenol of 50 corn hybrids.

Hybrid ¹	Damaged grains (%)	Crop Yield (kg/ha)	AFLA ³ (µg/kg)	FUM ⁴ (µg/kg)	ZEA ⁵ (µg/kg)	DON ⁶ (µg/kg)
1 ²	0.29	7,826 ^b	0.000 ^b	529.6 ^b	0.00	0.00
2	0.84	7,933 ^a	0.382 ^a	361.6 ^b	0.00	0.00
3	0.30	7,704 ^b	0.510 ^a	272.1 ^b	0.00	0.00
4	0.54	7,495 ^b	0.000 ^b	319.4 ^b	0.00	0.00
5	0.35	7,606 ^b	0.000 ^b	265.1 ^b	0.00	0.00
6	0.21	7,738 ^b	0.000 ^b	1,347 ^a	135.28	0.00
7	0.99	7,688 ^b	0.000 ^b	0.0 ^b	0.00	0.00
8	0.38	7,605 ^b	0.000 ^b	0.0 ^b	0.00	0.00
9	0.32	6,569 ^d	0.000 ^b	0.0 ^b	0.00	0.00
10	0.26	7,704 ^b	0.000 ^b	0.0 ^b	0.00	0.00
11	0.48	6,426 ^d	0.000 ^b	0.0 ^b	0.00	0.00
12	0.96	8,134 ^a	0.000 ^b	397.6 ^b	0.00	0.00
13	0.17	7,143 ^c	0.000 ^b	39.7 ^b	0.00	0.00
14	0.42	8,870 ^a	0.000 ^b	347.9 ^b	0.00	0.00
15	0.24	7,549 ^b	0.000 ^b	41.0 ^b	0.00	0.00
16	0.33	8,749 ^a	0.000 ^b	503.3 ^b	0.00	0.00
17	0.42	7,469 ^b	0.000 ^b	0.0 ^b	47.82	0.00
18	0.54	7,267 ^b	0.000 ^b	246.9 ^b	0.00	0.00
19	0.53	7,261 ^b	0.000 ^b	0.0 ^b	0.00	0.00
20	0.75	6,984 ^c	0.000 ^b	0.0 ^b	0.00	0.00
21	0.71	7,823 ^b	0.484 ^a	0.0 ^b	0.00	0.00
22	1.16	8,336 ^a	0.000 ^b	189.5 ^b	0.00	0.00
23	0.35	7,510 ^b	0.481 ^a	207.0 ^b	0.00	0.00
24	0.37	7,185 ^c	0.413 ^a	302.0 ^b	0.00	0.00
25	0.52	7,107 ^c	0.000 ^b	0.0 ^b	0.00	0.00
26	0.48	8,011 ^a	0.000 ^b	451.8 ^b	0.00	0.00
27	0.52	6,543 ^d	0.000 ^b	267.0 ^b	0.00	0.00
28	1.12	7,082 ^c	0.000 ^b	1,278 ^a	0.00	0.00
29	0.67	7,628 ^b	0.000 ^b	174.2 ^b	0.00	0.00
30	0.21	7,546 ^b	0.000 ^b	44.0 ^b	6.78	126.97
31	0.36	7,480 ^b	0.000 ^b	0.0 ^b	0.00	0.00
32	0.43	8,115 ^a	0.000 ^b	39.8 ^b	0.00	0.00
33	0.30	8,180 ^a	0.000 ^b	39.7 ^b	0.00	0.00
34	0.39	8,468 ^a	0.000 ^b	0.0 ^b	0.00	0.00
35	0.45	6,997 ^c	0.000 ^b	143.0 ^b	0.00	0.00
36	0.13	6,842 ^c	0.000 ^b	0.0 ^b	0.00	0.00
37	0.53	7,584 ^b	0.000 ^b	570.0 ^b	0.00	66.44
38	0.58	5,935 ^d	0.000 ^b	80.2 ^b	6.81	93.91
39	0.45	6,191 ^d	0.000 ^b	480.7 ^b	0.00	0.00
40	0.66	6,756 ^c	0.000 ^b	149.9 ^b	7.19	168.38
41	0.37	6,146 ^d	0.000 ^b	39.9 ^b	0.00	0.00
42	0.85	7,347 ^b	0.000 ^b	79.5 ^b	0.00	0.00
43	0.28	8,095 ^a	0.000 ^b	201.3 ^b	0.00	0.00
44	0.40	6,080 ^d	0.000 ^b	0.0 ^b	0.00	0.00
45	1.00	6,617 ^d	0.000 ^b	40.1 ^b	0.00	0.00
46	0.79	8,055 ^a	0.000 ^b	272.5 ^b	0.00	0.00
47	0.20	7,418 ^b	0.000 ^b	39.8 ^b	0.00	0.00
48	0.47	6,836 ^c	0.000 ^b	106.5 ^b	0.00	0.00
49	0.42	7,275 ^b	0.000 ^b	0.0 ^b	0.00	0.00
50	1.00	7,479 ^b	0.000 ^b	0.0 ^b	0.00	0.00
CV (%)	86.6	6.14	550	160	864	645
P-value	0.440	0.001	0.621	0.001	0.522	0.576

330 ¹Corn hybrid; ²Number assigned to the corn hybrid. ³AFLA: Aflatoxins (B₁+B₂+G₁+G₂) (µg/kg); ⁴FUM: Fumonisins (B₁+B₂)
 331 (µg/kg). ⁵ZEA: Zearalenone (µg/kg). ⁶DON: Deoxynivalenol (µg/kg). ^{a-c} Means followed by different letters in the same
 332 column differ by the Scott-Knott test at 5% significance.
 333

334 Table 3 - Nutrient composition, AMA inclusion, and cost of a starter feed for broilers
 335 obtained from 50 corn hybrids.

Hybrid ¹	Starch (%)	Av. P ³ (%)	EE ⁴ (%)	AME _n ⁵ (kcal/kg)	CP ⁶ (%)	Dig. Lys ⁷ (%)	Dig. Met+ Cys ⁸ (%)	Dig. Thr ⁹ (%)	AMA ¹⁰ (kg/t)	R\$/t feed ¹¹
1 ²	62.9 ^b	0.047	4.20 ^c	3,308 ^c	7.79 ^e	0.204 ^b	0.311 ^d	0.248 ^d	0.92	1,669
2	64.5 ^a	0.047	3.90 ^e	3,296 ^d	6.63 ^j	0.185 ^d	0.275 ^g	0.213 ⁱ	0.79	1,717
3	63.3 ^b	0.047	4.28 ^c	3,319 ^b	7.58 ^f	0.196 ^c	0.303 ^e	0.242 ^e	0.46	1,665
4	63.6 ^b	0.043	3.85 ^e	3,298 ^d	7.30 ^g	0.191 ^d	0.290 ^f	0.232 ^g	0.49	1,689
5	62.9 ^b	0.050	4.39 ^b	3,318 ^b	7.87 ^e	0.206 ^b	0.314 ^d	0.251 ^d	0.46	1,654
6	64.9 ^a	0.043	3.78 ^e	3,300 ^d	7.19 ^h	0.192 ^d	0.290 ^f	0.228 ^g	1.79	1,707
7	63.2 ^b	0.050	3.42 ^g	3,274 ^g	8.35 ^c	0.209 ^a	0.321 ^c	0.263 ^b	0.00	1,664
8	62.7 ^b	0.047	3.94 ^e	3,293 ^e	8.34 ^c	0.209 ^a	0.326 ^c	0.264 ^b	0.00	1,650
9	63.2 ^b	0.047	4.01 ^d	3,305 ^c	8.03 ^d	0.206 ^b	0.316 ^d	0.254 ^c	0.00	1,654
10	63.4 ^b	0.047	3.87 ^e	3,293 ^e	8.08 ^d	0.202 ^b	0.317 ^d	0.255 ^c	0.00	1,659
11	63.1 ^b	0.050	3.34 ^g	3,271 ^g	8.62 ^b	0.213 ^a	0.333 ^b	0.270 ^a	0.00	1,656
12	62.7 ^b	0.047	3.95 ^e	3,299 ^d	7.98 ^d	0.207 ^a	0.319 ^d	0.252 ^c	0.54	1,665
13	63.4 ^b	0.050	3.96 ^e	3,302 ^d	8.21 ^c	0.203 ^b	0.323 ^c	0.259 ^b	0.18	1,650
14	62.9 ^b	0.050	4.11 ^d	3,309 ^c	7.69 ^f	0.203 ^b	0.307 ^e	0.244 ^e	0.25	1,666
15	63.8 ^a	0.050	3.90 ^e	3,298 ^d	7.71 ^f	0.197 ^c	0.305 ^e	0.244 ^e	0.18	1,672
16	64.3 ^a	0.040	3.83 ^e	3,299 ^d	7.33 ^g	0.194 ^c	0.295 ^f	0.231 ^g	0.60	1,688
17	64.1 ^a	0.050	3.79 ^e	3,296 ^d	7.46 ^g	0.191 ^d	0.292 ^f	0.237 ^f	0.00	1,681
18	64.8 ^a	0.047	3.49 ^f	3,289 ^e	7.68 ^f	0.196 ^c	0.303 ^e	0.241 ^e	0.46	1,684
19	64.6 ^a	0.043	3.81 ^e	3,299 ^d	6.89 ⁱ	0.189 ^d	0.283 ^g	0.221 ^h	0.00	1,695
20	63.1 ^b	0.050	3.50 ^f	3,271 ^g	7.90 ^e	0.203 ^b	0.313 ^d	0.250 ^d	0.00	1,681
21	63.4 ^b	0.047	3.54 ^f	3,283 ^f	8.13 ^d	0.204 ^b	0.318 ^d	0.256 ^c	0.11	1,666
22	63.7 ^a	0.047	4.09 ^d	3,304 ^c	6.87 ⁱ	0.189 ^d	0.274 ^g	0.220 ^h	0.21	1,697
23	63.5 ^b	0.050	3.96 ^e	3,296 ^d	7.87 ^e	0.199 ^c	0.303 ^e	0.248 ^d	0.71	1,675
24	61.6 ^c	0.050	4.08 ^d	3,296 ^d	7.69 ^f	0.208 ^a	0.301 ^e	0.245 ^e	0.75	1,681
25	64.1 ^a	0.050	3.76 ^e	3,290 ^e	7.42 ^g	0.192 ^d	0.297 ^f	0.236 ^f	0.00	1,685
26	63.2 ^b	0.047	3.88 ^e	3,296 ^d	7.75 ^e	0.203 ^b	0.307 ^d	0.246 ^e	0.57	1,677
27	63.9 ^a	0.050	3.66 ^f	3,290 ^e	8.02 ^d	0.200 ^b	0.312 ^d	0.252 ^c	0.23	1,668
28	63.1 ^b	0.050	3.39 ^g	3,266 ^g	8.29 ^c	0.216 ^a	0.319 ^d	0.261 ^b	1.71	1,690
29	62.8 ^b	0.047	4.24 ^c	3,311 ^c	7.61 ^f	0.198 ^c	0.295 ^f	0.242 ^e	0.42	1,668
30	63.4 ^b	0.050	3.60 ^f	3,281 ^f	8.45 ^c	0.211 ^a	0.325 ^c	0.266 ^b	0.18	1,656
31	64.5 ^a	0.050	3.51 ^f	3,286 ^e	7.99 ^d	0.198 ^c	0.311 ^d	0.251 ^d	0.00	1,669
32	63.6 ^b	0.047	3.57 ^f	3,287 ^e	7.91 ^e	0.198 ^c	0.313 ^d	0.249 ^d	0.18	1,671
33	64.5 ^a	0.047	3.62 ^f	3,288 ^e	7.07 ^h	0.186 ^d	0.281 ^g	0.225 ^g	0.18	1,702
34	63.2 ^b	0.047	4.07 ^d	3,307 ^c	7.81 ^e	0.202 ^b	0.303 ^e	0.247 ^d	0.00	1,661
35	64.4 ^a	0.050	3.46 ^f	3,278 ^f	7.95 ^d	0.203 ^b	0.310 ^d	0.251 ^d	0.40	1,679
36	63.8 ^a	0.050	3.59 ^f	3,286 ^e	8.17 ^c	0.201 ^b	0.323 ^c	0.257 ^c	0.00	1,662
37	63.3 ^b	0.050	3.86 ^e	3,291 ^e	7.81 ^e	0.203 ^b	0.305 ^e	0.247 ^d	0.64	1,678
38	62.1 ^c	0.050	4.88 ^a	3,336 ^a	7.88 ^e	0.203 ^b	0.308 ^d	0.252 ^c	0.37	1,642
39	63.1 ^b	0.050	4.54 ^b	3,327 ^a	8.32 ^c	0.208 ^a	0.321 ^c	0.264 ^b	0.58	1,632
40	64.4 ^a	0.047	4.45 ^b	3,329 ^a	7.30 ^g	0.193 ^d	0.294 ^f	0.233 ^g	0.20	1,664
41	63.9 ^a	0.047	3.06 ^h	3,255 ^h	8.85 ^a	0.210 ^a	0.346 ^a	0.276 ^a	0.18	1,658
42	61.9 ^c	0.050	3.67 ^f	3,278 ^f	8.25 ^c	0.209 ^a	0.324 ^c	0.260 ^b	0.37	1,667
43	62.4 ^c	0.047	4.41 ^b	3,320 ^b	7.57 ^f	0.201 ^b	0.300 ^e	0.242 ^e	0.65	1,666
44	64.2 ^a	0.043	3.35 ^g	3,279 ^f	7.80 ^e	0.194 ^c	0.308 ^d	0.244 ^e	0.00	1,681
45	63.9 ^a	0.047	3.58 ^f	3,283 ^f	7.75 ^e	0.197 ^c	0.305 ^e	0.244 ^e	0.18	1,680
46	63.5 ^b	0.050	3.79 ^e	3,288 ^e	7.62 ^f	0.200 ^b	0.292 ^f	0.240 ^e	0.47	1,687
47	64.4 ^a	0.050	3.85 ^e	3,297 ^d	7.70 ^f	0.195 ^c	0.303 ^e	0.243 ^e	0.18	1,673
48	63.3 ^b	0.050	3.86 ^e	3,289 ^e	8.09 ^d	0.204 ^b	0.316 ^d	0.255 ^c	0.19	1,665
49	64.4 ^a	0.047	3.61 ^f	3,288 ^e	7.85 ^e	0.197 ^c	0.315 ^d	0.248 ^d	0.00	1,670
50	64.7 ^a	0.047	3.50 ^f	3,279 ^f	7.46 ^g	0.193 ^d	0.303 ^e	0.237 ^f	0.00	1,690
CV (%)	1.04	9.03	3.22	0.21	2.46	2.51	2.45	2.71	-	-
P-value	0.001	0.604	0.001	0.001	0.001	0.001	0.001	0.001	-	-

336 ¹Corn hybrid; ²Number assigned to the corn hybrid. ³Av. P (Available phosphorus = Total P – Phytic P); ⁴Ether Extract (%);
 337 ⁵Apparent metabolizable energy corrected for N (kcal/kg); ⁶Crude Protein (%); ⁷Digestible lysine (%); ⁸Digestible methionine
 338 + cysteine (%); ⁹Digestible threonine (%); ¹⁰Antimycotoxin additive inclusion in the diet (kg/t); ¹¹Cost of feed formulation of
 339 starter diet (R\$/t); a-f Means followed by different letters in the same column differ by the Scott-Knott test at 5% significance.

6 ARTIGO 2

Este capítulo é apresentado em formato de artigo de pesquisa denominado “A Three-Year Study on the Nutritional Composition and Occurrence of Mycotoxins of Corn Varieties with Different Transgenic Events Focusing on Poultry Nutrition” publicado em 2024 no periódico *Veterinary Sciences*, disponível em: <https://doi.org/10.3390/vetsci11020097>.

2 A three-year study on the nutritional composition and 3 occurrence of mycotoxins of corn varieties with different 4 transgenic events focusing on poultry nutrition

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13 **Simple Summary:** Annually, various corn hybrids are introduced to the market for cultivation.
14 Companies engaged in developing these technologies aim to enhance genetic traits, with a focus on
15 creating productive hybrids capable of addressing the challenges of agriculture. The global corn
16 market predominantly concerns livestock feed production, particularly for poultry farming, which
17 seeks nutrient-rich raw materials with high digestibility for broilers. Nevertheless, when comparing
18 the diverse transgenic technologies of corn, even when cultivated under the same conditions,
19 significant differences were observed. Surprisingly, in the present study, the most productive corn
20 transgenic technology in the field exhibited increased contamination by mycotoxins and a lower
21 content of some important nutrients for poultry. This outcome highlights the critical need for a
22 comprehensive assessment of the implications of transgenic technologies for nutritional
23 composition and agricultural product safety, especially when intended for animal feed.
24 Consequently, we concluded that the integration of nutritional considerations into the genetic
25 improvement of transgenic corn, along with detailed information about resistance to *Fusarium*,
26 holds great significance and may yield positive outcomes in the future. This approach ensures the
27 production of nutritionally balanced, mycotoxin-safe, and economically viable livestock feed.

28 **Abstract:** Corn is one of the most produced cereals in the world and plays a major role in poultry
29 nutrition. As there is limited scientific information regarding the impact of transgenic technology on
30 the quality and nutrient composition of the grains, this study investigated the effect of three major
31 transgenic corn varieties—VT PRO3[®], PowerCore[®] ULTRA, and Agrisure[®] Viptera 3—on the field
32 traits, nutrient composition, and mycotoxin contamination of corn grains cultivated in southern
33 Brazil during three consecutive harvests. VT PRO3[®], while demonstrating superior crop yield,
34 showed susceptibility to mycotoxins, particularly fumonisins. In contrast, PowerCore[®] ULTRA, with
35 the lowest yield, consistently exhibited lower levels of fumonisins. VT PRO3[®] had higher AMEn
36 than the other varieties, while PowerCore[®] ULTRA had the highest total and digestible amino acid
37 contents over the three years. The study's comprehensive analysis reveals the distinct impact of
38 transgenic corn technologies on both productivity and nutritional levels. Balancing the crops yield,
39 mycotoxin resistance, and nutritional content of corn is crucial to meet the demands of the poultry
40 feed industry. Such insights are essential for decision-making, ensuring sustainability and efficiency
41 in agricultural production as well as meeting the demands of the poultry industry.

42 **Keywords:** transgenic corn; poultry nutrition; mycotoxins; digestible amino acids; agronomic traits.

1. Introduction

Corn ranks among the most globally cultivated cereals, with the production of more than 1200 million tons in the 2022/2023 harvest, mainly concentrated in the United States, China, and Brazil [1]. In Brazil, corn is the second most produced grain, following soybeans. For the 2023/2024 harvest, the estimated production in Brazil will exceed 118 million tons [2]. In the Brazilian market, the diversity of corn cultivars is substantial, whereby 98, 259, and 98 different cultivars were available for commercialization in the 2020, 2021, and 2022 harvests, respectively [3–5]. Remarkably, the presence of transgenic cultivars has increased, accounting for 76%, 71%, and 95% of the total for the corresponding harvests. Among the transgenic cultivars, VT PRO3[®], PowerCore[®] ULTRA, and Agrisure[®] Viptera 3 have emerged as the primary transgenic events, together representing 46% of available technologies in 2020, 64% in 2021 and 56% in 2022 [3–5].

Over the last decade, corn has consistently constituted approximately 80% of the total volume in the global trade of cereal grains, which includes corn, sorghum, barley, and oats [1]. The relevance of corn for the feed industry is evident, as around 70% of the corn marketed in Brazil is intended for animal nutrition [5], with it being mainly consumed by the poultry and swine industries. Corn is considered a high nutritional value ingredient, which contributes approximately 65% of the metabolizable energy and 20% of the protein to a broiler's diet [6]. In addition to its recognized nutritional value, corn is also known as a natural source of carotenoids and xanthophyll [7], important pigments for the poultry industry, with them being deposited into the poultry skin and egg yolk. Despite being marketed as a commodity, there is substantial variability in its nutritional characteristics caused by several factors such as seed genetics, endosperm texture, cultivation location, climatic conditions, post-harvest management, and storage [8,9]. The current scenario emphasizes the importance of understanding the nutritional nuances of corn and the main factors involved.

The genetic improvement of corn has mainly been targeting high-productivity cultivars, with resistance to root lodging, and specific pathogens. However, there is an information gap concerning resistance to *Fusarium* in the corn hybrids currently marketed. In the 2020, 2021, and 2022 harvests, 82%, 99%, and 96% of hybrids, respectively, lacked information on *Fusarium* resistance [3–5]. Various strains of filamentous fungi, including *Aspergillus*, *Fusarium*, and *Penicillium*, are prevalent contaminants in corn crops. These fungal groups produce mycotoxins, secondary metabolites associated with well-documented toxic, mutagenic, and carcinogenic effects, leading to significant impacts on animal health as well as economic losses [10–12]. South American countries typically have high occurrences of fumonisins in corn, mycotoxins produced by *Fusarium* fungi that were found to contaminate more than 90% of the corn samples evaluated in different studies [13–15].

The management of mycotoxicological contamination in the animal feed chain involves several strategies. Reducing the moisture content of grains before storage as well as controlling and monitoring humidity and temperature during storage can significantly reduce the production of mycotoxins [11]. Other common strategies are the utilization of organic acids to reduce fungal contamination in grains and feeds [12], as well as the inclusion of antimycotoxin additives in the diet, which are capable of reducing the absorption of mycotoxins by the animals' gastrointestinal tract [16].

Since there are no certified corn transgenic technologies regarding nutritional quality and susceptibility to mycotoxin-producing fungi, this study aims to fill part of this gap by analyzing the differences among the major transgenic events available for cultivation in the Southern region of Brazil (VT PRO3[®], PowerCore[®] ULTRA, and Agrisure[®] Viptera 3) with respect to agronomic traits, nutrient composition, and contamination by mycotoxins.

2. Materials and Methods

95 2.1. *Classification of corn types*

96 Different commercial corn hybrids of each transgenic technology were chosen based
97 on their commercialization rate in the region of the study and grouped into three
98 categories of transgenic events: VT PRO3[®], PowerCore[®] ULTRA, and Agrisure[®] Viptera 3.
99 A total of 87 corn samples were evaluated (VT PRO3[®] = 30, PowerCore[®] ULTRA = 42,
100 Agrisure[®] Viptera 3 = 15) in 2020, 80 corn samples were evaluated (VT PRO3[®] = 44,
101 PowerCore[®] Ultra = 36) in 2021, and 48 corn samples were evaluated (VT PRO3[®] = 28,
102 PowerCore[®] ULTRA = 20) in 2022. The Agrisure[®] Viptera 3 technology did not have
103 commercial representation in 2021 and 2022 and, therefore, was not evaluated in these
104 two years. To maintain confidentiality, the designations of the corn hybrids were kept
105 undisclosed.

106 2.2. *Field experiments*

107 The samples of corn from the three years of the study were obtained from
108 experimental field plots cultivated at the Agricultural Research Center of the Cooperativa
109 Agroindustrial Consolata (COPACOL), located in the state of Paraná, Brazil (24°37'01.800"
110 S, 53°18'02.000" W, 580 m altitude). The region features dystrophic red latosol as its
111 predominant soil type. Fertilization of crops was guided by chemical analyses and the
112 nutritional requirements of the soil. Meteorological information such as precipitation, air
113 temperature (°C), and relative humidity (%) was obtained over the three years of
114 cultivation by a weather station positioned 50 m away from the experimental plots. The
115 recorded data corresponded to the months when corn cultivation took place each year.
116

117 Corn crops from the three years were cultivated in a consolidated no-till system,
118 under the same soil type. The field trials were arranged in a randomized block design,
119 with each corn hybrid being a block with three replications by corn hybrid in 2020 and
120 four replications by corn hybrid in both 2021 and 2022. In 2020, cultivation took place in
121 the second half of January, with experimental plots containing four corn rows spaced 0.68
122 m apart and extending 14 m in length. In 2021 and 2022, cultivation occurred in the initial
123 half of February, and the experimental plots contained four corn rows spaced 0.70 m
124 apart and extending 6 m in length.

125 Treatment of seeds was implemented consistently during the three-year period,
126 employing 300 mL/ha of thiodicarb + imidacloprid (Cropstar, Bayer, São Paulo, SP,
127 Brazil). Insecticides and herbicides were administered following the guidelines provided
128 by the manufacturers. These included 250 mL/ha of thiamethoxam + lambda-cyhalothrin
129 (Engeo- Pleno, Syngenta, São Paulo, SP, Brazil), 2 L/ha of mesotrione + atrazine (Calaris,
130 Syngenta, São Paulo, SP, Brazil), 150 mL/ha of lambda-cyhalothrin + chlorantraniliprole
131 (Ampligo, Syngenta, São Paulo, SP, Brazil), and 100 mL/ha of spinetoram (Exalt, Corteva,
132 Barueri, SP, Brazil), applied at the vegetative growth stages V1, V2, V3, and V5,
133 respectively. Harvesting took place in the second half of June 2020 and the first half of
134 July 2021 and 2022. The central two rows of each plot were harvested utilizing a
135 Wintersteiger[®] experimental plot harvester.

136 The mass of grains and moisture content were automatically determined by the Easy
137 Harvest weighing system (Wintersteiger, Ried im Innkreis, OÖ, Austria), with the
138 assistance of data collection systems Grain Gage[®] (HarvestMaster, Logan, UT, USA)
139 coupled with the harvesting system. The crop yield of the plots was calculated in kg/ha
140 and adjusted for 13% moisture. Damaged grains were classified according to MAPA
141 recommendations [17] and the percentage was obtained by the equation: [weight of
142 damaged grains (g)/weight of the sample (g)] × 100.
143

2.3. Quantification of mycotoxins by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS)

After harvest, the samples were dried in a forced-air oven. A temperature of 55 °C was maintained for a period of 12 h, aiming to reduce the moisture content of the samples to approximately 13%. The dried samples (± 1 kg) were sent to the Laboratory of Mycotoxicological Analysis at the Federal University of Santa Maria, Brazil. The samples were ground at 1 mm in an ultracentrifugal mill, model ZM 200 (RETSCH®, Haan, NRW, Germany), homogenized, and subsequently analyzed for the presence and concentration of mycotoxins.

2.3.1. Chemical reagents

Analytical standards for aflatoxins (AF), fumonisins (FUM), deoxynivalenol (DON), and zearalenone (ZEA) were obtained from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile, ammonium acetate, formic acid, and methanol (HPLC grade) were acquired from JT Baker (Center Valley, PA, USA). Ultra-pure water was obtained from a Milli-Q Advantage A10 Water Purification System (Merck KGaA, Darmstadt, HE, Germany).

2.3.2. Aflatoxins (AFB_1 , AFB_2 , AFG_1 , and AFG_2)

The method described by Mallmann et al. [18] was conducted for AF analyses. A sample of 5 g was mixed with 20 mL of an acetonitrile:water solution (84:16, *v/v*) and shaken for 60 min on a shaking table. The resulting extract was centrifuged (Eppendorf 5804R) at 2500 rpm, 20 °C, for 5 min. Then, 60 μ L was diluted in 840 μ L of a methanol:water solution (1:1, *v/v*) in a vial, and 20 μ L of the obtained solution was then injected into an HPLC Infinity Series 1200 instrument (Agilent, Palo Alto, CA, USA) coupled to a 5500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA). This system was equipped with an electrospray ionization (ESI) source in positive mode. Chromatographic separation was carried out at 30 °C using an Eclipse XDB-C8 column (4.6 \times 150 mm, particle size 5 μ m) (Agilent). The mobile phases consisted of water:ammonium acetate (99:1, *v/v*) and methanol:water:ammonium acetate (95:4:1, *v/v/v*).

2.3.3. Deoxynivalenol and Zearalenone

For the assessment of DON and ZEA, the method described by Berthiller et al. [19] was applied. In this procedure, a 3 g sample was combined with 24 mL of a methanol:water mixture (70:30, *v/v*) and stirred for 20 min on an orbital shaker. Following this, the resultant extract was submitted to centrifugation at 2500 rpm, 20 °C, for 5 min. Subsequently, 40 μ L of the centrifuged extract was diluted in 960 μ L of a methanol:water:ammonium acetate solution (90:9:1, *v/v/v*) in a vial. A 10 μ L aliquot of this solution was introduced into an HPLC Infinity Series 1200 instrument (Agilent Technologies, Santa Clara, CA, USA) coupled to a 5500 QTRAP mass spectrometer (Applied Biosystems, Waltham, MA, USA), featuring an ESI source in positive mode. Chromatographic separation was carried out at 40 °C using a Zorbax SB-C18 column (4.6 \times 150 mm, particle diameter of 5 μ m). The mobile phases were methanol:water:ammonium acetate (90:9:1, *v/v/v*) and water:ammonium acetate (90:10, *v/v*).

2.3.4. Fumonisins (FB_1 and FB_2)

The analyses of FUM were performed according to the method of Mallmann et al. [18]. A 3 g sample was added to a Falcon tube with 15 mL of a solution with acetonitrile and water in a 1:1 ratio (*v/v*). The tube was shaken for 20 min using an orbital shaker. Afterward, the resultant mixture was centrifuged at 2500 rpm, 20 °C, for 5 min, and 20 μ L was diluted in 980 μ L of a solution containing acetonitrile, water, and formic acid in a

196 50:40:10 ratio (*v/v/v*). Then, 10 μ L of the obtained solution was introduced into an HPLC
197 Infinity Series 1200 apparatus (Agilent), connected to an API 5000 mass spectrometer
198 (Applied Biosystems) featuring an ESI source in positive mode. Chromatographic
199 separation was conducted at 40 °C using an Eclipse XDB-C8 column (4.6 \times 150 mm,
200 particle diameter of 5 μ m). The mobile phases comprised acetonitrile and formic acid
201 (95:5, *v/v*) and water and formic acid (95:5, *v/v*).
202

203 2.3.5. Parameters of methods performance

204 The quantification limits (LOQ) and detection limits (LOD) of each mycotoxin were
205 determined by evaluating the signal-to-noise ratio (LOQ = 10/1; LOD = 3/1). The recovery
206 rate (%) of each method (AF, FUM, DON, and ZEA) was based on the mean concentration
207 obtained from corn-fortified samples with three different levels of the target analyte
208 (mycotoxin) with seven replicates each. The linearity of the analytical curves from each
209 mycotoxin was examined by utilizing the coefficient of determination (R^2), which was
210 computed following triplicate injections of the analytical curves at seven distinct
211 concentration levels.
212

213 2.4. Near infrared spectroscopy nutritional predictions

214 For predictions using near-infrared spectroscopy (NIRS), samples were milled at
215 0.5 mm in an ultra-centrifugal mill, placed in plastic bags, and left for 15 min to reach
216 room temperature (between 18 °C and 22 °C) and humidity (between 40% and 60%).
217 Subsequently, manual homogenization of each sample was performed for two minutes
218 using circular movements. Nutritional predictions were performed by reading the spectra
219 of the samples in a Bruker® instrument, model Tango-R, with a wavelength range of 3952–
220 11,536 cm^{-1} , using the calibration curves from the AMINONRG® and AMINONir®
221 programs (Evonik Nutrition & Care GmbH, Hanau, Germany). The following variables
222 were predicted: dry matter (DM) (%), crude protein (CP) (%), ether extract (EE) (%), ash
223 (%), total P (%), phytic P (%), total and digestible (dig.) amino acids (AA, %), and
224 apparent metabolizable energy (AMEn) (kcal/kg) for poultry. For study and comparison
225 purposes, all values were adjusted to an 87% DM basis.
226

227 2.5. Statistical analysis

228 The statistical analysis was conducted using SAS software, version 9.4, 2015 (SAS
229 Institute, Cary, NC, USA). The normality of the data was tested through the use of the
230 Shapiro–Wilk test prior to other analyses. The contamination data of all mycotoxins from
231 the three years were transformed by $\log_{10}(x + 1)$. Data were subjected to ANOVA using
232 the GLIMMIX procedure. Means of mycotoxin contamination, field data, and nutritional
233 variables from different transgenic technologies were compared using Tukey’s test.
234 Significance was accepted at $p < 0.05$. The following statistical model was utilized:
235

$$236 Y_{ijk} = \mu + \tau_i + \beta_j + \varepsilon_{ijk}$$

237 where Y_{ijk} represents the observed response of the i -th transgenic technology in the j -th
238 commercial hybrid and k -th replicate; μ is the overall mean; τ_i is the fixed effect of the i -th
239 transgenic technology; β_j is the random effect of the j -th commercial hybrid; and ε_{ijk} is the
240 residual error.
241

242 3. Results

243 3.1. Meteorological data

244 The analysis of meteorological data in 2020, 2021, and 2022 is represented in Figure 1.
245 Daily average temperature (°C) and relative humidity (%) are presented on a monthly
246 basis, while precipitation is expressed in cumulative millimeters (mm) per month.
247

The annual average temperature indicated overall stability among the years (21 °C in 2020, 20 °C in 2021, and 21 °C in 2022). In 2020, the monthly maximum and minimum averages were 25.1 and 17.4 °C, observed in February and July, respectively. In 2021, March exhibited the highest monthly average at 24.3 °C, whereas a minimum average of 16.5 °C was measured in June. For 2022, February had the highest monthly average at 25.9 °C, and the minimum average of 16.2 °C was measured in June.

In 2020, the average relative humidity was 69%, increasing to 72% in 2021 and reaching a maximum of 79% in 2022. The maximum and minimum averages for the periods were, respectively: 84% in June and 60% in April 2020; 84% in June and 62% in July 2021; and 87% in June and 66% in February 2022. Regarding accumulated precipitation, there was notable variation among the years, with 568 mm in 2020, 412 mm in 2021, and 824 mm in 2022. Monthly distribution also varied, as shown in the circle charts of Figure 1.

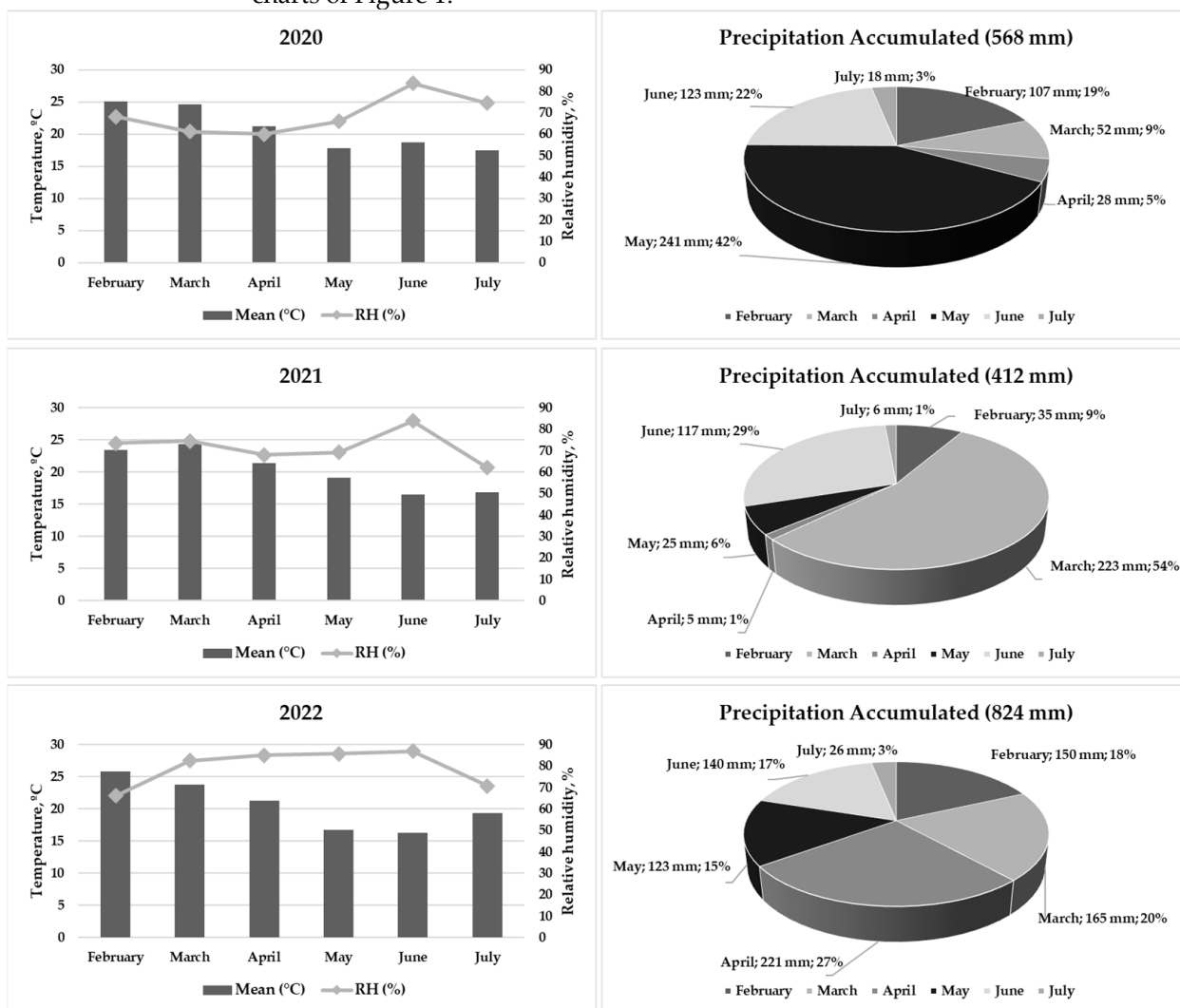


Figure 1. Climatic conditions during the cultivation of different transgenic technologies of corn in 2020, 2021 and 2022.

3.2. Damaged grains, crop yield and mycotoxins contamination

The R^2 of the analytical curves for mycotoxin analyses presented values greater than 0.99. The LOD and LOQ (in $\mu\text{g}/\text{kg}$) for the evaluated mycotoxins were, respectively: 0.4 and 1 for AFB₁; 0.6 and 1 for AFB₂, AFG₁, and AFG₂; 10 and 125 for FB₁; 20 and 125 for FB₂; 50 and 200 for DON; and 3 and 20 for ZEA. The results for the damaged grains, crop yield, and mycotoxin concentration for different corn transgenic events from 2020, 2021, and 2022 are presented in Table 1.

In 2020, a significant difference in crop yield was observed among the three technologies, with VT PRO3® exhibiting a higher yield (9029 kg/ha) compared to PowerCore® ULTRA (8591 kg/ha) ($p = 0.0411$). Agrisure® Viptera 3 presented intermediate productivity results (8767 kg/ha). Additionally, the occurrence of damaged grains was not different among corn transgenic events ($p = 0.6283$). The means of AF were not different among the transgenic technologies ($p > 0.05$) whereas DON and ZEA did not occur (<LOQ). However, total FUM (FB₁ + FB₂) was significantly higher in VT PRO3® (1180 µg/kg) compared to PowerCore® ULTRA (280.8 µg/kg) and Agrisure® Viptera 3 (8.33 µg/kg) ($p = 0.0001$).

In 2021, an increase in the percentage of damaged grains was observed in the VT PRO3® technology (1.66%) compared to PowerCore® ULTRA (0.75%) ($p = 0.0005$). Similarly to the previous year, crop yield was higher in VT PRO3® (5085 kg/ha) than in PowerCore® ULTRA (4166 kg/ha) ($p = 0.0002$). Regarding mycotoxins, total FUM levels were again higher in VT PRO3® (1657 µg/kg) compared to PowerCore® ULTRA (414.0 µg/kg) ($p = 0.0008$), with a difference greater than 1000 µg/kg. The means of AF, DON, and ZEA were not different between the two corn transgenic technologies ($p > 0.05$).

Table 1. Damaged grains, crop yield, and mycotoxin concentration in the different transgenic technologies of corn: 2020, 2021, and 2022.

2020					
Item	Transgenic Technology			SEM	<i>p</i> -value
	VT PRO3®	PowerCore® ULTRA	Agrisure® Viptera 3		
Damaged grains, %	0.21	0.15	0.22	0.034	0.6283
Crop yield, kg/ha	9,029 ^a	8,591 ^b	8,767 ^{ab}	85.81	0.0411
Total aflatoxins ¹ (µg/kg)	1.49	0.29	0.28	0.393	0.3518
Deoxynivalenol (µg/kg)	<LOQ ³	<LOQ	<LOQ	.	.
Total fumonisins ² (µg/kg)	1,180 ^a	280.8 ^b	8.33 ^b	94.22	0.0001
Zearalenone (µg/kg)	<LOQ	<LOQ	<LOQ	.	.
2021					
Item	Transgenic Technology			SEM	<i>p</i> -value
	VT PRO3®	PowerCore® ULTRA	Agrisure® Viptera 3		
Damaged grains, %	1.66 ^a	0.75 ^b	0.75 ^b	0.134	0.0005
Crop yield, kg/ha	5,085 ^a	4,166 ^b	4,166 ^b	127.2	0.0002
Total aflatoxins (µg/kg)	0.256	0.194	0.194	0.054	0.5705
Deoxynivalenol (µg/kg)	21.75	19.00	19.00	7.634	0.8591
Total fumonisins (µg/kg)	1,657 ^a	414.0 ^b	414.0 ^b	190.0	0.0008
Zearalenone (µg/kg)	11.80	1.56	1.56	2.728	0.0717
2022					
Item	Transgenic Technology			SEM	<i>p</i> -value
	VT PRO3®	PowerCore® ULTRA	Agrisure® Viptera 3		
Damaged grains, %	2.23	3.35	3.35	0.363	0.1304
Crop yield, kg/ha	9,411 ^a	8,806 ^b	8,806 ^b	107.8	0.0045
Total aflatoxins (µg/kg)	0.800	0.580	0.580	0.178	0.5491
Deoxynivalenol (µg/kg)	138.8 ^b	481.0 ^a	481.0 ^a	70.28	0.0147
Total fumonisins (µg/kg)	2,566 ^a	990.6 ^b	990.6 ^b	317.2	0.0127
Zearalenone (µg/kg)	131.7	345.5	345.5	57.57	0.0766

^{a-b} Means with different superscript letter differ ($p < 0.05$) based on Tukey's honestly significant difference test. ¹ Sum of aflatoxins B₁, B₂, G₁, and G₂. ² Sum of fumonisins B₁ and B₂. ³ LOQ, limit of quantification.

In 2022, crop yield differences persisted, with VT PRO3® presenting higher performance (9411 kg/ha) compared to PowerCore® ULTRA (8806 kg/ha) ($p = 0.0045$). Additionally, a difference was observed in the concentration of DON, with it being higher

in the PowerCore® ULTRA technology (481.0 µg/kg) compared to the VT PRO3® (138.8 µg/kg) ($p = 0.0147$). Regarding total FUM, consistent with the previous years' results, VT PRO3® (2566 µg/kg) presented higher means than PowerCore® ULTRA (990.6 µg/kg) ($p = 0.0127$), representing a difference greater than 1500 µg/kg.

3.3. Proximal composition and phosphorus values

The nutritional composition results for the different transgenic events of corn in the 2020, 2021, and 2022 crops are presented in Table 2. In 2020, although CP showed only a slight tendency among the technologies ($p = 0.0603$), CF and EE were statistically different ($p = 0.0104$ and $p = 0.0001$, respectively). Crude fiber and EE were higher in the VT PRO3® technology than in PowerCore® ULTRA and Agrisure® Viptera 3. Other components, such as ash and P, were not different among the corn technologies ($p > 0.05$).

Table 2. Nutrient composition in different transgenic technologies of corn: 2020, 2021, and 2022.

2020					
Variable	Transgenic Technology			SEM	<i>p</i> -value
	VT PRO3®	PowerCore® ULTRA	Agrisure® Viptera 3		
Crude protein, %	8.21	8.44	8.44	0.046	0.0603
Crude fiber, %	2.12 ^a	2.03 ^b	2.09 ^{ab}	0.014	0.0104
Ash, %	1.14	1.15	1.13	0.006	0.3168
Ether extract, %	4.03 ^a	3.77 ^b	3.69 ^b	0.030	0.0001
Total P, mg/kg	1,965	1,959	1,963	13.11	0.9761
Phytic P, mg/kg	1,474	1,469	1,472	9.83	0.9750
2021					
Variable	Transgenic Technology			SEM	<i>p</i> -value
	VT PRO3®	PowerCore® ULTRA			
Crude protein, %	9.29 ^b		10.02 ^a	0.082	0.0001
Crude fiber, %	1.99		2.00	0.021	0.8422
Ash, %	1.19 ^b		1.27 ^a	0.008	0.0001
Ether extract, %	3.75 ^a		3.58 ^b	0.033	0.0113
Total P, mg/kg	2,012 ^b		2,094 ^a	15.70	0.0084
Phytic P, mg/kg	1,509 ^b		1,571 ^a	11.78	0.0084
2022					
Variable	Transgenic Technology			SEM	<i>p</i> -value
	VT PRO3®	PowerCore® ULTRA			
Crude protein, %	7.95 ^b		8.87 ^a	0.093	0.0001
Crude fiber, %	1.96		1.91	0.022	0.3069
Ash, %	1.19 ^b		1.23 ^a	0.009	0.0326
Ether extract, %	3.70 ^a		3.36 ^b	0.038	0.0001
Total P, mg/kg	1,970 ^b		2,045 ^a	18.83	0.0480
Phytic P, mg/kg	1,477 ^b		1,533 ^a	14.14	0.0483

^{a-b} Means with different superscript letter differ ($p < 0.05$) based on Tukey's honestly significant difference test.

In 2021, there were significant variations in the concentrations of CP, ash, and EE between the corn technologies. Crude protein was higher in PowerCore® ULTRA (10.02%) compared to VT PRO3® (9.29%) ($p = 0.0001$). Ether extract was higher in VT PRO3® (3.75%) compared to PowerCore® ULTRA (3.58%) ($p = 0.0113$). The technologies also differed in their ash values ($p = 0.0001$), with VT PRO3® showing the lowest content. Furthermore, PowerCore® ULTRA presented higher levels of total ($p = 0.0480$) and phytic ($p = 0.0483$) P compared to VT PRO3®.

Table 3. Total and digestible amino acids and metabolizable energy for poultry in different transgenic technologies of corn: 2020, 2021, and 2022.

2020					
Variable	Transgenic Technology			SEM	<i>p</i> -value
	VT PRO3®	PowerCore® ULTRA	Agrisure® Viptera 3		
Total Met+Cys, %	0.354	0.359	0.363	0.0015	0.0700
Dig ¹ . Met+Cys, %	0.326	0.331	0.334	0.0014	0.0915
Total Lys, %	0.231	0.230	0.231	0.0009	0.8598
Dig. Lys, %	0.210	0.210	0.211	0.0008	0.9845
Total Thr, %	0.291	0.298	0.299	0.0015	0.0954
Dig. Thr, %	0.259	0.265	0.266	0.0013	0.1082
Total Trp, %	0.061	0.061	0.062	0.0002	0.7187
Dig. Trp, %	0.051	0.050	0.050	0.0001	0.1465
Total Arg, %	0.379	0.383	0.385	0.0017	0.4068
Dig. Arg, %	0.338	0.341	0.342	0.0014	0.4358
Total Val, %	0.390	0.399	0.401	0.0020	0.0808
Dig. Val, %	0.371	0.380	0.378	0.0019	0.1122
Total Ile, %	0.281 ^b	0.290 ^a	0.291 ^a	0.0018	0.0338
Dig. Ile, %	0.276 ^b	0.285 ^a	0.288 ^a	0.0017	0.0376
Total Leu, %	1.021 ^b	1.064 ^a	1.070 ^a	0.0079	0.0252
Dig. Leu, %	0.950 ^b	0.990 ^a	0.995 ^a	0.0074	0.0255
Total His, %	0.241	0.246	0.246	0.0011	0.1089
Dig. His, %	0.233	0.238	0.239	0.0011	0.0677
Total Phe, %	0.386 ^b	0.412 ^a	0.414 ^a	0.0029	0.0269
Dig. Phe, %	0.368 ^b	0.383 ^a	0.386 ^a	0.0027	0.0193
AMEn ² , kcal/kg	3,340 ^a	3,330 ^b	3,326 ^b	1.4952	0.0007
2021					
Variable	Transgenic Technology		SEM	<i>p</i> -value	
	VT PRO3®	PowerCore® ULTRA			
Total Met+Cys, %	0.367 ^b	0.384 ^a	0.0029	0.0043	
Dig. Met+Cys, %	0.334 ^b	0.350 ^a	0.0026	0.0025	
Total Lys, %	0.242 ^b	0.251 ^a	0.0014	0.0030	
Dig. Lys, %	0.213 ^b	0.221 ^a	0.0013	0.0020	
Total Thr, %	0.321 ^b	0.345 ^a	0.0028	0.0001	
Dig. Thr, %	0.277 ^b	0.297 ^a	0.0023	0.0001	
Total Trp, %	0.064 ^b	0.067 ^a	0.0003	0.0005	
Dig. Trp, %	0.053 ^b	0.057 ^a	0.0005	0.0016	
Total Arg, %	0.406 ^b	0.426 ^a	0.0028	0.0004	
Dig. Arg, %	0.361 ^b	0.379 ^a	0.0025	0.0004	
Total Val, %	0.430 ^b	0.462 ^a	0.0036	0.0001	
Dig. Val, %	0.400 ^b	0.430 ^a	0.0034	0.0001	
Total Ile, %	0.319 ^b	0.347 ^a	0.0031	0.0001	
Dig. Ile, %	0.307 ^b	0.333 ^a	0.0029	0.0001	
Total Leu, %	1.169 ^b	1.288 ^a	0.0137	0.0001	
Dig. Leu, %	1.076 ^b	1.185 ^a	0.0127	0.0001	
Total His, %	0.256 ^b	0.272 ^a	0.0020	0.0002	
Dig. His, %	0.244 ^b	0.257 ^a	0.0019	0.0005	
Total Phe, %	0.453 ^b	0.498 ^a	0.0052	0.0001	
Dig. Phe, %	0.417 ^b	0.459 ^a	0.0048	0.0001	
AMEn, kcal/kg	3,328 ^a	3,317 ^b	1.8655	0.0014	
2022					

Variable	Transgenic Technology		SEM	p-value
	VT PRO3®	PowerCore® ULTRA		
Total Met+Cys, %	0.349 ^b	0.367 ^a	0.0038	0.0226
Dig. Met+Cys, %	0.310 ^b	0.338 ^a	0.0034	0.0001
Total Lys, %	0.240	0.248	0.0016	0.1480
Dig. Lys, %	0.209 ^b	0.217 ^a	0.0015	0.0071
Total Thr, %	0.285 ^b	0.304 ^a	0.0032	0.0014
Dig. Thr, %	0.241 ^b	0.266 ^a	0.0020	0.0001
Total Trp, %	0.060 ^b	0.063 ^a	0.0004	0.0117
Dig. Trp, %	0.050	0.051	0.0002	0.0909
Total Arg, %	0.371 ^b	0.388 ^a	0.0033	0.0089
Dig. Arg, %	0.325 ^b	0.348 ^a	0.0029	0.0001
Total Val, %	0.381 ^b	0.407 ^a	0.0041	0.0017
Dig. Val, %	0.347 ^b	0.386 ^a	0.0038	0.0001
Total Ile, %	0.278 ^b	0.309 ^a	0.0036	0.0014
Dig. Ile, %	0.260 ^b	0.295 ^a	0.0035	0.0001
Total Leu, %	0.983 ^b	1.088 ^a	0.0171	0.0017
Dig. Leu, %	0.873 ^b	1.021 ^a	0.0151	0.0001
Total His, %	0.239 ^b	0.248 ^a	0.0025	0.0012
Dig. His, %	0.220 ^b	0.239 ^a	0.0024	0.0001
Total Phe, %	0.388 ^b	0.427 ^a	0.0063	0.0017
Dig. Phe, %	0.356 ^b	0.401 ^a	0.0060	0.0001
AMEn, kcal/kg	3,329 ^a	3,311 ^b	1.9347	0.0001

^{a-b} Means with different superscript letter differ ($p < 0.05$) based on Tukey's honestly significant difference test. ¹ Ileal digestible amino acids for poultry (predicted using AMINONIR® calibration curves). ² AMEn= apparent metabolizable energy for poultry (predicted using AMINONRG® calibration curves).

Differences in nutritional characteristics between corn transgenic technologies were also observed in 2022, with this being consistent with the results observed in the previous years. PowerCore® ULTRA had a higher concentration of CP and ash and higher p -values than VT PRO3® ($p < 0.05$), whereas VT PRO3® had the highest concentration of EE ($p = 0.0001$).

3.4. Amino acids and metabolizable energy for poultry

In 2020, corn transgenic events exhibited significant differences in certain total and dig. AA and in the AMEn, as observed in Table 3. Total and dig. Ile, Leu, and Phe were higher in PowerCore® ULTRA compared to VT PRO3® and Agrisure® Viptera 3 ($p = 0.0338$ and $p = 0.0376$; $p = 0.0252$ and $p = 0.0255$; and $p = 0.0269$ and $p = 0.0193$, respectively). Additionally, AMEn differed significantly among technologies, being higher in VT PRO3® than in PowerCore® ULTRA and Agrisure® Viptera 3 ($p = 0.0007$). In 2021, all the total and dig. AA was significantly different between the corn technologies ($p < 0.05$), with higher concentrations in PowerCore® ULTRA compared to VT PRO3®. In addition, AMEn was higher in VT PRO3® compared to PowerCore® ULTRA ($p = 0.0014$). In 2022, the PowerCore® ULTRA technology had a higher concentration of most of the total and dig. AA than VT PRO3® ($p < 0.05$), with the exception of total Lys ($p = 0.1480$) and dig. Trp ($p = 0.0909$). Furthermore, AMEn was significantly higher in VT PRO3® compared to PowerCore® ULTRA ($p = 0.0001$).

4. Discussion

The maximization of productivity per hectare in corn cultivation assumes significant relevance, given the evolution of human and animal nutrition, as well as the concern for environmental preservation [20]. Efficiency in the utilization of cultivated land plays a crucial role in meeting global food needs [21]. When it comes to corn intended for poultry

355 feed, it is therefore important to consider materials with high safety, nutritional
356 concentration, and nutrient digestibility [22].

357 Agricultural production is intrinsically dependent on climatic conditions.
358 Climatic fluctuation has negative impacts on crop development, grain yield, and quality,
359 influencing processes such as vegetative development, flowering, and grain maturation,
360 as well as the incidence of pests and diseases [8]. The analysis of meteorological data
361 collected in the experimental field over the three years of the present study enabled the
362 identification of variability among the years and the discussion of the potential impact of
363 these conditions on crop yield and grain quality. It has already been demonstrated that
364 the meteorological variables from different years exert some influence on the nutritional
365 and mycotoxicological composition of different corn hybrids [23,24]. Data from 2020,
366 2021, and 2022 reveal challenging climatic conditions for corn cultivation in the region
367 where the present study was conducted.

368 Temperature is crucial for the corn cycle and should range between 24 °C and
369 30 °C from emergence to the flowering period. A daily average temperature of 21 °C is
370 optimal for the highest grain yield, according to a study by EMBRAPA [25]. Despite the
371 stability of the annual mean temperature over the three years of study, monthly
372 variations were recorded, with maximum temperatures exceeding 25 °C in February and
373 March and minimums below 17 °C in June and July. The optimal relative humidity range
374 for corn cultivation is between 60% and 80% [26]. This variable plays a crucial role in
375 plant transpiration, soil water availability, and the occurrence of fungal diseases. Our data
376 indicated that the average relative humidity in the study region was within the expected
377 range over the three years analyzed (69% in 2020, 72% in 2021, and 79% in 2022).
378 However, values above 80% in June for all three years may have compromised crop
379 health and grain quality and favored some mycotoxins' occurrence. Notable variations in
380 accumulated precipitation were observed over the three years (568 mm in 2020, 412 mm
381 in 2021, and 824 mm in 2022), potentially impacting corn production, as well as fungal
382 development favored by high humidity levels.

383 Overall, fluctuations in temperature, high relative humidity, and irregular
384 precipitation were observed and are possibly related to the increase in FUM concentration
385 from 2020 to 2022 as well as the higher levels of DON and ZEA observed in 2022, which
386 could be explained by the higher precipitation observed in this last year. In 2020 and 2021,
387 the lower temperature variation, coupled with humidity below 80%, except in June of
388 both years, may have alleviated fungal stress, consequently leading to values below the
389 LOQ for ZEA and DON in 2020 and low concentrations of these mycotoxins in 2021.
390 Additionally, the temperature variation between April and May of 2022, coupled with an
391 average humidity close to 90%, may have served as a stress factor for *Fusarium* fungi,
392 triggering the production of FUM, DON, and ZEA in that year.

393 Data from the present study demonstrated that distinct transgenic technologies
394 applied to corn influenced crop yield, the incidence of damaged grains, and the con-
395 centration of mycotoxins during the 2020, 2021, and 2022 harvests. Notably, the VT PRO3®
396 technology demonstrated superior yield in all years of the study, possibly re- lated to the
397 three Bt genes of this technology (Cry1A.105, Cry2Ab2, and Cry3Bb1) [27], which together
398 promote resistance to insect pests (*Spodoptera frugiperda*, *Diatraea saccharalis*, *Helicoverpa*
399 *zea*, *Elasmopalpus lignosellus*, and *Diabrotica speciosa*), in addition to herbicide tolerance
400 (glyphosate) [5]. However, a higher concentration of fumonisins, mycotoxins mainly
401 produced by *Fusarium verticillioides* and *F. proliferatum*, was observed. This result may
402 indicate a higher susceptibility of VT PRO3® to infection by fumonisins-producing fungi.
403 In contrast to VT PRO3®, the PowerCore® ULTRA technology exhibited a lower yield but
404 a low concentration of total fumonisins. This technology carries four Bt genes (Cry1F,
405 Cry1A.105, Cry2Ab2, and Vip3Aa20) [28], which provide resistance to different insect
406 pests (*S. frugiperda*, *D. saccharalis*, *Helicoverpa armigera*, *H. zea*, *Elasmopalpus lignosellus*,

407 *Agrotis ipsilon*, *S. eridania*, and *S. cosmioides*), in addition to herbicide tolerance
408 (glyphosate) [5].

409 In 2022, PowerCore® ULTRA had a high concentration of DON, a type B
410 trichothecene mainly produced by *Fusarium graminearum* and *F. culmorum*. This result
411 suggests a possible higher susceptibility of PowerCore® ULTRA to DON-producing
412 species. Alternatively, Agrisure® Viptera 3 presented intermediate results among the three
413 transgenic events, with comparable crop yield to PowerCore® ULTRA and low
414 concentration of total fumonisins. Agrisure® Viptera 3 has three Bt genes (Cry1Ab,
415 Vip3Aa20, and Cp4-EPSPS) [29], conferring resistance to various insect pests (*S.*
416 *frugiperda*, *D. saccharalis*, *H. zea*, *E. lignosellus*, and *A. ipsilon*) and herbicide tolerance
417 (glyphosate) [5]. In 2020, this transgenic technology presented, numerically, the lowest
418 concentration of total fumonisins among the evaluated events. This finding suggests a
419 possible resistance against fumonisins-producing *Fusarium* species.

420 The mycotoxicological results from the present study emphasize the imperative
421 need to monitor the factors related to FUM production in different transgenic corn. This
422 group of mycotoxins has a significant prevalence in Brazilian and South American corn,
423 as evidenced by various prior surveys [13,30]. Poultry exposed to fumonisins typically
424 manifest mild to moderate toxicity, characterized by notable changes such as liver
425 pathology, increased intestinal permeability, and decreased growth performance [31,32].

426 The insertion of genes in transgenic events of corn, intended to improve
427 agronomic traits such as resistance to herbicides, insects, abiotic stresses, and diseases,
428 may affect its nutritional composition compared to conventional corn [33]. The results
429 regarding concentrations of CP, CF, ash, and EE in the present study revealed differences
430 in nutritional characteristics among transgenic technologies within each year and a
431 consistent pattern over the years, especially regarding CP and EE. In 2020, despite the
432 numerical difference, CP was the same among the technologies, while CF and EE had
433 higher concentrations in the VT PRO3® technology. In 2021 and 2022, all components
434 were significantly different between the transgenic events, except for CF. Consistent
435 results on nutrient compositions were observed over the years in the present study;
436 PowerCore® ULTRA presented higher levels of CP, ash, and P values compared to VT
437 PRO3® in 2021 and 2022 whereas VT PRO3® had the highest EE content during the three
438 years. Such differences can be attributed to environmental and genetic factors and their
439 interactions, influencing the metabolism and composition of corn grains [34]. Piovesan et
440 al. [35] and Vieira et al. [36] observed that protein is influenced by the corn hybrid,
441 production year, cultivation region, and meteorological data. Variations in the
442 concentrations of CP, CF, ash, and ether extract among corn technologies highlight the
443 need for constant monitoring of corn nutritional composition since different corn varieties
444 are consumed at poultry feed mills on a daily basis. In addition, results from the present
445 study demonstrate that the choice of one transgenic technology can thus directly
446 influence the nutritional density of the final feed.

447 According to Cowieson [6], corn can represent around 65% and 20% of the energy
448 and protein supplies in broiler starter diets, respectively. Therefore, any difference
449 observed in the composition of this ingredient impacts the cost of feed formulation. The
450 results of total and digestible amino acids as well as metabolizable energy indicated
451 remarkable variations among the transgenic technologies. These differences can be
452 attributed not only to the genetic characteristics but also tissue structure of corn grains [9].
453 In 2020, a significant difference in the dig. Ile, Leu, and Phe were observed among corn
454 technologies, with PowerCore® ULTRA and Agrisure® Viptera 3 standing out with the
455 highest levels. In 2021, reinforcing the data obtained in 2020, all dig. AA in the
456 PowerCore® ULTRA technology was higher compared to the VT PRO3® technology. In
457 2022, only dig. Trp was not different between the transgenic events, while the other dig.
458 AA had the highest concentration in PowerCore® ULTRA, corroborating the findings of
459 2020 and 2021. Additionally, VT PRO3® presented the highest levels of AMEn in the three

460 years of the study. It is possible that the high AMEn levels in VT PRO3® are related to the
461 higher EE content in the grains of this transgenic technology, which was also observed
462 during the three years of the present study.

464 5. Conclusions

465 The quality of corn is influenced by climatic, technological, and genetic factors. The
466 transgenic technologies evaluated herein have played distinct roles, with VT PRO3®
467 standing out in crop yield but also showing potential susceptibility to fumonisin
468 contamination. In spite of the lower crop yield, PowerCore® ULTRA had lower
469 concentrations of total fumonisins. The variation in nutritional characteristics among corn
470 technologies over the years and notable differences in digestible amino acids and
471 metabolizable energy highlight the importance of constant evaluations of corn nutritional
472 composition to optimize the feed efficiency of poultry. The differences among corn
473 technologies, both in productivity and nutritional levels, demonstrate the importance of
474 incorporating the “nutritional content” bias in the selection and improvement process of
475 corn hybrids/genetics.

476 In conclusion, selecting transgenic events for corn production intended for poultry
477 feed formulation should be based not only on crop yield but also on the quality of grains,
478 the presence of mycotoxins, and specific nutritional characteristics. This well-informed
479 decision-making is essential to ensure sustainability and efficiency in agricultural
480 production and to meet the demands of the poultry industry.

481 **Author Contributions:** Conceptualization, J.K.V. and C.T.S.; methodology, J.K.V. and H.V.P.;
482 formal analysis, J.K.V., C.T.S. and H.V.P.; investigation, J.K.V., C.T.S. and H.V.P.; resources, A.O.M.,
483 D.T. and C.A.M.; data curation, J.K.V. and C.T.S.; writing—original draft preparation, J.K.V. and
484 C.T.S.; writing—review and editing, J.K.V., C.T.S., A.O.M., D.T. and C.A.M.; supervision, A.O.M.,
485 D.T. and C.A.M.; project administration, C.A.M.; funding acquisition, C.A.M. and H.V.P. All
486 authors have read and agreed to the published version of the manuscript.

487 **Funding:** This research received no external funding.

488 **Institutional Review Board Statement:** Not applicable.

489 **Informed Consent Statement:** Not applicable.

490 **Data Availability Statement:** Data related to this research is available within the article.

491 **Acknowledgments:** The authors acknowledge the Copacol cooperative for providing the experi-
492 mental field and assisting in the project’s development. J.K. Vidal and C.T. Simões are grateful to
493 the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) for
494 providing their graduate fellowships. The present study was carried out with the support of the
495 CAPES/PROEX – Funding Code 001.

496 **Conflicts of Interest:** The authors have no conflict of interest regarding the content of this
497 article.

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CONSIDERAÇÕES FINAIS

A qualidade nutricional do milho, juntamente com sua produtividade, desempenha um papel crucial na produção de rações e na eficiência geral da produção de proteína animal. Os estudos detalhados sobre os híbridos de milho destacaram não apenas a ampla variação na produtividade entre eles, mas também revelaram diferenças significativas nos teores de nutrientes essenciais, como proteína bruta e aminoácidos digestíveis. A variabilidade das características nutricionais destaca a necessidade de uma abordagem integrada na seleção de híbridos de milho, considerando não apenas a maximização da produtividade, mas também a densidade nutricional e a segurança alimentar das rações avícolas.

A análise dos estudos ressaltou a correlação entre a produtividade dos híbridos de milho e os teores de nutrientes, destacando a importância de equilibrar a busca por altos rendimentos com a manutenção da qualidade nutricional do grão. Essa relação sugere que a seleção de híbridos com maior produtividade pode não garantir automaticamente uma ração de melhor qualidade, sendo necessário considerar cuidadosamente os aspectos nutricionais durante o processo de escolha.

Além disso, os estudos identificaram a presença de micotoxinas em alguns híbridos de milho, destacando a importância da resistência a esses contaminantes na seleção de materiais genéticos. A contaminação por micotoxinas representa um sério risco para a saúde animal e pode comprometer a qualidade dos produtos finais, enfatizando ainda mais a importância de garantir a segurança alimentar ao selecionar híbridos de milho.

Considerando a constante evolução do mercado agrícola e as mudanças nas condições climáticas e de cultivo, é essencial que futuras pesquisas explorem mais a fundo a relação entre produtividade, qualidade nutricional e a presença de micotoxinas no milho. Esses estudos devem abranger diversas regiões e condições ambientais para fornecer uma compreensão abrangente dos fatores que influenciam essas características. Em última análise, é vital adotar uma abordagem holística na seleção de híbridos de milho, visando otimizar não apenas a produtividade, mas também a qualidade e segurança nutricional do grão, a fim de promover uma produção de proteína animal sustentável e eficiente.

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