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INFLUÊNCIA DA TEXTURA DO ENDOSPERMA NAS CARACTERÍSTICAS FÍSICAS, NA COMPOSIÇÃO NUTRICIONAL E NA CONCENTRAÇÃO DE MICOTOXINAS EM GRÃOS DE MILHO CULTIVADOS NO BRASIL

Santa Maria, RS
2024

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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 **Doutora em Medicina Veterinária.**

Orientador: Prof. Dr. Carlos Augusto Mallmann

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Aprovada em 23 de fevereiro de 2024:

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RESUMO

INFLUÊNCIA DA TEXTURA DO ENDOSPERMA NAS CARACTERÍSTICAS FÍSICAS, NA COMPOSIÇÃO NUTRICIONAL E NA CONCENTRAÇÃO DE MICOTOXINAS EM GRÃOS DE MILHO CULTIVADOS NO BRASIL

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Esta tese teve o objetivo de avaliar a influência da textura do endosperma sobre as características físicas, a composição nutricional e a concentração de micotoxinas em diferentes tipos de milho cultivados no Brasil. Foram cultivados, no estado do Paraná, milhos de diferentes texturas de endosperma nos anos de 2020, 2021 e 2022. Em 2020, 213 amostras foram separadas em 4 grupos de acordo com a textura do endosperma: dentado, duro, semidentado e semiduro. A produtividade e os grãos avariados foram avaliados e a composição nutricional e os valores de energia foram preditos por espectroscopia no infravermelho próximo. Os dados foram submetidos à ANOVA e as médias foram separadas pelo teste de Tukey a 5% de significância. O percentual de amido foi maior ($P < 0,01$) no milho dentado do que no milho duro. O milho dentado apresentou o maior percentual de grãos avariados ($P < 0,01$). O milho duro teve a menor produtividade ($P < 0,001$); no entanto, apresentou os maiores teores de proteína bruta (PB) e de aminoácidos (AA) totais e digestíveis ($P < 0,001$). Os valores de energia não foram diferentes entre os tipos de milho ($P > 0,05$). Em 2021, 216 amostras de milho foram separadas em milho dentado, duro, semidentado e semiduro. Foi avaliada a contaminação micotoxicológica do milho cultivado nos anos de 2020 e 2021. As micotoxinas foram quantificadas por cromatografia líquida acoplada à espectrometria de massas. Os dados foram submetidos à ANOVA e as médias separadas pelo teste de Tukey a 5% de significância. Em 2020, o milho duro apresentou a menor concentração de fumonisinas B_1 (FB_1), enquanto o milho dentado teve a maior concentração de FB_1 e FB_2 ($P < 0,05$). Em 2021, as maiores médias de FB_1, FB_2 e diacetoxiscirpenol ($P < 0,05$) foram observadas no milho dentado. Os milhos dentado e semi-dentado apresentaram a maior concentração de nivalenol ($P < 0,05$). Em 2022, 80 amostras de milho foram utilizadas para avaliar a relação entre a dureza dos grãos, a sua composição nutricional e a concentração de micotoxinas. A dureza dos grãos foi determinada em um texturômetro, onde dez grãos de cada amostra foram colocados individualmente na plataforma e comprimidos até a quebra. Após, os grãos de milho foram separados em três grupos diferentes de acordo com sua dureza: Grupo 1 (< 52 kgf), grupo 2 ($52 \leq$ kgf < 54) e grupo 3 (≥ 54 kgf). A dureza correlacionou-se negativamente ($P < 0,05$) com as fumonisinas e o amido, e positivamente ($P < 0,05$) com PB e AA totais. O Grupo 3 apresentou menor concentração de fumonisinas e maior PB e AA que os Grupos 1 e 2 ($P < 0,05$). Portanto, conclui-se que a textura do endosperma do milho influencia as características de campo, a composição nutricional e a contaminação micotoxicológica nos grãos de diferentes tipos. Ainda, os resultados do presente estudo sugerem que quanto maior a dureza do grão, maior pode ser o seu teor de PB e AA, e menores podem ser os níveis de contaminação por fumonisinas.

Palavras-chave: Endosperma. Fumonisinas. Milho. Nutrição animal. Textura.

ABSTRACT

INFLUENCE OF ENDOSPERM TEXTURE ON PHYSICAL CHARACTERISTICS, NUTRITIONAL COMPOSITION, AND MYCOTOXIN CONCENTRATION IN CORN GRAINS CULTIVATED IN BRAZIL

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This thesis aims to evaluate the influence of endosperm texture on the nutritional composition, physical characteristics, and mycotoxin concentration in different types of corn cultivated in Brazil. Corn with different endosperm textures were cultivated in the state of Paraná in 2020, 2021, and 2022. In 2020, 213 samples were separated into 4 groups according to the endosperm texture: dent, flint, semident, and semiflint. Crop yield and damaged grains were determined. Nutritional composition and energy values were predicted by near-infrared spectroscopy. Data were subjected to ANOVA, and means were separated by Tukey's test at 5% significance. Starch was higher ($P < 0.01$) in dent corn than in flint corn. Dent corn showed the highest percentage of damaged grains ($P < 0.01$). Flint corn had the lowest crop yield ($P < 0.001$); however, it had the highest levels of crude protein (CP) and total and digestible amino acids (AA) ($P < 0.001$). Energy values were not different among corn types ($P > 0.05$). In 2021, 216 corn samples were also separated into dent, flint, semident, and semiflint. Mycotoxin contamination of corn cultivated in 2020 and 2021 was evaluated. Mycotoxins were quantified by liquid chromatography coupled with mass spectrometry. Data were subjected to ANOVA, and means were separated by Tukey's test at 5% significance. In 2020, flint corn had the lowest concentration of fumonisins B_1 (FB_1), while dent corn had the highest concentration of FB_1 and FB_2 ($P < 0.05$). In 2021, dent corn showed the highest averages of FB_1, FB_2, and diacetoxyscirpenol ($P < 0.05$). Dent and semi-dent corns had the highest concentration of nivalenol ($P < 0.05$). In 2022, 80 corn samples were used to assess the relationship between grain hardness, nutritional composition, and mycotoxins concentration. Grain hardness was determined using a texture analyzer, where ten grains from each sample were individually placed on the platform and compressed until breakage. Then, corn grains were separated into three different groups according to their hardness: Group 1 (< 52 kgf), Group 2 ($52 \leq$ kgf < 54), and Group 3 (≥ 54 kgf). Hardness negatively correlated ($P < 0.05$) with fumonisins and starch, and positively correlated ($P < 0.05$) with CP and total AA. Group 3 had lower fumonisin concentration and higher CP and AA than Groups 1 and 2 ($P < 0.05$). Therefore, it is concluded that the endosperm texture influences field characteristics, nutritional composition, and mycotoxin contamination in grains of different corn types. Furthermore, results of this study suggest that the higher the grain hardness, the higher its CP and AA content, and the lower its fumonisins contamination levels.

Keywords: Animal Nutrition. Corn. Endosperm. Fumonisins. Texture.

SUMÁRIO

1 INTRODUÇÃO

O milho é a segunda cultura mais produzida no Brasil e, estima-se que na safra de 2022/2023 foram produzidas cerca de 137 milhões de toneladas do grão no país (USDA, 2024), que é o terceiro maior produtor mundial desse grão, seguindo os Estados Unidos e a China (USDA, 2024). Segundo o levantamento da Companhia Nacional do Abastecimento (CONAB, 2024), na safra de 2022/23 foram exportadas mais de 56 milhões de toneladas de milho produzido no Brasil, um recorde em comparação às safras anteriores e que concretiza o país como segundo maior exportador do grão no mundo, ficando atrás apenas dos Estados Unidos (FAO, 2024).

Grande parte do milho produzido no Brasil é destinado para a alimentação animal (EMBRAPA, 2022), em que o milho é o principal ingrediente utilizado na formulação de rações para aves e suínos. Para atender esta demanda, o mercado disponibiliza anualmente uma variedade de híbridos de milho com diferentes características e aptidões, conferindo ao cereal importante adaptação a diferentes climas, solos e altitudes. Na safra 2019/2020, um total de 196 cultivares de milho foram ofertados ao mercado, sendo 155 de híbridos, representando 79% do total das cultivares (EMBRAPA, 2020). No último levantamento divulgado pela EMBRAPA (EMBRAPA, 2022) constatou-se o lançamento de 98 novos híbridos disponibilizados para os produtores na safra 2022/2023.

O endosperma é o maior tecido do grão de milho, sendo constituído principalmente por amido, que é armazenado em estruturas denominadas grânulos. O endosperma do milho se distribui no grão de duas formas distintas: farinácea e vítrea (PIOVESAN *et al*., 2011), sendo que o mesmo grão possui as duas formas de endosperma e a proporção entre elas é o que confere o grau de dureza ao grão. Logo, em relação a textura do endosperma, os grãos de milho podem ser classificados principalmente em duro, quando possuem maior proporção de endosperma vítreo (CORREA *et al*., 2002), ou dentado, quando possuem maior proporção de endosperma farináceo (XU *et al*., 2019).

Entre as cultivares disponíveis para comercialização no Brasil, encontram-se os grãos de milho dos tipos duro, semiduro, dentado e semidentado. A utilização dos diferentes tipos de milho disponíveis no mercado brasileiro é distinta e varia de acordo com o mercado ao qual o milho é destinado, sendo o milho semiduro o mais cultivado, representando 43,8% desse uso, seguido pelo milho semidentado com 31,6%. Em seguida, tem-se o milho duro (8,2%) e, por último, o milho dentado (3%) (EMBRAPA, 2022).

De acordo com Mallmann e Dilkin (2007), climas tropicais e subtropicais podem favorecer a contaminação do milho por várias espécies de fungos produtores de micotoxinas. As micotoxinas são metabólitos secundários tóxicos naturalmente produzidos por diferentes linhagens de fungos filamentosos, como os gêneros *Aspergillus*, *Claviceps*, *Fusarium* e *Penicillium*, durante o crescimento do cereal no campo, assim como durante o armazenamento dos grãos. As micotoxinas possuem efeitos nefrotóxicos, hepatotóxicos e imunossupressores que já estão bem documentados. Consequentemente, a contaminação micotoxicológica do milho pode ter um grande impacto na saúde animal, além de causar prejuízos econômicos para a cadeia de produção de grãos e para cadeia de produção animal (MUNKVOLD *et al*., 2019).

Diversos fatores como a genética dos grãos, o solo, a irrigação, a localização e as condições climáticas podem levar a variações na qualidade do grão (LIU *et al*., 2019), na composição de nutrientes (MALLMANN *et al*., 2019; VARGAS *et al*., 2023), na contaminação micotoxicológica (TYSKA *et al*., 2022) e, consequentemente, nos custos de formulação das rações (GEHRING *et al*., 2012; MALLMANN *et al*., 2019). Dessa forma, a medida que se amplia o conhecimento acerca dos fatores que podem causar variabilidade no milho, otimiza-se o aproveitamento desse ingrediente nas cadeias produtivas que o consomem.

Programas de seleção genética de milho têm buscado desenvolver híbridos com maior produtividade, resistência a pragas, menores custos de produção e alta tolerância às oscilações climáticas (QAIM; MATUSCHKE, 2005). No entanto, o potencial nutricional deste ingrediente bem como a susceptibilidade à contaminação por micotoxinas também deveriam ser considerados fatores de seleção. Neste contexto, Alves *et al*. (2014) avaliaram milhos de diferentes genótipos e concluíram que, aqueles que apresentavam características mais produtivas à campo, possuíam menor conteúdo de extrato etéreo e maior concentração de amilose nos grãos. Outros autores também já observaram uma correlação negativa entre a produtividade do milho e o teor nutricional dos grãos, com impacto principalmente na concentração de proteína bruta e lipídios (ÁLVAREZ-IGLESIAS *et al*., 2021; DUVICK, 2005).

A relação entre a textura do endosperma do milho, a digestibilidade dos nutrientes e o desempenho animal já foi avaliada em estudos anteriores (CÓRDOVA-NOBOA *et al*., 2021; NGONYAMO-MAJEE *et al*., 2008). Entretanto, poucas informações estão disponíveis na literatura quanto à influência da textura do endosperma na composição nutricional, na digestibilidade de nutrientes para aves e suínos, assim como na ocorrência de micotoxinas no milho. Uma vez que existem diferentes tipos de milho produzidos mundialmente e o cereal é o principal ingrediente utilizado nas rações, determinar a composição nutricional e a

susceptibilidade à micotoxinas em diferentes tipos de milho são pontos chave para iniciar um processo de desenvolvimento de genótipos de milho baseado não só no rendimento à campo, mas também na qualidade nutricional dos grãos. A melhor caracterização desse ingrediente é uma prática que poderá proporcionar seu melhor aproveitamento pela indústria de rações, devendo também ser considerada no desenvolvimento de novas variedades de milho.

Portanto, o objetivo desta tese de doutorado foi realizar três grandes estudos para avaliar a influência da textura do endosperma sobre a composição nutricional, o rendimento à campo e as características físicas dos grãos, bem como sobre a concentração de micotoxinas em grãos de milho de diferentes tipos cultivados no Brasil em safras distintas (2020, 2021 e 2022). Também foi avaliada a relação da dureza dos grãos de milho com a sua composição nutricional e a concentração de micotoxinas.

2 REVISÃO BIBLIOGRÁFICA

2.1 ESTRUTURA E COMPOSIÇÃO DO MILHO

Na sua estrutura, o grão de milho é dividido basicamente em três camadas: o pericarpo, que é a camada mais externa; o gérmen, que constitui o eixo embrionário; e o endosperma, tecido de reserva (MAGALHÃES *et al*., 2002). O pericarpo constitui aproximadamente 7% do peso do grão de milho e esse tecido subdivide-se em epicarpo, mesocarpo e endocarpo, formados principalmente por células fibrosas (GARCIA-LARA; CHUCK-HERNANDEZ; SERNA-SALDIVAR, 2019). A principal função do pericarpo é proteger ou defender o grão de fatores estressantes vindos do meio externo. Já o gérmen é constituído por duas partes principais: o embrião, também conhecido como eixo embrionário, e o cotilédone. O gérmen constitui cerca de 12% do peso do grão de milho e contém cerca de 85% da gordura total, especialmente os triglicerídeos, e a maior parte das albuminas e globulinas do grão. O gérmen também se destaca como o tecido com maior concentração de minerais no grão (GARCIA-LARA; CHUCK-HERNANDEZ; SERNA-SALDIVAR, 2019), especialmente de fósforo, o qual é predominantemente armazenado na forma de fósforo fítico (ANGEL *et al*., 2002).

A maior parte do grão de milho é constituída pelo endosperma, que representa cerca de 80-82% do peso do grão (GARCIA-LARA; CHUCK-HERNANDEZ; SERNA-SALDIVAR, 2019). O endosperma é composto em sua maior parte por amido, um polissacarídeo formado pela união de moléculas de α-1,4 e α-1,6 de amilose e amilopectina, sendo o principal nutriente energético do grão (ILDIZ *et al*., 2019). Este tecido divide-se em camada aleurona e endosperma amiláceo. O endosperma amiláceo subdivide-se em vítreo e farináceo. As células do endosperma amiláceo são constituídas por uma parede celular fina e grânulos de amido embebidos em uma matriz proteica (SERNA-SALDIVAR, 2010). O endosperma também contém cerca 74% do teor de proteína do grão, além de ß-caroteno e a maior parte das vitaminas hidrossolúveis do milho (GARCIA-LARA; CHUCK-HERNANDEZ; SERNA-SALDIVAR, 2019).

Embora o milho seja visto como um ingrediente homogêneo, que apresenta mínimas variações na sua composição, as suas variedades podem apresentar diferenças importantes na sua composição, causadas por fatores distintos. De modo geral, com relação à sua composição nutricional, as proteínas correspondem a aproximadamente 10% da massa seca dos grãos de milho, o amido corresponde de 70 a 80%, os açúcares solúveis de 1 a 4% e o óleo de 3 a 6% (BICUDO *et al*., 2006). Os valores de energia bruta do grão de milho podem variar entre 3.933

kcal/kg no milho dentado, 3.987 kcal/kg no milho *nutridense* (NRC, 2012) e 3.901 kcal/kg em milho com 7,86% de proteína bruta (ROSTAGNO *et al*., 2017).

2.2 TEXTURA DO ENDOSPERMA E DIFERENTES TIPOS DE MILHO

Existem duas formas de classificação do endosperma: vítreo e farináceo. O endosperma vítreo é formado por grânulos de amido compactos e poligonais ligados à matriz proteica do endosperma e está relacionado a dureza do grão (PIOVESAN *et al.*, 2011). Já no endosperma farináceo, os grânulos de amido são esféricos e apresentam maiores espaços entre eles (DOMBRINK-KURTZMAN; BIETZ, 1993). A relação entre os dois endospermas é denominada vitreosidade e determina a textura, ou grau de dureza dos grãos (PIOVESAN *et al*., 2011).

Geralmente, a matriz proteica de armazenamento está ligada à superfície dos grânulos de amido e pode afetar a textura do endosperma (COLEMAN; LARKINS, 2009; ZHANG; GAO; DONG, 2011). Desta forma, os endospermas vítreo e farináceo apresentam uma textura diferente em função da interação entre o amido e a proteína (GAYRAL *et al*., 2016; KLJAK *et al*., 2018). No endosperma vítreo, os grânulos de amido estão bem compactados devido à presença de uma matriz proteica abundante, já no endosperma farináceo, os grânulos de amido estão dispostos de maneira mais desorganizada devido à menor concentração de proteína (XU *et al*., 2019; ZHANG; GAO; DONG, 2011).

Diferentes pesquisas já observaram que o teor de proteína é maior no endosperma vítreo do que no farináceo (GAYRAL *et al*., 2016; KLJAK *et al*., 2018; XU *et al*., 2019). O maior teor de proteína no endosperma vítreo está associado a um aumento do teor de α-zeína (GAYRAL *et al*., 2016). As zeínas representam cerca de 80% das proteínas do milho e são a principal proteína de armazenamento no endosperma do grão (RANDALL *et al*., 2005).

O peso, o teor de proteínas, a esfericidade do grão, a arquitetura do tecido celular e a adesão entre as proteínas e o amido influenciam a dureza do grão (BLANDINO; SACCO; REYNERI, 2013; SCHUTYSER; VAN DER GOOT, 2011). A dureza do grão é um dos fatores mais importantes para determinar a utilização final do milho, especialmente para o desempenho da moagem a seco. O endosperma vítreo na região periférica do endosperma do milho tem um tamanho de célula menor, um teor de proteína maior e uma adesão mais forte entre a proteína e o amido do que o endosperma farináceo na região central do endosperma do grão, tais características fazem com que o endosperma vítreo tenha uma maior resistência à moagem (BLANDINO; SACCO; REYNERI, 2013).

A relação entre os endospermas vítreo e farináceo determina a dureza do grão, e consequentemente, o tipo de milho. Dessa maneira, o milho do tipo duro apresenta uma maior proporção de endosperma vítreo, ao passo que o milho do tipo dentado possui maior proporção de endosperma farináceo (PHILIPPEAU *et al*., 1999). Dentre os tipos de milho utilizados no Brasil, o milho semiduro apresenta o maior percentual de cultivo, com 43%, seguido pelo milho semidentado, com 31%. Já o milho duro representa 8% e, por último, o milho dentado, com apenas 3% do milho cultivado no país. Cerca de 13% dos grãos de milho cultivados no Brasil não possuem informação quanto a sua textura (EMBRAPA, 2022).

Por constituírem a maior parte do grão, os componentes do endosperma são decisivos na definição das propriedades químicas e físicas do milho (SILVA *et al*., 2000). A textura do grão de milho já foi positivamente associada a sua concentração de proteína, quando os grãos de milho dentado, milho pipoca e milho doce foram comparados por Zhang, Gao e Dong (2011). Esses autores observaram que quanto maior a porcentagem de endosperma vítreo, maior o teor de proteína do grão. Além disso, Álvarez-Iglesias *et al*. (2021) investigaram os valores nutricionais de grãos de milho com diferentes texturas de endosperma e relataram que os híbridos de milho do tipo duro apresentaram maior teor de proteína do que os híbridos do tipo dentado. Zhang e Xu (2019) avaliaram o teor de amido em diferentes cultivares de milho e observaram uma maior concentração deste polissacarídeo no endosperma do milho dentado comparado ao milho duro. Similarmente, Ildiz *et al*. (2019) observaram um maior teor de amido no endosperma do milho dentado do que do milho duro, enquanto que o teor de gordura foi muito semelhante entre os dois tipos de milho.

2.3 MILHO NA NUTRIÇÃO DE AVES E SUÍNOS

Aproximadamente 70% da produção brasileira de milho é destinada à nutrição animal (EMBRAPA, 2022), sendo utilizado principalmente nas indústrias de aves e suínos, onde o ingrediente tem elevada inclusão nas dietas. O Brasil é um dos líderes mundiais na produção de proteína animal, ocupando a segunda posição no ranking dos produtores e a primeira dentre os exportadores de carne de frango, e sendo o quarto maior produtor e exportador de carne suína no mundo (ABPA, 2023).

O milho é a principal fonte de energia em rações à base de milho e soja. De acordo com Cowieson (2005), diferenças entre amostras de milho podem chegar a uma variabilidade de mais de 400 kcal/kg de energia metabolizável para aves. Dozier *et al*. (2011) indicaram que variações na energia metabolizável podem resultar em mudanças economicamente importantes na conversão alimentar do lote de aves. Neste contexto, uma estimativa precisa do valor

energético do milho se torna um ponto chave para que os animais utilizem adequadamente a contribuição do milho na ingestão diária de energia, tornando possível a economia associada a fontes energéticas mais caras, como a gordura (STEFANELLO *et al.,* 2019).

A proteína é o segundo componente de maior importância nos grãos de milho, e a concentração de aminoácidos atua como um indicador da qualidade do grão (ALVES; FILHO, 2017). Embora esse cereal possua baixos teores de proteína bruta em comparação ao trigo e a cevada, o milho é responsável por fornecer em torno de 20% do total da proteína presente nas rações para frangos de corte (COWIESON, 2005). Rodrigues *et al*. (2003) observaram que há variação no teor de proteína de milhos de diferentes variedades, assim como na digestibilidade dos nutrientes e nos valores energéticos das rações de aves, que variaram em função da composição dos milhos utilizados. Ainda, de acordo com Gehring *et al*. (2012), a variabilidade na qualidade do milho pode afetar a conversão alimentar, alterando a utilização de nutrientes e de energia pelas aves. Por outro lado, no estudo realizado por Moore *et al*. (2008), não foram identificadas correlações entre as propriedades químicas e físicas do milho e o desempenho de frangos de corte e galinhas poedeiras. Essas correlações não foram consideradas suficientemente robustas para justificar a utilização dessas características como critérios de seleção do milho para a alimentação de aves.

É importante ressaltar que o custo de fornecer energia e proteína na dieta mantém-se elevado e representa uma grande porcentagem do custo de produção da carne de frangos de corte e de suínos (DONAHUE; CUNNINGHAM, 2009; ANGEL *et al*., 2011). Desta forma, embora os estudos realizados apresentem resultados distintos, o monitoramento da variabilidade nutricional do milho pode ser uma decisão importante para o melhor aproveitamento do ingrediente e a redução dos custos atrelados a nutrição de aves e suínos.

Dentre as características dos grãos de milho cultivados no Brasil, a textura do endosperma pode apresentar importante influência na nutrição animal. Stefanello *et al*. (2023) encontraram maior energia digestível ileal e energia metabolizável aparente em frangos de corte alimentados com dieta a base de milho semiduro do que a base dos milhos semi-dentado e *waxy* (alto amido). O milho semiduro também apresentou maior digestibilidade do extrato etéreo e menor digestibilidade do nitrogênio em comparação com o milho semidentado. Zhao *et al*. (2016) relataram maior ganho de peso e melhor conversão alimentar de frangos de corte de 1 a 42 dias alimentados com dietas que incluíam milho duro em comparação aos frangos alimentados com dietas com milho dentado. Em contrapartida, Kaczmarek *et al*. (2013) observaram que milhos com maior dureza podem afetar negativamente o desempenho de

frangos de corte na fase de crescimento, devido à baixa digestibilidade de nutrientes, principalmente da proteína e do amido.

Avaliando suínos, Piovesan *et al*. (2011) demonstraram que os valores de energia digestível e metabolizável em leitões foram influenciados pelos tipos de milho, sendo superiores em dietas a base de milho semidentado em comparação com o milho duro. A digestibilidade do amido e da proteína bruta não foram diferentes entre os grupos alimentados com os dois tipos de milho. Por outro lado, resultados de experimentos realizados com suínos em crescimento demonstraram a existência de uma relação negativa entre a dureza do grão e a digestibilidade do milho (CANTARELLI *et al*., 2007), onde dietas com milho dentado proporcionaram maior digestibilidade de matéria seca e proteína bruta e maior energia digestível do que dietas com milho duro.

Na indústria de rações, a escolha do tipo de milho também pode impactar os custos no processo de moagem dos grãos. Do ponto de vista tecnológico, a estrutura do amido controla amplamente as propriedades de cozimento e qualidade de processamento do grão (GRIEBEL *et al.,* 2019). Segundo Biliaderis (2009), a gelatinização do amido altera as propriedades reológicas e a funcionalidade dos produtos amiláceos, que são importantes para a qualidade do produto final. Assim, o tipo e a qualidade do amido presentes no milho são cruciais no processo de produção de ração. De acordo com Hofferber *et al*. (2010), grãos de milho duro elevam o consumo de energia e aumentam o tempo de moagem, já as cultivares de grãos semiduros são mais facilmente trituradas, proporcionando uma economia nos custos energéticos para produção de rações (FACTORI *et al*., 2008).

2.4 UTILIZAÇÃO DA ESPECTROSCOPIA DE INFRAVERMELHO PRÓXIMO PARA PREDIÇÃO DE NUTRIENTES DO MILHO

Existem múltiplos procedimentos químicos descritos para determinação da composição bromatológica e energética dos cereais. No entanto, as análises químicas tradicionalmente utilizadas (por exemplo, método de combustão para quantificação de proteínas), são, em geral, destrutivas, pouco sustentáveis, de custo e tempo de execução elevados (FALADE *et al*., 2017; ILDIZ *et al*., 2019). Em contrapartida, a espectroscopia de infravermelho próximo (NIRS) vem sendo extensamente explorada por ser uma técnica não destrutiva, que requer uma quantidade mínima de amostra e não requer o seu pré-processamento. A técnica também não necessita de reagentes químicos (NGONYAMO-MAJEE *et al*., 2008), que muitas vezes aumentam o custo da análise e se tornam um desafio quanto ao descarte correto de resíduos.

Os equipamentos com tecnologia NIRS efetuam análises de componentes orgânicos através do princípio de emissão de radiação eletromagnética. A técnica do espectrômetro é uma integração de espectroscopia e de computação de dados. A região do espectro eletromagnético está compreendida pelo infravermelho próximo, que varia entre 780 e 2.500 nm (SKOOG *et al*., 2001). Os espectros são obtidos por reflectância, onde a radiação incidente atravessa a amostra e, em seguida, é refletida, atravessando novamente a amostra e sendo captada pelo detector, gerando um espectro (SILVA, 2011). Os componentes presentes nos alimentos absorvem energia na região do infravermelho e a informação contida no espectro gerado pode ser empregada na estimativa da concentração de determinada substância na amostra.

O NIRS vem sendo amplamente utilizado pela indústria de nutrição animal com o objetivo de diminuir o tempo e o custo de análises laboratoriais (ALVES *et al*., 2014), auxiliando na formulação de rações e no processo de tomada de decisão em tempo real. De acordo com Melo-Durán *et al*. (2021), a tecnologia NIRS tornou-se uma ferramenta analítica importante no campo da nutrição animal, devido à sua praticidade, facilidade de uso, baixo custo e rapidez na obtenção de resultados. No entanto, a precisão do resultado depende de um banco de dados detalhado de amostras e das calibrações desenvolvidas a partir desse banco de dados.

A técnica tem resolução e precisão suficientes para avaliar mudanças sutis na composição das mais diversas matérias primas com considerável rapidez, proporcionando um rápido retorno do resultado. Neste contexto, Melo-Durán *et al*. (2021) utilizaram com sucesso a tecnologia NIRS para avaliar a variabilidade nutricional entre 16 variedades de milho e conseguiram observar diferenças nos níveis de umidade, proteína bruta, amido e cinzas.

2.5 MICOTOXINAS NO MILHO

Micotoxinas são substâncias tóxicas resultantes do metabolismo secundário de fungos filamentosos de diferentes gêneros como *Fusarium, Aspergillus, Penicillium, Alternaria* e *Claviceps,* podendo ser produzidas nos cereais à campo, após a colheita e durante o armazenamento e o processamento dos grãos (OPLATOWSKA-STACHOWIAK *et al*., 2015). Apesar de ter distribuição global, esses metabólitos predominam em regiões de clima tropical, como o Brasil, onde o desenvolvimento fúngico e a produção de micotoxinas em matérias-primas e alimentos são favorecidos pelas condições ambientais, principalmente temperatura e umidade (MALLMANN; DILKIN, 2007). As micotoxinas podem ser produzidas inicialmente à campo e, caso o grão apresente umidade elevada, o crescimento fúngico pode continuar após

a colheita e as toxinas podem acumular-se antes da secagem e do armazenamento dos grãos (MALLMANN *et al*., 2018).

Os principais gêneros de fungos responsáveis pela produção de micotoxinas encontradas no milho são *Aspergillus e Fusarium*. As aflatoxinas (AFLA) são produzidas por fungos do gênero *Aspergillus,* principalmente *A. flavus e A. parasiticus*. Os fungos do gênero *Fusarium* produzem principalmente as fumonisinas (FUM), a zearalenona (ZEA) e os tricotecenos, como o deoxinivalenol (DON) (OLIVEIRA *et al*., 2017). As ocratoxinas (OTA), produzidas por fungos dos gêneros *Aspergillus* e *Penicillium*, possuem menor ocorrência no milho (GRUBER-DORNINGER *et al*., 2019). O milho pode ser acometido pela contaminação de mais de um fungo, sendo que cada fungo é capaz de produzir diferentes metabólitos, podendo ocorrer a contaminação simultânea de mais de uma micotoxina (GRENIER; OSWALD, 2011).

Rodrigues e Naehrer (2012) investigaram a ocorrência de AFLA, ZEA, DON, FUM e OTA em amostras de milho provenientes da América do Sul e encontraram positividades de 25, 43, 17, 92 e 12%, respectivamente, sendo que a média de contaminação encontrada para cada micotoxina foi de 2 µg/kg, 75 µg/kg, 37 µg/kg, 2.966 µg/kg e 16 µg/kg, respectivamente. Avaliando a presença de diversas micotoxinas em amostras de grão de milho provenientes da região sul do Brasil, Oliveira *et al*. (2017) constataram que todas as amostras estavam contaminadas com FUM, sendo que a maior média de contaminação foi detectada no estado do Paraná (3.153 µg/kg). Ainda, ZEA foi a segunda micotoxina de maior ocorrência, sendo detectada em 73.6% das amostras, enquanto DON foi detectada em 48% das amostras. As aflatoxinas (AFB_1 e AFB_2) estavam presentes em amostras provenientes dos três estados da região sul do Brasil e sua média de contaminação (AFB_1 + AFB_2) foi maior nas amostras de Santa Catarina (49,9 µg/kg).

Tyska *et al*. (2022) avaliaram a contaminação micotoxicológica do milho em diferentes países da América Latina e concluíram que as FUM foram as micotoxinas mais prevalentes, sendo detectadas em 91,6% das amostras, com média de 1.723 µg/kg, seguidas por ZEA com 15.3% de ocorrência e média de 8 µg/kg. Nesta investigação, o país que apresentou as maiores médias das duas micotoxinas foi a Argentina, ao passo que Bolívia e Brasil apresentaram as menores médias de FUM e ZEA, respectivamente.

A presença concomitante de micotoxinas de *Fusarium* como DON e ZEA em cereais é esperada (RODRIGUES; NAEHRER, 2012), uma vez que o principal patógeno no Brasil, *Fusarium graminearum*, produz ambas as toxinas (GERALDO *et al*., 2006). Gruber-dorninger *et al*. (2019) avaliaram a co-ocorrência de algumas micotoxinas no milho em uma avaliação micotoxicológica global e encontraram presença concomitante de DON e FUM em 49% das

amostras, DON e ZEA em 39% e ZEA e FUM em 37% das amostras. Também foi constatada uma co-ocorrência de 24% para AFB_1 e FUM e de 15% para AFB_1 e DON. A ocratoxina apresentou baixa co-ocorrência com as demais micotoxinas.

Além das micotoxinas mais conhecidas, alguns autores já relataram a ocorrência de metabólitos de menor prevalência em grãos de milho. Mahdjoubi *et al*. (2020) relataram a ocorrência das micotoxinas T-2, Citrinina (CIT) e Fusarenon-X em 100%, 83% e 80% das amostras de milho avaliadas na Algeria, respectivamente. Em contrapartida, Abdallah *et al*. (2017) encontraram uma alta prevalência de FUM nas amostras de milho avaliadas e uma baixa prevalência das demais micotoxinas investigadas, como DON, OTA, ZEA, 3-Acetil-deoxinivalenol, 15-Acetil-deoxinivalenol, nivalenol e CIT. Oliveira *et al*. (2017) observou uma baixa ocorrência de ácido ciclopiazônico (CPA) e CIT em amostras de milho do sul do Brasil, com ocorrências de 5.3 e 4.4% respectivamente.

A ocorrência de CIT parece ser maior em países da Europa e África, como evidenciado no estudo de Warth *et al*. (2012) conduzido em Moçambique, onde a micotoxina foi encontrada em cerca de 46% das amostras de milho avaliadas. Já em uma investigação realizada em cereais não processados na Croácia, a CIT foi detectada em 49% das amostras, com média de 66,8 µg/kg (PLEADIN *et al*., 2017). Embora haja uma maior possibilidade de co-ocorrência entre CIT e OTA, no estudo de Pleadin *et al*. (2017), a CIT apresentou uma ocorrência média 15 vezes superior à de OTA nos cereais milho, trigo, cevada e aveia. Neste levantamento, os autores encontraram ocorrências de CIT em 27, 42 e 45% das amostras de milho avaliadas nos anos 2014, 2015 e 2016, respectivamente, enquanto OTA apresentou ocorrências de 3, 8 e 7% nos mesmos anos.

Possíveis razões para que haja divergência de resultados entre os estudos realizados em diferentes países podem ser as condições de armazenamento em cada região e as diferenças climáticas entre elas, o que pode contribuir para o desenvolvimento de diferentes fungos e, consequentemente, a produção de distintos grupos de micotoxinas no milho. Até o presente momento, não foram encontrados na literatura estudos que investigassem a ocorrência e a contaminação de micotoxinas em grãos de milho de diferentes texturas de endosperma.

2.6 EFEITOS DAS MICOTOXINAS EM AVES E SUÍNOS

As micotoxicoses podem ser definidas pelo conjunto de alterações fisiológicas causadas em humanos e animais que ingeriram micotoxinas, e podem ser divididas em agudas ou crônicas. As micotoxicoses agudas ocorrem quando os indivíduos consomem doses moderadas e altas de micotoxina, podendo desencadear sinais clínicos e um quadro patológico específico

dependente da micotoxina ingerida, da susceptibilidade da espécie, das condições individuais do organismo e da interação ou não com outros fatores. As lesões mais comumente encontradas são os danos hepáticos, hemorragias, nefrites, necrose das mucosas digestivas e morte (MALLMANN; SIMÕES, 2022).

A micotoxicose crônica é mais frequente na realidade da produção animal e ocorre quando existe um consumo de doses baixas de alguma micotoxina. Nestes casos, os animais podem apresentar redução da eficiência reprodutiva, diminuição do ganho de peso e piora na conversão alimentar, podendo ser confundidos com deficiências de manejo e com outras doenças (DILKIN, 2021). Segundo Bryden (2012), os níveis de contaminação por micotoxinas em alimentos destinados a nutrição animal geralmente não são altos o suficiente para causar uma doença aguda, mas podem resultar em perdas econômicas em decorrência de mudanças sutis no crescimento, no desempenho produtivo e imunossupressão.

2.6.1 Fumonisinas

As fumonisinas são micotoxinas produzidas por fungos do gênero *Fusarium*, especialmente o *F. verticillioides* e *F. proliferatum*, mas também são produzidas por algumas espécies de *Alternia spp*. Mundialmente, espera-se encontrar uma importante ocorrência de FUM no milho, fato que já é bastante consolidado na literatura (COLOMA *et al*., 2019; KROUT-GREENBERG *et al*., 2013; MENDES DE SOUZA *et al*., 2013; TYSKA *et al*., 2022). Por esse motivo, essa se torna uma das micotoxinas de maior preocupação na produção de aves e suínos, exigindo um monitoramento constante da sua ocorrência.

A fumonisina B_1 (FB_1) causa edema pulmonar em suínos (HASCHEK *et al*., 2001), levando a quadros respiratórios perceptíveis a campo. Além disso, está relacionada ao comprometimento da integridade da barreira intestinal em suínos (BOUHET *et al*., 2004; LALLÈS *et al*., 2009) e a mudanças de conformação na camada mucosa do duodeno de aves (ANTONISSEN *et al*., 2015).

Em ensaios *in vitro*, Bouhet *et al*. (2004) avaliaram o efeito da FB_1 sobre uma linhagem celular epitelial intestinal de suínos (IPEC-1) onde a toxina reduziu a resistência elétrica transepitelial e bloqueou a proliferação de células intestinais. Em ensaios *ex vivo* utilizando explantes intestinais de suínos, Lalles *et al*. (2009) demonstraram um aumento na permeabilidade do intestino delgado após 2h de exposição à FB_1. O impacto de FB_1 sobre o epitélio intestinal também foi verificado *in vivo* utilizando suínos intoxicados com baixas doses durante 5 semanas (BRACARENSE *et al*., 2012), com surgimento de lesões histológicas nas

vilosidades, maior expressão de citocinas pró-inflamatórias e redução na expressão de proteínas de adesão e oclusão intestinal.

2.6.2 Aflatoxinas

As aflatoxinas são resultantes do metabolismo secundário de fungos da espécie *Aspergillus*, sobretudo *A. flavus* e *A. parasiticus*, os quais são contaminantes naturais de grãos comumente utilizados em dietas para aves e suínos, e causam preocupação em saúde animal devido ao seu alto potencial carcinogênico e hepatotóxico (MALLMANN; DILKIN, 2007). A Agência Internacional de Pesquisa de Câncer classifica a AFB_1 no grupo I de agentes carcinogênicos naturais (IARC, 2012).

Os impactos da contaminação por AFLA presentes em ingredientes utilizados na alimentação são amplamente estudados na produção de aves e suínos, pois além de afetar a saúde animal, podem representar perdas no desempenho produtivo. Os sinais clínicos variam entre ascite, hepatite, anemia, icterícia e diarreia hemorrágica, com diminuição do consumo alimentar e do crescimento (FINK-GREMMELS; VAN DER MERWE, 2019).

Atualmente, o maior risco que essas micotoxinas representam à produção animal decorre do efeito de baixas concentrações nas rações, insuficientes para desencadear intoxicações agudas, mas capazes de alterar o desempenho animal (LOPES *et al*., 2006), piorando a conversão alimentar e causando imunodepressão, prejudicando assim a lucratividade do sistema produtivo e representando perdas econômicas significativas (BINTVIHOK *et al*., 2006; ENSLEY; RADKE, 2019).

2.6.3 Tricotecenos

Os tricotecenos pertencem a um grupo de micotoxinas de grande importância imunossupressora. Assim, a capacidade inibitória destas micotoxinas sobre a síntese proteica e a interação com a membrana celular podem influenciar na sua capacidade supressora do sistema imune (DILKIN, 2021). Em intoxicações agudas, efeitos como degeneração e necrose podem ser observados em células de rápida divisão, como na mucosa intestinal, baço, timo, medula óssea e nódulos linfáticos (MALLMANN; DILKIN, 2007). Antonissen *et al*. (2014) avaliaram o impacto dos tricotecenos sobre a susceptibilidade dos animais a outras doenças, concluindo que, de forma geral, os tricotecenos afetam negativamente a resposta imune e aumentam a severidade de doenças que causam importante impacto em avicultura, como coccidiose, salmonelose e enterite necrótica.

Os tricotecenos são bastante tóxicos para aves e suínos, especialmente os do tipo A, tendo o DAS e a toxina T-2 como principais metabólitos. Segundo Dilkin (2021), os sinais clínicos da intoxicação por DAS e T-2 compreendem diminuição do consumo alimentar, especialmente em suínos, e diminuição no ganho de peso. Além disso, casos de diarreia sanguinolenta, acompanhada de palidez no bico, crista e barbela também são bastante frequentes. Os tricotecenos também induzem formação de lesões no trato gastrointestinal, sendo as lesões na cavidade oral bastante características das intoxicações causadas por toxina T-2 (MALLMANN; SIMÕES, 2022).

Classificado como um tricoteceno tipo B, DON tem a capacidade de comprometer diferentes funções intestinais, como diminuir a área de superfície das vilosidades, interferir na modulação dos transportadores de nutrientes, aumentar a permeabilidade intestinal e aumentar o número de células em apoptose nas vilosidades intestinais (DUARTE *et al*., 2021; LESSARD *et al*., 2015; OSSELAERE *et al*., 2013). Assim, atua causando diminuição do consumo ou recusa alimentar, vômitos, diarreia, diminuição da eficiência alimentar e diminuição do ganho de peso (ENSLEY; RADKE, 2019). Frangos de corte expostos a DON também podem apresentar menores títulos de anticorpos para vacinas de Newcastle e bronquite infecciosa, assim como redução no tamanho da Bursa de Fabricius (AWAD *et al*., 2013).

2.6.4 Zearalenona

A zearalenona é um metabólito fúngico estrogênico não esteroide produzido por várias espécies de fungos do gênero *Fusarium*, incluindo *F. culmorum, F. graminarum* e *F. crookwellense.* Em suínos, os principais sinais clínicos e lesões causadas pela ZEA são a síndrome de hiperestrogenismo (vulvovaginite) e *splayleg* em leitões recém-nascidos (MALLMANN; SIMÕES, 2022). O hiperestrogenismo caracteriza-se principalmente pelo avermelhamento e aumento da vulva e desenvolvimento precoce da glândula mamária em fêmeas suínas. Ainda, sinais clínicos como edema de vulva e útero, prolapso vaginal, pseudo-gestação, manifestações de estro permanente e infertilidade podem ser observados em fêmeas suínas submetidas à alimentação contaminada com ZEA (AOYAMA *et al*., 2009).

Embora a intoxicação por ZEA em suínos seja mais perceptível nas fêmeas, em machos é possível observar o crescimento das glândulas mamárias e edema do prepúcio, podendo chegar ao ponto de dificultar a micção. Ocorre também a atrofia testicular, com comprometimento da qualidade e do volume espermático e, consequentemente, diminuição da capacidade de fecundação. A redução da libido também é observada com frequência em lotes intoxicados (ENSLEY; RADKE, 2019).

As aves são mais resistentes a ZEA do que os suínos, entretanto, já foram relatadas alterações como redução da produção de ovos e redução da fertilidade em animais intoxicados (WU *et al*., 2021). Segundo Dilkin (2021), aves que consumem alimentos com altas concentrações de ZEA podem apresentar diminuição do consumo alimentar, piora na conversão alimentar, cistos no peritônio e oviduto, edema cloacal e diminuição do tamanho dos testículos.

2.6.5 Ácido ciclopiazônico

O ácido ciclopiazônico é uma micotoxina produzida por espécies de *Aspergillus* e *Penicillium*. Muitas vezes é produzido por cepas aflatoxigênicas de *Aspergillus flavus* e, portanto, pode ser encontrada em alimentos juntamente com AFLA (GALLAGHER *et al*., 1978; BRYDEN, 2012). Esta micotoxina é conhecida por causar desordens gastrointestinais e neurológicas nos animais que a consomem. De acordo com Ostry *et al*. (2018) os órgãos mais afetados incluem os rins, o fígado, o coração e o trato gastrointestinal, que apresenta alterações degenerativas e necrose.

Galinhas poedeiras apresentam redução do consumo de ração e queda na produção de ovos após a ingestão de CPA (SUKSUPATH, 1993). A toxicidade do CPA também já foi evidenciada principalmente através do aumento dos pesos relativos do proventrículo e aumento da atividade da creatina quinase presente no sangue de frangos de corte (GENTLES *et al*., 1999). Em outro estudo, a contaminação da ração de frangos de corte por CPA resultou em distúrbios renais e hepáticos (AKBARI *et al*., 2012). Da mesma maneira, em suínos intoxicados com CPA, foram observadas alterações principalmente no trato gastrointestinal, fígado e rins, com nefrite intersticial, atrofia das vilosidades intestinais, gastrite e hepatite necróticas (LOMAX; COLE; DORNER, 1984).

2.6.6 Ocratoxina A

Apesar de ser uma micotoxinas de baixa ocorrência no milho, a ocratoxina A é considerada um potente agente nefrotóxico, hepatotóxico e imunossupressor. Seu principal impacto na produção avícola é a diminuição da qualidade da casca dos ovos em galinhas poedeiras, além de provocar queda na produção de ovos (BRYDEN, 2012). Isto ocorre devido a lesões renais que alteram a reabsorção de minerais e a síntese de algumas enzimas (DILKIN, 2021). Os principais sinais clínicos da ocratoxicose em suínos são relacionados ao aumento da ingestão de água e poliúria, além de diminuição da ingestão de alimentos, piora da conversão alimentar e do ganho de peso quando os alimentos ingeridos estão contaminados com doses moderadas a altas da toxina (MALLMANN; SIMÕES, 2022). Além disso, KUMAR *et al*.

(2014) observaram uma ação sinérgica entre OTA e CIT em coelhos intoxicados com baixas concentrações das duas micotoxinas, com apoptose das células renais, o que desempenhou um papel importante na patogênese da nefrotoxicidade.

2.6.7 Citrinina

A citrinina é uma micotoxina produzida por fungos dos gêneros *Aspergillu*s, *Monascus* e *Penicillium*. Em geral, a CIT possuí efeitos nefrotóxicos (KUMAR *et al*., 2014; MEHDI; CARLTON; TUITE, 1981) e é frequentemente detectada em alimentos como cereais, produtos à base de cereais, nozes, ervas e especiarias (OSTRY; MALIR; RUPRICH, 2013; PLEADIN *et al*., 2017). Em geral, a toxicidade mediada pela CIT está relacionada ao estresse oxidativo e à alteração de enzimas antioxidantes (KUMAR *et al*., 2014; SINGH *et al*., 2016).

Meerpoel *et al*. (2020) observaram baixa ação nefrotóxica desta micotoxina, porém foi detectada degeneração mitocondrial nos rins de aves e suínos intoxicados com níveis crescentes de CIT, o que pode levar a uma maior toxicidade em casos de exposição crônica. Os autores também observaram um importante acúmulo da micotoxina em tecidos (músculo, pele, rins e fígado) de aves e suínos.

Esta micotoxina também pode apresentar ação aditiva e sinérgica à da OTA, onde o principal órgão alvo são os rins (GRENIER; OSWALD, 2011; KUMAR *et al*., 2014; OSTRY; MALIR; RUPRICH, 2013). Além disso, a CIT permanece por mais tempo no organismo dos suínos do que das aves, sendo mais rapidamente absorvida e mais lentamente eliminada, o que pode levar a uma sensibilidade maior desta espécie em comparação com as aves (MEERPOEL *et al*., 2020).

2.7 IMPACTO ECONÔMICO DAS MICOTOXINAS

A contaminação micotoxicológica dos alimentos pode causar um grande impacto na saúde animal, bem como perdas econômicas nas cadeias de produção de grãos e de proteína animal devido à redução do rendimento e do valor das culturas e à diminuição do desempenho dos animais de produção.

Diversas investigações já avaliaram este impacto, encontrando custos atrelados às micotoxinas de cerca de 0,5 a 1,5 bilhões de dólares por ano nos Estados Unidos (EUA), com as AFLA representando o maior impacto (sem incluir os custos com saúde humana) e com perdas de 225 milhões de dólares por ano relacionadas à contaminação do milho (CAST, 2003). Neste cenário, os custos somente com as análises de AFLA podem atingir 20-30 milhões de dólares por ano.

As perdas associadas a DON nos EUA foram estimadas em 655 milhões de dólares por ano (CAST, 2003), principalmente devido ao trigo contaminado. Além disso, Wu (2007) estimou que as perdas econômicas nos EUA causadas pela contaminação de FUM na alimentação animal poderiam variar entre 1 e 20 milhões de dólares em um ano normal e entre 31 e 46 milhões em um ano com grave desafio de *Fusarium*. Ainda nos EUA, a contaminação por FUM no DDGS, ingrediente utilizado em rações e subproduto da indústria do etanol de milho, pode contribuir com perdas na produção de suínos superiores a 147 milhões de dólares por ano (WU; MUNKVOLD, 2008).

Em outras regiões do mundo, o impacto de contaminações por AFLA foi estimado em 400 milhões de dólares por ano no Sudeste Asiático, com a maior parte dos custos atribuída aos efeitos na saúde humana (CARDWELL; MILLER, 1996). Além disso, as micotoxinas são responsáveis por 39% das rejeições fronteiriças na União Europeia (UE) aos produtos vindos da África (CHILAKA *et al*., 2022). Wu (2008) estimou que uma média de 7.000 a 11.000 euros são perdidos em cada rejeição que ocorre na fronteira da UE.

No Brasil, os prejuízos econômicos causados pelas micotoxinas ainda são pouco calculados e divulgados. Entretanto, a julgar pelos índices de produção e pelos resultados de ocorrência de algumas micotoxinas, é possível que estes números sejam bastante expressivos. Além disso, as perdas econômicas podem ser significativamente mais elevadas devido aos efeitos aditivos ou sinérgicos da co-ocorrência de micotoxinas nos ingredientes utilizados para a alimentação animal.

3 HIPÓTESES CIENTÍFICAS

Hipótese 1:

Há efeito da textura do endosperma sobre a composição nutricional de diferentes tipos de milho cultivados no Brasil.

Hipótese 2:

Há efeito da textura do endosperma sobre a ocorrência e média de contaminação de micotoxinas em diferentes tipos de milho cultivados no Brasil.

Hipótese 3:

Há efeito da textura do endosperma sobre o rendimento a campo e a qualidade dos grãos de diferentes tipos de milho cultivados no Brasil.

Hipótese 4:

Há correlação entre a dureza dos grãos, a composição nutricional e a contaminação por micotoxinas no milho.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Avaliar a influência da textura do endosperma sobre a composição nutricional, o rendimento à campo, e a concentração de micotoxinas em grãos de milho de diferentes tipos cultivados no Brasil.

4.2 OBJETIVOS ESPECÍFICOS

Avaliar a composição nutricional e as características físicas e agronômicas de quatro tipos de milho (duro, semiduro, dentado e semidentado) cultivados no Brasil, com ênfase na nutrição de aves e suínos.

Avaliar a ocorrência e concentração de micotoxinas em quatro tipos de milho (duro, semiduro, dentado e semidentado) cultivados no Brasil em duas safras consecutivas.

Correlacionar o grau de dureza dos grãos de milho com a composição nutricional e a contaminação micotoxicológica.

5 ARTIGO 1

Este capítulo é apresentado em formato de artigo de pesquisa denominado “Assessment of field traits, nutrient composition and digestible amino acids of corns with different endosperm textures for poultry and swine.” publicado em 2023 no periódico *Animal Feed Science and Technology,* disponível em: https://doi.org/10.1016/j.anifeedsci.2022.115510.

Assessment of field traits, nutrient composition and digestible amino acids of corns with different endosperm textures for poultry and swine

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Abbreviations: AA, amino acids; ADF, acid detergent fiber; AME, apparent metabolizable energy; CP, crude protein; DE, digestible energy; dig., digestible; DM, dry matter; EE, ether extract; GE, gross energy; ME, metabolizable energy; NDF, neutral detergent fiber; NIRS, near infrared spectroscopy; PCA, principal component analysis; TGW, thousand grains weight.

ABSTRACT

An experiment was conducted to evaluate field traits and nutrient composition of four corn types presenting distinct endosperm textures. A total of 213 samples of different corn hybrids from a field experiment conducted in Brazil were separated into 4 groups according to the endosperm texture classification: dent (n=30), flint (n=51), semi-dent (n=60) and semi-flint (n=72). Crop yield, thousand grains weight and damaged grains were determined. Nutritional composition as well as digestible amino acids (AA) and metabolizable energy values for poultry and swine of the four corn types were predicted by near infrared spectroscopy. Data were submitted to analysis of variance and mean differences of corn types were separated by Tukey's test at 5% of significance. Principal component analysis was performed for the main nutritional variables and damaged grains. Starch concentration was greater ($P < 0.01$) in dent than in flint corn. Dent corn presented higher percentage of damaged grains ($P < 0.01$) than the other types. Flint corn had the lowest crop yield ($P < 0.001$); however, this corn texture presented the higher crude protein, total Cys, Thr, Arg, Ile, Leu, Val, His, Gly, Ser, Pro, Ala and Glu contents than the remaining three types ($P < 0.001$). The flint type had also the highest ($P < 0.0001$) content of most of digestible AA for swine and poultry. Energy values were not different among corn types ($P > 0.05$). In conclusion, field traits and nutritional composition of corn vary depending on the characteristic of its endosperm, and such differences should be considered by the corn and feed production chains.

Keywords: Animal nutrition; Endosperm texture; NIRS; Starch.

1. Introduction

Corn (*Zea mays* L.) is one of the most cultivated crops worldwide and represents an important energy source in animal nutrition. It is the second most grown cereal in Brazil, with a production exceeding 87 million tons in the 2020/2021 harvest (CONAB, 2021). Brazil is the third largest global producer of corn, behind only the USA and China; it is also the world's third biggest corn exporter, having traded more than 27 million tons from its 2020/2021 harvest (USDA, 2022).

Grains of the dent, flint, semi-dent and semi-flint types can be found among the varieties of corn and this classification is made according to the endosperm texture, which is also greatly important in animal nutrition. Corn endosperm is related to agronomic traits, nutritive values (Zurak et al., 2020) and grain quality as well as it affects shipping and processing characteristics of the grain (Zhang et al., 2011); it is basically composed of granules of starch that can be arranged in floury and vitreous forms (Piovesan et al., 2011).

The floury endosperm comprises spherical starch granules, which are more loosely packed and adhered to a low protein content, whereas the vitreous endosperm presents compact and polygonal starch granules linked to the protein matrix of the endosperm, being associated with grain hardness (Piovesan et al., 2011). The ratio between floury and vitreous endosperms in corn grains, also called vitreousness, is used to assess its type, which ranges from flint to dent. Corn hybrids classified as flint have a greater proportion of vitreous endosperm, while those classified as dent have a higher proportion of floury endosperm (Xu et al., 2019).

The relationship between corn endosperm texture and digestible nutrients and animal performance has already been evaluated in previous studies (Ngonyamo-Majee et al., 2008; Giuberti et al., 2013; Córdova-Noboa et al., 2021). However, there is little

information available in the literature regarding the influence of endosperm texture on nutrient composition in order to improve precision in poultry and swine feed formulations.

Corn genetic selection programs have searched for hybrids with higher yields, resistance to pests, lower production costs and high tolerance to climatic fluctuations (Qaim and Matuschke, 2005). Nevertheless, the nutritional potential of this ingredient should also be considered a selection factor. Since distinct corn types are produced worldwide, and considering this as the major ingredient in poultry and swine diets (Gehring et al., 2012), a thorough determination of its composition is essential for the development of genotypes based not only on field production, but also taking its nutritional value for the industry into account.

There is a lack of peer-reviewed data characterizing corn types and evaluating crop yield and nutritional values as a function of the endosperm characteristics. Therefore, this research aimed to assess the nutritional composition and physical and agronomic traits of four types of corn with different endosperm textures grown in Brazil, focusing on poultry and swine nutrition.

2. Material And Methods

2.1. Classification of corn types

Grain texture classification was based on the registration of each commercial corn hybrid in the Brazilian National Cultivar Registry (RNC), which is available on the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) website (BRASIL, 2021). The material was separated into four groups according to the grain endosperm texture: dent (n=30), flint (n=51), semi-dent (n=60) and semi-flint (n=72). Different

commercial corn hybrids of each texture were used, totalizing 213 samples. The commercial names of the hybrids have been withheld to ensure confidentiality.

2.2. Field experiment

Corn samples were sourced from experimental field plots conducted in the Agricultural Research Center (24º37´18"S, 53º18´20"W, 580 m altitude) of the Cooperativa Agro-industrial Consolata (COPACOL), which is located in the western region of Paraná State, Southern Brazil. The soil in the region is classified as dystroferric red latosol.

The crops were planted in the second half of January 2020 in a consolidated no-tillage system under the same soil type and agroclimatic and production conditions. The field experiment was designed in randomized blocks with three replications of each corn hybrid, and the experimental plots consisted of four corn rows spaced 0.68 m apart with 10 m in length (27.2 m^2). Fertilization of the crops was based on nutritional requirements and chemical analyses of the soil. Phytosanitary management was conducted according to the technical recommendations on pest, disease and weed control for each crop. Harvesting was performed in June 2020 with the aid of a Wintersteiger® experimental plot harvester-classic model. The two central lines were harvested by 10 m in length, totaling 13.6 m^2 of useful plot.

Data on rainfall, daily minimum, maximum and average air temperatures (°C) and relative humidity (%) during the experimental period were collected at the weather station located in the Agricultural Research Center, 50 m away from the experimental plots. The accumulated precipitation was 582 mm, with an average of 3.37 mm/day. The average daily temperature was 20.9°C, with maximum and minimum averages of 28.5°C and 9.93°C being detected in February and June, respectively. The average relative humidity

for the entire period was 68.8%; maximum and minimum daily averages of 99.3% and 43.4% were recorded in April and March, respectively.

2.3 Measurement of field traits

Crop yield was calculated in kg/ha and reported at 12% moisture. Thousand grains weight (TGW) was determined by randomly selecting 1,000 grains from the overall sample and weighing them on a digital electronic scale; the resulting value was then corrected to 12% moisture and expressed in g. Grains or pieces of grain that were scorched, flat or immature, frosted, fermented, sprouted and moldy were considered damaged, following the recommendations of MAPA (BRASIL, 2011), and the percentage of damaged grains was obtained according to the equation: [weight of damaged grains (g)/weight of the sample (g)]*100.

2.4. NIRS nutritional predictions

Corn samples (500 g) were milled with a 0.5 mm sieve in an ultra-centrifugal mill (RETSCH®, model ZM 200) and then homogenized. Nutritional analyses were carried out by reading the samples spectra via NIRS. The spectral data were originated from a Bruker® equipment, model Tango-R, using the calibration curves from the AMINONRG® and AMINONir® advanced programs (Evonik Nutrition & Care GmbH, Hanau, Germany). The wavelength ranged from 3,952 to 11,536 cm^{-1}, and the rotating sphere macro sample was the cell type used for reading solid samples. Subsequently, the corn samples were predicted for the following variables: dry matter (DM, g/kg); crude protein (CP, g/kg); ether extract (EE, g/kg); ash (g/kg); crude fiber (g/kg); starch (g/kg); total P (mg/kg), phytic P (mg/kg); acid detergent fiber (ADF, g/kg); neutral detergent fiber (NDF, g/kg); total and digestible (dig.) amino acids (AA, g/kg) for poultry and swine;

149 gross energy (GE, MJ/kg); digestible energy (DE, MJ/kg) for growing pigs and sows,
150 apparent metabolizable energy (AME_n, MJ/kg) for poultry and metabolizable energy
151 (ME, MJ/kg) for growing pigs and sows. Values of such variables are expressed on an
152 88% DM basis.
153
154 *2.5 NIRS calibration models*
155 For the bromatological predictions, several reference methods were used to build
156 the calibration models. The DM was determined by the oven method (105°C for 16h).
157 The Dumas combustion method was applied to measure CP content. The Soxhlet
158 apparatus was used for fats, and lipids were extracted with hexane; after evaporation of
159 the solvent, the remaining residue is the EE fraction. The determination of ash was carried
160 out through the muffle furnace at 550°C for at least 4h until complete removal of organic
161 compounds. Carbohydrates were retrieved in two fractions of the proximate analysis. The
162 fraction which is not soluble in a defined concentration of alkalis and acid was defined as
163 crude fiber. The Van Soest method was used to obtain ADF and NDF values. Starch was
164 determined by polarimetric measurement according to the Ewers method ISO 10520. To
165 determine total P, samples were treated by microwave pressure digestion and determined
166 by inductively coupled plasma mass spectrometry. Phytic P contents were estimated
167 during the NIR prediction based on total P multiplied by the phytic P factor taken from
168 the L'Institut National de la Recherche Agronomique (INRA) tables.
169 Calibration models for total AA were developed from the reference technique based
170 on ion exchange chromatography analysis. Prediction equations for digestible AA were
171 based on several *in vivo* experiments and expressed as standardized ileal digestibility for
172 both poultry and swine. Energy prediction equations were developed based on the
173 correlations between the proximate composition and the measured DE, ME or NE of feed

ingredients for pigs (Noblet et al., 1994) and between proximate composition and measured AME_n for poultry (WPSA, 1989).

2.6. Statistical analysis

Normality of the data was tested by Shapiro-Wilk test prior to other analyses. Data were submitted to analysis of variance using the mixed GLM procedure of Statgraphics® software (Statgraphics Centurion 15.2.11, Manugistics Inc., Rockville, MD, USA). Significance was accepted at 5%, and different means of corn types were separated by applying the Tukey test. In order to summarize the information from the data into a smaller set of indices that can be easily visualized and analyzed, the principal component analysis (PCA) was performed for the main nutritional variables and damaged grains using the Unscrambler® software (Unscrambler 9.7, CAMO, Oslo, Norway).

3. Results

Differences among the four types of corn regarding field traits and nutrient composition are given in Table 1. Dent corn had a higher percentage of damaged grains ($P < 0.01$) than the other types, whereas flint corn showed the lowest crop yield ($P < 0.001$). Corn type had no effect on TGW ($P > 0.05$). Flint corn presented the greatest CP content ($P < 0.001$); moreover, its EE was higher compared to dent corn ($P < 0.05$). The lowest CP ($P < 0.001$) content was observed in the semi-dent type. Starch content was greater in dent than in flint corn ($P < 0.01$). Flint corn had higher ash ($P < 0.05$) and ADF ($P < 0.01$) than the semi-dent corn. There was no effect ($P > 0.05$) of corn type on total P, phytic P and NDF concentration.

The effect of corn endosperm textures was also evaluated in the predicted energy values for poultry and swine (Table 2). No difference ($P > 0.05$) was observed in GE,

199 AME for poultry, as well as in DE and ME for growing pigs and sows among the four
200 corn types ($P > 0.05$).

201 The effects of corn endosperm texture on total and digestible AA for poultry and
202 swine are shown in Tables 3 and 4, respectively. Flint corn had higher ($P < 0.001$) total
203 Cys, Thr, Arg, Ile, Leu, Val, His, Gly, Ser, Pro, Ala and Glu than the remaining three
204 types. The greatest concentration of total Lys ($P < 0.001$) was found in dent and flint corn
205 textures, while the lowest total Lys was obtained in semi-dent corn (Table 3).

206 Digestible AA for poultry also varied among the corn textures (Table 4) where the
207 digestible Cys, Thr, Arg, Ile, Leu, Val, His, Phe, Gly, Ser, Pro, Ala and Glu were greater
208 ($P < 0.0001$) in flint than in the other types. Furthermore, digestible Met and Lys for
209 poultry were higher in dent and flint corn ($P < 0.0001$) compared to semi-dent and semi-
210 flint corn. The lowest content of most of digestible AA for swine was observed in semi-
211 dent corn, whereas the flint corn had the highest digestible AA contents ($P < 0.0001$).
212 The exceptions were digestible Met, which was similar between the dent and flint types;
213 digestible Lys, which was similar among the dent, flint and semi-flint types, and
214 digestible Phe that was similar between the flint and semi-flint types (Table 4).

215 The compositional difference among the four corn types could also be noticed when
216 performing principal component analysis, as shown in the PCA loadings and scores plots
217 (PC1 *vs.* PC2) in Fig. 1 and 2, respectively. The PC1 expresses 56% of the variance in
218 the dataset, while PC2 expresses 17% of variance, explaining 73% of the total variance.

219 In the Fig. 1, the distribution of the analyzed variables along PC1 (X axis) and PC2
220 (Y axis) is illustrated. It demonstrates that total AA and CP are clearly separated from
221 starch, which is in an opposite quadrant. Furthermore, results of total AA and CP are
222 explained by PC1, while starch is better represented by PC2. Damaged grains are in a
223 quadrant opposite to total AA, CP and starch, thus indicating a possible negative

correlation among these variables. Moreover, GE, ADF and NDF are better explained by PC2 than by PC1. The Fig. 2 allows to discriminate the samples belonging to each group of corn, represented by different colors and formats and, although total separation of corn types was not observed, the scores plot evidences the discrimination of the samples belonging to the flint (red circle) and semi-dent (green circle) groups.

4. Discussion

Corn is one of the leading grain crops around the world, and its economic relevance lies in its broad use in feed mills and ethanol industries. Quality of this grain can be affected by numerous factors, such as soil, harvest conditions, genotype, growing location and processing conditions (Gehring et al., 2012; Liu et al., 2020). Maximizing yield concomitantly with the physical quality of the grain should be considered to achieve significant improvements in both agricultural and animal production (Alves and Filho, 2017). The current study on the physical quality of grains demonstrated that the corn classified as dent yielded more than the other types; nonetheless, it presented the highest percentage of damaged grains. In addition, in spite of the low crop yield, flint corn had lower percentage of damaged grains than the remaining three types. Such findings can indicate that high yield is not necessarily associated with better grain quality.

The corn grain consists mainly of starch, whose chief site of storage (98-99% of total starch) is the endosperm (Ildiz et al., 2019). The current data demonstrates a greater starch content in dent than in flint corn. The present outcomes are in parallel with those attained by Zhang and Xu (2019) when evaluating the starch content of distinct corn cultivars; the authors observed a statistically higher starch content in the floury endosperm from dent corn than in the flint type. Still, Álvarez-Iglesias et al. (2021) did

not observe difference in starch concentration between dent and flint corn, but these endosperm types presented higher concentration of starch than waxy and opaque hybrids.

As corn is the main energy source in corn-based feeds (Cowieson, 2005), knowledge on the ME of this ingredient has been essential to obtain a balanced diet, thus meeting the nutrient demands in feed formulations. Dozier et al. (2011) indicated that variations in ME might translate to economically important changes in poultry feed conversion. Besides, Cowieson (2005) stated that differences between corn samples could yield a variability of more than 1.7 MJ/kg in metabolizable energy corrected for N retention for poultry. In this study, gross energy, DE and ME values for growing pigs and sows and AME_n values for poultry were not statistically different among corn textures. However, it would be of great value to predict the possible differences among corn types in terms of nutrient digestibility, energy values and starch content to improve precision in feed formulations; with that, costs would be reduced and animal growth performance would be enhanced.

It is important to point out that the corn genetic improvement with the sole goal of increasing grain productivity could result in an indirect selection of plants with lower content of protein (Duvick, 2005). Álvarez-Iglesias et al. (2021) stated that nutrient content can be improved in corn, but negative effects on agronomic traits should be considered; these authors observed that grain yield and plant height had negative correlations with protein and lipid contents of the grains. The current findings are in line with the above considerations: flint corn was the type to show the greatest CP and AA contents, but its crop yield was the lowest, being 11.14% less productive than dent corn, which was the most productive type and yielded 8,243 kg/ha. However, in spite of the greatest yield, dent corn presented lower CP content than flint corn.

Protein is the second main component in corn grains, and AA content acts as an indicator of grain quality (Alves and Filho, 2017). Grain texture has already been positively associated with protein content by Zhang et al. (2011). The authors observed that the higher the percentage of vitreous endosperm in the grain, the higher the protein content. Additionally, Álvarez-Iglesias et al. (2021) investigated the nutritional values of corn grains with diverse endosperms and reported that flint hybrids had higher protein content than dent hybrids. The overall outcomes of the present assessment evidenced higher CP and AA contents in flint corn than in the other types.

As feeds are accountable for the highest cost in poultry and swine meat production chain, it is highly necessary to improve the nutrient and energy utilization. In this context, feed formulation for such animals not only considers CP and total AA of ingredients. Requirements of ME, dig. AA, minerals and vitamins for each species and phase are taken into account. With respect to AA, feed formulation for any phase of poultry and swine development considers requirements of digestible Lys, Met+Cys, Thr, Val, Arg, Ile, Leu and Trp.

The NRC (1994) lists the essential AA for poultry as Arg, Gly, His, Leu, Ile, Lys, Met, Cys, Phe, Thr, Trp and Val. In the present research, flint corn had the highest results for dig. AA for poultry, except for dig. Lys and Met, which were similar between the dent and flint types. The essential AA for swine established by NRC (2012) are His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val. In the current study, flint corn showed the highest dig. AA for swine, with exception of dig. Lys, Met and Phe. Adequate dietary intake of dig. AA may vary depending on the feed ingredients, thus knowledge on the composition of Brazilian corn types could help animal nutritionists to use feed additives in a way to match the requirements of each species and phase more closely.

296 The present results on corn composition are representative of those currently found
297 in industrial settings because NIRS, despite being a secondary analytical methodology, is
298 a real-time technique widely used to predict the animal feed components (Alves et al.,
299 2014) and accounts for the majority of the ingredient analyses conducted in feed mills on
300 a daily basis. It is a non-destructive screening tool that allows reducing the time and cost
301 of analyses (Tyska et al., 2021), besides eliminating the use of chemicals that can
302 potentially harm the environment. Some factors can be considered limiting for the
303 construction of robust prediction models, such as the reference methodology used to
304 construct the calibration curves, the representativeness of the samples and the variability
305 in terms of the concentration of the parameters. All these factors are important and if there
306 is a failure in some of them, the model may not respond adequately. Finally, the NIRS
307 calibration curves used in the present study allow the prediction of information not only
308 used for the classification of grains, but also for formulation of diets for poultry and swine,
309 as dig. AA and ME for these species.
310 The principal component analysis conducted in the present study provided an easier
311 visualization of the compositional differences among the four types of corn. Ildiz et al.
312 (2019) clearly discriminated the differences between two varieties of corn (yellow dent
313 and purple flint) using the PCA scores plot. Although there was no clear separation
314 between the four types of corn tested herein, it was possible to observe a slight difference
315 between flint and semi-dent corns. The differences in hybrids within the same type of
316 corn may be the main reason why more differences were not found among the studied
317 corn types.
318 Results on nutrient composition of different types of corn can be variable depending
319 on an assortment of factors. In countries with extensive cultivation areas, as Brazil, such
320 variability is likely to be remarkable. Nevertheless, there is still a lack of scientific

information concerning the influence of endosperm texture on the characteristics described herein. To the best of the authors' knowledge, this is the first scientific work aimed at evaluating the differences among corn types with respect to field traits, nutrient composition and digestible amino acids for poultry and swine.

5. Conclusions

The data reported herein indicated that field traits and nutritional composition of corn vary depending on the characteristic of its endosperm. In spite of the lowest crop yield, flint corn presented higher protein and amino acid contents than the dent, semi-dent and semi-flint textures. The present findings fill part of the information gap in the available literature by revealing variations in nutrient content, digestible amino acids and field traits of distinct corn types. Further evaluations are needed to better understand such results, especially regarding the correlation between field data and grain nutritional composition.

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CRediT authorship contribution statement

C.T. Simões: Conceptualization, Methodology, Data Curation, Formal analysis, Writing - Original Draft, Writing - Review & Editing **J.K. Vida**l: Conceptualization, Methodology, Investigation **D. Tyska:** Investigation, Formal analysis, Data Curation **A.O. Mallmann:** Conceptualization, Visualization, Writing - Review & Editing **T.**

Madalosso: Conceptualization, Methodology, Investigation **C.A. Mallmann:** Supervision, Project administration, Writing - Review & Editing.

Declaration of Competing Interest

None.

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444

Table 1

Damaged grains, crop yield, thousand grains weight and nutrient composition of four types of corn.

Item	Types of corn				SEM	P-value
	Dent	Semi-dent	Flint	Semi-flint		
Damaged grains, %	0.54[a]	0.29[b]	0.20[b]	0.31[b]	0.025	0.0051
Crop yield, kg/ha	8,243[a]	8,201[a]	7,324[b]	7,957[a]	70.27	0.0001
TGW[a], g	292	304	302	303	1.95	0.5376
Crude protein, g/kg	81.82[b]	79.29[c]	84.41[a]	82.08[b]	0.335	0.0001
Ether extract, g/kg	37.83[b]	38.68[ab]	40.00[a]	38.96[ab]	0.240	0.0483
Crude fiber, g/kg	21.57[a]	20.54[b]	21.21[ab]	21.16[ab]	0.099	0.0120
Ash, g/kg	11.39[ab]	11.32[b]	11.62[a]	11.43[ab]	0.036	0.0288
Starch, g/kg	647.0[a]	644.7[ab]	640.8[b]	643.5[ab]	0.634	0.0018
ADF[b], g/kg	29.18[ab]	28.63[b]	30.05[a]	29.55[ab]	0.135	0.0015
NDF[c], g/kg	95.33	96.33	98.17	97.20	0.366	0.1331
Total P, mg/kg	1,964	1,916	1,966	1,936	8.00	0.0528
Phytic P, mg/kg	1,473	1,437	1,474	1,452	6.00	0.0617

[a-c] Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[a]TGW = Thousand grains weight, g.

[b]ADF = Acid detergent fiber, g/kg.

[c]NDF = Neutral detergent fiber, g/kg.

Table 2

Energy values of four types of corn for poultry and swine.

Energy values, MJ/kg	Types of corn				SEM	P-value
	Dent	Semi-dent	Flint	Semi-flint		
Gross Energy	16.45	16.46	16.50	16.48	0.006	0.2001
DE[a] Growing pigs	14.75	14.73	14.75	14.74	0.004	0.2321
DE Sows	15.34	15.33	15.34	15.33	0.004	0.1968
ME[b] Growing pigs	14.39	14.38	14.40	14.39	0.004	0.1752
ME Sows	14.89	14.88	14.90	14.88	0.005	0.2098
AME_n[c] Poultry	13.93	13.96	13.97	13.96	0.004	0.2194

[a]DE = digestible energy (predicted using AMINONRG® calibration curves).

[b]ME = metabolizable energy (predicted using AMINONRG® calibration curves).

[c]AME_n = apparent metabolizable energy (predicted using AMINONRG® calibration curves).

Table 3

Total amino acids composition of four types of corn.

Amino acid, g/kg	Types of corn				SEM	P-value
	Dent	Semi-dent	Flint	Semi-flint		
Methionine	1.671[ab]	1.625[b]	1.715[a]	1.664[b]	0.007	0.0001
Cysteine	1.81[bc]	1.77[c]	1.86[a]	1.82[b]	0.006	0.0001
Lysine	2.30[a]	2.23[b]	2.31[a]	2.27[ab]	0.006	0.0001
Threonine	2.89[b]	2.81[c]	2.99[a]	2.91[b]	0.011	0.0001
Tryptophan	0.61[a]	0.59[b]	0.61[a]	0.60[ab]	0.001	0.0001
Arginine	3.72[bc]	3.66[c]	3.84[a]	3.75[b]	0.012	0.0001
Isoleucine	2.80[b]	2.70[c]	2.91[a]	2.81[b]	0.013	0.0001
Leucine	10.1[bc]	9.80[c]	10.7[a]	10.2[b]	0.058	0.0001
Valine	3.86[b]	3.76[c]	4.00[a]	3.89[b]	0.015	0.0001
Histidine	2.37[bc]	2.32[c]	2.47[a]	2.39[b]	0.009	0.0001
Phenylalanine	3.97[b]	3.81[c]	4.12[a]	3.98[ab]	0.021	0.0001
Glycine	3.04[b]	2.98[c]	3.11[a]	3.05[b]	0.008	0.0001
Serine	3.91[b]	3.79[c]	4.06[a]	3.94[b]	0.018	0.0001
Proline	7.47[bc]	7.28[c]	7.82[a]	7.56[b]	0.035	0.0001
Alanine	6.09[b]	5.88[c]	6.34[a]	6.13[b]	0.029	0.0001
Asparagine	5.24[a]	5.05[b]	5.35[a]	5.22[a]	0.020	0.0001
Glutamine	15.02[b]	14.53[c]	15.60[a]	15.17[b]	0.079	0.0001

[a-c] Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

Table 4

Digestible amino acids of four types of corn for poultry and swine.

Digestible amino acids	Types of corn				SEM	P-value
	Dent	Semi-dent	Flint	Semi-flint		
Dig. AA for poultry, g/kg						
Methionine	1.59^{ab}	1.54^{c}	1.62^{a}	1.58^{bc}	0.006	0.0001
Cysteine	1.61^{bc}	1.58^{c}	1.66^{a}	1.62^{b}	0.005	0.0001
Lysine	2.10^{a}	2.03^{b}	2.10^{a}	2.06^{ab}	0.006	0.0001
Threonine	2.57^{b}	2.50^{c}	2.66^{a}	2.59^{b}	0.010	0.0001
Tryptophan	0.50^{ab}	0.49^{b}	0.52^{a}	0.49^{b}	0.001	0.0001
Arginine	3.31^{bc}	3.26^{c}	3.41^{a}	3.34^{b}	0.011	0.0001
Isoleucine	2.74^{b}	2.65^{c}	2.85^{a}	2.76^{b}	0.013	0.0001
Leucine	9.46^{bc}	9.10^{c}	9.93^{a}	9.54^{b}	0.054	0.0001
Valine	3.67^{b}	3.57^{c}	3.80^{a}	3.69^{b}	0.015	0.0001
Histidine	2.29^{bc}	2.25^{c}	2.40^{a}	2.32^{b}	0.009	0.0001
Phenylalanine	3.69^{b}	3.54^{c}	3.83^{a}	3.70^{b}	0.019	0.0001
Glycine	2.65^{b}	2.60^{c}	2.70^{a}	2.65^{b}	0.007	0.0001
Serine	3.84^{b}	3.71^{c}	3.98^{a}	3.86^{b}	0.017	0.0001
Proline	7.55^{bc}	7.35^{c}	7.90^{a}	7.64^{b}	0.035	0.0001
Alanine	5.66^{b}	5.47^{c}	5.90^{a}	5.70^{b}	0.027	0.0001
Asparagine	4.87^{a}	4.70^{b}	4.97^{a}	4.85^{a}	0.019	0.0001
Glutamine	14.75^{b}	14.22^{c}	15.38^{a}	14.84^{b}	0.078	0.0001
Dig. AA for swine, g/kg						
Methionine	1.46^{ab}	1.41^{b}	1.49^{a}	1.44^{b}	0.006	0.0001
Cysteine	1.50^{bc}	1.47^{c}	1.55^{a}	1.51^{b}	0.005	0.0001
Lysine	1.72^{a}	1.67^{b}	1.73^{a}	1.70^{ab}	0.005	0.0001
Threonine	2.31^{b}	2.25^{c}	2.39^{a}	2.33^{b}	0.009	0.0001
Tryptophan	0.47^{ab}	0.46^{c}	0.47^{a}	0.46^{bc}	0.001	0.0001
Arginine	3.32^{bc}	3.26^{c}	3.41^{a}	3.34^{b}	0.011	0.0001
Isoleucine	2.40^{b}	2.32^{c}	2.50^{a}	2.42^{b}	0.012	0.0001
Leucine	9.06^{bc}	8.71^{c}	9.51^{a}	9.14^{b}	0.052	0.0001
Valine	3.28^{b}	3.19^{c}	3.40^{a}	3.30^{b}	0.014	0.0001
Histidine	2.06^{bc}	2.02^{c}	2.15^{a}	2.08^{b}	0.008	0.0001
Phenylalanine	3.45^{b}	3.31^{c}	3.58^{a}	3.46^{ab}	0.019	0.0001

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

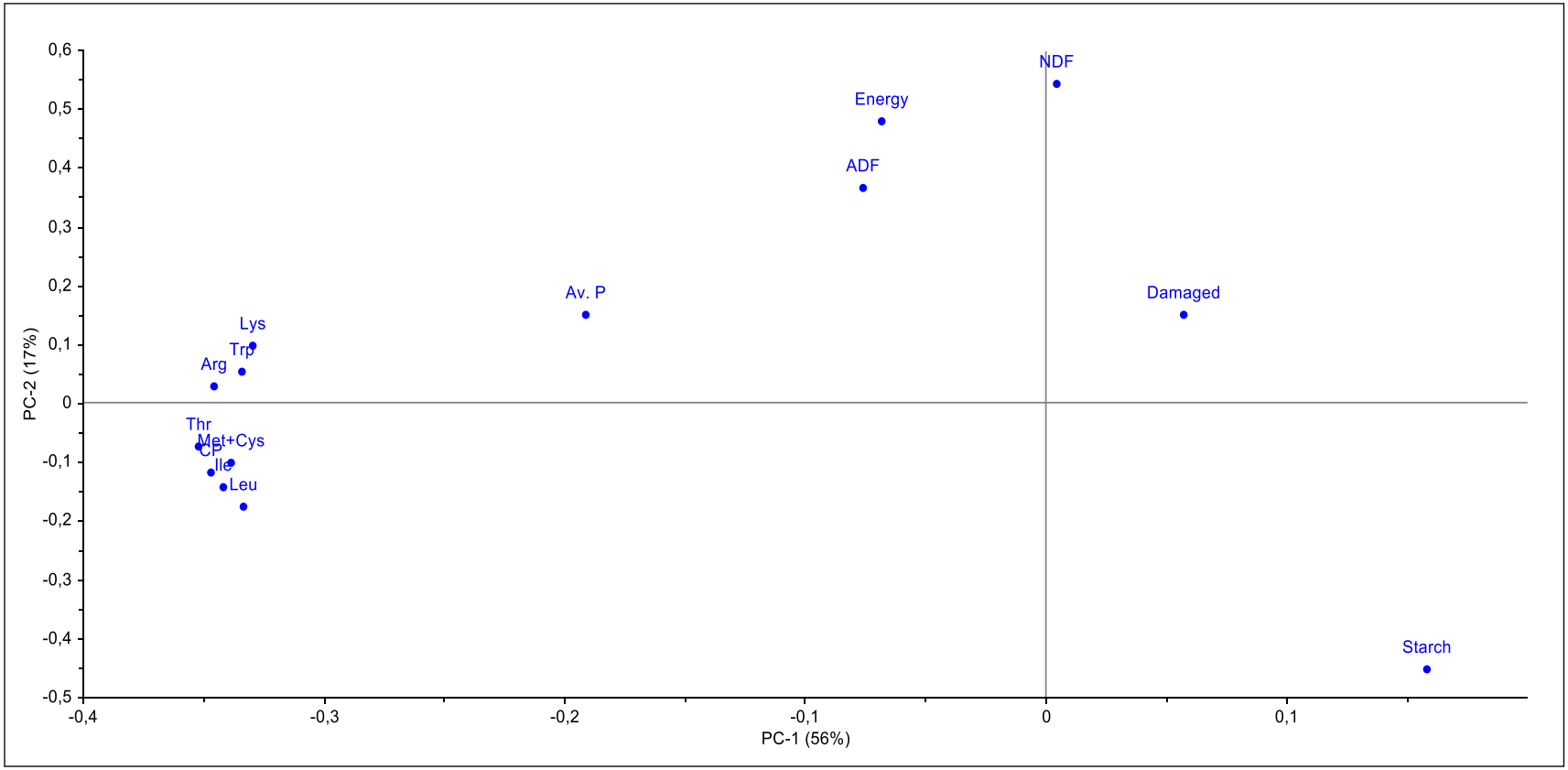

Fig. 1. PCA loadings plot of PC2 vs. PC1 principal components obtained from NIRS prediction of four types of corn (n = 213). Distribution of the analyzed variables along PC1 and PC2. Variables used in the PCA analysis: damaged grains (Damaged), Av. P (Available phosphorus = Total P – Phytic P) in mg/kg, Gross Energy (Energy) in MJ/kg, CP, ADF, NDF, total AA and starch expressed in g/kg.

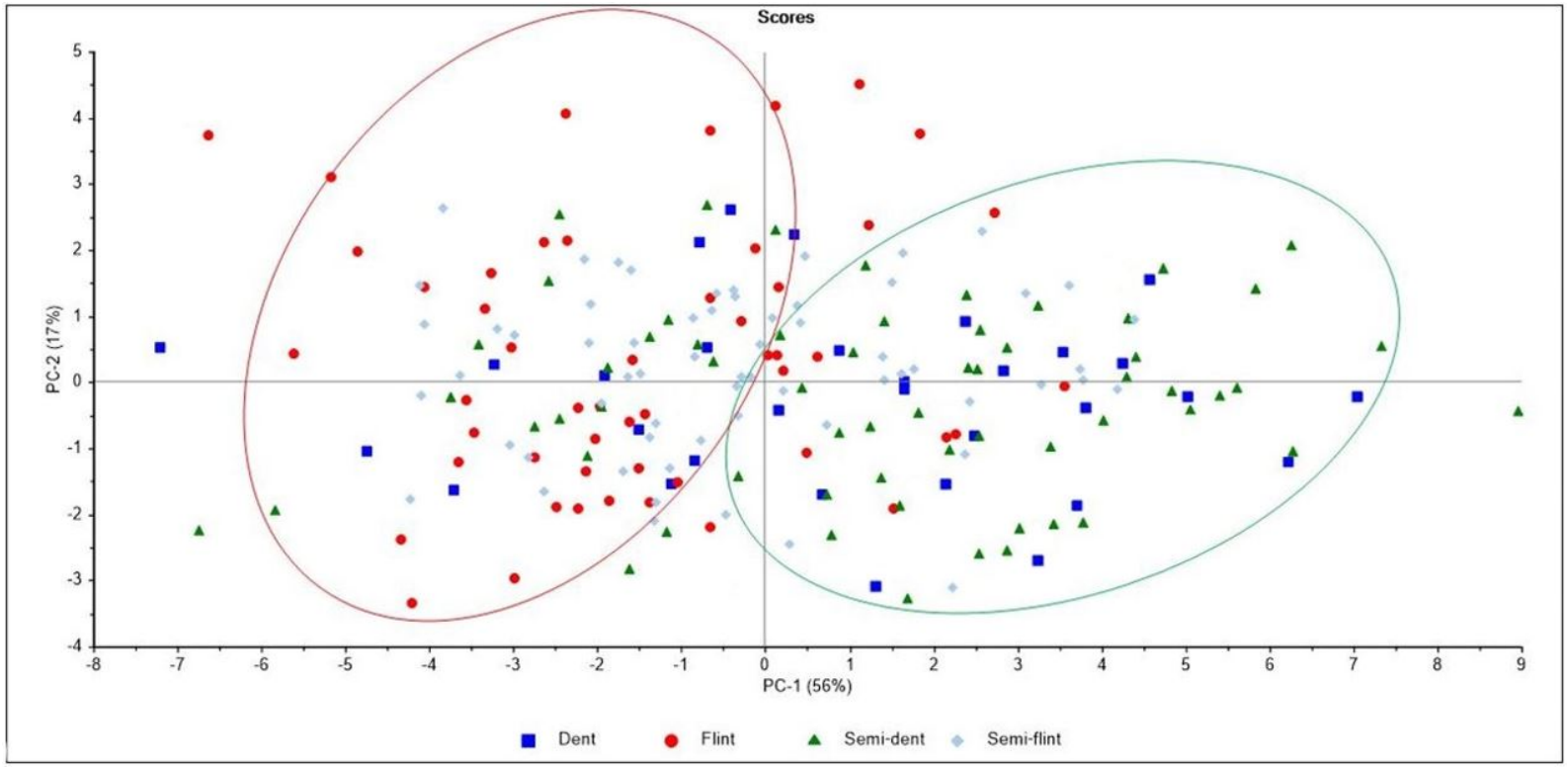

Fig. 2. PCA scores plot of PC2 vs. PC1 principal components obtained from NIRS prediction of four types of corn (n=213). Distribution of the corn types along PC1 and PC2.

6 ARTIGO 2

Este capítulo é apresentado em formato de artigo de pesquisa denominado “A two-year study on the occurrence and concentration of mycotoxins in corn varieties with different endosperm textures” publicado em 2023 no periódico *Journal of the Science of Food and Agriculture,* disponível em: https://doi.org/10.1002/jsfa.12801.

A two-year study on the occurrence and concentration of mycotoxins in corn varieties with different endosperm textures

MYCOTOXINS IN DIFFERENT TYPES OF CORN

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ABSTRACT

Background: Mycotoxin monitoring in cereal grains has great importance in the food and feed industries. This study evaluated mycotoxins contamination in corns with different endosperm textures in two years of cultivation. Samples of dent, semi-dent, flint, and semi-flint corns from field experiments were analyzed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).

Results: Occurrences of fumonisins B_1 (FB_1) and B_2 (FB_2) in 2020 were 45.72% (mean 270 μg kg^{-1}) and 35.89% (94.97 μg kg^{-1}), respectively, and 68.98% (446 μg kg^{-1}) and 45.83% (152 μg kg^{-1}) in 2021. Aflatoxin B_1 occurred 11.96% (0.16 μg kg^{-1}) in 2020 and 11.11% (0.13 μg kg^{-1}) in 2021. In 2020, deoxynivalenol (DON) and zearalenona (ZEA) presented occurrences of 1.28% and 1.70%, with means of 4.08 μg $kg^{-1}$and 2.45 μg kg^{-1}, respectively. In 2021, results were 8.33% (31.00 μg kg^{-1}) for DON and 8.79% (4.38 μg kg^{-1}) for ZEA. Citrinin, diacetoxyscirpenol, and fusarenon-X did not occur in 2020 but presented 1.66%, 0.83%, and 2.50% positive rates in 2021, respectively. In 2020, flint corn presented the lowest concentration of FB_1 whereas dent corn presented the highest concentration of FB_1 and FB_2 ($P < 0.05$). In 2021, dent corn presented the highest means of FB_1, FB_2, and diacetoxyscirpenol ($P < 0.05$). Dent and semi-dent presented the highest concentration of nivalenol ($P < 0.05$).

Conclusion: The endosperm texture influenced mycotoxins contamination in corn grains, especially FB_1 and FB_2, which had the highest concentration in dent corn in the two years of this study.

Keywords: dent, flint, fumonisin, HPLC-MS/MS.

1. INTRODUCTION

Corn (*Zea mays* L.) is one of the most cultivated crops worldwide, being widely used in both animal and human feeding due to its nutritional value. The grain is the second most grown cereal in Brazil and the country is the third largest global corn producer and exporter.[1] In countries with extensive cultivation areas, as Brazil, the quality of this grain can vary depending on several factors such as weather conditions, soil, cultivation practices and grain genotypes.

The endosperm is the storage tissue of corn and its structure has been related to agronomic traits, nutritive values, and grain quality.[2,3] It is basically composed of starch granules which can be arranged in floury and vitreous forms. The floury endosperm comprises loosely packed round starch granules, whereas the vitreous endosperm presents compact and polygonal starch granules linked to the protein matrix of the endosperm.[4] The vitreousness is defined by the ratio between floury and vitreous endosperms in the grains, which is considered to assess the type of the corn. Dent, semi-dent, flint, and semi-flint corn types have been cultivated in Brazil and other countries; for instance, corn grains classified as dent have a higher proportion of floury endosperm, while those classified as flint have a greater proportion of vitreous endosperm.[5]

Due to the intense globalization, corn is commercialized worldwide and the safety of corn-based products has become an issue of widespread interest.[6] Tropical and subtropical climates may favor corn contamination by several mold species, which can damage grains, causing losses in its quality and safety. Thus, the monitoring of mycotoxin contamination is of great value for the assessment of dietary exposure related to corn consumption. Mycotoxins are toxic secondary metabolites naturally produced by different lines of filamentous fungi, such as *Aspergillus, Alternaria*, *Claviceps, Fusarium* and *Penicillium* genera, during cereal growth in the field and postharvest, storage and processing.[7]

Main aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) are classified as carcinogenic and fumonisin B_1 as potentially carcinogenic in humans.[8] Still, AFB_1 is considered the most carcinogenic natural known compound. Furthermore, the nephrotoxic, hepatotoxic and immunosuppressive effects caused by most of mycotoxins are well documented. Therefore, mycotoxins contamination in corn can cause great impact in human and animal health as well as economic losses for both grain and animal production chains due to reduced crop yields, loss of crop value and decrease on performance of production animals.[9]

Within this context, the susceptibility of different types of corn to mycotoxins contamination should be better elucidated and considered as a factor of selection for the utilization of this ingredient and also in the development of new corn varieties. The aim of the present study was to evaluate the effects of different endosperm textures on the occurrence and concentration of mycotoxins in different types of corn produced in Brazil during two consecutive harvests.

2. MATERIALS AND METHODS

2.1 Classification of corn types

Grain texture classification was based on the registration of each commercial corn hybrid in the Brazilian National Cultivar Registry (RNC) from the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) website.[10] The material was separated into four groups according to the grain endosperm texture: dent, semi-dent, flint, and semi-flint. Different commercial corn hybrids of each texture were used in 2020 and 2021. In 2020, a total of 234 corn samples (dent = 36, semi-dent = 66, flint = 57 and semi-flint = 75) were provided, whereas in 2021, a total of 216 corn samples (dent = 36, semi-dent = 56, flint = 44 and semi-flint = 80) were obtained. The commercial names of the hybrids have been withheld to ensure confidentiality.

2.2. Field experiments

Corn samples of both years were sourced from experimental field plots cultivated in the Agricultural Research Center (24º37´18"S, 53º18´20"W, 580 m altitude) of the Cooperativa Agro-industrial Consolata (COPACOL), located in the Paraná State, Brazil. The soil type in the region is dystroferric red latosol. Fertilization of the crops was based on chemical analyses and nutritional requirements of the soil. Data on rainfall, daily average air temperatures (°C) and relative humidity (%) during the two harvests were collected at the weather station located 50 m away from the experimental plots.

Crops of each year were planted in a consolidated no-tillage system under the same soil type and agroclimatic and production conditions. The two field experiments were designed in randomized blocks with three replications of each corn hybrid in 2020 and four replications of each corn hybrid in 2021. In 2020, the crop was planted in the second half of January and the experimental plots consisted of four corn rows spaced 0.68 m apart with 2.72 m in width by 10 m in length. In 2021, the crop was planted in the first half of February and the experimental plots consisted of four corn rows spaced 0.70 m apart with 2.72 m in width by 14 m in length.

Seed treatment was carried out in the same manner for both years with thiodicarb + imidacloprid (Cropstar®, Bayer, Brazil; 300 mL ha^{-1}). For both years, insecticides and herbicides were applied as recommended by the manufacturers, with thiamethoxam + lambda cyhalothrin (EngeoPleno®, Syngenta, Brazil; 250 mL ha^{-1}), mesotrione + atrazine (Calaris®, Syngenta, Brazil; 2 L ha^{-1}), lambda cyhalothrin + chlorantraniliprole (Ampligo®, Syngenta, Brazil; 150 mL ha^{-1}), and spinetoram (Exalt®, Corteva, Brazil; 100 mL ha^{-1}), being applied at V1, V2, V3, and V5 vegetative growth stages, respectively. Harvestings occurred in the second half of June 2020 and in the first half of July in 2021. For both years, the two central lines of

each plot were harvested with the aid of a Wintersteiger® experimental plot harvester-classic model.

2.3. Mycotoxins quantification by HPLC-MS/MS

The sample preparation was carried out following the same methodology in 2020 and 2021. After harvested, samples were dried in a forced air oven at 55 °C for 12 h and sent to the Laboratory of Mycotoxicological Analysis, at Federal University of Santa Maria, Brazil. Samples with 1kg were milled using a 1 mm sieve in an ultra-centrifugal mill, homogenized and then analyzed for the presence and concentration of mycotoxins.

2.3.1 Chemical reagents

Analytical standards for aflatoxins (AF), reported as AFB_1, AFB_2, AFG_1, AFG_2, fumonisins (FUM) B_1 (FB_1) and B_2 (FB_2), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), diacetoxyscirpenol (DAS), 3-acetildeoxynivalenol (3-Ac-DON) ,15-acetildeoxynivalenol (15-Ac-DON), nivalenol (NIV), fusarenon-X (FUSA-X), T-2 toxin (T-2), HT-2 toxin (HT-2), cyclopiazonic acid (CPA), and citrinin (CIT) were acquired from Sigma Aldrich (St. Louis, MO, USA). Methanol, acetonitrile, formic acid, and ammonium acetate (HPLC Grade) were purchased from J.T. Baker (Center Valley, PA, USA). Ultrapure water was obtained from a Milli-Q® Advantage A10 Water Purification System.

2.3.2 Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2)

Analyses of AF were performed using the method described by Mallmann *et al*.[11]. A 5g sample was mixed with 20 mL of acetonitrile:water solution (84:16, v/v) and shaken in a shaking table for 60 min. The resulted extract was spun (Eppendorf − 5804R) at 1,258 x*g*, 20 °C, for 5 min, and then 60 µL were diluted with 840 µL of a methanol:water (1:1, v/v) solution. The obtained solution was placed in a vial for posterior injection of 20 µL into a 1200 Series

Infinity HPLC (Agilent, Palo Alto, U.S.) coupled to a 5500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, U.S.) equipped with an Electrospray ionization (ESI) source in positive mode. Chromatographic separation was performed at 30 °C using an Eclipse XDB-C8 column (4.6´150 mm, 5 µm particle diameter) (Agilent, Palo Alto, CA, U.S.). The mobile phase gradient was composed of methanol:water:ammonium acetate (95:4:1, v/v/v) and water:ammonium acetate (99:1, v/v).

2.3.3 Fumonisins (FB_1 and FB_2)

The FUM were analyzed according to the methodology described by *Mallmann et al.*[11]. A 3g sample was mixed with 15 mL of acetonitrile:water solution (1:1, v/v) and vortexed for 20 min in an orbital shaker. The extract was spun at 1,258 x*g*, 20 °C, for 5 min, and 20 µL were diluted in 980 µL of an acetonitrile:water:formic acid solution (50:40:10, v/v/v). 10 µL of the obtained solution was injected into a 1200 Series Infinity HPLC (Agilent) coupled to an API mass spectrometer 5000 (Applied Biosystems) equipped with an ESI source in positive mode. Chromatographic separation was done at 40 °C using an Eclipse XDB-C8 column (4.6´150 mm, 5 µm particle diameter). The mobile gradient elution phases were composed water:formic acid (95:5, v/v) and acetonitrile:formic acid (95:5, v/v).

2.3.4 Zearalenone and deoxynivalenol

For ZEA and DON analyses, a method proposed by Berthiller *et al.*[12] was adapted. A sample containing 3g was mixed with 24 mL of a methanol:water (70:30, v/v) solution and vortexed for 20 min using an orbital shaker. The resulted extract was spun at 1,258 x*g*, 20 °C, for 5 min, and then 40 µL were diluted in 960 µL of a methanol:water:ammonium acetate solution (90:9:1, v/v/v). A total of 10 µL of the obtained solution was injected into a 1200 Series Infinity HPLC coupled to a 5500 QTRAP mass spectrometer, equipped with an ESI source in

positive mode. Chromatographic separation was carried out at 40 °C with a Zorbax SB-C18 column (4.6′150 mm, 5 μm particle diameter). The mobile gradient elution phases were composed of water:ammonium acetate (90:10, v/v) and methanol:water:ammonium acetate (90:9:1, v/v/v).

2.3.5 Ochratoxin A

A method described by Mallmann *et al.*[11] was used for the analysis of OTA. A 3g sample was mixed with 12 mL of an acetonitrile/water solution/acetic acid (700:290:10, v/v) solution and vortexed in an orbital shaker for 10 min. The extract was spun at 1,258 x*g*, 20 °C, for 5 min, and then 100 μL were diluted in 900 μL of an acetonitrile/water solution (1:1, v/v). The obtained solution was placed in a vial for posterior injection of 10 μL into an Agilent 1200 Series Infinity HPLC coupled to an API mass spectrometer 5000, equipped with an ESI source in positive mode. Chromatographic separation was performed at 40 °C with a Zorbax SB-C18 column (4.6×150 mm, 5 μm). The mobile gradient elution phases were composed of water/formic acid (95:5, v/v) and acetonitrile/formic acid (95:5, v/v).

2.3.6 Diacetoxyscirpenol, 3-Acetildeoxynivalenol, 15-Acetildeoxynivalenol, Nivalenol, Fusarenon-X, T-2 toxin and HT-2 toxin

These TRCs were analyzed via a method proposed by Mallmann *et al.*[11], with adaptations. A sample of 5g was mixed with 20 mL of an acetonitrile/water solution (84:16, v/v), and shaken in a shaking table for 1 h. Then, the extract was spun at 1,258 x*g*, 20 °C, for 5 min and 5 mL was evaporated under a nitrogen flow at 65 °C. Resuspension of the extract was carried out in an acetonitrile/water/acetic acid (840:160:5, v/v) solution; then it was shaken for 1 min. Next, 20 μL from the eluate was subsequently diluted in 980 μL of methanol/water (1:1, v/v) solution, and 10 μL was injected into an Agilent 1200 Infinity HPLC system coupled to an

API mass spectrometer 5000, equipped with an ESI source in positive and negative mode. The mobile gradient elution phases consisted of water/ammonium acetate (99:1, v/v) and methanol/water/ammonium acetate (90:9:1, v/v/v). Chromatographic separation was conducted at 40 °C with the Zorbax SB-C8 column (4.6 × 150 mm, 5 µm).

2.3.7 – Citrinin

For CIT analyses, a method proposed by Oliveira *et al*.[13] was adapted. A 5g sample was mixed with 20 mL of acetonitrile:water:ammonium acetate solution (395:100:5, v/v) and shaken in a shaking table for 90 min. The extract was spun at 1,258 x*g*, 20 °C, for 5 min. After that, 100 µL of each resulted extract were diluted in 900 µL of dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v) and 20 µL of the obtained solution were injected into a 1200 Series Infinity HPLC coupled to a 5500 QTRAP mass spectrometer, equipped with an ESI source in positive mode. Chromatographic separation was done at 40 °C using a Gemini-C18 column (4.6´150 mm, 5 µm particle diameter). The mobile gradient elution phases consisted of methanol/water/ammonium acetate/acetic acid (50:445:2.5:2.5, v/v/v/v) and methanol/water/ammonium acetate/acetic acid (485:10:2.5:2.5, v/v/v/v).

2.3.8 – Cyclopiazonic acid

A method described by Martins-Junior *et al*.[14] was adapted for the analysis of CPA. A 3g sample mixed with 20 mL of acetonitrile:water solution (1:1, v/v) and vortexed for 20 min in an orbital shaker. The extract was spun at 1,258 x*g*, 20 °C, for 5 min, and 100 µL of the resulted extract were then diluted in 900 µL of an acetonitrile:water solution (1:1 v/v), and 20 µL of the obtained solution was injected into a 1200 Series Infinity HPLC coupled to an API mass spectrometer 5000 equipped with an ESI source in positive mode. Chromatographic separation was done at 30 °C using a Luna-C18 column (4.6´150 mm, 5 µm particle diameter).

The mobile phase gradient was composed of solutions of water:formic acid (990:10, v/v) and acetonitrile:formic acid (990:10, v/v).

2.5 Method performance parameters

The limit of quantification (LOQ) and the limit of detection (LOD) were established by means of the signal-to-noise ratio (LOQ = 10/1, LOD = 3/1). Seven spiked replicates of each analyte were analyzed in three different concentration levels so that recovery (%) could be estimated. Linearity of analytical curves was evaluated through the coefficient of determination (R^2); it was calculated after triplicate injections of analytical curves at seven different concentration levels. Analytical curves with a R^2 higher than 0.99 were used. The LOD and LOQ (in μg kg^{-1}) for the assessed mycotoxins were, respectively: 0.4 and 1 for AFB_1; 0.6 and 1 for AFB_2, AFG_1 and AFG_2; 10 and 125 for FB_1; 20 and 125 for FB_2; 3 and 20 for ZEA; 50 and 200 for DON; 0.1 and 2.5 for OTA; 80 and 100 for DAS, 3-Ac-DON,15-Ac-DON, NIV, FUSA-X, T-2 and HT-2; 5 and 10 for CIT; and 5 and 10 for CPA.

2.6 Statistical analysis

Statistical analysis was conducted using the software SAS (SAS Institute). Results of mycotoxins occurrence and concentration of the total amount of corn samples in the two harvests (2020 and 2021) were submitted to descriptive statistics. The contamination of all mycotoxins that presented positive occurrence in each year were transformed by $\log_{10}(x+1)$ and then submitted to analysis of variance using the GLIMMIX procedure. For this analysis, the four corn types (dent, semi-dent, flint and semi-flint) were considered as independent variables. Significance was accepted at 5% and different contamination means of corn types were compared by the Tukey's test.

3. RESULTS

The weather conditions in the two years of evaluation are presented in monthly charts in Fig. 1. Average daily temperature (°C) and relative humidity (%) are presented by month while rainfall (precipitation) is presented as accumulated millimeters (mm) per month. In 2020, the average daily temperature during the crop cultivation was 20.9 °C, with maximum and minimum means per month of 25.1 °C and 17.8 °C, detected in February and May, respectively. The average relative humidity for the entire period was 68.8%; monthly maximum and minimum of 83.6% and 59.9% were recorded in June and April, respectively. The accumulated precipitation during the crop cultivation was 582 mm, with the maximum precipitation detected in May (241 mm) and the minimum in April (28 mm).

In 2021, the average daily temperature during the crop cultivation was 20.3 °C, with maximum and minimum per month of 24.3 °C and 16.5 °C, detected in March and June, respectively. The average relative humidity for the entire period was 71.8%; maximum and minimum of 83.9% and 62.1% were recorded in June and July, respectively. The accumulated precipitation during the crop cultivation was 412 mm, with the maximum precipitation detected in March (223 mm) and the minimum in April (5 mm).

Results of descriptive statistics on mycotoxins occurrence and concentration on the total amount of corn samples are shown in Tables 1 and 2, for 2020 and 2021 harvests respectively. Occurrences and means of FB_1 and FB_2 in 2020 were 45.72% (mean 270 μg kg^{-1}) and 35.89% (mean 94.97 μg kg^{-1}), respectively. In 2021, these results were higher than the previous year, with means of 446 μg $kg^{-1}$and 152 μg $kg^{-1}$for FB_1 and FB_2, respectively. The positive rate in 2021 was 68.98% for FB_1 and 45.83% for FB_2.

Aflatoxin B_1 presented similar results in both years of evaluation: 11.96% of positive rate (mean 0.16 μg kg^{-1}) in 2020 and 11.11% (mean 0.13 μg kg^{-1}) in 2021. AFB_2 presented low positive rate (0.42%) and mean (0.05 μg kg^{-1}) in 2020 while this metabolite did not occur in 2021. In 2020, DON and ZEA presented occurrences of 1.28% and 1.70%, with means of 4.08

μg kg^{-1} and 2.45 μg kg^{-1}, respectively. In 2021, the occurrences and means of these two mycotoxins were higher, being 8.33% (mean 31.00 μg kg^{-1}) for DON and 8.79% (mean 4.38 μg kg^{-1}) for ZEA. Despite CIT, DAS, and FUSA-X did not occur in 2020, these mycotoxins presented 1.66%, 0.83%, and 2.50% of positive rate and means of 0.82 μg kg^{-1}, 0.66 μg kg^{-1}, and 2.66 μg kg^{-1} in 2021, respectively. Finally, OTA, CPA, T2, and HT2 mycotoxins did not occur in any of the years.

Mycotoxins occurrences (%) in corn samples from the two years of evaluation are presented in Fig. 2. The presented mycotoxins had similar positive rates between 2020 and 2021, being the FUM the most prevalent metabolites in both harvests. In 2021, FUM occurrences were higher than in 2020, with an increase of 23.26% for FB_1 and 9.94% for FB_2. The occurrences of DON and ZEA also presented higher rates in 2021 than in 2020, increasing 7.05% for DON and 7.09% for ZEA. Still, from 2020 to 2021 the occurrences of CIT and NIV increased 1.66 and 3.14%, respectively.

Mycotoxins that presented positive occurrences were considered to analyze the mean concentration differences among the four corn types (Table 3). In 2020, flint corn presented the lowest concentration of FB_1 whereas dent corn presented the highest ($P < 0.0001$). Still, corns classified as dent presented higher concentration of FB_2 than corn with semi-dent, flint, and semi-flint textures ($P < 0.05$). Means of AFB_1, DON, ZEA, NIV, 3-Ac-DON, and 15-Ac-DON were not different among types of corn in 2020. In 2021, dent corn presented the highest means of FB_1 and FB_2 ($P < 0.05$). In addition, concentration of NIV was higher in semi-dent than in flint and semi-flint types ($P < 0.05$). Dent corn presented higher concentration of DAS than the other three types ($P < 0.05$). Furthermore, means of AFB_1, DON, ZEA, FUSA-X, 3-Ac-DON, 15-Ac-DON, and CIT were not different among corn types in 2021.

4. DISCUSSION

Fumonisins contamination in corn is of great impact due to their adverse and toxic effects in animals and humans, in addition to economic losses for grain producers and industrial processors. In a case study regarding fumonisin in US corn intended for animal diets, Wu[15] estimated that economic losses through fumonisin in animal feed could range from US$ 1 to 20 million in a normal year and from US$ 31 to US$ 46 million in a year with severe *Fusarium* contamination in crops. Results of FUM detected in the total amount of samples evaluated in the present study were lower than the results found in the survey by Oliveira *et al*.[13], when FB_1 and FB_2 were detected in 100% of corn samples from Paraná State, in Brazil, and the mean contamination of total FB (FB_1 + FB_2) was 3,153 μg kg^{-1}. Ono *et al*.[16] analyzed 870 harvested corn samples and FB_1 was reported in 100% of the samples, while FB_2 was detected in 73.7% of the samples. The lower FUM occurrence and concentration presented herein compared to previous investigations can be explained, in addition to the expected differences among different harvests, by the fact that the samples provided for the current investigation were cultivated in experimental plots and cultivated under suitable agricultural practices.

The occurrence of AF was previously reported as 15.2% in corn samples from Brazil[17] and 19.2% in corn samples from Paraná state in 2017.[13] The slight low concentration of AF found in the present study compared to previous investigations could be related to the absence of a storage period that would enable these mycotoxins production, since corn was harvested and samples were dried, sent to the laboratory and analyzed in a short period.

The frequency of DON contamination found in the present study (1.28% in 2020 and 8.33% in 2021) did not differ from a previous study conducted by Souza *et al*. [18] in Brazil. These authors reported DON in 4% of corn samples, with a maximum level of 30 μg kg^{-1}. Besides the most known mycotoxins, results from the present study indicate that NIV contamination in corn produced in Brazil must be monitored, since this mycotoxin was present in 6.86% and 10% of the samples from 2020 and 2021, respectively. Oliveira *et al*. [13] found

superior results from those of the present study, where 75.6% of the corn samples from the south region of Brazil were contaminated with NIV.

The remaining mycotoxins investigated herein did not present great results in terms of occurrence and concentration levels. Abdallah *et al*.[19] also observed low prevalence of OTA, ZEA, 3-Ac-DON, 15-Ac-DON, and CIT in corn samples. In contrast, Mahdjoubi *et al*.[20] reported the occurrence of T-2, CIT, and FUS-X mycotoxins in 100%, 83%, and 80% of the corn samples evaluated in their study conducted in Algeria, respectively. Possible reasons for divergence of results between studies conducted in different countries may be the cultivate conditions in each region, the climatic differences between them and the types of grain cultivated, which can contribute to the fungal development and production of mycotoxins in corn.

Similar to the results from the current investigation, previous studies have shown differences on mycotoxins occurrences and mean concentration between different years of evaluation, especially with regard to FUM concentration.[6, 21] Thus, the distinct results on FUM occurrences among 2020 and 2021 could be correlated to the differences observed in the climatic conditions between the experimental harvests.

In the present study, it was possible to observe a difference in the rainfall between the two years; 2020 had 170 mm higher accumulated precipitation during the crop cultivation than 2021. An important drought was observed around the corn silking stage in 2021, with only 5 mm of accumulated precipitation observed in the third month of cultivation (April), while, in 2020 the accumulated precipitation around this period was 52 mm (May). In one report from the United States, FUM concentrations were observed to be inversely proportional to rainfall before silking (correlation coefficient between FUM level and mean monthly precipitation: -0.779).[22] Still, de La Campa *et al.*[23] observed that the highest levels of FUM in their study were measured at locations with dry and warm weather around silking and the lowest levels were

measured at locations with rainfall or adequate moisture during this period. Considering this, it is possible that the lower the rainfall during the silking stage of a corn crop, the higher could be the FUM concentration in the harvested grains and this impact can be observed through the results from the present study.

The differences in FUM contamination between distinct types of corn were significant in the two years of this survey. These results are possibly related to their differences in the proportion of floury to vitreous endosperms, since corns classified as dent presented higher means of FUM concentration than the other types, and the flint corn, with the most vitreous endosperm, presented the lowest FB_1 concentration in 2020. The present outcomes are in parallel with the statement of Huntington[24], who defended that starch granules from the floury endosperm may be more susceptible to external forces, because the protein matrix that surrounds them is relatively thin, with blank spaces and lower density. The structural difference of the dent grain endosperm, compared to corns with a higher proportion of vitreous endosperm, may be related to the higher levels of FUM observed in dent corn. Moreover, according to Sampietro *et al*.[25], the tolerance to cracking of grains indirectly contributes to resistance against pathogens by preventing wounds that can act as infection spots for *F. verticillioides* and other mycotoxigenic fungi.

The main purposes of the present study were to determine if the endosperm texture could exert influence in the occurrence of multiple mycotoxins in different types of corn and to assess whether the results between two years of harvest in the same area were different. The data reported herein indicated differences on mycotoxins occurrence in corns cultivated in the same region but in two different years, which are probably due to climatic fluctuations, especially the rainfall. Moreover, our results suggest that the higher the proportion of floury endosperm in corn grains the higher could be the FUM levels in it, since dent corns presented higher concentrations of FB_1 and FB_2 than flint corns in both cultivation years. The results from the

present study fill part of the information gap in published data by revealing variations in mycotoxins concentration, especially fumonisins, in different types of corn.

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Table 1. Mycotoxins occurrence and concentration (μg kg^{-1}) in samples of corn cultivated in 2020

Mycotoxin[†]	Number of analyses	Positive rate, %	Maximum (μg kg^{-1})	Mean (μg kg^{-1})	Standard deviation	Mean of positive samples (μg kg^{-1})	Standard deviation
AFB_1	234	11.96	2.20	0.16	0.45	1.34	0.34
AFB_2	234	0.42	1.20	0.05	0.08	1.20	.
AFG_1	234	0.00	.	< LOQ[‡]	.	< LOQ	.
AFG_2	234	0.00	.	< LOQ	.	< LOQ	.
OTA	126	0.00	.	< LOQ	.	<LOQ	.
CIT	126	0.00	.	< LOQ	.	<LOQ	.
CPA	126	0.00	.	< LOQ	.	< LOQ	.
ZEA	234	1.70	428	2.45	28.47	155	191
FB_1	234	45.72	4,810	270	511	592	620
FB_2	234	35.89	1,670	94.97	188	265	233
DON	234	1.28	529	4.08	40.16	345	165
DAS	102	0.00	.	< LOQ	.	< LOQ	.
3-Ac-DON	102	5.88	216	12.88	51.24	214	1.37
15-Ac-DON	102	3.92	231	6.27	33.13	156	69.09
T-2	102	0.00	.	< LOQ	.	< LOQ	.
HT-2	102	0.00	.	< LOQ	.	< LOQ	.
FUSA-X	102	0.00	.	< LOQ	.	< LOQ	.
NIV	102	6.86	316	10.98	44.15	157	73.92

[†]AFB_1 = aflatoxin B_1; AFB_2 = aflatoxin B_2; AFG_1 = aflatoxin G_1; AFG_2 = aflatoxin G_2; OTA = ochratoxin A; CIT = citrinin; CPA = cyclopiazonic acid; ZEA = zearalenone; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; DON = deoxynivalenol; DAS = diacetoxyscirpenol; 3-Ac-DON = 3-Acetildeoxynivalenol; 15-Ac-DON = 15-Acetildeoxynivalenol; T-2 = T-2 toxin; HT-2 = HT-2 toxin; FUS-X = fusarenon-X; NIV = nivalenol.

[‡]LOQ = limit of quantification.

Table 2. Mycotoxins occurrence and concentration (μg kg^{-1}) in samples of corn cultivated in 2021

Mycotoxin †	Number of analyses	Positive rate, %	Maximum (μg kg^{-1})	Mean (μg kg^{-1})	Standard deviation	Mean of positive samples (μg kg^{-1})	Standard deviation
AFB_1	216	11.11	2.20	0.13	0.37	1.13	0.31
AFB_2	216	0.00	.	< LOQ‡	< LOQ	< LOQ	< LOQ
AFG_1	216	0.00	.	< LOQ	< LOQ	< LOQ	< LOQ
AFG_2	216	0.00	.	< LOQ	< LOQ	< LOQ	< LOQ
OTA	120	0.00	.	< LOQ	< LOQ	< LOQ	< LOQ
CIT	120	1.66	81.00	0.82	7.58	49.70	44.68
CPA	120	0.00	.	< LOQ	< LOQ	< LOQ	< LOQ
ZEA	216	8.79	185	4.38	18.05	47.90	39.31
FB_1	216	68.98	3,740	446	610	660	641
FB_2	216	45.83	1,530	152	249	328	276
DON	216	8.33	1,330	31.00	131	361	292
DAS	120	0.83	80.00	0.66	7.30	80.00	.
3-Ac-DON	120	3.33	112	3.52	19.21	106	3.78
15-Ac-DON	120	5.83	607	17.05	79.86	289	183
T-2	120	0.00	.	< LOQ	< LOQ	< LOQ	< LOQ
HT-2	120	0.00	.	< LOQ	< LOQ	< LOQ	< LOQ
FUSA-X	120	2.50	125	2.66	16.95	107	15.24
NIV	120	10.00	683	33.28	122	335	224

†AFB_1 = aflatoxin B_1; AFB_2 = aflatoxin B_2; AFG_1 = aflatoxin G_1; AFG_2 = aflatoxin G_2; OTA = ochratoxin A; CIT = citrinin; CPA = cyclopiazonic acid; ZEA = zearalenone; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; DON = deoxynivalenol; DAS = diacetoxyscirpenol; 3-Ac-DON = 3-Acetildeoxynivalenol; 15-Ac-DON = 15-Acetildeoxynivalenol; T-2 = T-2 toxin; HT-2 = HT-2 toxin; FUS-X = fusarenon-X; NIV = nivalenol.

‡LOQ = limit of quantification.

Table 3. Means of mycotoxins concentration (μg kg^{-1}) in different types of corn – 2020 and 2021

Mycotoxins, μg kg^{-1}	Types of corn				SEM	*P*-value
	Dent	Semi-dent	Flint	Semi-flint		
2020						
AFB_1	0.05	0.16	0.14	0.18	0.029	0.7191
FB_1	712[a]	259[b]	150[c]	252[bc]	32.12	0.0001
FB_2	230[a]	84.7[b]	60.4[b]	92.3[b]	11.85	0.0001
DON	0.00	0.00	5.52	6.53	2.520	0.6460
ZEA	0.00	6.48	0.40	1.52	1.786	0.9089
NIV	14.05	13.00	15.73	13.00	4.414	0.5000
3-Ac-DON	22.68	8.88	19.52	0.00	5.123	0.4126
15-Ac-DON	12.83	0.00	2.66	9.62	3.312	0.5585
2021						
AFB_1	0.12	0.09	0.13	0.14	0.026	0.3011
FB_1	1,019[a]	377[b]	454[b]	390[b]	43.84	0.0395
FB_2	358[a]	131[b]	146[b]	133[b]	17.89	0.0256
DON	23.19	26.69	24.19	37.60	9.379	0.9911
ZEA	5.56	7.21	0.62	3.80	1.286	0.1074
NIV	25.17[a]	67.31[a]	0.00[b]	7.25[b]	11.04	0.0372
FUSA-X	7.81	0.00	2.40	2.09	1.535	0.6381
3-Ac-DON	0.00	7.24	3.86	2.21	1.739	0.7036
15-Ac-DON	7.31	19.63	25.59	13.62	7.339	0.4597
DAS	5.00[a]	0.00[b]	0.00[b]	0.00[b]	0.661	0.0163
CIT	0.00	2.89	0.00	2.89	0.690	0.3478

[a-c] Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

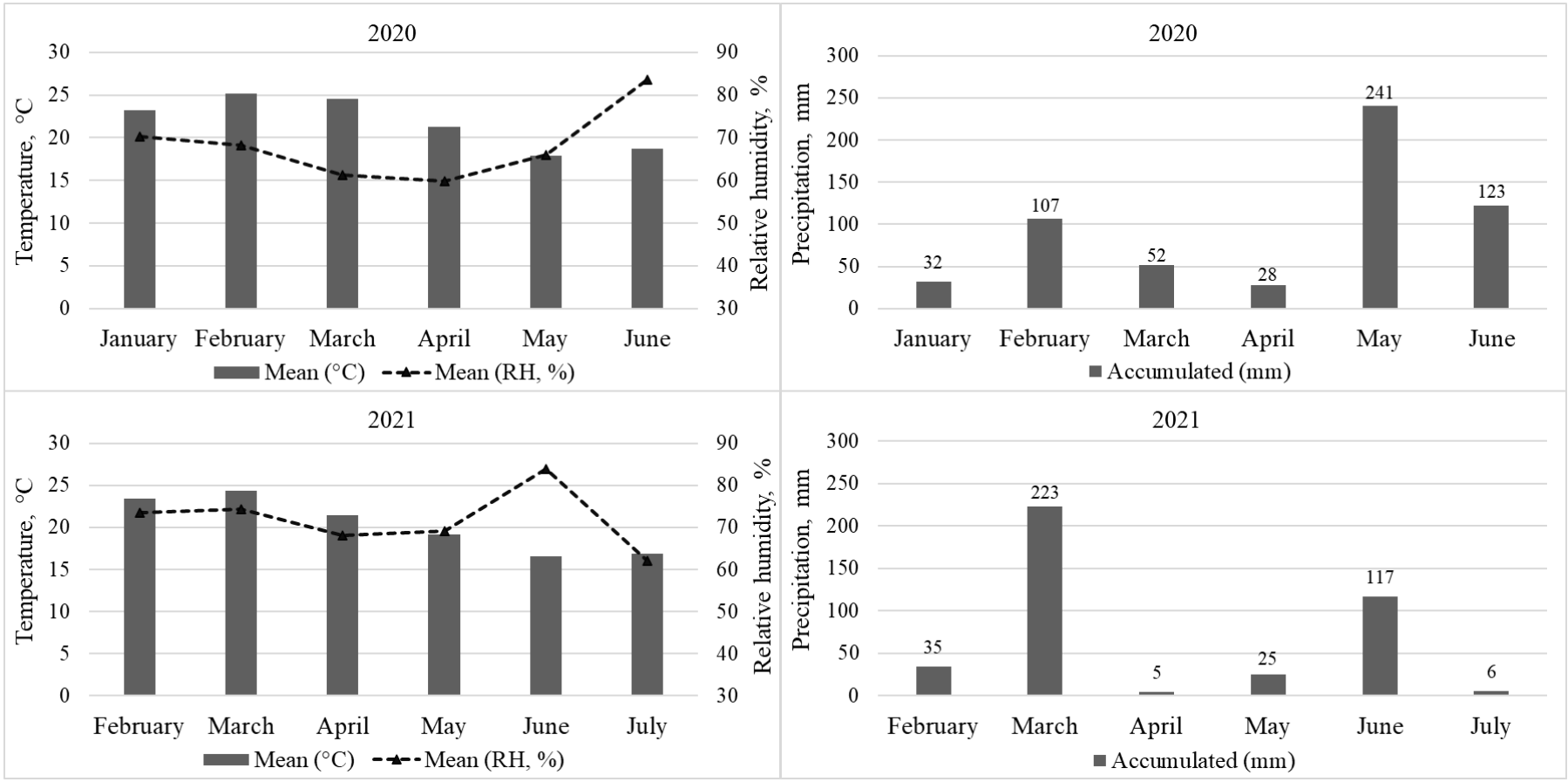

Fig. 1. Climatic conditions during the crop's cultivation of different types of corn in 2020 and 2021.

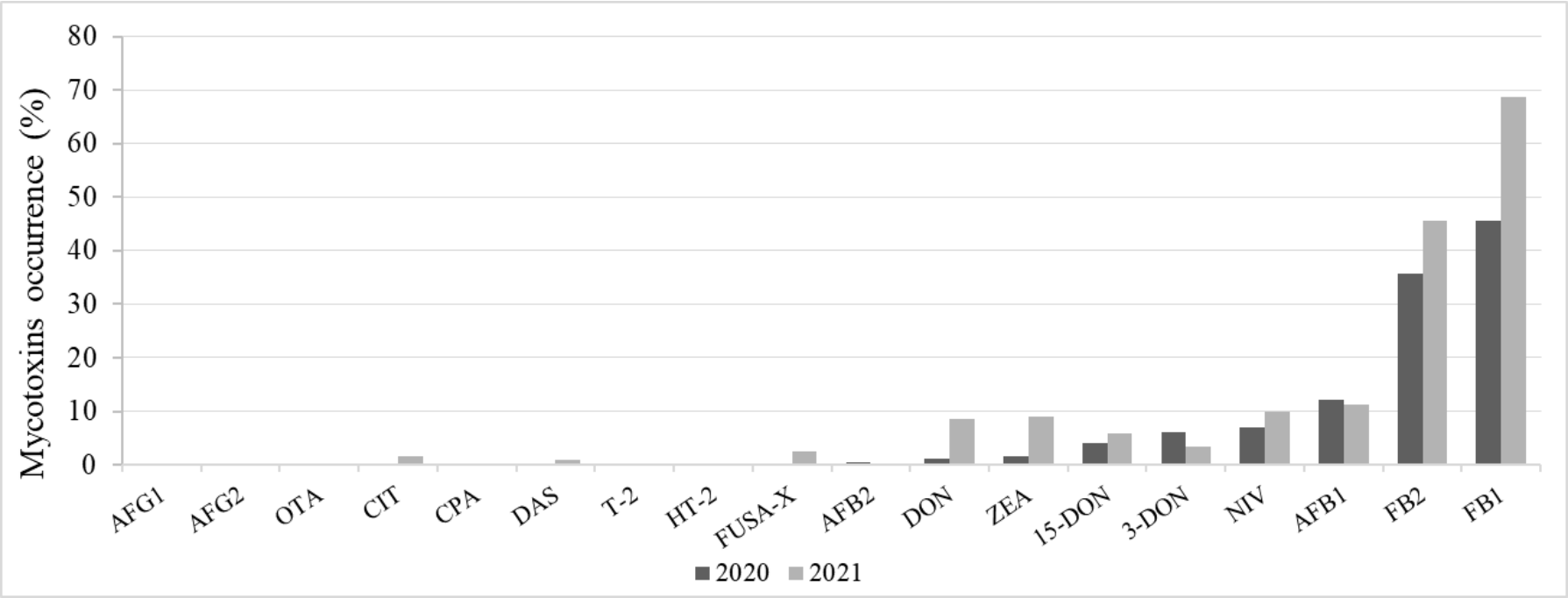

Fig. 2. Mycotoxins occurrences (%) in corn samples in 2020 and 2021.

7 ARTIGO 3

Este capítulo é apresentado em formato de artigo *short communication* denominado “A study on the relationship of corn grain hardness with mycotoxins contamination and nutrients composition” que será submetido ao periódico *Journal of Cereal Science.*

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A study on the relationship of corn grain hardness with mycotoxins contamination and nutrients composition

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1. Introduction

Corn (*Zea mays* L.) is one of the most cultivated crops in the world, with a global production exceeding 1.15 billion tons in the 2022/2023 harvest season (USDA, 2024). The grain is commercialized worldwide and its quality and safety is a matter of common interest. Corn and other cereal grains cultivated especially in countries with tropical and subtropical climates are frequently contaminated by mycotoxins, which are toxic secondary metabolites naturally produced by different lines of filamentous fungi, such as *Aspergillus*, *Fusarium* and *Penicillium*. The toxic and carcinogenic effects caused by most of mycotoxins are well documented and commonly related to a great impact on human and animal health as well as economic losses (CAST, 2003, Munkvold et al., 2019).

Considering that distinct corn varieties are produced worldwide, an investigation on the factors involved in the composition and quality of corn is essential for the development of new genotypes and might be helpful to continuously improve the utilization of this valuable ingredient. Previous studies already correlated the hardness of corn and wheat grains with their physicochemical properties (Lee et al., 2005, Saenz et al., 2021, Kaliniewicz et al., 2023); however, there is still a lack of published data assessing the interaction of corn hardness not only with the nutritional composition but also with mycotoxins contamination. Therefore, this study was conducted to evaluate the relationship between corn grain hardness and the concentration of mycotoxins, nutrient values and energy composition.

2. Materials and methods

A total of 80 samples of different corn hybrids were obtained from the same experimental field plots conducted in Parana state, southern Brazil. The commercial corn hybrids used in the present study are classified as dent (n = 6), flint (n = 10), semi-dent

(n = 28) and semi-flint (n = 36) types based on their registration in the Brazilian National Cultivar Registry (MAPA, 2024). After harvested, samples were dried in a forced air oven at 55 °C for 12 h and sent to the Laboratory of Mycotoxicological Analysis (LAMIC) at Federal University of Santa Maria, Brazil.

2.1. Mycotoxins quantification by HPLC-MS/MS

Samples with 1 kg were milled using a 1 mm sieve in an ultra-centrifugal mill and homogenized. Mycotoxins analyses were performed using the methods described by Mallmann et al. (2020).

For total aflatoxins (AFT = AFB_1 + AFB_2 + AFG_1 + AFG_2), a 5g sample was added to a Falcon tube with 20 mL of acetonitrile:water solution (84:16, v/v) and shaken in a shaking table at 120 rpm for 60 min. The resulted extract was spun (Eppendorf − 5804R) at 2,500 rpm, 20 °C, for 5 min, and then 60 µL were diluted with 840 µL of a methanol:water (1:1, v/v) solution. The mobile phases were composed of methanol:water:ammonium acetate (95:4:1, v/v/v) and water:ammonium acetate (99:1, v/v). For total fumonisins (FBT = FB_1 + FB_2), a 3g sample was added to a Falcon tube with 15 mL of acetonitrile:water solution (1:1, v/v) and vortexed for 20 min in an orbital shaker. The extract was spun at 2,500 rpm, 20 °C, for 5 min, and 20 µL were then diluted in 980 µL of an acetonitrile:water:formic acid solution (50:40:10, v/v/v). The mobile phases were composed of water:formic acid (95:5, v/v) and acetonitrile:formic acid (95:5, v/v).

Zearalenona (ZEA) and deoxynivalenol (DON) analyses were conducted with 3g of sample that were added into a tube with 24 mL of methanol:water (70:30, v/v) solution and vortexed for 20 min using an orbital shaker. The extract was spun at 2,500 rpm, 20 °C, for 5 min, and then 40 µL were diluted in 960 µL of methanol:water:ammonium

acetate solution (90:9:1, v/v/v). The mobile phases were composed of water:ammonium acetate (90:10, v/v) and methanol:water:ammonium acetate (90:9:1, v/v/v).

For AFT, FBT, DON and ZEA, finished samples were injected in a 1200 Series Infinity HPLC (Agilent, Palo Alto, US) coupled to a 5500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, U.S) equipped with an Electrospray ionization (ESI) source in positive mode. For AFT and FBT, chromatographic separation was performed at 30 °C using an Eclipse XDB-C8 column (Agilent, Palo Alto, CA, US) and for DON and ZEA, chromatographic separation was carried out at 40 °C with a Zorbax SB-C18 column (Agilent). The limits of detection (LOD) and quantification (LOQ) (in μg/kg) were, respectively: 0.4 and 1 for AFB_1; 0.6 and 1 for AFB_2, AFG_1 and AFG_2; 10 and 125 for FB_1; 20 and 125 for FB_2; 3 and 20 for ZEA; and 50 and 200 for DON.

2.1. NIRS nutritional predictions

Samples (500 g) were milled with a 0.5 mm sieve in an ultra-centrifugal mill (RETSCH®, model ZM 200), placed in plastic bags and left for 15 min to reach room temperature (18 °C to 22 °C) and humidity (40% to 60%). Subsequently, manual homogenization of each sample was performed for two min using circular movements. Nutritional analyses were carried out by reading the samples spectra via NIRS. The spectral data were originated from a Bruker® equipment, model Tango-R, using the calibration curves from the AMINONRG® and AMINONir® programs (Evonik GmbH, Hanau, DE). The wavelength ranged from 3,952 to 11,536 cm^{-1}, and the rotating sphere macro sample was the cell type used for solid samples. Subsequently, the corn samples were predicted for the following variables: dry matter (DM, %), crude protein (CP, %), ether extract (EE, %), ash (%); crude fiber (%), starch (%), total P (%), total amino acids (AA, %), and gross energy (GE, kcal/kg).

2.3 Texture analysis of corn

The hardness of corn was determined on a texture analyzer (TA.XT plus, Stable Micro Systems Ltd., UK) using a cylindrical probe (36 mm diameter). Ten randomly chosen corn grains from each sample were individually placed horizontally on the platform and compressed (1 mm distance) until breakage at a test speed of 2 mm/s with a trigger force of 20kg. Hardness was calculated as the applied force in kg required to break the corn grain by compression (kgf). After texture analyses, corn grains were separated in three different groups according to their hardness: Group 1 (< 52 kgf), group 2 (52 ≤ kgf < 54) and group 3 (≥ 54 kgf).

2.4 Statistical analysis

Statistical analysis was conducted using the software SAS v.9.4. Data from corn groups were subjected to ANOVA using the GLM procedure and means were separated by the Tukey's test at 5% significance. Mycotoxins means were transformed by log10(x+1) prior to ANOVA. The Pearson's correlation test was performed among corn hardness, mycotoxins, and nutrient composition.

3. Results and Discussion

The different corn hybrids used in the present study are classified as dent, flint, semi-dent and semi-flint types, and presented the average hardness of 52.04, 54.50, 53.03, and 53.37 kgf, respectively. The type of corn is classified according to its endosperm texture. The endosperm is divided into floury, which comprises loosely packed spherical starch granules, and the vitreous endosperm, which presents compact and polygonal starch granules linked to the protein matrix (Piovesan et al., 2011). Corn grains have both floury and vitreous endosperms and their ratio (vitrousness) determines the hardness of the grain.

The average corn hardness observed in the present study was 53.5 kgf, with minimum and maximum values of 49.7 and 56.6 kgf, respectively. Corn samples were

separated in 3 groups according to the hardness (group 1: < 52 kgf; group 2: 52 ≤ kgf < 54; and group 3: ≥ 54 kgf). The effects of corn hardness on nutritional composition can be observed in Table 1. According to previous researches (Lee et al., 2005, Alvarez-Iglesias et al., 2021, and Simões et al., 2023a), the hardness of the corn grain is expected to affect its protein content. Similarly, in the present study, group 3 presented higher ($P < 0.05$) CP, Met, Cys, Thr, Iso, Leu, and Val than groups 1 and 2. There was no effect ($P > 0.05$) of corn hardness on starch, GE, total P, EE, ash, and crude fiber contents.

Contamination of AFT, DON and ZEA were similar ($P > 0.05$) among corn hardness groups; however, group 3 presented lower ($P < 0.05$) FBT contamination than groups 1 and 2 (Table 1). The endosperm texture of corn grains has already been associated with mycotoxins occurrence by Simões et al. (2023b). The authors observed that dent corn (with higher proportion of floury endosperm) presented higher concentration of FB_1 and FB_2 than flint corn (with higher proportion of vitreous endosperm) in two consecutive harvests. These findings agree with the present study, where corns with hardness higher than 54 kgf presented lower FBT concentration than corns with hardness lower than 52 kgf. The structural difference in the endosperm among the evaluated grains herein may be related to the higher levels of FBT observed in corns with lower hardness. Huntington (1997) reported that the protein matrix surrounding starch granules from the floury endosperm is relatively thin, with blank spaces and lower density and it may be more susceptible to external forces, as fungi colonization in the grain. Moreover, according to Sampietro et al. (2013) the resistance to cracking, which should be higher as the grain hardness increase, indirectly contributes to resistance against pathogens such as *F. verticillioides* and other mycotoxigenic fungi.

The Pearson's correlation values obtained among different variables are presented in Figure 1. Corn hardness had a negative correlation ($P < 0.05$) with FBT (-0.39),

indicating that corns with harder texture may present higher resistance to fumonisins contamination. Ponce-Garcia et al. (2020) found a negative correlation (-0.56) between corn hardness and colony-forming units (CFU) of *F. verticillioides*, however, these authors did not find correlation between the hardness of the grain and the concentration of fuminisins. Hardness also presented a negative correlation ($P < 0.05$) with starch (-0.23) and a positive correlation ($P < 0.05$) with CP (0.43), total Met (0.46), Lys (0.35), Thr (0.44) and Trp (0.35). There was no significant correlation ($P > 0.05$) between corn hardness and values of AFT, DON, ZEA, and total P.

Overall, results from the present study suggested that the higher the hardness of the grain, the higher might be its protein and amino acids content and the lower its fumonisins contamination levels. Such findings can be used as supplementary information in corn genetic programs, thereby contributing to improve the utilization of this important ingredient.

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Table 1. Effect of corn hardness on nutrient composition and mycotoxins average.

Item, %	Corn hardness group[1]				
	Group 1	Group 2	Group 3	SEM	*P*-value
Starch	66.7	66.3	65.9	0.149	0.1312
Total P	0.202	0.208	0.208	0.001	0.3964
Ether extract	3.51	3.68	3.39	0.038	0.0646
Ash	1.22	1.25	1.25	0.007	0.4063
Crude fibre	1.92	2.03	1.98	0.020	0.1574
Crude protein	8.42[b]	8.52[b]	9.05[a]	0.081	0.0014
Methionine	0.168[b]	0.171[b]	0.183[a]	0.001	0.0008
Cystine	0.184[b]	0.186[b]	0.200[a]	0.001	0.0001
Lysine	0.241[b]	0.248[ab]	0.251[a]	0.001	0.0185
Threonine	0.294[b]	0.298[b]	0.316[a]	0.002	0.0012
Tryptophan	0.062[b]	0.063[ab]	0.064[a]	0.001	0.0315
Arginine	0.377[b]	0.385[ab]	0.400[a]	0.002	0.0025
Isoleucine	0.288[b]	0.293[b]	0.314[a]	0.003	0.0008
Leucine	1.029[b]	1.040[b]	1.151[a]	0.014	0.0003
Valine	0.393[b]	0.399[b]	0.423[a]	0.003	0.0008
Gross energy, kcal/kg	3,993	4,007	3,999	2.400	0.0641
[2]AFT, µg/kg	0.43	1.19	0.45	0.140	0.2383
[3]DON, µg/kg	177	149	371	51.11	0.3644
[4]FBT, µg/kg	1,234[a]	2,126[a]	621[b]	177.7	0.0038
[5]ZEA, µg/kg	173	129	281	50.17	0.8521

[a-b] Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Group 1: < 52 kgf; Group 2: 52 ≤ kgf < 54; and group 3: ≥ 54 kgf

[2]AFT: Total aflatoxin ($AFB_1 + AFB_2 + AFG_1 + AFG_2$).

[3]FBT: Total fumonisin ($FB_1 + FB_2$).

[4]DON: Deoxynivalenol.

[5]ZEA: Zearalenone.

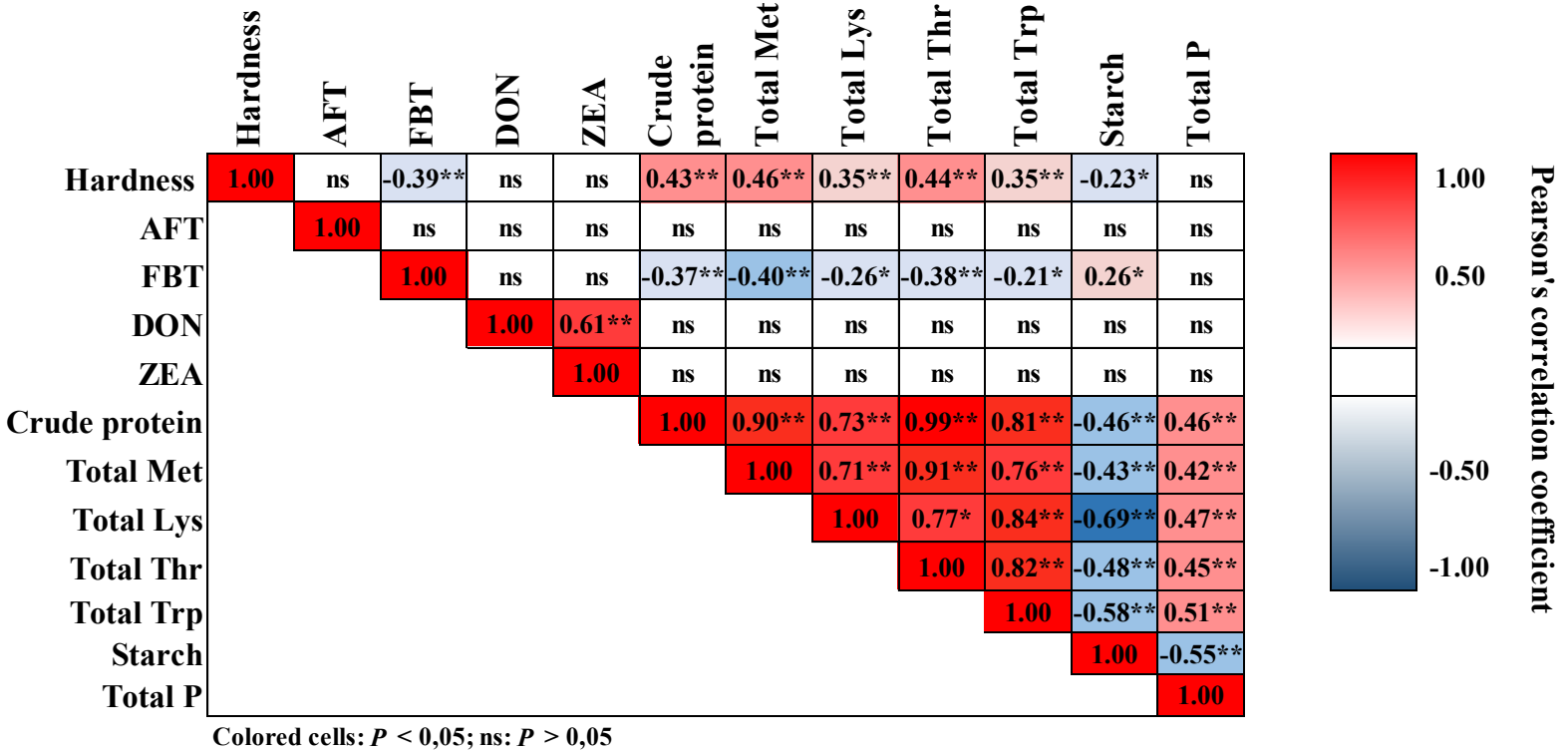

Figure 1. Correlation between corn hardness and other variables.
*$P \leq 0.05$; **$P \leq 0.01$; ns: $P > 0.05$.

8 CONSIDERAÇÕES FINAIS

Como demonstrado ao longo destes estudos, a textura do grão de milho influenciou grande parte das variáveis estudadas, em especial a contaminação dos grãos por fumonisinas, que foi significativamente mais elevada no milho dentado. Além disso, os resultados indicam que o milho duro, apesar de apresentar um menor rendimento a campo, possuí maior concentração de proteína bruta e aminoácidos do que os milhos dentado, semidentado e semiduro. Estes resultados sugerem que um alto rendimento à campo não está necessariamente associado a uma melhor qualidade dos grãos e tais características podem apresentar importante influência na utilização dessa matéria-prima na nutrição animal.

Embora já tenham sido desenvolvidos trabalhos comparando diferentes híbridos de milho, pouco conhecimento tem sido gerado correlacionando a composição, o rendimento à campo e a ocorrência de micotoxinas em milhos com diferentes texturas de endosperma. As diferenças encontradas entre os tipos de milho, assim como o impacto observado da dureza do grão sobre algumas variáveis investigadas, compõem um material técnico e científico com informações que podem ser utilizadas pelos diferentes segmentos da cadeia produtiva e consumidora de milho. Um diferencial importante deste trabalho reside no fato de que foram cultivadas as genéticas de milho mais comercializadas na região do estudo em cada safra, sob as condições de manejo adotadas na realidade do campo, trazendo ao trabalho uma abordagem mais próxima da realidade da agroindústria brasileira.

Em saúde pública e na medicina veterinária preventiva, a ciência desempenha um papel importante no fornecimento de informações cruciais sobre as micotoxinas e sua toxicidade, além de dados de ocorrência e avaliação de novas metodologias de diagnóstico. Com um aperfeiçoamento científico contínuo e estabelecendo uma comunicação e parceria eficazes entre os centros de investigação, as universidades e a indústria, é possível reduzir os impactos negativos causados pela contaminação por micotoxinas nos alimentos de consumo animal.

O conhecimento da matriz nutricional dos ingredientes utilizados na formulação de rações, neste caso dos diferentes tipos de milho, é essencial para que sejam produzidas rações que atendam às exigências nutricionais de cada espécie animal, otimizando também os custos de formulação. Sendo o milho o principal componente das dietas formuladas no Brasil para aves e suínos, conhecer a sua composição, nas mais diferentes genéticas comercializadas, é um ponto chave para que a indústria brasileira de proteína animal mantenha sua posição de destaque no cenário mundial.

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