

FUNGUS DAMAGE EFFECT ON PHYSICAL-CHEMICAL CHARACTERISTICS OF CORN GRAINS

(efeito dos danos causados por fungos sobre as características físico-químicas de grãos de milho)

Paula Carvalho Leal¹, Andréia Massuquetto¹, Marley Conceição dos Santos¹, Luis Daniel Giusti Bruno²,
Everton Luis Krabbe³, Ananda Portella Felix¹, Alex Maiorka¹.

¹Universidade Federal do Paraná, Setor de Ciências Agrárias, Departamento de Zootecnia, Curitiba-PR, Brasil.

²Universidade Estadual do oeste do Paraná, Centro de Ciências Agrárias, Campus Marechal Cândido Rondon-PR, Brasil.

³Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA Suínos e Aves, Concórdia-SC, Brasil.

*Corresponding author: marleyconceicao@gmail.com

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ABSTRACT - The objective of this study was to analyze the physical-chemical characteristics of corn lots with different ratios of damaged to intact grains, considering the interaction between them. Recently harvested corn grains were selected, and grains attacked by fungi (fermented and moldy) and intact grains were separated to establish the following grain mixtures (treatments): 1 - 0% fermented/moldy and 100% intact grains; 2 - 10% fermented/moldy and 90% intact grains; 3 - 20% fermented/moldy and 80% intact grains; 4 - 30% fermented/moldy and 70% intact grains; and 5 - 40% fermented/moldy and 60% intact grains. Samples from each treatment were analyzed for dry matter (DM), ash (A), crude protein (CP), crude fiber (CF), ether extract (EE), and gross energy (GE) contents, water activity (aw), density, and hardness. Chemical composition and density data were submitted to analysis of regression, hardness values were compared by Tukey test ($P < 0.05$), and Pearson's correlations ($P < 0.05$) between chemical and physical parameters were calculated. Increasing ratios of moldy corn relative to intact corn reduced DM, CF, EE, and GE contents, increased A, CP, Wa values, and reduced the density of the lot. Moldy grains presented lower hardness values compared with intact corn. This study shows that fungus damage affects corn grain physical-chemical properties.

Key words - chemical composition; density; fungi; hardness; water activity.

RESUMO - O objetivo deste trabalho foi analisar as características físico-químicas de lotes de milho com diferentes proporções de grãos danificados e grãos intactos, considerando a interação entre eles. Foram selecionados grãos de milho colhidos recentemente, e foram separados os grãos atacados por fungos (fermentados e mofados) e os intactos para estabelecer as seguintes misturas de grãos (tratamentos): 1 - 0% fermentados/mofados e 100% grãos intactos; 2 - 10% de grãos fermentados/mofados e 90% de grãos intactos; 3 - 20% de grãos fermentados/mofados e 80% de grãos intactos; 4 - 30% de grãos fermentados/mofados e 70% de grãos intactos; e 5 - 40% de grãos fermentados/mofados e 60% de grãos intactos. Amostras de cada tratamento foram analisadas para matéria seca (MS), matéria mineral (MM), proteína bruta (PB), fibra bruta (FB), extrato etéreo (EE) e conteúdo de energia bruta (EB), atividade de água (Aw), densidade e dureza. Os dados de composição química e densidade foram submetidos à análise de regressão, os valores de dureza foram comparados pelo teste de Tukey ($P < 0,05$) e as correlações de Pearson ($P < 0,05$) entre os parâmetros químicos e físicos foram calculadas. O aumento das proporções de milho mofado em relação ao milho intacto reduziu os conteúdos de MS, FB, EE e EB, aumentou os valores de MM, PB, aw e reduziu a densidade do lote. Os grãos mofados apresentaram valores de dureza inferiores em comparação com o milho intacto. Este

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estudo mostra que os danos dos fungos afetam as propriedades físico-químicas dos grãos de milho.

Palavras-chave - bromatologia; densidade, fungos, dureza, atividade de água.

INTRODUCTION

Corn is the main feedstuff of Brazilian broiler diets. Therefore, good corn quality is essential to formulate diets that allow birds to express their maximum genetic potential to achieve the best live performance and to ensure food safety. Different physical and chemical properties may be used to define corn grain quality.

Corn grains with undesirable or questionable physical quality are often found in feed mills, and require the correction of feed formulas, which, however, is rarely made (Rodrigues, 2009). Moreover, the nutritional value of corn grains is related to their chemical composition, which is essential for dietary balance (D'Agostini et al., 2004).

Several factors influence grain composition, such as origin, variety, processing, climate, soil, presence of pests, etc. These factors may also affect corn physical properties, such as density, hardness, and grinding resistance (Moore et al., 2008).

Poultry diets may contain a diverse microbiota derived from multiple environmental sources. When corn is physically damaged, there is a larger surface area of contact of the contaminating microorganisms with the substrates needed for their development, accelerating their replication, and leading to the production of harmful toxins (Maciorowski et al., 2007). This vulnerability to contamination is explained by the high nutritional quality of corn grain, which consists predominantly of starch (> 600 g/kg) (Weurding et al., 2001) but it also contains considerably amounts of fat, which is an important substrate for fungal species.

The nutritional value of grains contaminated with fungi (mycotoxin-producing or not) is reduced to changes in their chemical composition, as the bioavailability of some nutrients is decreased (Rostagno, 1993), particularly of fat (Krabbe, 1995).

Therefore, the objective of the present study was to evaluate the effects of fungal infestation on the physical-chemical characteristics of corn grains.

MATERIAL AND METHODS

Corn grains were selected immediately after harvesting (no storage period) from a same field located in the Campos Gerais region, state of Parana, Brazil, in order to prevent possible regional and cultivar differences. Grains were separated in grains attacked by fungi (fermented/moldy grains) and healthy grains (intact grains), according to Brazilian regulations (Administrative Ruling 845 and 11; MAPA, 1976, 1996).

The grains considered moldy were those that more than 25% of their total area presented color changes or visible fermentation in the entire germ area or any part of the endosperm. Fermented grains presented dark spots of any size, visible to the naked eye, in up to 25% of its area (approximately corresponding to the germ area). In this experiment these two categories of grains were considered as one, as they both have the same origin: fungi attack. Intact grains did not show any changes and normal color and were considered healthy. Grains with any other type of damage, such as those cracked and soft, were excluded from the experiment.

The five treatments consisted of different ratios of fermented/moldy (no defined proportion) and intact grains, as follows: 1 – 0% fermented/moldy and 100% intact grains; 2 - 10% fermented/moldy and 90% intact grains; 3 - 20% fermented/moldy and 80% intact grains; 4 - 30% fermented/moldy and 70% intact grains; and 5 - 40% fermented/moldy and 60% intact grains.

Each treatment included six replicates of a 500 g sample each. Corn samples were homogenized, ground to 0.5 mm particle size, and analyzed for dry matter (DM), ash (A), crude protein (CP), crude fiber (CF), and acid-hydrolysis ether extract (AHEE) contents, according to the AOAC (1995). Gross energy (GE) was determined in a bomb calorimeter (Ika Werke®, model C2000 basic). Water activity (aw) was read in intact, non-ground grains in a digital device (Rotronic®, model Hygropalm AW1) in a temperature-controlled room (between 21°C and 25°C).

Density was determined in a specific equipment (Motomco®) coupled to a scale (Marte®), which weighs a volume 1325 mL and automatically delivers the density value of the sample. Hardness was analyzed in 50 grains of each corn class (intact, fermented and moldy grains) using a hardness tester (Nova Ética®, model 298 DGP), and the results are expressed in kilograms-force (kgf).

Data were submitted to analysis of variance (ANOVA), using SAS (2004) statistical package. When results were significant, analysis of regression was applied to chemical, density, and water activity data. Means were compared by the test of Tukey ($P < 0.05$). Chemical and physical parameters were submitted to Pearson correlation analysis ($P < 0.05$).

RESULTS AND DISCUSSION
Table 1 - Physical-Chemical characteristics of corn grains with different ratios of damaged to intact grains.

| | Fermented and moldy grains (%) | | | | | R ² |
|---------------------------|--------------------------------|-------|-------|-------|-------|----------------|
| | 0 | 10 | 20 | 30 | 40 | |
| Dry Matter % | 89.9 | 89.7 | 89.6 | 89.5 | 89.4 | 0.993 |
| Density kg/m ³ | 747 | 742 | 730 | 724 | 718 | 0.980 |
| Ash % | 0.86 | 0.88 | 0.90 | 0.91 | 0.92 | 0.969 |
| Crude Protein % | 6.52 | 6.54 | 6.55 | 6.60 | 6.66 | 0.907 |
| Crude Fiber % | 1.447 | 1.419 | 1.345 | 1.190 | 1.117 | 0.947 |
| Ether Extract % | 3.930 | 3.585 | 3.430 | 3.270 | 3.210 | 0.925 |
| Gross Energy kcal/kg | 4038 | 4018 | 3950 | 3948 | 3946 | 0.818 |
| Water Activity (aw) | 0.651 | 0.654 | 0.659 | 0.658 | 0.665 | 0.915 |

0– Intact corn (0% fermented/moldy and 100% intact grains); 10 - 10% fermented/moldy and 90% intact grains; 20 - 20% fermented/moldy and 80% intact grains; 30 - 30% fermented/moldy and 70% intact grains; and 40 - 40% fermented/moldy and 60% intact grains.

Dry matter: $Y = -0.011X + 86.4$ ($P < 0.05$); Ash: $Y = 0.001X + 0.8984$ ($P < 0.05$); Crude protein: $Y = 0.003X + 6.504$ ($P < 0.05$); Crude Fiber: $Y = -0.008X + 1.481$ ($P < 0.05$); Ether Extract: $Y = -0.017X + 3.835$ ($P < 0.05$); Gross Energy: $Y = -2.55X + 4031$ ($P < 0.05$); Density: $-0.7517X + 747$ ($P < 0.05$); aw: $0.0003X + 0.6511$ ($P < 0.05$).

Dry matter content was reduced as the percentage of fermented/moldy grains increased (Table 1), which is consistent with the findings of Dilkin et al. (2000). According to Buiate et al. (2006), the DM content of moldy corn is reduced because fungi utilize its nutrient reserves. The corn density was also reduced as the percentage of fermented/moldy grains increased; demonstrating that corn nutritional value is proportional to its density (Pereira et al., 2008). Silva et al. (2008) showed that higher-density corn presents higher energy values due to its higher starch content.

The progressive increase in ash and CP contents (Table 1) as the proportion of fermented and moldy grains increase is justified by a proportional reduction in the content of other nutrients, such as starch (Silva et al., 2008; Rodrigues, 2009), and fat (Krabbe, 1995).

Limitations of the analysis may justify the progressive reduction in CF content as the ratio of fermented/moldy grains increased (Table 1). Some soluble components, such as resistant starch, may be retained as insoluble fiber, and are accounted as CF (Mañas et al., 1994).

The highest ratio of grains infested with fungi reduced AHEE content in the analyzed corn samples (Table 1). This effect was previously detected by Rodrigues (2009), who also observed EE content reduction in the presence of moldy corn grains. This may be explained by the fact that fat is the main target of fungi (Krabbe, 1995). Fat readily

provides a large amount of the energy required by these microorganisms, in addition of being concentrated in the germ, which is easily attacked by fungi.

Results showed that GE content decreased as the ratio of fermented/moldy grains increased in the analyzed samples (Table 1). Fungus infestation reduces the energy values of feedstuffs, particularly due to their consumption of fat. However, corn mixtures with ratios of 20, 30, and 40% of fermented/moldy grains presented very close dry matter values, which may be attributed to the fact that the *aw* content is more important for mold growth than the moisture itself.

Total water content or humidity of feedstuffs is represented by free water (*aw*) and that bound to the substrate. Free water affects food stability as it participates in chemical, physical, and biological reactions (Timmons, 2006), determining the development, death, survival, sporulation, and production of toxins by different microorganisms (Marcinkowski, 2006). The increase in free-water content in the evaluated corn grain mixtures, as measured by *aw* (Table 1) was proportional to the ratio of moldy grains, because the higher the presence of free water in the grain, the higher the activity, respiration, and free water production by fungi.

The results of the correlation between chemical (on DM basis) and physical parameters are shown in Table 2. Chemical parameters were correlated with physical parameters, except for CP x EE and GE, and CF x GE ($P > 0.05$). The presence of fermented and moldy grains was correlated ($P < 0.05$) with all analyzed parameters and was positive for A and CP contents and *aw*, and negative for DM, CF, AHEE, and GE contents and density. These results support the regression analysis findings.

Table 2 - Correlations between physical and chemical parameters of corn grains.

| | DM | A | CP | CF | EE | GE | D | <i>aw</i> |
|----|----------|---------|--------|---------|---------------------|---------------------|---------|---------------------|
| FM | -1.00*** | 0,98** | 0,95* | -0,97** | -0,96** | -0,90* | -0,99** | 0,95* |
| DM | - | -0,98** | -0,95* | 0,96* | 0,96* | 0,89* | 0,98** | -0,97** |
| A | - | - | 0,89* | -0,93* | -0,99** | -0,95* | -0,99** | 0,94* |
| CP | - | - | - | -0,98** | -0,85 ^{NS} | -0,74 ^{NS} | -0,92* | 0,90* |
| CF | - | - | - | - | 0,89* | 0,83 ^{NS} | 0,96** | -0,88* |
| EE | - | - | - | - | - | 0,92* | 0,95* | -0,89* |
| GE | - | - | - | - | - | - | 0,95* | -0,87 ^{NS} |
| D | - | - | - | - | - | - | - | -0,95* |

FM: % of fermented and moldy grains; DM: dry matter; A: ash; CP: crude protein; CF: crude fiber; EE: ether extract; GE: gross energy; D: density; *Wa*: water activity.

There was a negative and highly significant correlation between the presence of grains attacked by fungi with DM content, also showing that this parameter was strongly affected by microorganisms. The higher the DM content, the higher the density, and CF, AHEE, and GE contents, and the lower the A and CP contents and aw of the samples. When A was correlated with the other chemical and physical parameters, the results were opposite to those found for DM.

The CP level of the analyzed samples was positively correlated ($P < 0.05$) only with the increasing presence of fungus-attacked grain, A content, and aw. There was a positive correlation ($P < 0.05$) between AHEE content and GE values: as AHEE content decreased with the increasing ratio of damaged grains, more energy was lost.

The positive correlation determined showed that grains with higher GE content are denser. On the other hand, there was a negative correlation between density and aw, showing that mixtures with higher ratios of fungus-attacked grains presented lower density and higher aw, which promotes fungus development.

Hardness results of intact, fermented, and moldy grains, shown in Table 3, indicate that intact and fermented grains were harder than moldy grains ($P < 0.05$).

Table 3 - Corn grains hardness as a function of its physical quality.

| Grain physical quality | Hardness (kgf) ¹ |
|------------------------|-----------------------------|
| Intact corn grains | 19.16 ^a |
| Fermented corn grains | 18.35 ^a |
| Moldy corn grains | 13.80 ^b |

¹Means in the same column are different by the test of Tukey ($P < 0.05$).

The "vitreous" aspect of corn endosperm is related with its amylopectin content and hardness. According to Batal and Parsons (2004), softer corn grains contain a relatively higher proportion of amylopectin, and therefore, are easier to grind. However, in the present study, the lower hardness of moldy corn grains was due to the physical destruction of the grain caused by fungus attack, particularly to the endosperm, which is the hardest part of the grain. The hardness of the intact grains was not different from that of fermented grains possibly because the latter presented a less advanced stage of fungus proliferation than the moldy grains, i.e., the contaminated area frequently did not extend beyond the germ (the "soft" part of the grain) and did not affect the endosperm.

It must be noted that the fermented and moldy grains presented different chemical and physical characteristics compared with intact grains, demonstrating that fungi consume nutrients and, consequently, changes grain structure and composition.

CONCLUSION

The regression analysis indicates that in the presence of each 1% of fermented/moldy grains, corn dry matter, crude fiber, and acid-hydrolysis ether extract contents are reduced in 0.011%, 0.008 %, and 0.017 %, respectively, and ash and crude protein increased 0.001% and 0.003%, respectively. In addition, the presence of each 1% of fermented/moldy grains reduces corn gross energy content in 2.55 kcal/kg and density in 0.752 kg/m³ and increases water activity in 0.0003. Moldy corn grains present lower hardness compared with fermented and intact corn grains.

INFORMATIVE NOTES

All experimental procedures complied with the Ethics Committee on the Use of Animals of the Federal University of Paraná.

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